The International Journal of Life Cycle Assessment
https://doi.org/10.1007/s11367-023-02209-2

LCA FOR ENERGY SYSTEMS AND FOOD PRODUCTS

Life cycle assessment of microalgal cultivation medium: biomass, glycerol, and beta-carotene production by *Dunaliella salina* and *Dunaliella tertiolecta*

Gleison de Souza Celente[1,2,3] · **Rosana de Cassia de Souza Schneider**[1,2] · **Jennifer Julich**[1] · **Tiele Medianeira Rizzetti**[1,2] · **Eduardo Alcayaga Lobo**[1] · **Yixing Sui**[3]

Received: 24 May 2023 / Accepted: 12 July 2023

Abstract

Purpose *Dunaliella* is a halophilic genus of microalgae with high potential in the global food market. The microalgal cultivation process contributes to not only economic impact but also environmental impact, especially regarding the artificial medium composition. In this context, a life cycle assessment was carried out to analyze the impacts associated with the components of the modified Johnson medium (MJM) and to predict the best scenarios to cultivate *Dunaliella tertiolecta* and *Dunaliella salina* for biomass, glycerol, and beta-carotene production.

Method Two chains were analyzed separately: (1) *Dunaliella salina* (strain DF 15) cultivated in 8 scenarios combining different nitrogen (0.1 and 1.0 g L^{-1} KNO_3) and magnesium (1.1–2.3 g L^{-1} $MgCl_2.6H_2O$) concentrations to produce biomass, glycerol, and beta-carotene and (2) *Dunaliella tertiolecta* (strain CCAP 19/30) cultivated in 5 scenarios combining different nitrogen (0.1 and 1.0 g L^{-1} KNO_3) and salt (116.9–175.4 g L^{-1} sea salt) concentrations to produce biomass and glycerol. In addition, we evaluated the potential of cultivating these species to reduce the carbon footprint of the proposed scenarios.

Results and discussion For *D. salina*, S_5 (1 g L^{-1} KNO_3, 1.1 g L^{-1} $MgCl_2.6H_2O$) had the lowest environmental damage for biomass (74.2 mPt) and glycerol production (0.95 Pt) and S_3 (0.1 g L^{-1} KNO_3, 1.9 g L^{-1} $MgCl_2.6H_2O$) for beta-carotene (3.88 Pt). T_4 (1 g L^{-1} KNO_3, 116.9 g L^{-1} sea salt) was the best for *D. tertiolecta* for biomass (74 mPt) and glycerol (0.49 Pt). “Respiratory inorganics,” “Non-renewable energy,” and “Global warming” were the most impacted categories. “Human health,” “Climate change,” and “Resources” had the highest share of all damage categories. All the scenarios presented negative carbon emission after proposing using brine as alternative salt source: S_5 was the best scenario (− 157.5 kg CO_2-eq) for *D. salina* and T_4 for *D. tertiolecta* (− 213.6 kg CO_2-eq).

Conclusion The LCA proved its importance in accurately predicting the optimal scenarios for MJM composition in the analyzed bioproducts, as confirmed by the Monte Carlo simulation. Although the absolute values of impacts and productivity cannot be directly compared to large-scale cultivation, the validity of the LCA results at this scale remains intact. Productivity gains could outweigh the impacts of “surplus” MJM components. Our study showcased the potential of combining *D. salina* and *D. tertiolecta* cultivation with CO_2 capture, leading to a more environmentally friendly cultivation system with a reduced carbon footprint.

Keywords Life cycle assessment · *Dunaliella salina* · *Dunaliella tertiolecta* · Modified Johnson medium · Medium optimization · Carbon footprint

1 Introduction

Microalgae have a long history of being proposed as a food source (Kay and Barton 1991). They offer an attractive alternative to conventional land plants due to their high protein content and less land requirement for their cultivation (Vanthoor-Koopmans et al. 2013). Microalgae are also known to produce valuable bioproducts for applications in the food industry (Dufossé et al. 2005; Matos 2017), such as glycerol (Monte et al. 2020) for emulsifiers, shorteners (Morrison 2000), and edible food packaging (Atta et al. 2022), and pigments for natural coloring (Dufossé et al. 2005). *Dunaliella* is a halophilic genus worldwide known

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Communicated by Tim McAllister.

Extended author information available on the last page of the article for its pharmaceutical and nutraceutical benefits owing to the production of active compounds (de Souza Celente et al. 2022). Their biomass use is commonly associated with obesity control (Melnikov et al. 2022), anti-inflammatory (Wang et al. 2022), and anti-cancer (Chen et al. 2021) activity. Currently, their use for human consumption is still limited in Europe. Yet, its generally recognized as safe (GRAS) status granted by the US Food and Drug Administration (US Food & Drug Administration 2020) and wide acceptance in Asia as a conventional food source indicate their promising global market potential. In 2020, the food and pharmaceutical sectors shared approximately 75% of the global *D. salina* market share, i.e., 67.9 M USD (Maia Research 2021).

Along with cultivation cost (Colusse et al. 2020) and restrictive/non-existent legislation (Harvey and Ben-Amotz 2020), consumer acceptability is a major bottleneck for commercializing microalgae-based products (Nova et al. 2020). This consumer behavior, known as food neophobia (i.e., repulsion for non-traditional food), can make it difficult for novel food products to be introduced to the market (García-Segovia et al. 2020). Fortunately, the scenario for microalgae as food has been steadily improving (García-Segovia et al. 2020) as "green labeling" largely aids in publicizing the benefits of microalgae consumption for human health and the environment. Nonetheless, such "green" marketing can also be misleading (Ihemezie et al. 2018). For instance, the cultivation process, especially regarding the usage of artificial medium, can contribute to a great environmental impact due to the consumption of non-renewable resources, such as fossil energy, water, and macronutrients (Chen et al. 2015). Thomassen et al. (2018) evaluated the environmental impact of different hypothetical large-scale scenarios to cultivate *D. salina* for beta-carotene, and cultivation had the greatest impact among all the steps analyzed: cultivation, pre-harvest, harvest, washing, drying, extraction, and purification. Unlike unconventional cultivation media such as wastewater, artificial and semi-artificial media using natural water and artificial nutrient supply offer better-controlled and replicable conditions for microalgae cultivation (Bauer et al. 2021). In this way, microalgal biomass and bioproduct productivities are often increased, which is a reliable approach to lessening the environmental, social, and economic impacts of artificial media (Chen et al. 2015; Bauer et al. 2021; Kabir et al. 2022). It can be expected that improving microalgal biomass and bioproduct productivities by optimizing the cultivation media could potentially reduce the environmental impacts.

Unlike an economic analysis, evaluating a certain process's environmental and social impact is not straightforward, requiring a more complex and dedicated tool. Life cycle assessment (LCA) is a widely accepted approach to categorizing environmental loads based on inputs and outputs. It helps to compare different scenarios to identify the best method for a common issue/goal and processes that still need improvement (Guinée 2002). LCA converts different aspects (inputs and outputs) into easier-to-interpret data, facilitating decision-making (Sun et al. 2019). However, LCA of *Dunaliella* sp. cultivation is addressed by only a few papers (e.g., Thomassen et al. (2018) and Keller et al. (2017)). So far, this is the first approach addressing different artificial medium compositions to optimize bioproduct yield by two *Dunaliella* species and improve the environmental aspect. In this context, an LCA was conducted to analyze the impacts of using artificial medium and predict the best scenarios to cultivate *D. salina* and *D. tertiolecta* for biomass, glycerol, and beta-carotene production on a laboratory-scale. Two chains were analyzed separately: (1) *D. salina* cultivated in eight scenarios combining different nitrogen and magnesium concentrations to produce biomass, glycerol, and beta-carotene, and (2) *D. tertiolecta* cultivated in five scenarios combining different nitrogen and salt concentrations to produce biomass and glycerol. Since *D. tertiolecta* is not carotenogenic, beta-carotene was not assessed for this species. In addition, the potential for carbon footprint reduction following this LCA has been evaluated, and the hypothetical use of brine from a desalination plant was analyzed.

2 Methodology

2.1 LCA goal, scope, and boundaries

A gate-to-gate assessment was performed for the cultivation of *D. salina* DF 15 and *D. tertiolecta* CCAP 19/30 to produce biomass, glycerol, and beta-carotene (only for *D. salina*) following ISO 14044 (2006) guidelines. The life cycle impact analysis (LCIA) was performed using SimaPro software version 8.5 considering 1 kg of biomass/bioproduct produced (functional unit). The method chosen for the LCIA was Impact 2002+ (Humbert et al. 2012). Table 1 shows the impact categories and the equivalent unit. The impact analysis was shown as normalized and non-normalized results. In normalized results, a value is attributed to each category quantifying the respective share to the overall damage. In non-normalized results, the value of 100% is attributed for a specific category to the scenario with the highest impact, and for the remaining scenarios, the impact is calculated relative to the former scenario. Only impact categories with values greater than 1×10^{-3} (after normalization) were selected for the figures and tables in the result section to improve readability (except for the Monte Carlo simulation), as their contribution was

Table 1 Analyzed impact categories, their equivalent units, and resulting damage categories using Impact 2002+ (SimaPro 2020)

Impact categories	Unit	Damage categories
Carcinogens	kg C_2H_3Cl eq	Human health
Non-carcinogens	kg C_2H_3Cl eq	
Respiratory inorganics	kg $PM_{2.5}$ eq	
Respiratory organics	kg C_2H_4 eq	
Ionizing radiation	Bq C_{-14} eq	
Ozone layer depletion*	kg CFC_{-11} eq	
Aquatic ecotoxicity	kg TEG water	Ecosystem quality
Terrestrial ecotoxicity	kg TEG soil	
Terrestrial acidification/ nutrification	kg SO_2 eq	
Land occupation	m^2 org.arable	
Aquatic acidification	kg SO_2 eq	
Aquatic eutrophication	kg PO_4 P-lim	
Global warming	kg CO_2 eq	Climate change
Non-renewable energy	MJ primary	Resources
Mineral extraction	MJ surplus	

*Also accounting for the "Ecosystem quality" damage category

irrelevant compared with the other categories (the complete list of categories is shown in Table 1).

Each chain (*D. salina* and *D. tertiolecta* cultivation) was assessed individually for its proposed scenarios (Tables 2 and 3) through a specific inventory of input/output, as the goal was to compare the scenarios within each chain. The LCA included only the impacts associated with the production of chemicals for the artificial medium (modified Johnson medium (MJM) (Borowitzka (1988); see Table 4 for details), retrieved directly from Ecoinvent 3.6 and Agri-footprint databases; the contribution of infrastructure and electricity for the equipment used for cultivation, biomass recovery, and bioproduct extraction were not included in this scope.

2.2 Lifecycle inventory (LCI)

The LCI data was derived from the Ecoinvent 3.6 and Agri-footprint databases, and the inputs and outputs were obtained from laboratory-scale experiments. Some components in the medium were not found in the LCA databases, requiring replacement by similar compounds (Table 4). Scenarios within the same chain were cultivated using the same cultivation equipment; thus, energy and infrastructure inputs were disregarded for better visualization and comparison between scenarios. Furthermore, as the cultivation was done on a laboratory-scale, the energy impact would be overestimated and would not reflect what would happen on a large-scale. The only variables were the chemical composition of the medium and productivity. The LCA was limited to the composition of the artificial medium and productivity influence. As will be discussed in the third section, NaCl (substitute of sea salt in the inventory) had a major contribution to most of the analyzed impact categories; thus, to validate this LCA results, the hypothetical use of brine from desalination plants was analyzed (provided in the Supplementary Material).

2.3 Carbon footprint

"Global warming" contribution was further discussed to identify the possibility of using the two species' biomass to fix carbon to compensate for the CO_2-eq emissions of the proposed scenarios and reduce the carbon footprint associated with cultivation in an artificial medium. It was considered that CO_2 could replace $NaHCO_3$ without changing the biomass yield based on our previous experiment (Celente et al. 2022). A 40% carbon content based on dry weight (DW) and a 1.83 g CO_2 captured per g of biomass ratio were hypothesized (Acién Fernández et al. 2012). The hypothetical assumption of replacing sea salt with brine from desalination plants was proposed and analyzed for the

Table 2 Different scenarios for *D. salina* cultivation and their respective inputs and outputs

Inputs/outputs	Scenarios							
	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Concentration of varied MJM components								
KNO_3	0.1	0.1	0.1	0.1	1	1	1	1
$MgCl_2.6H_2O$	1.1	1.5	1.9	2.3	1.1	1.5	1.9	2.3
Productivity								
Biomass (mg L^{-1} day^{-1} AFDW)	58	58	67	63	108	84	108	85
Glycerol (mg L^{-1} d^{-1})	5.31	5.71	7.94	6.09	8.21	8.05	8.47	5.86
Beta-carotene (mg L^{-1} d^{-1})	3.43	3.93	3.96	3.94	2.06	1.65	1.89	1.30

D. salina ($3.3–3.4 \times 10^4$ cell mL^{-1} initial cell density) was cultivated in 50-mL Erlenmeyer flasks containing 20 mL of MJM with the proposed modifications. The flasks were kept in a temperature-controlled chamber (Varicon Aqua, Worcester, UK) at 25 °C and approximately 100-µmol photons m^{-2} s^{-1} continuous LED white light for 18 (S_1–S_4) or 25 (S_5–S_8) days. Data were obtained from our previous (unpublished) experiments

Table 3 Different scenarios for *D. tertiolecta* cultivation and their respective inputs and outputs

Compounds (g L^{-1})	Scenarios				
	T_1	T_2	T_3	T_4	T_5
Concentration of varied MJM components					
KNO_3	0.1	0.1	0.1	1	1
Sea salt	116.9	175.4	233.9	116.9	175.4
Productivity					
Biomass (mg L^{-1} day^{-1} AFDW)	88	86	60	133	114
Glycerol (mg L^{-1} d^{-1})	4.28	6.26	7.39	20.28	19.32

D. tertiolecta (6.6–6.7 × 10^4 cell mL^{-1} initial cell density) was cultivated in 50-mL Erlenmeyer flasks containing 20 mL of MJM with the proposed modifications. The flasks were kept in an Algem® HT24 photobioreactor (Algenuity, Stewartby, Bedfordshire, UK; https://www.algenuity.com/; accessed October 24, 2022) at 25 °C; 200-µmol photons m^{-2} s^{-1} continuous LED white light, and 100 rpm agitation for 16 (T_1–T_3) or 18 (T_4 and S_5) days. Data were obtained from our previous (unpublished) experiments

best-predicted scenarios (S_3, S_5, and T_4) to quantify the possible reduction in CO_2 emission and damage categories.

2.4 Data processing

Graphs were generated using OriginPro 2022 software (OriginLab Corporation, Northampton, MA, USA), excepted for Fig. 1, which was created using the Canvas website. Linear correlation (*r*) analysis was performed using the PAST v. 4.07 software (HAMMER et al. 2001) with a significance level of $p \leq 0.05$ (all statistical requirements for using this parametric test were significantly proven). A positive linear correlation indicates that an increase in one variable results in an increase in the second; a negative linear correlation indicates that an increase in one variable results in a decrease in the second. Linear correlation coefficient (*r*) is an absolute value varying from − 1 to 1 (Taylor 1990), demonstrating the degree of linear association between two variables. Uncertainty analysis was performed using Monte Carlo simulation (normal distribution, 1000 interactions, and 95% confidence) for each category of the Impact 2002+ Method for the best scenarios for each product (biomass, glycerol, and beta-carotene) (McMurray et al. 2017).

Table 4 Chemical composition of the modified Jonhson medium and its substitute when necessary

Compounds	Concentration (g L^{-1})	Substitute in Ecoinvent or Agri-footprint databases
KH_2PO_4	0.035	Na_3PO_4
$MgSO_4.7H_2O$	0.5	
$CaCl_2.2H_2O$	0.2	
$MgCl_2.6H_2O$	1.5	$MgSO_4.7H_2O$ and KCl
KCl	0.2	
KNO_3	1	
$NaHCO_3$	0.84	K_2CO_3
Sea salt	87.7	NaCl
$FeCl_3.6H_2O$	0.00244	
$Na_2EDTA.2H_2O$	0.00189	
H_3BO_3	0.00061	
$MnCl_2.4H_2O$	0.000041	
$ZnCl_2$	0.000041	ZnO
$CuSO_4.5H_2O$	0.00006	
$CoCl_2.6H_2O$	0.000051	
$(NH_4)6Mo_7O_{24}.4H_2O$	0.00038	$MoO_3.nH_2O$

3 Results and discussion

3.1 LCIA of the *Dunaliella salina* chain

3.1.1 Biomass

The greatest impact for *D. salina* cultivation was "Respiratory inorganics" (31.0–55.2 mPt), followed by "Global warming" (20.6–35.3 m Pt), and "Non-renewable energy" (16.3–28.4 mPt; Fig. 2a). NaCl (62–77%; Supplementary Material, "*D. salina* – Impact" tab) was the main contributor to the three mentioned categories. Thomassen et al. (2018) reported that salt and nutrients were the biggest contributors to environmental impacts in *D. salina* cultivation. Interestingly, supplementation with less nitrogen (S_1–S_4, 0.1 g L^{-1} KNO_3) did not result in less impact: S_1–S_4 had its non-normalized impact ranging from 85.1 to 100% for all categories, while it varied between 55.9 and 78.5% for S_5–S_8 (1 g L^{-1} KNO_3; Fig. 2b). S_3 (0.1 g L^{-1} KNO_3, 1.9 g L^{-1} $MgCl_2.6H_2O$; 111 mPt) was the best scenario among the lower nitrogen concentration group and S_5 (1 g L^{-1} KNO_3, 1.1 g L^{-1} $MgCl_2.6H_2O$; 74 mPt) between all scenarios. S_4 was the worst scenario (0.1 g L^{-1} KNO_3, 2.3 g L^{-1} $MgCl_2.6H_2O$; 130 mPt; Fig. 2a–c).

Biomass productivity (Table 2) may explain the damage results for scenarios S_5–S_8 ($r = -1$, $p < 0.001$) but not for S_1–S_4 ($r = -0.78$, $p = 0.22$), indicating that the biomass yields at S_5–S_8 were high enough to compensate the impact caused by using more nitrogen. Nitrogen concentration was negatively correlated with the damage result ($r = -0.90$, $p = 0.002$), which indirectly correlates with biomass productivity: higher yield at higher nitrogen concentration. Magnesium concentration did not have enough impact to influence the results ($r = 0.08$, $p = 0.85$): for instance, it contributed less than 7% to the impact categories for S_8 where the highest amount of magnesium and nitrogen were used (Supplementary Material, "*D. salina* – Impact" tab). "Human health" had the highest share of all damage categories (34–60 mPt; Fig. 2c), which is a reflection of the "Respiratory

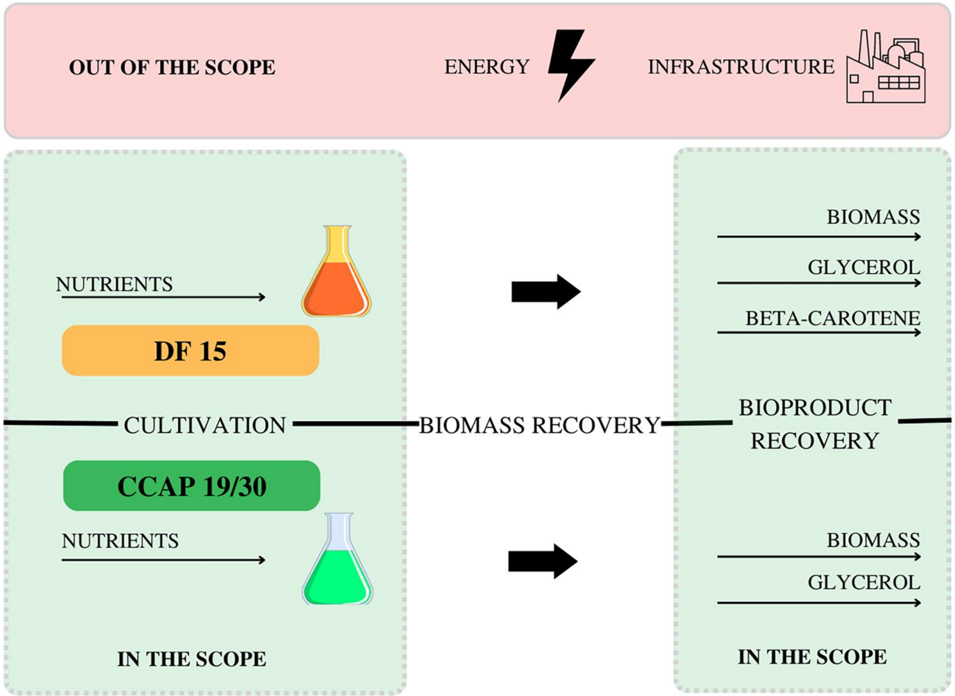

Fig. 1 Steps for obtaining the target bioproducts of *D. salina* and *D. tertiolecta*. Dashed green rectangles demonstrate the boundaries of the "gate-to-gate" microalgae cultivation system for producing biomass, glycerol, and beta-carotene on a laboratory-scale. Solid red rectangles demonstrate the exclusions of the scope

inorganics" impact category (Table 1). Thomassen et al. (2018) reported that "Respiratory inorganics" contributed more to "Human health" when assessing different cultivation scenarios for growing *D. salina* for beta-carotene production. "Climate change" (Global warming) and "Resources" (Non-renewable energy) were the second and third largest damage categories. "Ecosystem quality" scored < 6.5 mPt as none of the proposed scenarios showed significant potential to impact any of the impact categories (e.g., "ozone depletion," "aquatic toxicity," and "Land occupation") relevant to "Ecosystem quality."

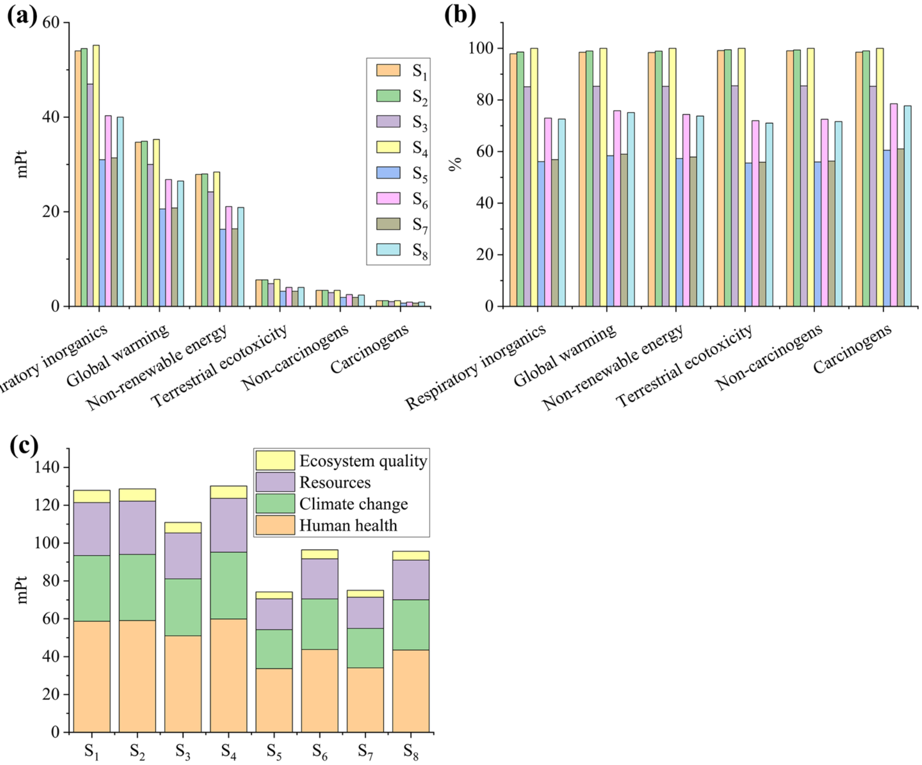

Fig. 2 Normalized (**a**) and non-normalized (**b**) impact categories for each scenario and single score system for damage categories considering biomass produced by *D. salina*. Columns are sorted from S_1 to S_8 (**c**)

3.1.2 Glycerol

D. salina is a good glycerol source, with productivity values between 5.31 and 8.47 mg L^{-1} d^{-1} (Table 2). Glycerol production is triggered to maintain the osmotic balance (Singh et al. 2019). The salt concentration (87.7 g L^{-1} sea salt) was the same for all *D. salina* scenarios; thus, glycerol production was proportional to biomass yield ($r=0.74$, $p=0.04$). As a result, the LCA result for glycerol was similar to that for biomass: lower nitrogen concentration induced lower biomass productivity (thus, lower glycerol yield), resulting in more impact/damage (Fig. 3). Among the scenarios S_1–S_4, S_3 was the least impactful (1.00 Pt). Overall, the least impactful scenario was S_5 (0.95 Pt). Unlike the biomass LCIA result, S_8 (1.36 Pt) was the most impactful scenario, presenting the lowest glycerol productivity (1.30 mg L^{-1} d^{-1}, Table 2). The glycerol productivity directly influenced the damage result ($r=-0.99$, $p<0.001$), while the concentration of nitrogen ($r=-0.50$, $p=0.21$) and magnesium ($r=0.16$, $p=0.71$) did not influence the results.

3.1.3 Beta-Carotene

Unlike the results from the biomass and glycerol analysis, the damage score demonstrates that the group with the lowest nitrogen concentration (S_1–S_4) had a smaller impact (2.0–2.2 Pt) than the group with the highest nitrogen concentration (S_5–S_8; 3.9–6.3 Pt; Fig. 4). Beta-Carotene production responds positively to nitrogen-limiting conditions (Han et al. 2019). Thus, S_1–S_4 offered the best condition for *D. salina* to produce it. S_8 presented the worst scenario concerning beta-carotene due to its lower productivity (1.30 mg L^{-1} d^{-1} beta-carotene) and higher nitrogen (1 g L^{-1} KNO_3) and magnesium (2.3 g L^{-1} $MgCl_2.6H_2O$) concentrations, while S_3 (3.94 mg L^{-1} d^{-1} beta-carotene; 0.1 g L^{-1} KNO_3; 1.9 g L^{-1} $MgCl_2.6H_2O$) performed better. Beta-Carotene productivity was 3.43–3.96 and 1.30–2.06 mg L^{-1} d^{-1} for the groups with the lowest and highest KNO_3 concentrations, respectively (Table 2). Beta-Carotene showed a negative linear correlation with the single damage score ($r=-0.97$, $p<0.001$). Nitrogen concentration presented a positive correlation ($r=0.91$, $p=0.002$), while magnesium and single damage score did not correlate ($p=0.21$–0.61).

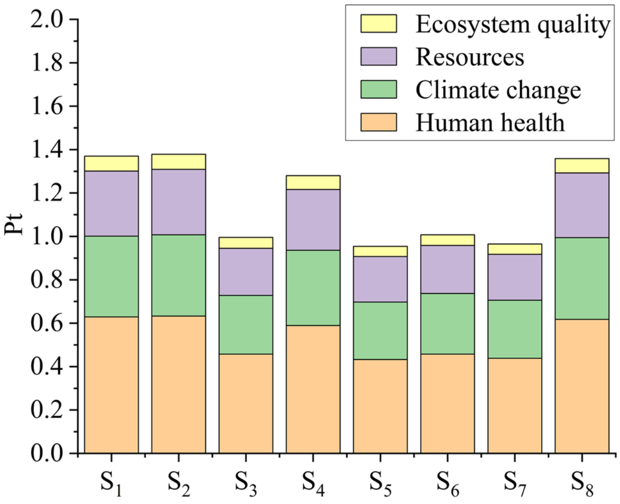

Fig. 3 Single score system for damage categories for glycerol production by *D. salina*

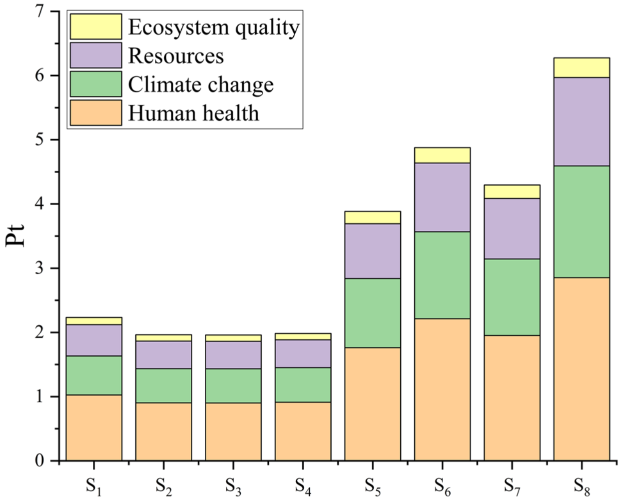

Fig. 4 Single environment damage score for beta-carotene production by *D. salina*

3.2 LCIA of the *Dunaliella tertiolecta* chain

3.2.1 Biomass

The greatest impact for *D. tertiolecta* cultivation was "Respiratory inorganics" (31.0–122.0 mPt), followed by "Global warming" (20.6–78.2 mPt) and "Non-renewable energy" (0.02–0.06; Fig. 5a). NaCl was the main contributor to the three mentioned categories (77–91%; Supplementary Material, "*D. tertiolecta* – Impact" tab), which agrees with Thomassen et al. (2018). Although *Dunaliella* species can cope with hypersaline environments (> 150 g L^{-1} salt concentration), biomass yield can be significantly impaired at salt concentrations greater than 135 g L^{-1} (Ishika et al. 2019). In the same way as the results of *D. salina* cultivation, the supplementation of less nitrogen (T_1–T_3, 0.1 g L^{-1} KNO_3) did not result in a smaller impact: T_1–T_3 had their impact varying between 35.1 and 100% for all categories, while it varied between 23.8 and 43.9% for T_4 and T_5 (1 g L^{-1} KNO_3; Fig. 5b). T_4 (1 g L^{-1} KNO_3, 116.9 g L^{-1} sea salt; 74.4 mPt) was the best scenario, while T_3 (0.1 g L^{-1} KNO_3, 233.9 g L^{-1} sea salt; 288.1 mPt) was the worst (Fig. 5a–c).

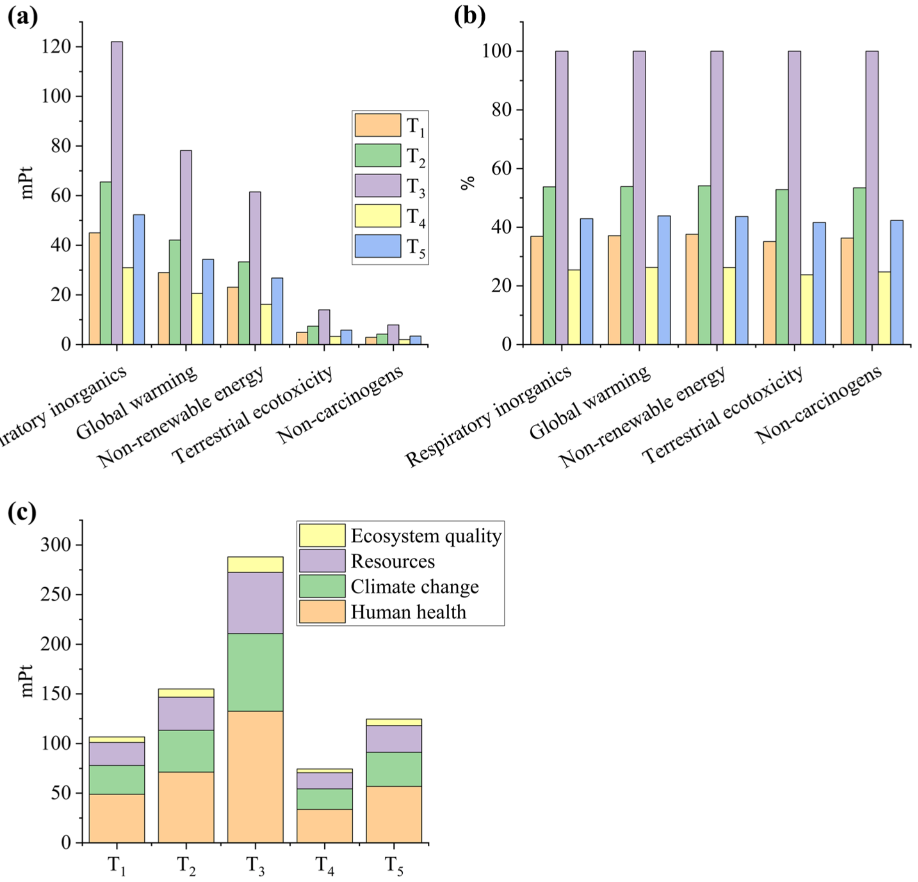

Fig. 5 Normalized (**a**) and non-normalized (**b**) impact categories for each scenario and single scoring system for damage categories considering biomass yielded by *D. tertiolecta*. Columns are sorted from T_1 to T_5 (**c**)

Overall, T_4 and T_5 had 1.3–1.5 times more biomass than their counterparts (T_1 and T $_2$, respectively), directly impacting the LCA: biomass productivity and damage results were negatively but not significantly correlated ($r = -0.85$, $p = 0.07$). Nitrogen concentration did not affect the LCA ($r = -0.55$, $p = 0.33$). This was expected because KNO_3 had a small share of the overall impact (< 9%, data not shown). Sea salt concentration, as expected, correlated positively with damage results ($r = 0.93$, $p = 0.02$). "Human health" (33.8–132.6 mPt; Fig. 5c), followed by "Climate change" (20.6–78.2 mPt), and "Resources" (16.2–61.5 mPt), presented the largest share of all damage categories. As identified for *D. salina*, "Ecosystem quality" (< 15.7 mPt) was the lowest damage category for *D. tertiolecta* cultivation.

3.2.2 Glycerol

Glycerol productivity varied between 4.28 (T_1) and 20.28 mg L^{-1} d^{-1} (T_4) for *D. tertiolecta* (Table 3). Its productivity results from glycerol content and biomass growth and is triggered to deal with osmotic shock (Singh et al. 2019; de Souza Celente et al. 2022). In our experiments, glycerol productivity and salt concentration correlated ($r = 0.99$, $p = 0.1$) for T_1–T_3. For T_4 and T_5, the correlation was impossible to calculate due to the limited amount of data; however, there was a small reduction in glycerol productivity at T_5 due to lower biomass yield. Overall, the results for glycerol were similar to the biomass results. T_4 was the least (0.49 Pt; Fig. 6), while T_3 was the most (2.34 Pt) impactful scenario. Although glycerol productivity was higher in T_3 (7.39 mg L^{-1} d^{-1}) than in T_1 (4.28 mg L^{-1} d^{-1}) and T_2 (6.26 mg L^{-1} d^{-1}; Table 3), it was not enough to compensate the impacts of MJM components. Glycerol productivity ($r = -0.98$, $p = 0.003$) and nitrogen concentration ($r = -0.99$, $p < 0.001$) were negatively correlated with damage results, while salt concentration was not correlated ($r = 0.40$, $p = 0.51$). As discussed above, NaCl is the main component of MJM contributing to impacts. This demonstrates that the linear correlation (r) is not enough to assess how the variables influence each other, and the LCIA is important to quantitatively identify a component's contribution.

3.3 Uncertainty analysis

Although LCA is a powerful tool for predicting the best scenarios and opportunities for improvement within a production chain, inaccuracies regarding the actual input values reflect on the output accuracy. If an LCA is expected to play a crucial role in decision-making, the quality and uncertainties of the results must be clear (Heijungs and Huijbregts

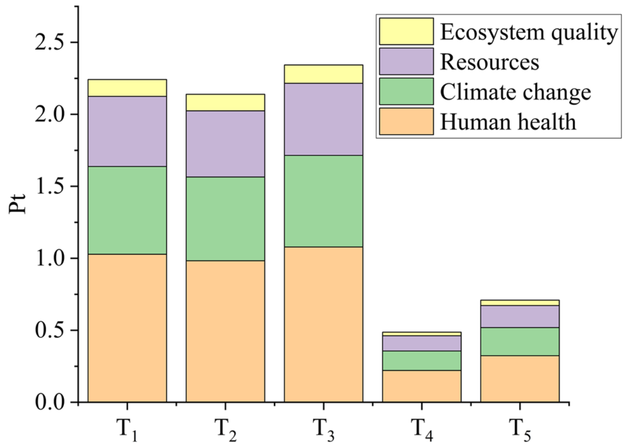

Fig. 6 Single scoring system for damage categories for glycerol production by *D. tertiolecta*

2004). These uncertainties propagate through the analysis and cannot be neglected, especially when many inputs are involved. The Monte Carlo simulation helps to assess the uncertainties of a given LCA scenario. It performs repeated interactions based on random input values within a specified probable range (Raynolds et al. 1999; Heijungs and Lenzen 2014).

S_3, S_5 (*D. salina* chain), and T_4 (*D. tertiolecta* chain) were chosen for the uncertainty analysis using Monte Carlo simulation because they were the best scenarios predicted by the LCA. As shown in Table 5, uncertainties were similar between the three scenarios. The variation coefficient (VC), i.e., the uncertainty, was higher for "ionizing radiation" (82.3–91.7%), "non-carcinogens" (65.0–69.0%), and "aquatic eutrophication" (50.5–65.8%), demonstrating that imprecision was high for the inputs that contributed to these categories. "Respiratory inorganics," "Non-renewable energy," and "Global warming," the three most impacted categories, presented the VC = 10.4–11.7%. The interpretation of a Monte Carlo simulation depends on the critical appreciation of the LCA practitioner; that is, conclusions are drawn based on the knowledge and judgment of those who analyze the results. However, the IPCC (2022) states that a VC of less than approximately 30% is considered reasonable. Thus, LCA predicted the three best scenarios with acceptable accuracy.

To analyze the quality of the LCA in predicting the best scenarios considering the role played by uncertainties, a comparison was performed using Monte Carlo simulation to compare the two best-predicted scenarios for each bioproduct. Regarding biomass and glycerol production by *D. salina* (Fig. 7a), S_5 had a minor impact for all categories in 100% of cases (i.e., interactions) simulated by the Monte Carlo. For beta-carotene (Fig. 7b), S_2 had a lower impact in 2.2% of cases for "respiratory inorganics," 0.7% for "ionizing radiation," and 0.1% for "global warming," "non-renewable energy," and "terrestrial acidification/nutrification;" that is, S_3 showed less uncertainty for beta-carotene production by *D. salina*. T_4 was the best scenario in 100% of cases for all impact categories for biomass and glycerol production by *D. tertiolecta* (Fig. 8a, b). These results demonstrate that the uncertainties were not impactful enough to discredit the LCA predictions: S_5 and S_3 are the best scenarios for biomass/glycerol and beta-carotene production by *D. salina*,

Table 5 Variation coefficient (%) resulting from the uncertainty analysis using Monte Carlo simulation (normal distribution, 1000 interactions, 95% confidence) for the proposed best scenarios

Impact categories	*D. salina*		*D. tertiolecta*
	S_3	S_5	T_4
Carcinogens	43.2	40.3	42.0
Non-carcinogens	65.0	69.0	68.1
Respiratory inorganics	**11.4**	**11.2**	**11.7**
Respiratory organics	**10.8**	**10.6**	**10.8**
Ionizing radiation	91.7	82.6	82.3
Ozone layer depletion	**19.2**	**19.2**	**19.3**
Aquatic ecotoxicity	41.6	37.8	44.5
Terrestrial ecotoxicity	38.2	38.7	44.0
Terrestrial acidification/nutrification	**10.5**	**9.5**	**10.2**
Land occupation	**19.8**	**17.3**	**20.0**
Aquatic acidification	**13.6**	**11.6**	**12.9**
Aquatic eutrophication	50.5	65.8	52.4
Global warming	**10.4**	**10.7**	**10.9**
Non-renewable energy	**11.1**	**11.3**	**11.6**
Mineral extraction	**12.3**	**11.1**	**11.3**
Average (± standard deviation)	29.9 ± 23.8	29.8 ± 24.0	30.1 ± 22.9

Values in bold represent reasonable variation coefficient (< 30%) according to Pörtner et al. (2022)

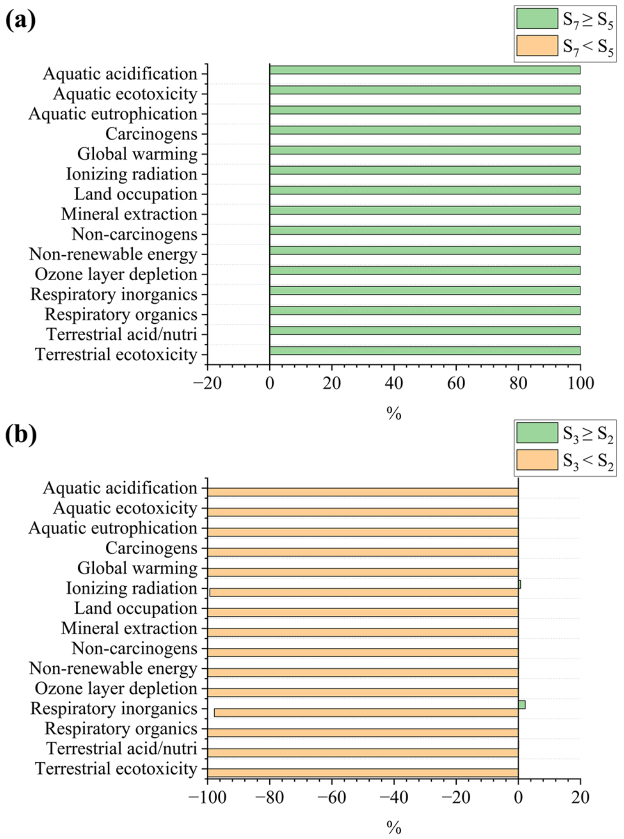

Fig. 7 Uncertainty analysis comparing the best-predicted scenarios for biomass/glycerol (**a**) and beta-carotene (**b**) produced by *D. salina*. **a** represents the production of biomass and glycerol as they had the same results. Terrestrial acid/nutri = terrestrial acidification/nutrification. "A" < "B" = the cases in which scenario "A" was better than "B"; "A" ≥ "B" = the cases in which scenario "A" was not better than "B"

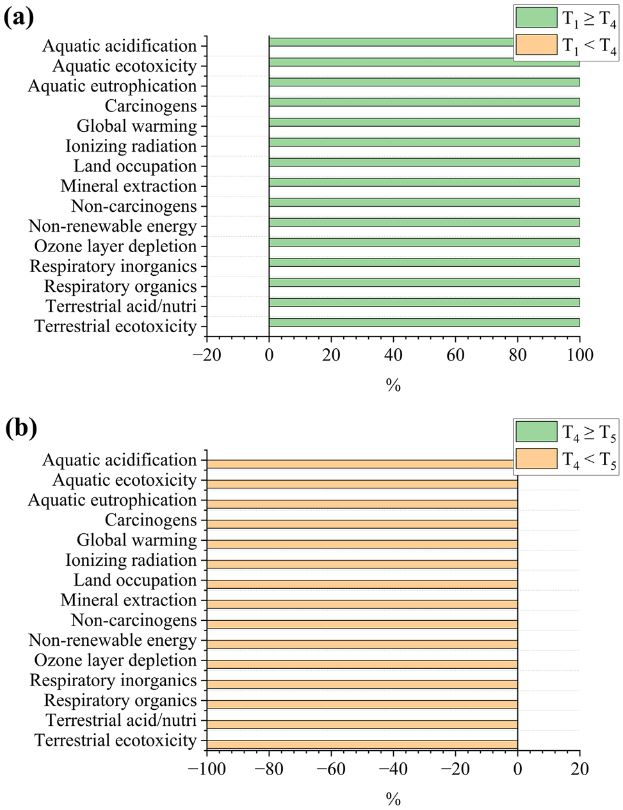

Fig. 8 Uncertainty analysis comparing the best-predicted scenarios for biomass (**a**) and glycerol (**b**) produced by *D. tertiolecta*. **a** represents the production of biomass and glycerol as they had the same results. Terrestrial acid/nutri = terrestrial acidification/nutrification. "A" < "B" = the cases in which scenario "A" was better than "B"; "A" ≥ "B" = the cases in which scenario "A" was not better than "B"

respectively, and T_4 is the best scenario for biomass and glycerol production by *D. tertiolecta*.

3.4 Perspectives and replicability of best scenarios

As demonstrated for the two analyzed chains (*D. tertiolecta* and *D. salina*), salt and deionized water had pronounced contributions to the LCA; however, environmental impacts can be significantly reduced by using brine from desalination plants and recycling the cultivation medium (Thomassen et al. 2018; Yildirim et al. 2022). Keller et al. (2017) calculated that the environmental impact contribution of brine to cultivate *D. salina* could be reduced by 99% by integrating *D. salina* cultivation with salt production or seawater desalination facilities. This is similar to our results: considering that brine from desalination plants could be used to supply salt for the cultivation of *D. salina* and *D. tertiolecta* (considering the productivity would be maintained), a reduction of over 69% in the damage categories could be obtained (Supplementary Material in the "*D. salina* – Impact" tab). Furthermore, combining species cultivation with flue gas mitigation can lessen the impacts associated with carbon supplementation, improve the MJM footprint, and reduce global warming (Collet et al. 2011). Wastewater can be used as an alternative culture medium to supply nutrients (de Souza Schneider et al. 2018), combining wastewater treatment and biomass yield (Celente et al. 2022); however, microalgae cultivation in wastewater limits biomass applications, especially in the food context.

Microalgae cultivation aiming at a single bioproduct yield has limited economic success (Merz et al. 2023). The co-production of multiple products could expand the applications of these species' biomass (e.g., food, feed, and biofuel), leading to a system with lower environmental and economic impacts (Sui and Vlaeminck 2020). For example, Keller et al. (2017) reported that the co-production of zeaxanthin, all-trans beta-carotene, and lutein, among other bioproducts, reduced 0.5 t CO_2 eq kg 9-cis beta-carotene^{-1} (around 2% reduction) in a *Dunaliella*-based biorefinery. In our experiments, cultivating a higher nitrogen concentration resulted in higher biomass and glycerol productivity by

D. salina, decreasing the overall impact. Nitrogen and protein accumulation are positively related (Uriarte et al. 1993; Sui et al. 2019), meaning the best scenario for biomass and glycerol production applies to protein yield. On the other hand, carbohydrate and lipid accumulation in *Dunaliella* spp. are increased under nitrogen-limited conditions (Uriarte et al. 1993; Yuan et al. 2019); thus, beta-carotene could be associated with the production of carbohydrates and lipids. While glycerol, protein, and beta-carotene can be used for food applications (Morrison 2000; Sui and Vlaeminck 2020), the remaining biomass can be used for biofuels (Karpagam et al. 2021): Mohamed et al. (2023) co-pyrolyzed sewage sludge with lignocellulosic and algal biomass to produce liquid and gaseous fuel, which significantly reduced global warming potential and achieved a high net positive energy balance.

Different parameters and cultivation protocols affect productivity, such as light intensity (Yuan et al. 2019), temperature (Mixson Byrd and Burkholder 2017), and growth phase (Sui et al. 2019), which were not in this scope. This shows that there is room for improvement in terms of optimizing the cultivation process. However, a new LCA would be required to predict the impacts and bottlenecks of the optimized system. In addition, energy must be considered when considering other variables unrelated to the medium composition, as exemplified by other authors (e.g., Hossain et al. (2019) and Porcelli et al. (2020)). For instance, Pérez-López et al. (2014) compared using natural sunlight and artificial lighting to cultivate *Haematococcus pluvialis* for astaxanthin production: sunlight greatly reduced the environmental impacts; however, lower productivity limited system improvement. Another crucial factor is the structure used to cultivate microalgae, such as raceways, open ponds, and photobioreactors, which not only affect productivity but also present different contributions to the environmental impact associated with materials. In this regard, novel and alternative materials are proposed to reduce costs and environmental impacts: for instance, Merz et al. (2023) repurposed and reused commercially available air-cushion packaging material as a low-cost, low-labor, and contamination-free photobioreactor (PBR) to cultivate *C. vulgaris*, *Nannochloropsis oculata*, and *Cyclotella cryptica* to produce biomass, lipid, and fucoxanthin with productivity compared favorably with traditional PBR. This not only offers an alternative to expensive conventional PBR but expends the lifetime of a material that otherwise would become waste (da Silva et al. 2021). The structure contribution to the LCA was not in the scope of our study.

Certainly, microalgal cultivation performance for bioproduct yield depends on complex biological, technological, physical, and geographic interactions (Jouannais et al. 2022), which does not always result in being environmentally friendly (Keller et al. 2017). Moreover, local legislation and the energy matrix also dictate microalgal bioproducts' economic and environmental characteristics. Thus, the path from biomass quality identification to large-scale production is highly uncertain (Jouannais et al. 2022). Experimental data were obtained from the cultivation of the two species in Erlenmeyer flasks, which does not mimic real large-scale cultivation, for example, raceways PBRs. However, biomass productivity (58–133 mg L^{-1} d^{-1}; Tables 2 and 3) used for this LCA was similar to previously reported for larger cultivation systems using artificial medium and could potentially provide comparable indications: García-González et al. (2005) produced 80 mg L^{-1} d^{-1} dry biomass of *D. salina* in f2 medium in an outdoor 55-L PBR; Kim et al. (2012) obtained 245 mg L^{-1} d^{-1} and 109 mg L^{-1} d^{-1} cultivating *D. salina* and *Dunaliella* sp. in D medium in a 12-L PBR; Zhu and Jiang (2008) produced 71 mg L^{-1} d^{-1} cultivating *D. salina* in an artificial medium in a PBR.

The data used for this LCA should not be used to calculate absolute impacts for large-scale cultivation of the two evaluated species; however, comparing these LCA results with large-scale production is still valid. The goal was to predict the best scenario regarding the composition of the MJM to produce biomass and bioproducts, which can be safely extrapolated to a large-scale cultivation system. Even if brine from a desalination plant were to be used, the contribution of each category to the total impact and damage score would be similar to the one identified in our proposed scenarios (Supplementary Material, "Damage and impact results" tab).

3.5 Carbon footprint assessment

Global warming has been reported as one of humanity's biggest problems (Acién Fernández et al. 2012): it has not only environmental impacts (Yoro and Daramola 2020) but also threats to human health and economic dynamics. As carbon dioxide is the main component of greenhouse gases (GHG) (Li et al. 2021), seeking more eco-friendly alternatives is a must to ensure the sustainability of our planet. Yadav et al. (2020) demonstrated through an LCA that the *Chlorella vulgaris* cultivation step (raceway open pond) was responsible for > 75% of the environmental impact related to GHG emissions. Although microalgae cannot be considered a tool to sequester CO_2 as their biomass cannot store it for a long period, they can capture approximately 1.83 g of CO_2 per g of biomass (based on a 40% carbon content in DW) (Acién Fernández et al. 2012), making them an important vector in the carbon flow.

Inorganic carbon as $NaHCO_3$ was used to cultivate both species in our experiments. However, we have demonstrated that *D. tertiolecta* can grow on atmospheric carbon as efficiently as on $NaHCO_3$ (Celente et al. 2022). Yadav et al. (2020) increased *C. vulgaris* biomass productivity by almost three times by supplying flue gas (CO_2, 10% v/v). Thus, it is possible to assume that the productivity obtained in our experiments could be maintained if CO_2 were to replace $NaHCO_3$. Figure 9 shows the CO_2-eq emissions concerning the MJM for the cultivation of the two species in different proposed scenarios and the hypothetical fixed CO_2 (1.83 g CO_2 g microalgal biomass^{-1} (Acién Fernández et al. 2012)) to obtain the same biomass productivity (Tables 2 and 3), considering that $NaHCO_3$ was to be replaced by atmospheric CO_2.

For *D. salina*, S_5 presented negative carbon emissions ($\Delta = -12$ kg CO_2 kg biomass^{-1}; $\Delta =$kg CO_2 emitted, kg CO_2 captured), while S_2 was the farthest ($\Delta = 207$ kg CO_2 kg biomass^{-1}). In the case of *D. tertiolecta*, T_4 showed negative CO_2-eq emission ($\Delta = -54$ kg CO_2 kg biomass^{-1}), demonstrating the potential of this species for a green economy. It is important to emphasize that our studies did not consider the impacts associated with energy consumption and infrastructure, which would contribute to more CO_2 emissions and require further analysis. Pérez-López et al. (2014) demonstrated that electricity contributed to 61% of the GHG potential of *H. pluvialis* cultivation (considering the Belgium matrix, highly dependent on fossil fuel). Nevertheless, the energy impact directly depends on the supply matrix; thus, using energy from renewable sources can notably decrease the impact (Porcelli et al. 2020).

However, if flue gas were to be used as an alternative CO_2 source, it would further improve the carbon footprint. Yadav et al. (2020) reduced GHG emissions by approximately 45–50% GHG emissions when using flue gas from a thermal to cultivate *C. vulgaris* in open ponds compared to no carbon supply. Additionally, NaCl contributed to 70–90% of the CO_2-eq emission (Supplementary Material, "*D. salina* – Impact" and "*D. tertiolecta* – Impact" tabs). Using NaCl as an input may have overestimated the environmental impacts for the proposed scenarios since obtaining sea salt (replacement in our experiments) does not require a purification step like NaCl. As demonstrated in the Sect. 3.4, NaCl significantly contributes to the LCA; if *D. salina* and *D. tertiolecta* were cultivated using brine from desalination plants, all the scenarios would present negative CO_2 emissions (Fig. 10). CO_2 emission would be reduced by 75%, 71%, and 78% for the best-proposed scenarios S_3, S_5 (*D. salina*), and T_4 (*D. tertiolecta*), respectively, further improving carbon footprint (further information can be found in Supplementary Material, "CO2 – net" tab). However, it is important to highlight that the calculated emissions consider only the contribution of components in the artificial media and exclude other contributions, such as electricity, infrastructure, and other steps relevant to microalgae cultivation and bioproduct recuperation.

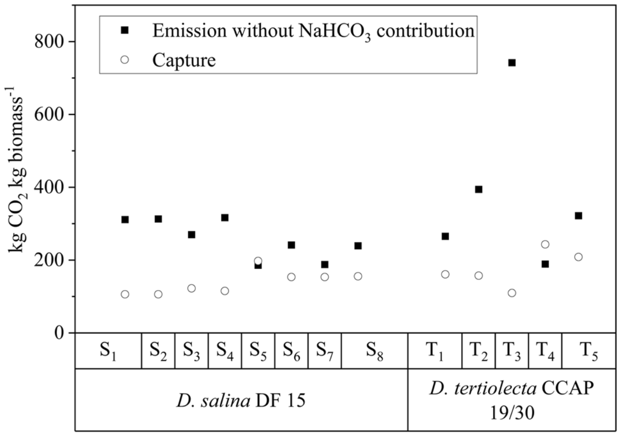

Fig. 9 CO_2-eq emission from the LCIA and the hypothetical CO_2 captured by *D. salina* and *D. tertiolecta* for the proposed scenarios

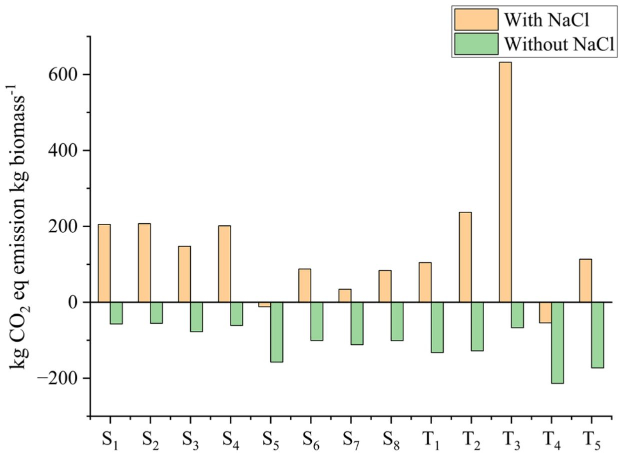

Fig. 10 CO_2-eq emissions by *D. salina* and *D. tertiolecta* for the proposed scenarios with and without NaCl contribution (assuming the use of brine from desalination plants). $NaHCO_3$ contribution was neglected in both conditions (with and without NaCl contribution) as carbon would be supplied by CO_2

4 Conclusions

The LCA was an important tool to accurately predict the best scenarios regarding the composition of the MJM for the analyzed bioproducts, proved by the Monte Carlo simulation. Although the data results from laboratory-scale experiments, the comparative LCA results still provide valuable indications for large-scale cultivation. Among the proposed scenarios for *D. salina*, S_5 (1 g L^{-1} KNO_3, 1.1 g L^{-1} $MgCl_2.6H_2O$) was the best in terms of environmental impacts for biomass and glycerol production, while S_3 (0.1 g L^{-1} KNO_3, 1.9 g L^{-1} $MgCl_2.6H_2O$) was the best for beta-carotene. T_4 (1 g L^{-1} KNO_3, 116.9 g L^{-1} sea salt) offered the best approach to cultivating *D. tertiolecta* for biomass and glycerol yield. When comparing both strains regarding biomass production, the best scenarios (S_5 and T_4) presented similar impact potential (approximately 74 mPt) as they presented similar productivities despite different medium compositions (refer to Tables 2 and 3). On the other hand, *D. tertiolecta* cleared performed better (damage score of 0.49 Pt for T_4) than *D. salina* (0.95 Pt for S_5) for glycerol production as the former produced almost 2.5 times more than the last (based on the best scenarios). Since *D. tertiolecta* does not produce significant amounts of beta-carotene, *D. salina* is the best candidate among the two species for this purpose. These results demonstrated that environmental impact assessment is not straightforward: increasing the nitrogen or magnesium concentrations does not necessarily result in more impact, as productivity can be significantly improved under these conditions and depends on the species. Overall, productivity could overcome the impacts of "surplus" artificial medium components. Our study also demonstrated the opportunity to combine *D. salina* and *D. tertiolecta* cultivation with CO_2 capture and use brine from desalination plants, which helps to achieve an eco-friendlier cultivation system with a lower carbon footprint. Nevertheless, the LCA provides useful information for decision-making as the cultivation step is often the most impactful step for "Human health" and "Resources" due to its high contribution to "Respiratory inorganics" and "Global warming".

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11367-023-02209-2.

Author contribution Conceptualization: Yixing Sui, Rosana de Cassia de Souza Schneider, and Gleison de Souza Celente; methodology: Yixing Sui, Rosana de Cassia de Souza Schneider, and Gleison de Souza Celente; formal analysis and investigation: Gleison de Souza Celente; writing, original draft preparation: Gleison de Souza Celente; writing, review and editing: Rosana de Cassia de Souza Schneider, Tiele Medianeira Rizzetti, Eduardo Alcayaga Logo, and Yixing Sui; funding acquisition: Yixing Sui; supervision: Yixing Sui and Rosana de Cassia de Souza Schneider; software: Rosana de Cassia de Souza Schneider and Jennifer Julich.

Funding This study was financed in part by the UKRI Science and Technology Facilities Council (STFC) Food Network+ (SFN) and Interreg 2 Seas Programme 2014–2020, co-funded by the European Regional Development Fund under subsidy contract no. ValgOrize 2S05017.

Data availability The datasets generated or analyzed during the current study are included in this published article and its Supplementary information file.

Declarations

Conflict of interest The authors declare no competing interests.

References

Acién Fernández FG, González-López C, Fernández Sevilla J, Molina Grima E (2012) Conversion of CO 2 into biomass by microalgae: how realistic a contribution may it be to significant CO 2 removal? Appl Microbiol Biotechnol 96:577–586

Atta OM, Manan S, Ul-Islam M, Ahmed AA, Ullah MW, Yang G (2022) Development and characterization of plant oil-incorporated carboxymethyl cellulose/bacterial cellulose/glycerol-based antimicrobial edible films for food packaging applications. Adv Compos Hybrid Mater pp 1–18

Bauer L, Ranglová K, Masojídek J, Drosg B, Meixner K (2021) Digestate as sustainable nutrient source for microalgae—challenges and prospects. Appl Sci 11(3):1056

Borowitzka M (1988) Algal growth media and sources of algal cultures. Cambridge University Press

Celente GDS, Rizzetti TM, RdCdS Schneider PJ, Harvey YS (2022) Organic carbon is ineffective in enhancing the growth of dunaliella. Fermentation 8(6):261

Chen G, Zhao L, Qi Y (2015) Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: a critical review. Appl Energy 137:282–291

Chen Q-H, Wu B-K, Pan D, Sang L-X, Chang B (2021) Beta-carotene and its protective effect on gastric cancer. World J Clin Cases 9(23):6591

Collet P, Hélias A, Lardon L, Ras M, Goy R-A, Steyer J-P (2011) Life-cycle assessment of microalgae culture coupled to biogas production. Biores Technol 102(1):207–214

Colusse GA, Mendes CR, Duarte ME, de Carvalho JC, Noseda MD (2020) Effects of different culture media on physiological features and laboratory scale production cost of Dunaliella salina. Biotechnology Reports 27:e00508

da Silva TR, de Azevedo ARG, Cecchin D, Marvilla MT, Amran M, Fediuk R, Vatin N, Karelina M, Klyuev S, Szelag M (2021) Application of plastic wastes in construction materials: a review using the concept of life-cycle assessment in the context of recent research for future perspectives. Materials 14(13):3549

de Souza Celente G, Rizzetti TM, Sui Y, de Souza Schneider RD (2022) Potential use of microalga Dunaliella salina for bioproducts with industrial relevance. Biomass Bioenerg 167:106647

de Souza Schneider RD, de Moura Lima M, Hoeltz M, de Farias Neves F, John DK, de Azevedo A (2018) Life cycle assessment of microalgae production in a raceway pond with alternative culture media. Algal Res 32:280–292

Dufossé L, Galaup P, Yaron A, Arad SM, Blanc P, Chidambara Murthy KN, Ravishankar GA (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16(9):389–406

García-González M, Moreno J, Manzano JC, Florencio FJ, Guerrero MG (2005) Production of Dunaliella salina biomass rich in 9-cis- β-carotene and lutein in a closed tubular photobioreactor. J Biotechnol 115(1):81–90

García-Segovia P, García Alcaraz V, Tárrega A, Martínez-Monzó J (2020) Consumer perception and acceptability of microalgae based breadstick. Food Sci Technol Int 26(6):493–502

Guinée JB (2002) Handbook on life cycle assessment: operational guide to the ISO standards. Springer Science & Business Media. Dordrecht, Netherlands

Hammer Ø, Harper DA (2001) Palaeontological statistics software package for education and data analysis. Palaeontol Electron 4(1):1. https://doc.rero.ch/record/15326/files/PAL_E2660.pdf. Accessed 17 July 2023

Han T, Lu H, Zhao Y, Xu H, Zhang Y, Li B (2019) Two-step strategy for obtaining dunaliella sp. biomass and β-carotene from anaerobically digested poultry litter wastewater. Int Biodeterior Biodegra 143:104714

Harvey PJ, Ben-Amotz A (2020) Towards a sustainable Dunaliella salina microalgal biorefinery for 9-cis β-carotene production. Algal Res 50:102002

Heijungs R, Huijbregts MA (2004) A review of approaches to treat uncertainty in LCA. International Congress on Environmental Modelling and Software. Germany

Heijungs R, Lenzen M (2014) Error propagation methods for LCA—a comparison. Int J Life Cycle Assess 19(7):1445–1461

Hossain N, Zaini J, Indra Mahlia TM (2019) Life cycle assessment, energy balance and sensitivity analysis of bioethanol production from microalgae in a tropical country. Renew Sustain Energy Rev 115:109371

Humbert S, Margni M, Jolliet O (2012) IMPACT 2002+: user guide. Draft for version Q 2

Ihemezie EJ, Ukwuaba IC, Nnaji AP (2018) Impact of 'green' product label standards on consumer behaviour: a systematic review analysis. Int J Acad Res Bus Soc Sci 8(9):666–684

Ishika T, Moheimani NR, Laird DW, Bahri PA (2019) Stepwise culture approach optimizes the biomass productivity of microalgae cultivated using an incremental salinity increase strategy. Biomass and Bioenergy 127:105274. https://doi.org/10.1016/j.biombioe.2019.105274

ISO 14044 (2006) International Organization for Standardization. Environmental Management. Life cycle Assessment. Requirements and Guidelines. Geneva, Switzerland

Jouannais P, Hindersin S, Löhn S, Pizzol M (2022) Stochastic LCA model of upscaling the production of microalgal compounds. Environ Sci Technol 56(14):10454–10464

Kabir SB, Khalekuzzaman M, Hossain N, Jamal M, Alam MA, Abomohra AE-F (2022) Progress in biohythane production from microalgae-wastewater sludge co-digestion: an integrated biorefinery approach. Biotechnol Adv 57:107933

Karpagam R, Jawaharraj K, Gnanam R (2021) Review on integrated biofuel production from microalgal biomass through the outset of transesterification route: a cascade approach for sustainable bioenergy. Sci Total Environ 766:144236

Kay RA, Barton LL (1991) Microalgae as food and supplement. Crit Rev Food Sci Nutr 30(6):555–573

Keller H, Reinhardt G, Gärtner S, Rettenmaier N, Goacher P, Mitchell R, Peñaloza D, Stahl S, Harvey PJ (2017) Integrated sustainability assessment of Dunaliella-based algae biorefinery concepts. D-Factory project reports

Kim W, Park JM, Gim GH, Jeong S-H, Kang CM, Kim D-J, Kim SW (2012) Optimization of culture conditions and comparison of biomass productivity of three green algae. Bioprocess Biosyst Eng 35:19–27

Li R, Wang Q, Liu Y, Jiang R (2021) Per-capita carbon emissions in 147 countries: the effect of economic, energy, social, and trade structural changes. Sustain Prod Consum 27:1149–1164

Maia Research (2021) Global Dunaliella salina market research report, segment by major players, types, applications and regions, 2016–2026

Matos ÂP (2017) The impact of microalgae in food science and technology. J Am Oil Chem Soc 94(11):1333–1350

McMurray A, Pearson T, Casarim F (2017) Guidance on applying the Monte Carlo approach to uncertainty analyses in forestry and greenhouse gas accounting. Winrock International: Arlington, VA, USA 26

Melnikov N, Kamari Y, Kandel-Kfir M, Barshack I, Ben-Amotz A, Harats D, Shaish A, Harari A (2022) β-Carotene from the alga Dunaliella bardawil decreases gene expression of adipose tissue macrophage recruitment markers and plasma lipid concentrations in mice fed a high-fat diet. Marine Drugs 20. https://doi.org/10.3390/md20070433.

Merz CR, Arora N, Welch M et al (2023) Microalgal cultivation characteristics using commercially available air-cushion packaging material as a photobioreactor. Sci Rep 13:3792

Mixson Byrd S, Burkholder JM (2017) Environmental stressors and lipid production in Dunaliella spp. II. Nutrients, pH, and light under optimal or low salinity. J Exp Mar Biol Ecol 487:33–44

Mohamed BA, O'Byle M, Li LY (2023) Co-pyrolysis of sewage sludge with lignocellulosic and algal biomass for sustainable liquid and gaseous fuel production: a life cycle assessment and techno-economic analysis. Appl Energy 346:121318

Monte J, Ribeiro C, Parreira C, Costa L, Brive L, Casal S, Brazinha C, Crespo JG (2020) Biorefinery of Dunaliella salina: Sustainable recovery of carotenoids, polar lipids and glycerol. Bioresour Technol 297:122509. https://doi.org/10.1016/j.biortech.2019.122509

Morrison LR (2000) Glycerol. Kirk-Othmer Encyclopedia of Chemical Technology. https://doi.org/10.1002/0471238961.0712250313151818.a01

Nova P, Martins AP, Teixeira C, Abreu H, Silva JG, Silva AM, Freitas AC, Gomes AM (2020) Foods with microalgae and seaweeds fostering consumers health: a review on scientific and market innovations. J Appl Phycol 32(3):1789–1802

Pérez-López P, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D, Murray P, Moane S, Feijoo G, Moreira MT (2014) Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. J Clean Prod 64:332–344

Porcelli R, Dotto F, Pezzolesi L, Marazza D, Greggio N, Righi S (2020) Comparative life cycle assessment of microalgae cultivation for non-energy purposes using different carbon dioxide sources. Sci Total Environ 721:137714

Pörtner HO, Roberts DC, Adams H et al. (2022) Climate change 2022: impacts, adaptation and vulnerability. IPCC. https://report.ipcc.ch/ar6/wg2/IPCC_AR6_WGII_FullReport.pdf. Accessed 17 July 2023

Raynolds M, Checkel MD, Fraser RA (1999) Application of Monte Carlo analysis to life cycle assessment. SAE transactions pp 1–9

SimaPro (2020) SimaPro database manual: Methods library. https://simapro.com/wpcontent/uploads/2020/10/DatabaseManualMethods.pdf. Accessed 17 July 2023

Singh P, Khadim R, Singh AK, Singh U, Maurya P, Tiwari A, Asthana RK (2019) Biochemical and physiological characterization of a halotolerant Dunaliella salina isolated from hypersaline Sambhar Lake, India. J Phycol 55(1):60–73

Sui Y, Muys M, Vermeir P, D'Adamo S, Vlaeminck SE (2019) Light regime and growth phase affect the microalgal production of protein quantity and quality with Dunaliella salina. Biores Technol 275:145–152

Sui Y, Vlaeminck SE (2020) Dunaliella microalgae for nutritional protein: an undervalued asset. Trends Biotechnol 38(1):10–12

Sun C-H, Fu Q, Liao Q, Xia A, Huang Y, Zhu X, Reungsang A, Chang H-X (2019) Lifecycle assessment of biofuel production from microalgae via various bioenergy conversion systems. Energy 171:1033–1045

Taylor R (1990) Interpretation of the correlation coefficient: a basic review. Journal of Diagnostic Medical Sonography 6(1):35–39

Thomassen G, Van Dael M, Van Passel S (2018) The potential of microalgae biorefineries in Belgium and India: an environmental techno-economic assessment. Biores Technol 267:271–280

US Food & Drug Administration (2020) Generally recognized as safe. https://www.fda.gov/. Accessed 4 Apr 2020

Uriarte I, Farías A, Hawkins AJS, Bayne BL (1993) Cell characteristics and biochemical composition of Dunaliella primolecta Butcher conditioned at different concentrations of dissolved nitrogen. J Appl Phycol 5(4):447–453

Vanthoor-Koopmans M, Wijffels RH, Barbosa MJ, Eppink MHM (2013) Biorefinery of microalgae for food and fuel. Biores Technol 135:142–149

Wang Y, Lu J, Qu H, Cai C, Liu H, Chu J (2022) β-Carotene extracted from Blakeslea trispora attenuates oxidative stress, inflammatory, hepatic injury and immune damage induced by copper sulfate in zebrafish (Danio rerio). Comp Biochem Physiol C Toxicol Pharmacol 258:109366

Yadav G, Dubey BK, Sen R (2020) A comparative life cycle assessment of microalgae production by CO2 sequestration from flue gas in outdoor raceway ponds under batch and semi-continuous regime. J Clean Prod 258:120703

Yildirim O, Tunay D, Ozkaya B (2022) Reuse of sea water reverse osmosis brine to produce Dunaliella salina based β-carotene as a valuable bioproduct: a circular bioeconomy perspective. J Environ Manage 302:114024

Yoro KO, Daramola MO (2020) Chapter 1 - CO2 emission sources, greenhouse gases, and the global warming effect. Advances in Carbon Capture. M. R. Rahimpour, M. Farsi and M. A. Makarem, Woodhead Publishing pp 3–28

Yuan Y, Li X, Zhao Q (2019) Enhancing growth and lipid productivity in Dunaliella salina under high light intensity and nitrogen limited conditions. Bioresour Technol Rep 7:100211. https://doi.org/10.1016/j.biteb.2019.100211

Zhu Y-H, Jiang J-G (2008) Continuous cultivation of Dunaliella salina in photobioreactor for the production of β-carotene. Eur Food Res Technol 227(3):953–959

Authors and Affiliations

Gleison de Souza Celente[1,2,3] · **Rosana de Cassia de Souza Schneider**[1,2] · **Jennifer Julich**[1] · **Tiele Medianeira Rizzetti**[1,2] · **Eduardo Alcayaga Lobo**[1] · **Yixing Sui**[3]

✉ Yixing Sui
Y.Sui@greenwich.ac.uk

[1] Environmental Technology Post-graduation Program, University of Santa Cruz do Sul, Avenida Independência, 2293, Santa Cruz do Sul, Rio Grande do Sul 96815-900, Brazil

[2] Centre of Excellence in Oleochemical and Biotechnological Products and Processes, University of Santa Cruz do Sul, Avenida Independência, 2293, Santa Cruz do Sul, Rio Grande do Sul 96815-900, Brazil

[3] School of Science, Faculty of Engineering and Science, Central Avenue, University of Greenwich, Chatham Maritime, Kent ME4 4TB, UK