POTENTIAL USE OF MICROALGA *Dunaliella salina* FOR BIOPRODUCTS WITH INDUSTRIAL RELEVANCE

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Acknowledgment

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by Brazilian National Council for Scientific and Technological Development (CNPq) – protocol 310228/2019-0.

ABSTRACT

Using microalgal technology has been getting attention over the last decades, mainly for primary use but also for generating high-value compounds. *Dunaliella salina* is one of the most important microalgae, and its biomass can be used to yield carotenoids, lipids, glycerol, carbohydrates, and proteins for biofuel, pharmaceuticals, and food generation. Many factors affect bioproduct yields, such as light regime and intensity, salinity, harvesting period, and media composition, which directly impact the feasibility of biorefineries. Although it has been addressed over the past decades, there is still a lack of consensus

regarding an effective method for biomass and bioproduct generation and recovery on an industrial scale. In this study, a bibliometric analysis over the five years is used to identify (I) the global distribution of research; (II) the bioproducts yielded by *D. salina*, and (III) the future perspective for the valorization of its biomass. China is the major contributor to research on *D. salina*, followed by India and the United States of America. Carotenoid production has been the major focus of the research, followed by protein, lipid, carbohydrate, and glycerol. The genetic engineering approach seems to carry out the future of *D. salina* to improve the generation of bioproducts, especially pigments and protein.

Keywords: *Dunaliella salina*, bioproduct, byproduct, microalgae, industrial relevance.

1. INTRODUCTION

Microalgal biotechnology has been focusing on four main pillars over the years: (1) strain development, (2) regulatory mechanisms of cell, (3) photobioreactor design, and (4) development of new bioproducts and markets [1]. Although the worldwide energetic demand has extensively encouraged microalgae studies for biofuel yield [2], concerns also arise regarding the economic feasibility of biofuel production by using microalgal biomass. The primary use of microalgae, such as biofuel, feed, and raw material production, is not economically feasible unless the yield of high-value compounds is addressed together. The production of byproducts, for instance, beta-carotene, bioactive and functional pigments, natural dyes, polysaccharides, antioxidants, and other algal extracts, increases the viability of the primary production with a smaller footprint [3].

D. salina is a halophilic green flagellate microalga, a member of the phylum Chlorophyta commonly found in saline environments, such as saline lakes [4] and coastal marine waters [5]. Its halophilic characteristics allow it to grow in hypersaline environments (> 150 g L^{-1} salinity) [6], which was observed in the early 1900s [7]. Many studies have been conducted to explore its different applications, such as toxicity assessment [8], biodiesel [9], biomethane [10], lipid [11], antioxidant and anticarcinogen production [12]; bioremediation [13]; wastewater treatment [14], and as a food source [15]. Among the various applications, *D. salina* has been extensively applied to produce high-value compounds, such as carotenoids, which started in the early 1980s [16, 17] and, more recently, for biofuel production. However, to the best of our knowledge, there has not been a bibliometric approach to identify the full potential of *D.*

salina on bioproduct production. In this study, a bibliometric analysis over the five years is used to identify (I) the global distribution of research; (II) the bioproducts yielded by *D. salina*, and (III) the future perspective for the valorization of its biomass.

2. BIBLIOMETRIC ANALYSIS METHODOLOGY

A bibliometric analysis over 2016–2021 was conducted using VosViewer v. 1.6.15, a software to create maps based on network data. In the generated visualization map, items closely related to each other are clustered in groups with different colors. In the network visualization, a weight is assigned to each item, as indicated by frames/circles, to represent its importance: the higher the weight, the bigger the circles/frames and the higher its importance. Links connect items, and a strength is associated with each link, representing the relatedness of two items. An individual item can be highlighted to show its links with other items; the same can be done with individual links to show the connection between two items. In the density visualization map, the larger the number of items surrounding a point and the higher their weights, the denser the color of that area.

The references for the VosViewer analysis were collected in March 2021 from the databases Web of Science (WoS), ScienceDirect, and Scopus and submitted to the Endnote, a software tool to manage references. Articles from Google Scholar were included for further discussion of the maps resulting from the bibliometric analysis. The references collection for the bibliometric analysis included only research articles in English, and the searched terms were included either within the title, abstract, or keywords to avoid unrelated articles. All duplicated references were removed by using the duplicated finder feature available on the Endnote software and by manually comparing the references. The terms used to collect the references and the number of references for each term and database are listed in Table 1. The coupled terms "*Dunaliella salina*" + "future" covered the period 2016–2021 to identify the prospective uses and challenges related to *D. salina*.

Table 1 - Publications sorted by database over the period 2016–2021

Term	WoS	Scopus	ScienceDirect	Total[a]
Dunaliella salina	423	415	122	542
Dunaliella salina + product	50	56	13	83

Dunaliella salina + future	17	15	3	20

[a] documents after duplication filtering

Before submitting the references to the bibliometric analysis, a thesaurus file was created with the OpenRefine software, a free java-based tool for loading and organizing data. An Excel text (tab-delimited) file extension was created with all the items of occurrence from the Vosviewer. This file was uploaded to the OpenRefine tool, and all the methods and keying functions were used to cluster items with the same meaning. After this first step, every item was examined to ensure all items were clustered. One thesaurus file was created and applied for every VosViewer map, except for the bibliometric analysis of the geographic distribution of the research, which had its thesaurus file.

The maps were created based on text data using the Endnote's references saved as a Research Information Systems (RIS) file, showing the co-occurrence items limited to the title and abstract fields. For the research distribution, which was based on bibliographic data and shown by countries, only the references from the WoS database were used due to VosVierwer limitations. In this case, the references collected by searching the term "*Dunaliella salina*" were used, and the relatedness of the items was based on the number of co-authored documents. However, since WoS showed the highest number of articles for the term beforementioned, the distribution map should present the actual research distribution.

3. RESEARCH WORLDWIDE

Using *D. salina* to generate compounds of high industrial relevance has extensively focused on beta-carotene. Nevertheless, the industrial potential of these microalgae stands out regarding other valuable compounds as well: the first granted patents on *D. salina* focused on, for instance, the production of glycerol [18], cosmetic compounds [19], protein, and carotene [20]. Feasible methods to harvest and extract these products have also gotten attention, as exemplified by several patents [21-24].

There are three major groups regarding the studies on *D. salina* (Fig. **1**). China, the United States of America, and Iran are the countries with the most co-authored documents in their respective groups (green, blue, and red). It is important to highlight a noteworthy connection between China and the United States, and the last with South Korea (evidenced by the thickness of the line connecting the countries in Fig. **1**b).

The strong collaboration between the two first regarding the studies on microalgae had been demonstrated before [1].

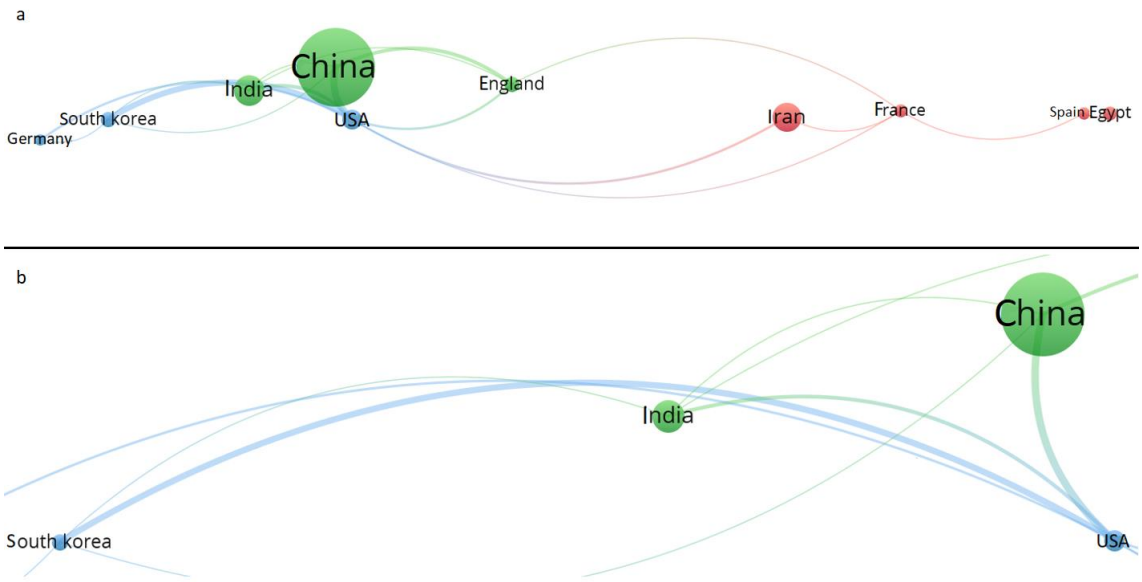


Fig. 1 Co-authorship map showing the top 10 countries with most research documents on *D. salina* on the WoS database within 2016–2021. The lines' width represents the strength between two items

China has the highest number of publications on *D. salina* (**Fig. 1**) and is the second country that published most research on microalgae, with the Chinese Academy of Sciences as the major contributor [1]. This academy comprises over one hundred research institutes, branch academies, universities, and supporting organizations nationally and in partnership with international collaborators, focusing strongly on environmental engineering, ecology, salt lakes, green technologies, and energy conversion studies [25]; which explains their interest in *D. salina*, being the second institution that most contributed to the *D. salina* studies over the past five years (Fig. ***2***). The *Nature Index*, compiled by Nature Research, ranks this academy as number one globally in the number of articles published [26]. The Ocean University of China is the fourth academic institution to conduct most papers on *D. salina* (Fig. ***2***), contributing to placing China first in the number of co-authored studies (Fig. **1**). The university focuses on oceanography and fisheries science and comprises the College of Marine Life Science; housing the Key Labs of Marine Biotechnology, Marine Genetics, and Breeding [27], which published some of the papers on *D. salina*.

Fig. 2 Distribution of articles on *D. salina* by organization indexed in the Web of Science database within 2016–2021. Box's size is proportional to the number of published documents

Centre National de la Recherche Scientifique (CNRS) in France is the youngest institute to comprise the top five that published the most research articles about *D. salina* on the WoS database. It was founded in the 2000s [28] and ranked fourth in the international ranking of scientific institutions organized by the journal Nature [26]. In 2015, the CNRS launched the AlgoSolis platform in partnership with the University of Nantes to focus on extracting compounds of industrial relevance and providing algal-based wastewater treatment methods [29]. National Research Centre is the largest multidisciplinary research and development center in Egypt and third in the number of published documents on *D. salina* (Fig. ***2***). The institution ran, among others, the international project named "Biodiesel production from algae as a renewable energy source" (end year: 2017); currently, the institution runs nationally the project named "Anti-tumors and anti-virus from Egyptian marine algae" [30]. Most of the research on *D. salina* came from the Plant Biochemistry Department, which provided studies on bioactive compounds yielded by this microalga. Vellore Institute of Technology, an Indian institution, houses the School of Bio-sciences and Technology and the Centre for NanoBiotechnology, which leads research in nanotoxicology, nanoaquaculture, nanobiosynthesis, environmental nanobiotechnology, and many other related research areas [31]. These departments conduct research on *D. salina* regarding the production of pharmaceuticals and toxicity assessment.

4. *D. Salina* PRODUCTION

The bibliometric analysis using the terms "*Dunaliella salina*" + "product" resulted in three well-defined clusters indicated by red, blue, and green colors. The red cluster suggests the primary uses of *D. salina*

biomass for food and biodiesel production (Fig. ***3***). The green cluster groups items related to cultivation methods, such as nitrogen (N) limitation, phosphate availability, light and salinity, and the response towards the exposure to these conditions (Fig.4). The blue cluster is related to the generation and extraction of biocompounds, especially those with pharmaceutical and nutraceutical potential (Fig. ***5***).

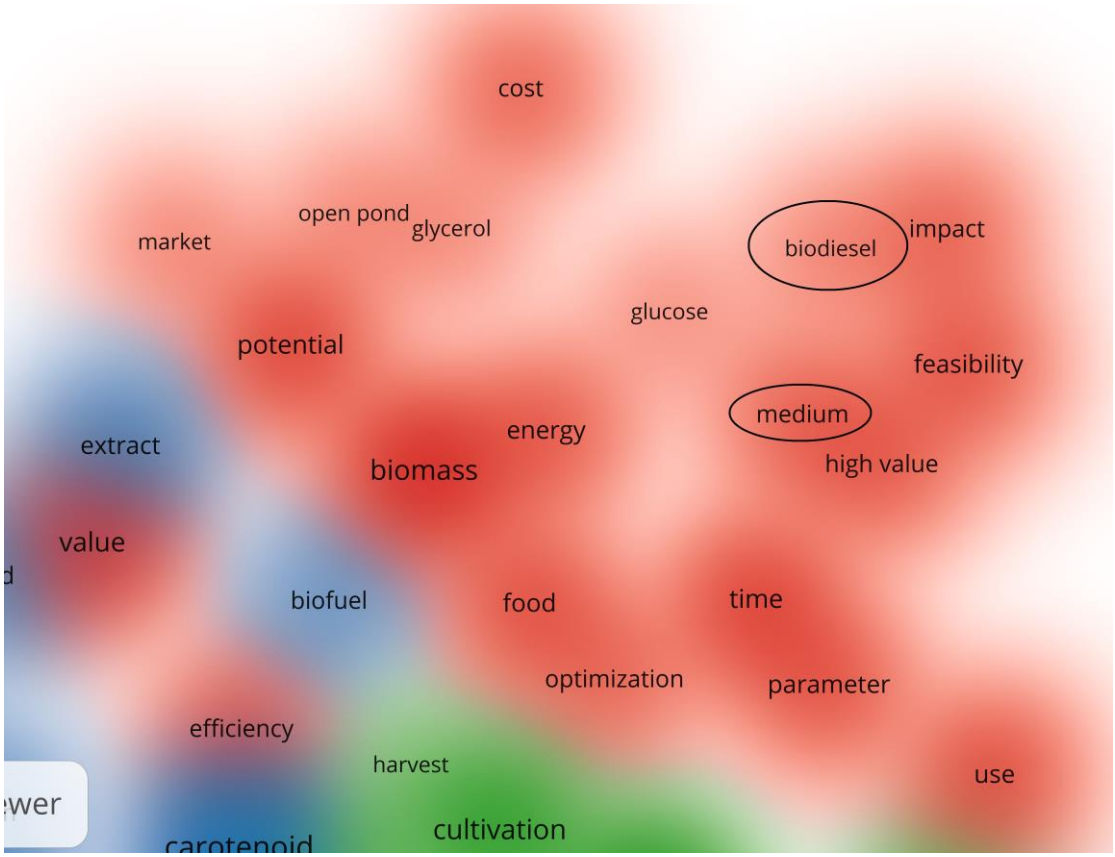


Fig. 3 Density visualization of the bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the items in the red cluster with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus

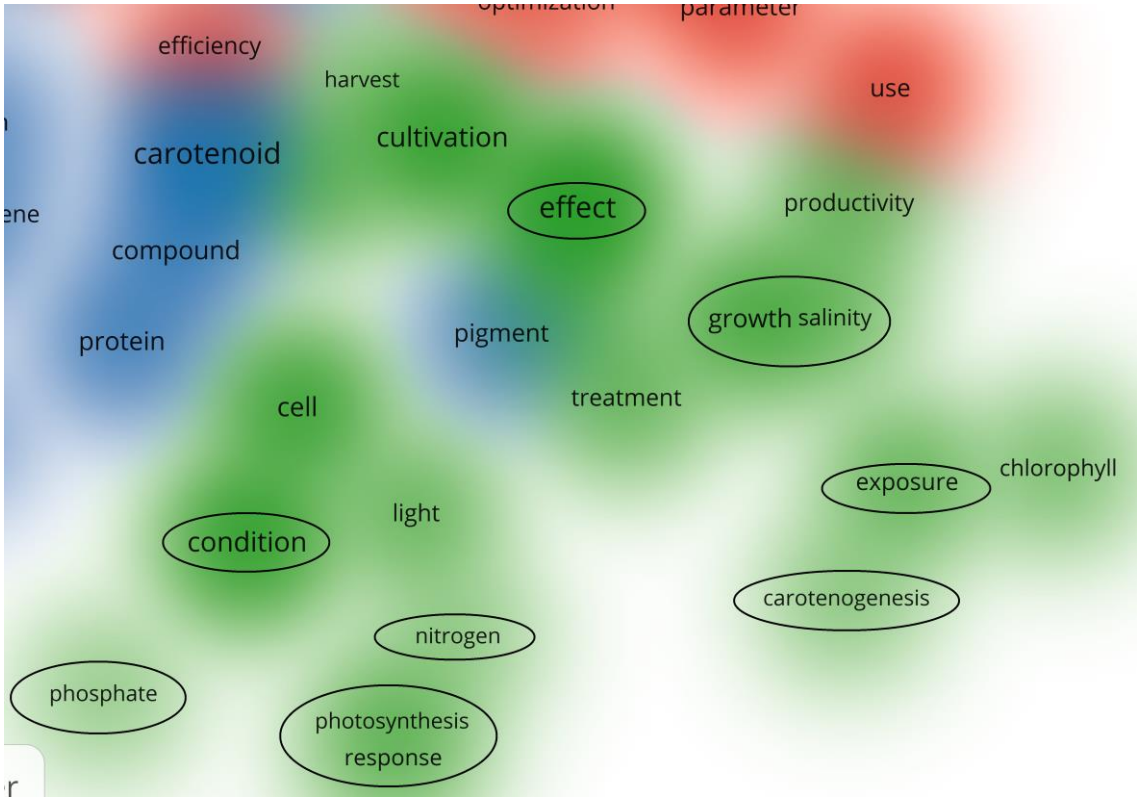


Fig.4 Density visualization of the bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the items in the green cluster with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus

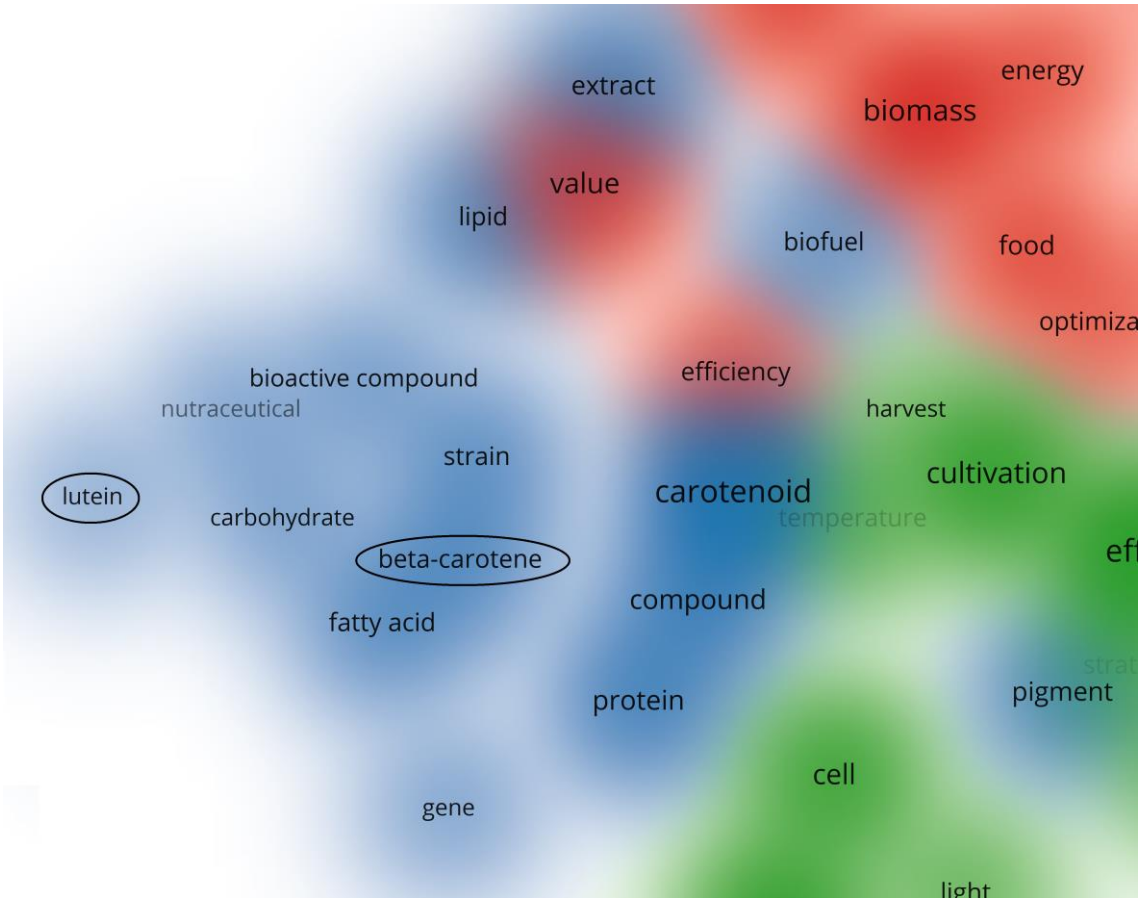


Fig. 5 Density visualization of the bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the items in the blue cluster with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus

As denoted by the item "value" (red cluster) in Fig. ***6***, it is noticeable that the production of *D. salina* and its application are associated to increase biomass value, especially in a biofuel and food context. Biomass conversion approaches this by coproducing compounds of industrial relevance, such as pigments, lipids, carbohydrates, fatty acids, and other bioactive compounds. The close links of cultivation condition, denoted by the items "condition", "temperature", "light", and "exposure" with "value", demonstrate its significance in promoting high-value bioproduct production. The item "strain" connects with "value", reflecting that different strains have different biochemical compositions and responses to cultivation conditions. It is also possible to notice the connection between the items "value" and "optimization". This implies that to increase the value of bioproducts, it is necessary to optimize the cultivation, harvest, and extraction of biochemical compounds. This is demonstrated by the vast production of articles regarding the optimization of culture and scalability [6, 32, 33]. Besides "harvest" and "extract", items related to the

culture condition are associated with the item "cost" (Fig. 7). This is expected since light regime, salinity, temperature, and medium composition remarkably impact energy costs. Thus, the optimal method for cultivating *D. salina* must be reached to guarantee the profitability of microalgal biorefinery.

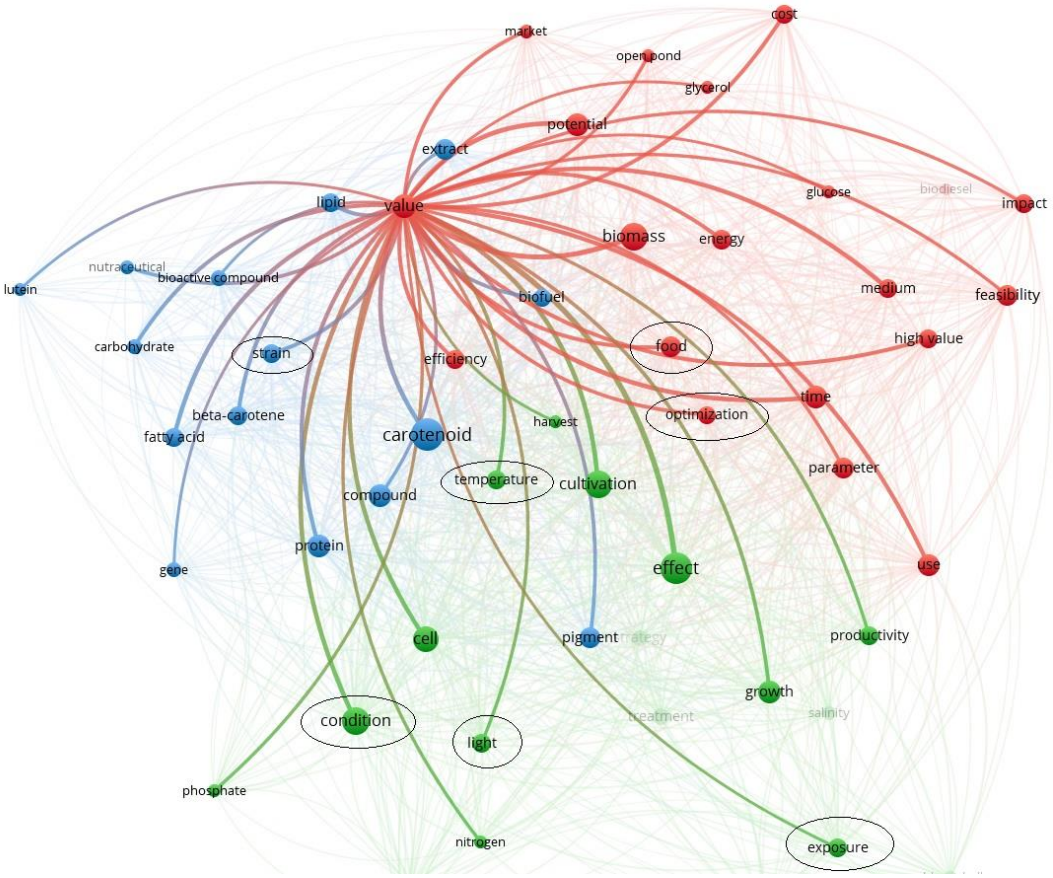


Fig. 6 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus, highlighting the item "value" and its connections

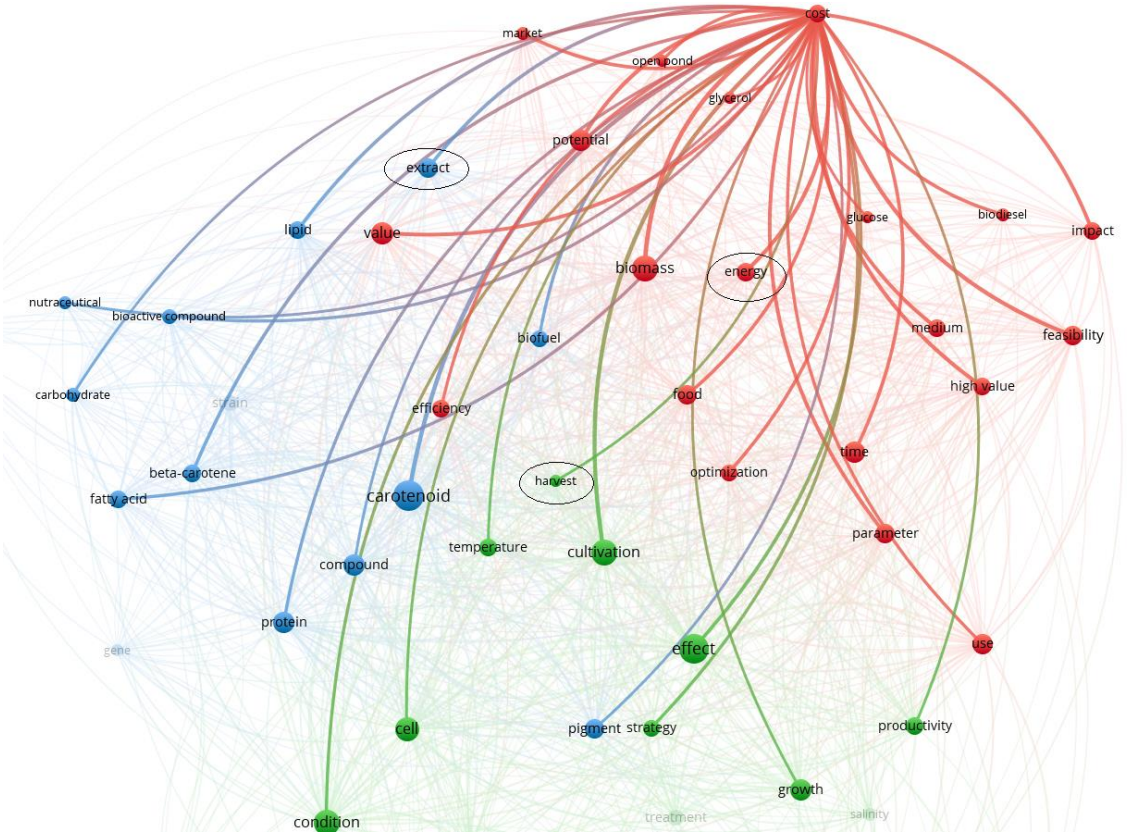


Fig. 7 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus, highlighting the item "cost" and its connections

4.1 HARVESTING METHODS AND GROWTH PHASE

The most common methods for biomass and bioproduct recovery seems to rely on centrifugation (e.g., [34-36]), flocculation and floatation (e.g., [37, 38]), filtration (e.g., [11]) and solvent extraction (e.g., [39, 40]). The first patent addressing a feasible method for harvesting *D. salina* biomass was published in 1985 [41], followed by a few more in the late 20th century [23, 42, 43]. The method for dewatering the microalgae biomass directly impacts the costs of operation. Hence, a suitable pretreatment must be assessed to reduce energy consumption. For instance, less efficient pretreatment, such as the sieving step, might require post-treatments for harvesting, and an intensive drying process without pretreatment may lead to higher costs and deterioration of the bioproduct. Nevertheless, the reported data on the subject is limited [44, 45].

Mouahid, Crampon, Toudji and Badens [44] analyzed different pretreatment methods for drying *D. salina* biomass combined with supercritical CO_2 extraction. Their results showed that airflow drying, followed by microwave, is the most suitable drying pretreatment to enhance beta-carotene extraction. In addition, they demonstrated the role of water as a cosolvent for the extraction of carotenoids instead of a barrier for diffusion. Tirado and Calvo [46] evaluated different cosolvent for supercritical CO_2 extraction of beta-carotene and applied the Hansen theory to choose the best option. Their findings predicted that adding ethanol as a cosolvent could reduce the bubble pressures and consequently increase the solubility of beta-carotene. This allowed the recovering of 25 g carotenoids per kg microalgae biomass in contrast to the 6 g carotenoids per kg microalgae biomass recovered by using only supercritical CO_2 extraction. Ethanol is also a reliable extract solvent preceding the recovery of polar lipids, glycerol, and some proteins [39].

Monte, Sá, Galinha, Costa, Hoekstra, Brazinha and Crespo [45] preconcentrated *D. salina* biomass through membrane processing (ultrafiltration) with recirculation before centrifugation. They reported a final concentration factor of 16.4, with an average permeate flux of 22 L m^{-2} h^{-1} and a minimal cell integrity loss of 13%. The total cost of ownership (sum of investment, energy, and maintenance cost) and energy demand were reduced by 52% and 45%, respectively, when preconcentrating by ultrafiltration.

Saponification and membrane processing (organophilic route) can be conducted to separate fractions of carotenoids from free fatty acids. Membrane processing followed by acetone extraction (hydroethanolic route) can recover glycerol and purify polar lipids from proteins and carbohydrates. Monte, Ribeiro, Parreira, Costa, Brive, Casal, Brazinha and Crespo [39] used n-heptane to recover carotenoids (85%) (organophilic route) while ethanol in water (68% v v^{-1}) was used to recover glycerol (86%), polar lipids (94%), proteins (95%) and carbohydrates (81%) (hydroethanolic route).

Rose, Maart, Phillips, Tucker, Cowan and Rowswell [47] evaluated the feasibility of cross-flow filtration as a harvesting approach to recover *D. salina* biomass. They noted the occurrence of filter blockage, cell damage, and flux loss after initiating the separation system, which could be explained by a drop in pressure. In addition, the abrasive features of the diatomaceous earth may have contributed to the negative results. Later, Monte, Bernardo, Sá, Parreira, Galinha, Costa, Casanovas, Brazinha and Crespo [11] evaluated the application of membrane and centrifugation for the preconcentration of *D. salina* biomass. This approach led to a reduction of 76% of the energy consumption compared to centrifugation

alone. Although this research aimed at harvesting carotenoid extraction, the preconcentration of the biomass applies to other desired purposes.

Flocculation is a useful method for biomass separation and has been reported as a low-cost and promising technique [48]. Cho, Hur, Lee, Ko, Lee, Kim, Kim, Chung, Kim and Oda [49] applied the dinoflagellate *Heterocapsa circularisquama* as a bioflocculant to recover *D. salina*, and they could increase both the quality and quantity of the recovered lipid. Although *D. salina* does not present autoflocculation features, this can be induced by increasing the pH of the medium, as demonstrated by Besson and Guiraud [50] and Ajala and Alexander [48]; however, the medium must be rich in magnesium (Mg) to trigger the flocculation of the cells. Ajala and Alexander [48] studied the use of plantain peel ash-derived alkalis to induce the flocculation of *D. salina*. They reached the maximum biomass concentration factor of 14 at 1% v v^{-1} flocculant dose, with 97.71% of biomass flocculated.

Sand-enhanced electro-flocculation (SEF) offers a cost-efficient harvesting approach. Xiong, Pang, Pan, Chika, Wang, Shi, Jia, Chen and Gao [51] increased the maximal recovery from 95.13% in 6 min to 98.09% in 4.5 min by using SEF with a 51.03% decrease in energy consumption compared to electro-flocculation. They also concluded that the flocculated medium could be further reused to cultivate *D. salina* by just supplementing N. Colloidal *D. salina* cells present a negative charge, stabilizing the suspension of the cells within the medium by forming an electrostatic barrier. Hence, positively charged sand bonds to the algal cell, forming larger and denser flocs that deposit on the bottom.

The culture phase is also a factor that must be considered to produce and recover compounds efficiently. Sui, Muys, Vermeir, D'Adamo and Vlaeminck [52] suggest the stationary phase to be the most suitable harvesting period, aiming to recover essential amino acids (EAA), as some pathways related to the biosynthesis of EAA are triggered in the later growth phase. Although high N availability boosts the production of proteins, the EAA are positively affected by short N starvation. This demonstrates that cultivating *D. salina* under N limitation might be a good approach to obtaining good protein quality [53]. The stationary phase seems to be the preferable culture phase for enhancing the quality of *D. salina* biomass for carotenoids and protein production [53-55]. However, when aiming to maximize protein quantity regardless of its quality, the exponential to linear growth phase with abundant N availability seems to be the optimal point for harvesting [53].

4.2 CULTIVATION AND MEDIUM INFLUENCE

The items "medium" and "condition", which are respectively grouped in the red (Fig. ***3***) and green clusters (Fig.4), most likely represent the different parameters related to microalgae culture, such as nutrient composition, light regime, and pH, among others. These parameters strongly affect biomass production and its content.

4.2.1 Light

Sui, Muys, Van de Waal, D'Adamo, Vermeir, Fernandes and Vlaeminck [53] compared different light intensities coupled with N conditions to optimize the co-production of protein and carotenoids. The results showed a 77% increase in intracellular protein under higher light intensity (110 µmol m^2 s^{-1}) in contrast to lower light intensity (70 µmol m^2 s^{-1}). The optimized cultivation condition (N starvation followed by higher illumination) resulted in an essential amino acid index (EAAI) of 1.1 and carotenoid content of 24 pg $cell^{-1}$. Gallego-Cartagena, Castillo-Ramirez and Martinez-Burgos [55] associated light intensity, N limitation, and higher sodium chloride (NaCl) concentration with high carotenogenic activity (9.67 ± 0.19 µg mL^{-1}) of *D. salina*. High light intensity triggers the photosynthetic mechanism of *D. salina* to protect it from any damage caused by light stress. The production of carotenoids is one of the most important responses to high light intensity, as this pigment filters the excessive light [55].

Zhang, Tang, Wang, Zhang, Zhou and Wang [56] assessed the impact of UV-B rays on *D. salina* biomass, protein, and glycerol content. UV-B triggers metabolic responses in microalgae, which yielded 51.36 pg glycerol per cell. They showed that UV-B markedly reduced cell density and increased protein and glycerol content. This suggests that UV-B radiation negatively impacted cell division, not cell size.

Besides illumination intensity, photoperiod is a tool to control the production of bioproducts. Sui, Muys, Vermeir, D'Adamo and Vlaeminck [52] cultivated *D. salina* under two photoperiods: 24-h continuous light and 12-h/12-h light/dark cycle. Under the light/dark cycle, the light-harvesting efficiency was increased, yielding 5 to 97% higher protein and 18 to 28% higher EAA mass on light energy throughout the growth. The biomass growth was also enhanced: 138% faster in the light phase of the light/dark cycle than in continuous light. During the dark phase in the light/dark cycle, there were no biomass and protein losses.

Pereira and Otero [54] evaluated how light quality influences growth, pigment content, and photosynthetic response of *D. salina* coupled with N starvation. They exposed the microalgae to 100% red,

100% blue, and a mix of 50% red and 50% blue light at 300 µmol photon m^2 s^{-1}. According to their results, the biomass concentration was the highest under 100% red and the lowest under 100% blue light. Blue light boosted carotenoid content, where Fv/Fm remained high. Overall, maximum carotenoid concentration was reached under the mix of red and blue light, so higher biomass production stimulated by the red light compensated for the lower carotenoid content. Xu and Harvey [57] found the red light to increase the *9-cis* beta-carotene compared to blue and white light; and reached over 2.5 *9-cis*/*all-trans* beta-carotene ratio within 48 h, independently of the light intensity. This follows their previous results, where they identified high-intensity red light to enhance the production of carotenoids [58]. Red light increases the isomerization rate of *all-trans* beta-carotene to *9-cis* beta-carotene relative to the rate of its destruction [57].

Fig. ***8*** demonstrates the links between the item "light" and different bioproducts, such as fatty acids, protein, and pigments.

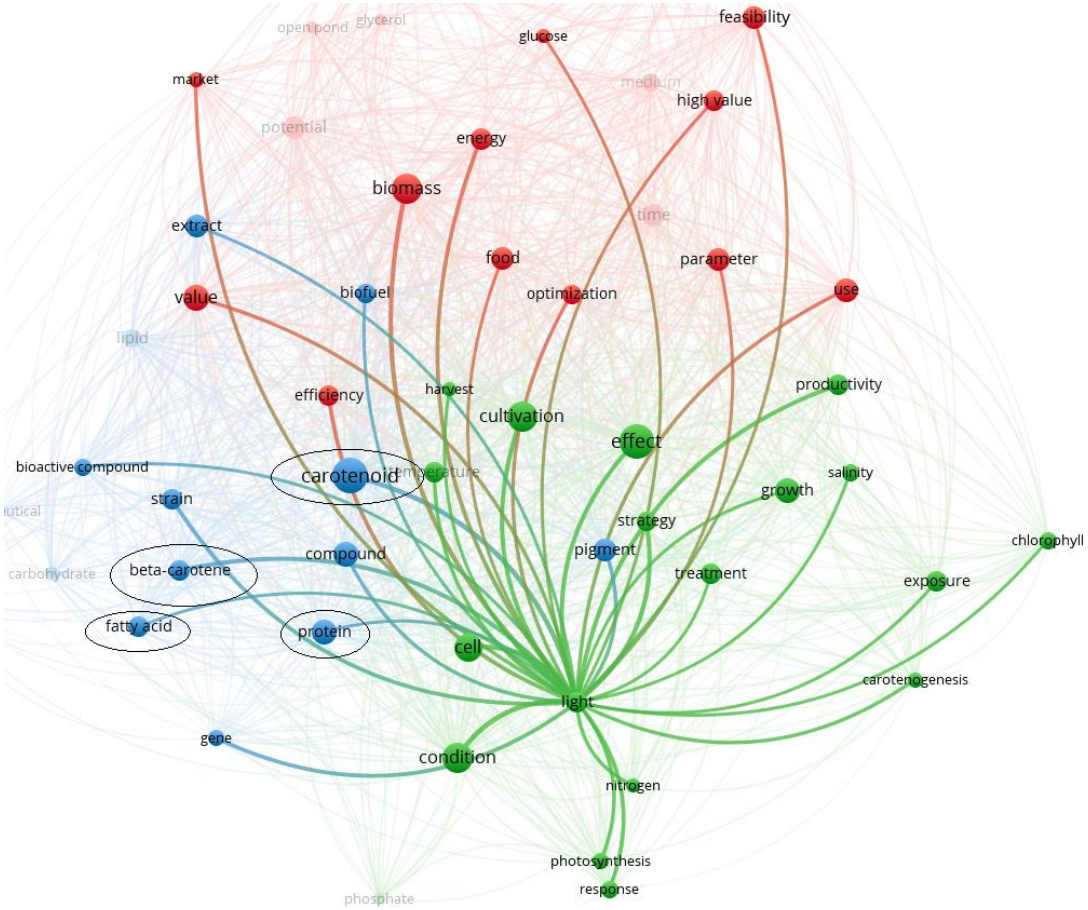


Fig. 8 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 69 items with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus, highlighting the item "light" and its connections

4.2.2 Salinity

Although *D. salina* can prevail within a range of salinity levels, from 32 mg L^{-1} [59] to 1500 g L^{-1} [60, 61], suboptimal salinities hamper biomass productivity and influences bioproducts yield. Salinity acclimatization triggers the stress defense system of *D. salina* and photosynthetic genes, which facilitate growth, pigment synthesis, and antioxidant capacity. This feature is reported to allow these microalgae to thrive under the presence of heavy metals [62]. Fig. ***9*** shows that "salinity" is related to "glycerol" and "protein", and further impacts the "biomass" yield and composition, e.g. "carbohydrate", "pigment", "carotenoid", "beta-carotene", and "lipid".

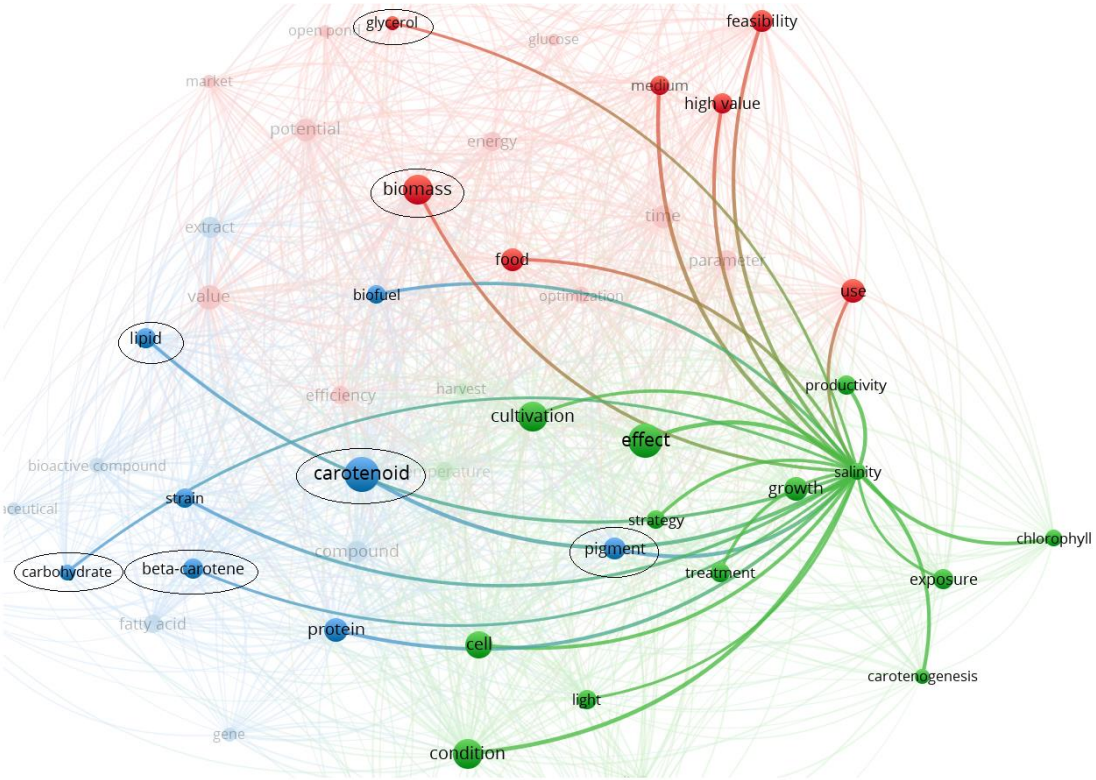


Fig. 9 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, Science Direct, and Scopus, highlighting the item "salinity" and its connections

Alkayal, Albion, Tillett, Hathwaik, Lemos and Cushman [63] found the biosynthesis of new proteins and maintenance of existing proteins as a response to salinity shock. Interestingly, many studies have been tracking the metabolic response of *D. salina* towards salinity stress (e.g. [64-66]), which explains the green cluster in (Fig.4) that groups the items "effect", "growth", "exposure", and "salinity".

Ishika, Moheimani, Laird and Bahri [6] cultivated *D. salina* throughout a range of salinity (125–145 g L^{-1}). Their findings pointed to the highest biomass productivity to be around 135 g L^{-1} salinity, which decreased towards higher salinities. In comparison, lipid content was almost 65% higher under 145 g L^{-1} compared to 135 g L^{-1}. This may be explained by changes in the lipid biosynthetic pathways related to the formation and storage of fatty acids. Despite the higher lipid content under high salinity, the lipid productivity slightly decreased towards higher salinities, ascribed to lower biomass productivity. However, Abomohra, El-Naggar, Alaswad, Elsayed, Li and Li [60] found the highest lipid and fatty acid methyl esters productivity at a salinity of 1500 g L^{-1}, which decreased towards both lower and higher salinities.

Intracellular glycerol in *D. salina* rapidly and positively responds to changes in extracellular salinity [64, 67, 68]. Wu, Lan, Cao, Yao, Qiao, Xu and Cao [69] assessed two different salinities (29.22 and 87.66 g L^{-1}) to produce glycerol. They reached approximately a maximum of 120 mg glycerol g wet $cell^{-1}$ in *D. salina* at the highest salinity. Singh, Khadim, Singh, Singh, Maurya, Tiwari and Asthana [61] reported similar results: intracellular glycerol was higher at higher salinities (4 M NaCl); however, at high salinities (3 and 4M), the glycerol leakage increased. This indicates that glycerol production is a mechanism that balances the salinity osmotically in the environment.

4.2.3 Medium composition

Phosphorus (P) and N strongly affect the growth and composition of *D. salina*, as shown by Chen, Tang, Kapoore, Xu and Vaidyanathan [70], and Pancha, Chokshi, George, Ghosh, Paliwal, Maurya and Mishra [71]. The items "nitrogen" and "phosphate" are grouped with "carotenogenesis", "response", and "photosynthesis" (Fig.4). Even though nutrient limitation can cease cell growth in microalgae, N deficiency is the most reported trigger of lipid accumulation when light and carbon (C) sources are abundant [72]. This is demonstrated by Riyazat Khadim, Mohanta, Singh, Maurya, Kumar Singh, Kumar Singh and Asthana [73], who obtained the highest lipid content (341.1 mg g^{-1} DW) at lower nitrogen (1.25 mM KNO_3) combined with phosphate deficiency. However, the same authors reported lower biomass productivity (13.12 mg L^{-1} d^{-1} DW) at the same conditions, which was overcome by adding 10.00 mM $NaHCO_3$, inducing an improvement of 1.7-fold and 2.25-fold in lipid content and biomass productivity, respectively.

Yuan, Li and Zhao [74] monitored the effect of N, sulfur (S), and P limitation, light intensity (100 and 800 µmol $m^{-2}s^{-1}$), and CO_2 concentration (1% and 10%) on *D. salina* growth and lipid accumulation. They

found that low light intensity benefits lipid accumulation under N depletion. However, lipid productivity was the highest under 10% CO_2 and high light intensity due to higher biomass generation.

Chen, Tang, Kapoore, Xu and Vaidyanathan [70] reported that recycled F/2 medium with N deficiency limits the production of chlorophylls (a and b) and total carotenoids in *D. salina*, despite continuing biomass growth under this condition. This can be related to the role played by N on the synthesis of pigments, which decreased with time (>45h) when N was unavailable. A positive relation between chlorophyll and vitamin deficiency was reported. N limitation hampered the protein productivity in *D. salina*, which can be associated with lower biomass production and inhibited protein metabolism; interestingly, P limitation seemed to enhance protein productivity. The effect of N on carotenoids in this research is contrary to the results of Sá, Monte, Brazinha, Galinha and Crespo [75], which showed enhanced carotenoid production in *D. salina* with N depletion. Gallego-Cartagena, Castillo-Ramirez and Martinez-Burgos [55] found similar results linked to carotenoid accumulation, the depletion of nutrients, mainly N, and excessive biomass production during the first seven days of culture. Nitrogen depletion had been reported to trigger metabolic responses in microalgae, such as the degradation of nitrogenous compounds and the accumulation of carbohydrates and proteins [71].

Nakas, Schaedle, Parkinson, Coonley and Tanenbaum [76] evaluated the relationship between C source and glycerol production by *D. salina*. They could yield 27.6 pg $cell^{-1}$ when using $NaHCO_3$ as a C source in contrast to 7.94 pg $cell^{-1}$ with 3% CO_2 as a C source. Nevertheless, the glycerol yield per liter of culture medium was slightly lower with $NaHCO_3$ (10.5 mg) compared to 3% CO_2 (12.6 mg) as a C source. This is likely associated with the higher pH in the medium enriched with $NaHCO_3$, which reached 9.5 after 5 days and inhibited growth.

The presence of heavy metals influences the productivity of *D. salina*. For instance, cadmium (Cd) can potentially reduce cellular pigment, total protein, and glutathione content, aside from weakening photosynthetic efficiency and antioxidant capacity. This effect can be mitigated by salinity acclimatization, which has been proved to increase the tolerance of *D. salina* towards Cd toxicity [62]. Bahador, Einali, Azizian-Shermeh and Sangtarash [77] demonstrated that nanoparticles of silver (Ag) with a dose of 2.7 ng L^{-1} boosted the total protein, chlorophyll, beta-carotene, hydrogen (H) peroxidation, carbohydrates, and free amino acids content in *D. salina*. Tolerance toward contaminants allows *D. salina* to thrive even in water contaminated with No. 0 diesel oils water-soluble fractions (WSFs), as shown by Liu, Tu, Li, Cai,

Huang and Zheng [78]. The protein and beta-carotene synthesis were promoted, increasing their cell content by approximately 3 folds when cultivated under 5 mg L^{-1} of WSF.

The productivity of *D. salina* biomass and lipid can be enhanced by adding supplements into the medium, such as inositols. Cho, Kim, Lim, Kim, Ha, Shin, Kim, Roh, Kim and Oda [79] compared the use of four inositols derivatives: myo-inositol, scyllo-inositol, D-chiro-inositol, and L-chiro-inositol. Their results demonstrated that myo-inositol (500 mg L^{-1}) promoted the highest biomass yield (1.48 times higher than the control) and affected the fatty acid methyl ester composition, inducing significantly higher production of linoleic, linolenic, and linolelaidic acids. Lipid productivity was positively affected by the deficiency of trace metals in the medium [70]; thus, supplementing inositols derivates coupled with trace metals deficiency seems to offer a potential method for lipid accumulation.

5. BIOPRODUCTS GENERATION

5.1 *Lipid*

Neutral, glyco, and phospholipids compose the total lipid in microalgae. They participate in energy storage and are important components of the external and chloroplast membrane and endoplasmic reticulum [71]. During stress events, microalgae accumulate lipid as an energy source, which may be exploited to enhance lipid production [13, 80]. *D. salina* is a great source of lipid and triacylglycerol (TAG) [9], and the lipid content can range from 7% to 60% depending on the cultivation method, harvesting phase (Table 2) [70], and strain [9]. Although nutrient deficiency and other stressful conditions can boost lipid content, cell growth can be hampered, lowering overall lipid productivity. This can be countermeasured by a two-step cultivation method, where the first step enhances cell growth with abundant nutrients, and the second step boosts lipid accumulation under nutrient limitation conditions [72]. However, higher costs come with the two-step cultivation approach, which needs to be considered [13]. Lipid production is majorly associated with biodiesel generation (Fig. ***3***), which strongly depends on the fatty acid profile. Nevertheless, lipid generation offers a significant source for the production of biogas [9], biolubricants [81], and pharmaceutical purposes as well [36].

Table 2 - Lipid content in *D. salina*

Lipid content (%)	Medium	Salinity (g L^{-1})	Light intensity (µmol m^{-2} s^{-1})	Photoperiod (L:D) (h)	Reference
32.5 ± 2.6	F/2 with increasing salinity	125–154	150	12:12	[6]
12.5–60	F/2	~21	150	24:0	[9]
22	F/2 (N starvation)	~21	300	n.e	[34]
7.89 ± 0.4	BG11	100	n.e	n.e	[36]

n.e: Not evaluated

5.2 Carbohydrate

Carbohydrate is a key component for energy storage, especially under nutrient limitation and starvation. When under N limitation or starvation, microalgae favor synthesizing excess metabolic C into starch, followed by TAG synthesis. Microalgae store carbohydrates mainly in their cell wall as cellulose and the cytoplasm as starch [71]. Since *D. salina* lacks a cell wall, the carbohydrate is mostly stored in the cytoplasm as glucose, galactose, ribose, and xylose [82]. Carbohydrates are key components for biofuel production [83, 84], making *D. salina* a potentially strong candidate with its high amounts of carbohydrates (over 50%) [82]. However, to the authors' knowledge, this is not the case largely since these microalgae can produce compounds with higher potential for industrial use. This is demonstrated in Fig. ***10***, where the frame size of the item "carbohydrate" is smaller than other items regarding bioproducts, such as "protein", "lipid", and "carotenoid".

Fig. 10 Comparison of the frame size of the items "carbohydrate", "protein", "lipid", and "carotenoid" generated by the bibliometric analysis. Frame size indicates the number of occurrences of an item, and it is ordered from less occurrence (carbohydrate) to more occurrence (carotenoid)

5.3 Protein

Extracts from microalgal protein are the source of functional biopeptides with potential use to tackle cancer cells [85]. Moreover, microalgal protein offers a great alternative to animal-based food to support the increasing demand for food [53]. The protein content in *D. salina* biomass can range from around 40% to 80% of its ash-free dry weight (AFDW) [86]. Protein synthesis depends directly on the bioavailability of N. Reductions of up to 60% in protein content have been documented when microalgae are exposed to an N-free medium. This indicates that the cell might use the N stored in nitrogenous compounds (for instance, protein) to maintain the intracellular N quota to keep its metabolic functions [71].

s Nitrate (NO_3^-) is assimilated into the cell and reduced by nitrate reductase into nitrite (NO_2^-) and then into ammonium (NH_4^+) by nitrite reductase. Next, NH_4^+ enters the tricarboxylic acid (TCA) cycle and is incorporated into glutamate/glutamine, which are intermediates for the further formation of the protein profile [87]. One aspect contributing to the enhancement of protein content is the luxury uptake of N, an overcompensatory mechanism. This is triggered when the microalgae are cultivated in an N-rich medium after experiencing N limitation [53].

5.4 Carotenoid

As represented by the items "beta-carotene" and "lutein" in Fig. ***5***, these two carotenoid forms are key bioproducts produced by *D. salina*. These microalgae accumulate massive amounts of carotenoids (around 10% of the AFDW [88]), especially beta-carotene [89], which was already targeted in 1987 [20], and in the late 90s [22, 90-92]. The yield may vary depending on the culture conditions (Table 3), which may be induced by light, salinity stress [55], nutrient limitation [14], and oxidative stress [77]. Han, Lu, Zhao, Xu, Zhang and Li [14] indicated salinity as the major factor regarding beta-carotene yield, followed by N and the light intensity.

Table 3 - Yield of total carotenoids and beta-carotene by *D. salina*

Total carotenoids	beta-carotene	Unit	Reference
~0.67		pg cells^{-1}	[93]
	33.8 ± 1.76		[78]
	~1.2		[77]

	~3.0		[94]
	3.3	mg g^{-1}	[36]
25			[46]
~160			[95]
~110 (blue light)			[54]
~80 (red light)			
~100 (blue and red light)			
0.115			[96]
	47.7		[97]
~38		mg L^{-1}	[98]
	4.02		[14]
0.01 ± 0.0002			[55]
0.002 ± 0.00008			
0.020 ± 0.003			[99]

The pathway for beta-carotene accumulation by *D. salina* is not well established since there are contradictions regarding the transcriptional regulation of phytoene synthase and phytoene desaturase, enzymes responsible for the catalization of geranylgeranyl pyrophosphate and phytoene. However, the regulatory mechanism starts with signal sensing and transduction of relevant environmental changes. The signal sensing might be approached by a UV-A photoreceptor, single-oxygen sensor, and plastoquinone redox state; as for the transduction step, there are few available data to explain this process [100].

Lutein is a xanthophyll oxygenated carotenoid applied as a food ingredient due to its high-value nutraceutical function (protection of the eye and cardiovascular health, antioxidant, infant brain development, decrease of the risk of cancer, and anti-inflammatory). The pathway shares the one for beta-carotene, with the difference that lycopene, a compound that results from phytoene desaturation, is converted into alpha-carotene instead of beta-carotene and later into lutein [101].

5.5 Glycerol

Glycerol is getting notorious as addictive for biodiesel; the item "glycerol" is linked to "biodiesel" in Fig. ***11***. Glycerol derivates present a hydrotropic feature, which allows the implementation of water into biofuels, named hydrofuels, which reduces NO_x emissions and optimizes biofuel production [102]. This might boost the interest in the glycerol generation by *D. salina* (over 50% of its AFDW [103]).

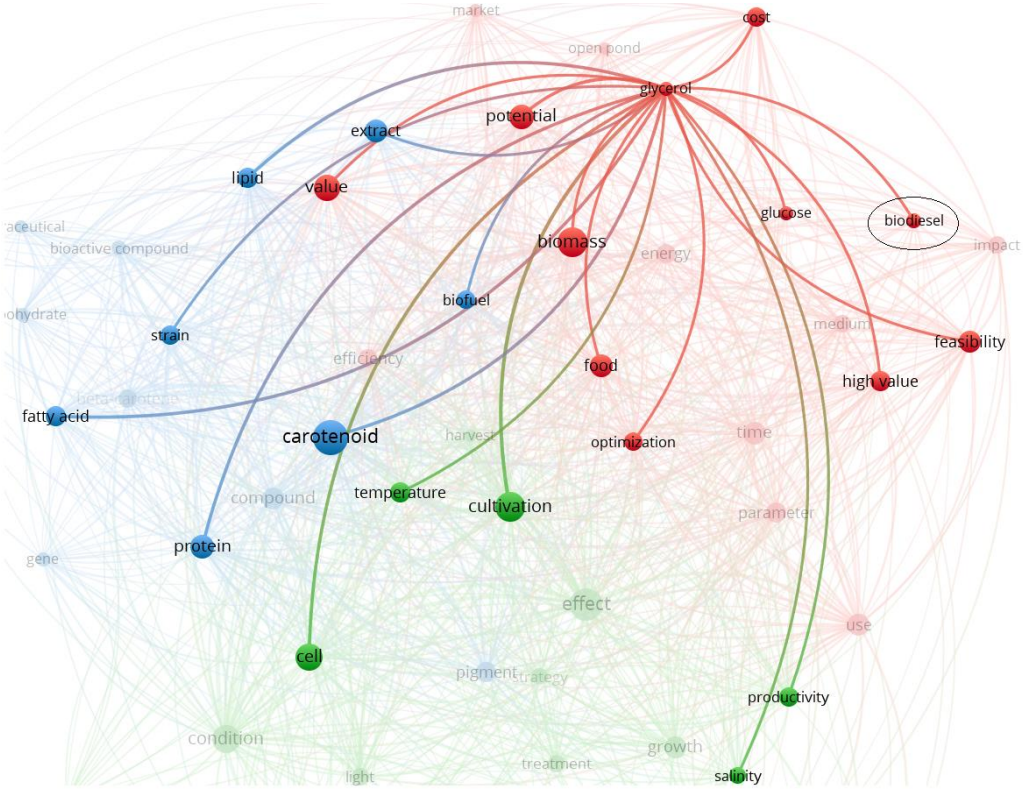


Fig. 11 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, ScienceDirect, and Scopus, highlighting the item "glycerol" and its connections

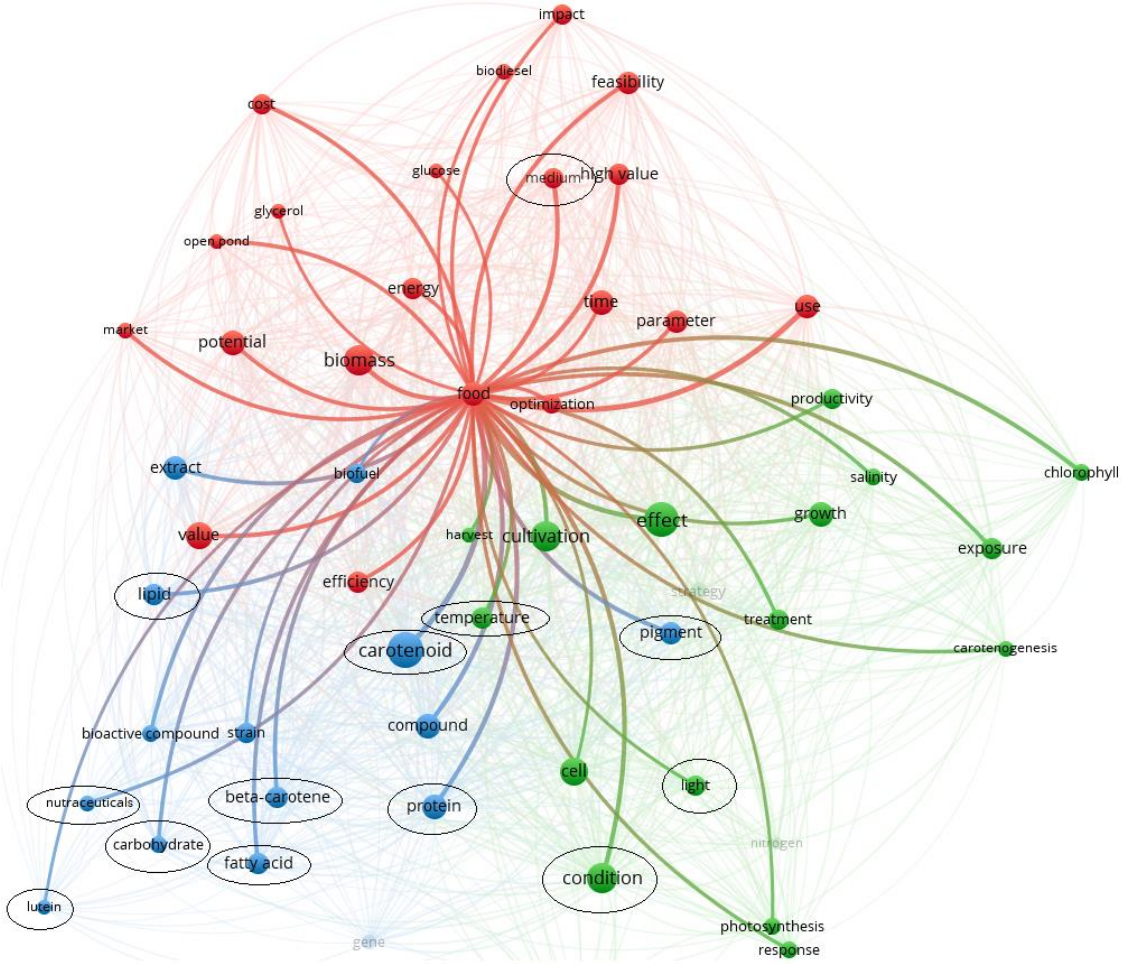
Metabolic pathways regarding glycerol production by *D. salina* are not clearly understood and are controversially under debate. So far, many researchers have been trying to fill the gaps over the years (e.g. [64, 69, 104-107]). Different pathways had been suggested and described in earlier studies [104, 105]. They all involved reducing dihydroxyacetone phosphate to glycerol-1-phosphate by nicotinamide adenine dinucleotide (NAD^+)-dependent glycerol-1-phosphate dehydrogenase and dephosphorylation to glycerol by glycerol-1-phosphatase. However, the dependence of the Mg^{2+}-glycerol 1-phosphate complex as substrate was postulated later [106]. He, Qiao, Bai, Zhang, Yang, Li and Cao [108] suggested that the NAD^+-dependent glycerol 3-phosphate dehydrogenase from *D. salina* catalyzes the step from

dihydroxyacetone phosphate to glycerol directly. This could explain the rapid synthesis of glycerol found in *D. salina* under oxidative conditions. Regardless of the mechanisms, *D. salina* unquestionably can produce high amounts of glycerol.

6. APPLICATIONS

6.1 Food

Using algae as food is not new; the oldest known use of algae as a food source dates to 14 thousand years ago in Chile [109]. Related to the *Dunaliella* genre, the first patent on microalgae as a food source was published in 1997 [110]. Notably, *Dunaliella* is among the few microalgae granted the Generally Recognized As Safe (GRAS) status by the Food and Drug Administration [111]. Before considering a microalgae species as a food source, one crucial factor that must be considered is the nutritional composition. This depends directly on the species, the culture, and environmental conditions, such as temperature and light regime [112]. Lipids, proteins, vitamins, and minerals are important constituents of human health and must be addressed [113]. The items "condition", "medium", "temperature", "light", "lipid", "fatty acid", "protein", "carotenoid", "beta-carotene", "pigment", and "nutraceuticals" represent these relations (



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492 Fig. ***12***).

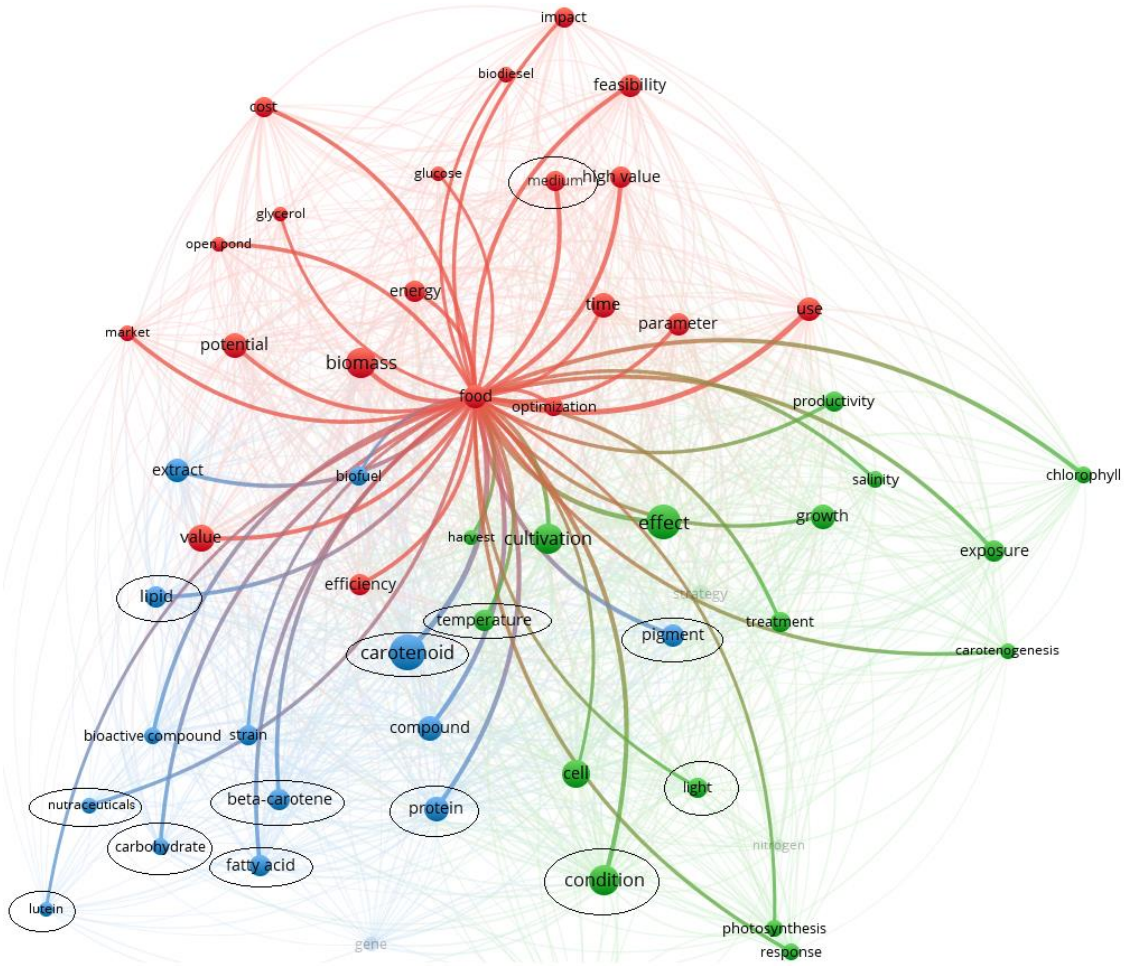

Fig. 12 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, ScienceDirect, and Scopus, highlighting the item "food" and its connections

D. salina can be used as an additive to food due to its lipophilic (carotenoids and alpha-tocopherol) and hydrophilic (glutathione and ascorbic acid) antioxidant compounds [114]. Abd El-baky, El Baz and El-Baroty [115] produced 3.83 and 25.41 mg g^{-1} of alpha-tocopherol (vitamin E) and ascorbic acid (vitamin C), respectively. Their findings were similar to their previous research (approximately 12 and 25 mg g^{-1} of vitamin E and C, respectively) [116]. *D. salina* produces protein both in high quantity (up to 80%) and high quality (EAAI $\geq$ 1). Sui, Muys, Van de Waal, D'Adamo, Vermeir, Fernandes and Vlaeminck [53] investigated a two-phase cultivation strategy to produce high-quality protein and carotenoid. They generated 22, 7, and 3 mg L^{-1} of protein, EAA, and carotenoid, respectively. They also observed the production of beta-carotene with high antioxidant pro-vitamin A activity, and EAAI of 1.3, making it a

suitable and sustainable food source. [86] Dolganyuk, Andreeva, Budenkova, Sukhikh, Babich, Ivanova, Prosekov and Ulrikh [117] evaluated the lipid composition of *D. salina*. Their results showed that the fatty acid profile, rich in saturated (34.67 ± 0.56% DW) and unsaturated (65.08 ± 0.22% DW) chains, can be used to create biologically active food supplements; and myristic, palmitic, oleic, stearic and linoleic acid can serve as feed additives for animal husbandry.

Boonyaratpalin, Thongrod, Supamattaya, Britton and Schlipalius [118] evaluated the effect of replacing astaxanthin by beta-carotene from *D. salina* as feed for *Penaeus monodon* (tiger shrimp). Their findings showed that beta-carotene achieved the same result (growth, pigmentation, survival, and health) as the more expensive astaxanthin. An increase in growth and pigmentation was also found in crayfish *Cherax tenuimanos* fed with carotenoid-rich *D. salina* [119]. Alishahi, Karamifar and Mesbah [120] fed *Astronotus ocellatus* with beta-carotene from *D. salina* and compared it to feeding with astaxanthin. Both additives increased the immunological system, growth, and skin carotenoid. A positive immunological response was also identified for *P. monodon* [121]. Guermazi, Elloumi, Ayadi, Bouain and Aleya [122] reached the greatest length (243 µm) for *Fabrea salina* when fed with *D. salina* biomass compared to *Isochrysis galbana* and *Saccharomyces cerevisiae.*

6.2 Pharmaceutical

D. salina can produce bioactive compounds (Fig. ***3***) with potential pharmaceutical purposes, especially for cancer and inflammatory disease treatment. The antioxidant potential of algae is mainly associated with four main classes of low-molecular-weight natural antioxidants: phenolic compounds, carotenoids, vitamins, and sulfated polysaccharides [12, 123]. Singh, Tiwari, Singh, Singh, Khadim, Singh, Laxmi, Srivastava, Hasan and Asthana [124] characterized the potential of the aqueous extract of *D. salina* in synthesizing gold nanoparticles (AuNP) against MCF-7 and MCF-10A, both breast cancer cell lines. The extract was composed of phenolics, flavonoids, and proteins, which comprised functional groups likely responsible for reducing and stabilizing AuNP. This result reveals an economically viable and eco-friendly approach to breast cancer. Zamani, Rastegari and Varamini [12] introduced the use of magnetic nanoparticles grafted with gum arabic (GA-MNPs) to deliver *D. salina* extract to treat MCF-7 and HeLa cancerous cell lines. The cytotoxicity test presented toxicity towards MCF-7 and HeLa after 72h exposure to *D. salina* extract, and the oral delivery with GA-MNPs helped reduce adverse gastric effects and maintain the extract's bioactive potency.

El-Baz, Salama and Hussein [125] assessed the use of carotenoids against thioacetamide (TAA)-induced hepatic fibrosis in rats. They showed a pronounced protective activity of *D. salina*, which can be attributed to the enhancement of accretion of extracellular matrix accumulation and the decrease of alpha-SMA and collagen I. Carotenoids also presented therapeutic efficiency on obesity-associated cardiac dysfunction in rats by attenuating fibrotic cardiac tissue and congesting myocardial blood vessels [126]. Madkour and Abdel-Daim [127] attributed carotenoids in *D. salina* to the hepatoprotective effect against paracetamol overdose.

Chuang, Ho, Liao and Lu [128] demonstrated the benefic use of *D. salina* to treat leukemia by acting as an antileukemia and immunomodulatory agent, prolonging the survival of leukemic mice. The immunomodulatory effect of *D. salina* seems to be linked to the excretion of pentasaccharides [129]. Khayyal, El-Baz, Meselhy, Ali and El-Hazek [36] investigated the potential protective effect of *D. salina* against intestinal injury in rats and found a reduction in the severity of intestinal mucositis induced by oral doses of *D. salina* extract. The content of total fatty acids were 7.32 ± 0.04 mg g^{-1} DW, of which C16:0 (4.58 ± 0.02 mg g^{-1}) was the major fatty acid, followed by C18.3 (0.66 ± 0.02 mg g^{-1}), C18:1 (0.63 ± 0.03 mg g^{-1}), C18:2 (0.54 ± 0.01 mg g^{-1}), C16:1 (0.52 ± 0.00 mg g^{-1}) and C18:0 (0.38 ± 0.06 mg g^{-1}). Total fatty acids may have contributed to the protective effect of *D. salina*.

D. salina extracts present antibacterial and anti-adherent properties, and their use to prevent bacterial infections in humans has been documented. Jafari, Mobasher, Najafipour, Ghasemi, Mohkam, Ebrahimi and Mobasher [130] proved the antibacterial and antibiofilm potential of *D. salina* extracts on biofilm formed by *Streptococcus mutans*, which is believed to be the most important agent in dental caries. Medina-Jaritz, Carmona-Ugalde, Lopez-Cedillo and Leon F [131] had previously documented similar results against several pathogens such as *Proteus vulgaris, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella typhi* ATCC 6534, *E. coli* ATCC 8739, *S. aureus* ATCC 25923, and *B. subtilis* ATCC 6635. Herrero, Ibanez, Cifuentes, Reglero and Santoyo [132] tested different solvents (hexane, petroleum ether, and water) and temperatures (40, 100, and 160°C) to recover *D. salina* extracts to test against microorganisms of importance for the food industry (*E. coli*, *S. aureus*, *Candida albicans*, and *Aspergillus niger*). Their studies showed that the best antimicrobial activity was obtained using petroleum ether and hexane at 160 °C. Besides, they identified fifteen volatile compounds (beta-cyclocitral, alpha- and beta-

564 ionone, neophytadiene, phytol, among others) and several fatty acids (mostly palmitic, alpha-linolenic, and
565 oleic acids), which can be associated with antimicrobial activity.

566 *6.3 Biofuel*

567 Using microalgae biomass for biofuel production has been discussed over the years as a suitable
568 approach to supply the increasing energy demand. Marine microalgae could be economically feasible for
569 biofuel production, although the desalination of the substrate would be needed. This can be achieved by
570 desalination techniques that demand low energy consumption [60, 133]. Although the viability of
571 microalgae for biofuel generation is questionable, the interest has been consistently pointed out. Fig. ***13***
572 shows a close relation between feasibility and biofuel production of *D. salina*. This is denoted by the link
573 among the item "biofuel" and the items "cost", "efficiency", "productivity", and "feasibility". Most of the
574 concerns are related to its feasibility and scalability. For instance, the method for lipid extraction varies
575 during the biorefinery process [134]. The costs associated with biomass harvest, lipid extraction, and its
576 conversion into advanced biofuel can account for up to 60% of all costs for biodiesel production due to
577 energy demand [135], also implied by the item "energy" (Fig. ***7***). Some costs associated with biofuel
578 production can be counterposed by the generation of byproducts, such as carotenoids. This might explain
579 the association among the items "biofuel", "value", and "carotenoid" (Fig. ***13***).

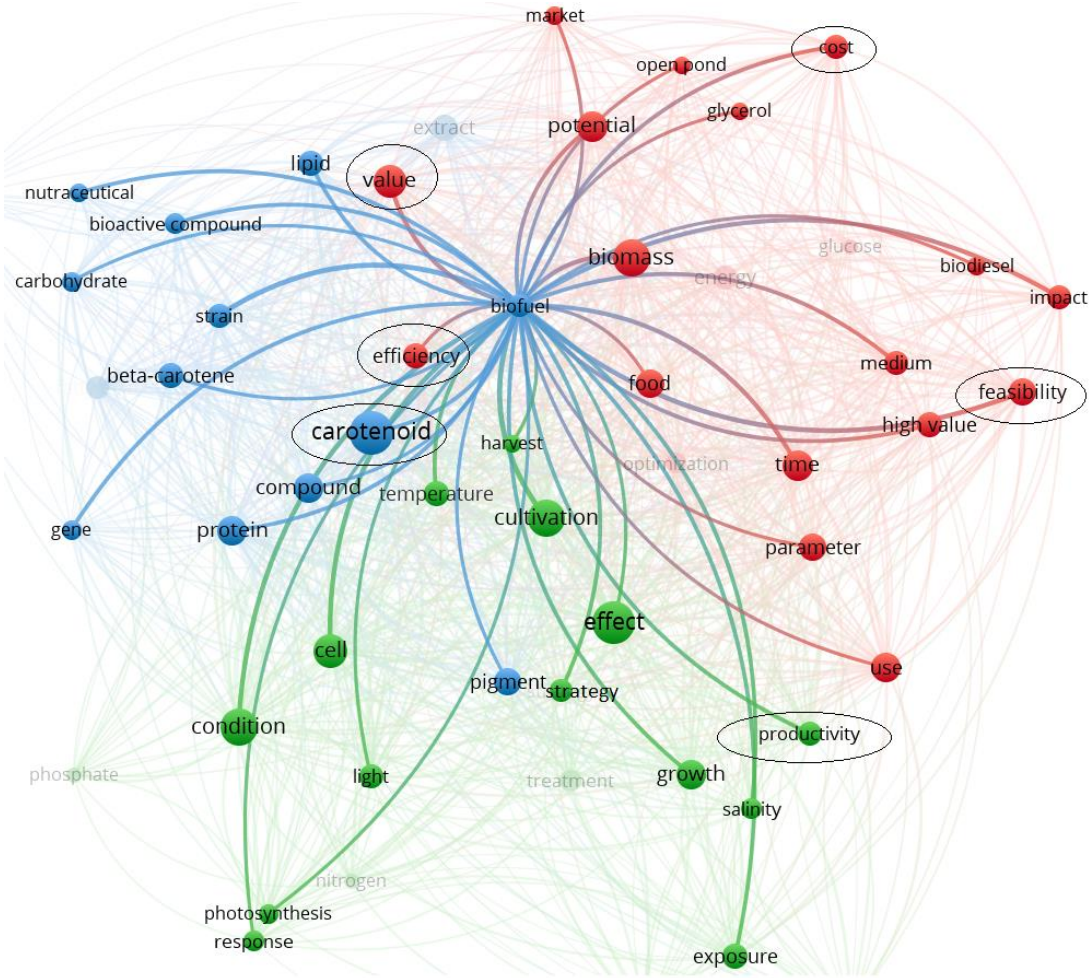

Fig. 13 Bibliometric analysis of the terms "*Dunaliella salina*" + "product" showing the 54 items with the most occurrence within 2016–2021 on the databases WoS, ScienceDirect, and Scopus, highlighting the item "biofuel" and its connections

One use of microalgae as biofuel aims to generate biogas, which is possible by converting the carbon in the biomass into methane. The lack of a cell wall makes this species suitable for methane (CH_4) production. Considering the higher contribution of lipids, compared to carbohydrates and proteins, for CH_4 generation and that *D. salina* can produce up to 60% of lipid content [9], the use of this species is promising. Fernandez-Rodriguez, Rincon, Fermoso, Jimenez and Borja [136] coupled *D. salina* biomass and olive mill solid waste (OMSW) with different ratios to produce CH_4. Their results demonstrated that a 25%/75% (*D. salina*/ OMSW) mixture (C:N ratio of 26.7) showed a substrate biodegradability and CH_4 yield of 71.5% and 330 mL CH_4 g VS^{-1}, respectively.

Roberts, Heaven and Banks [137] assessed the effect of using *D. salina* biomass cultivated under low and high sulfate (SO_4) conditions (4.7 g SO_4 L^{-1}) for anaerobic digestion. The authors observed a yield of

0.233 and 0.193 L CH_4 g^{-1} volatile solids at low and high SO_4 medium, respectively; there was a rise in hydrogen sulfide (H_2S) at high SO_4 medium. The inhibition of CH_4 and yield of H_2S under higher SO_4 concentration may be explained by the competition for available electron acceptors between methanogens and sulfate-reducing bacteria. González-González, Astals, Pratt, Jensen and Schenk [34] assessed the suitability of an integrated biorefinery of biodiesel and biogas production using *D. salina*. In contrast to the results of Roberts, Heaven and Banks [137], the authors reached a methane yield of 0.364 CH_4 L g^{-1} volatile solids and a recovery of 21% from the lipid content by conducting a solvent-free method.

Glycerol is readily available for bioethanol production through fermentation. Nakas, Schaedle, Parkinson, Coonley and Tanenbaum [76] converted 95.9% of the available glycerol (47.5 µmol mL^{-1}) presented in *D. salina* biomass into 2.17 g L^{-1} of total solvent (n-butanol; 1,3-propanediol; ethanol, acetate), where ethanol production was 0.05 g L^{-1}. In addition, bioethanol can be produced from the hydrolysis and fermentation of carbohydrates [138]; thus, it is possible to use *D. salina* to produce this biofuel, considering that it reaches over 50% of carbohydrate content [82].

Photosynthesizer microorganisms can convert water into H_2 through solar energy harvesting. This process is driven by either the enzyme hydrogenase or nitrogenase [139]. Hydrogenase activity is essential during nutrient deprivation, especially under S deprivation [140]. However, even under the S deprivation condition and though *D. salina* culture became anaerobic in the light (condition necessary to activate the hydrogenase pathway), this species could not yield H_2. This supports the prediction of the lack of hydrogenase enzyme by *D. salina* [141].

Nevertheless, it is possible to convert microalgae biomass into H_2 by anaerobic acetone-butanol fermentation using bacteria, mainly *Clostridium acetobutylicum* [142]. The composition of the substrate dictates the efficiency of H_2 yield, and it highly depends on the carbohydrate content [143]. Chen, Qu, Xiao and Miao [144] obtained H_2 yields of 192.35 and 183.02 mL g^{-1} volatile solid (VS) with algae residue of *Dunaliella primolecta* and *Dunaliella tertiolecta*. Considering that the carbohydrate contents of the former *Dunaliella* species were found to be 20.99 ± 0.56 and 20.38 ± 0.37% ww^{-1} prior to lipid extraction by the same authors and that *D. salina* can reach up to 57.8% ww^{-1} of carbohydrate content [82], it is unquestionable the potential use of *D. salina* for H_2.

7. FUTURE PERSPECTIVES

Microalgae have been used in various applications ranging from biofuels, biofertilizers, biopolymers, and bioremediation to human nutrition, animal feed, and cosmetics [1]. *D. salina* is remarkable in biotechnology and has been extensively used for different purposes owing to its versatility. Nevertheless, new application opportunities seem to be far from being exhausted. As shown in Fig. ***14***, the future of *D. salina* seems to rely on three distinct clusters.

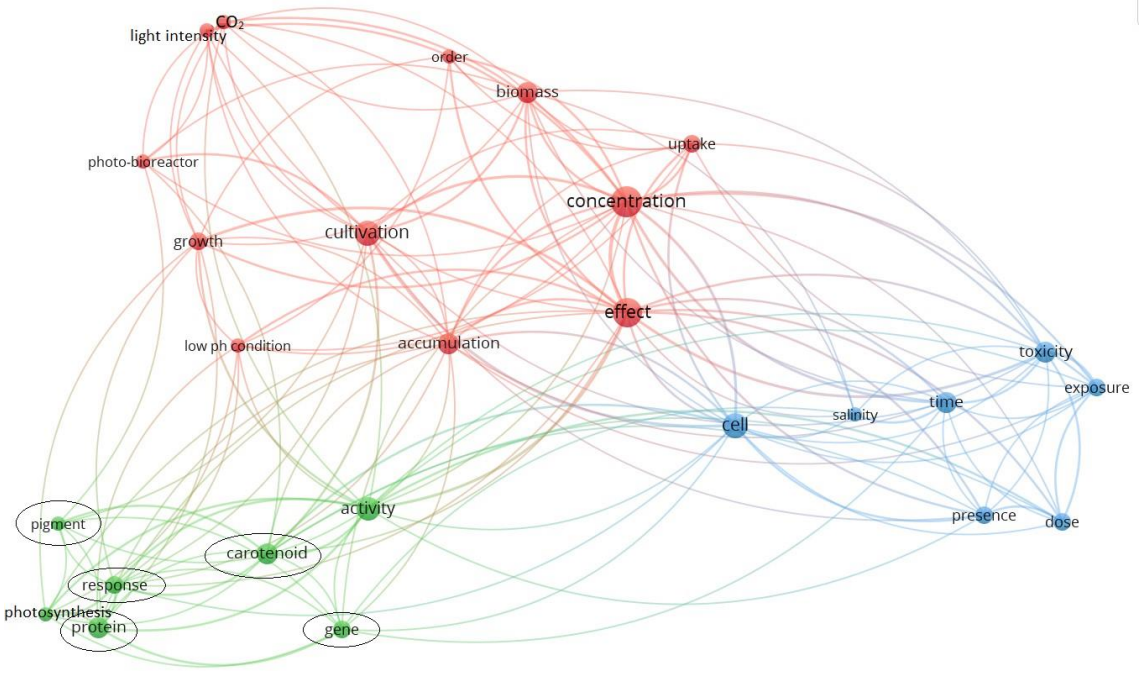

Fig. 14 Bibliometric analysis of the terms "*Dunaliella salina*" + "future" showing the 26 items with the most occurrence within 2016-2021

The green cluster is regarding the accumulation of proteins and pigments. The future of *D. salina* is assigned to the capacity to produce "pigment", "carotenoid", and "protein". This is probably due to the increasing demand for food alternatives and their use as feedstock for pharmaceutical products. Recently, Xu, Ibrahim, Wosu, Ben-Amotz and Harvey [145] identified the potential of new isolates of *D. salina*, namely DF15, DF 17, DF 40, UTEX 2538, and CCAP 19/30, for natural beta-carotene production at different light intensities (200 – 1500 µmol photons $m^{-2} \cdot s^{-1}$). According to their results, DF 15 and UTEX 2538 were the only strains not susceptible to photoinhibition, maintaining their photosynthetic efficiency even at high light intensity (1500 µmol photons $m^{-2} \cdot s^{-1}$) and accumulating large amounts of carotenoids

(around 20 and 13 pg cell^{-1} for DF15 and UTEXT 2538, respectively), especially beta-carotene. Later, Sui, Mazzucchi, Acharya, Xu, Morgan and Harvey [146] compared the strains DF15 and CCAP 19/30 regarding protein production and pigments: the first presented higher protein content (circa 2 times more than CCAP 19/30) and total beta-carotene from 12.3 to 12.9% AFDW, whereas the CCAP 19/30 barely showed any carotenoid production. These results prove the importance of assessing different strains to magnify bioproduct production, especially for industrial applications.

The items "gene" and "response" indicate that DNA research has been conducted. Some studies were conducted to identify new strains based on their genome sequencing (represented by the term "gene" in Fig. ***14***) (for example, [147] and [148]). Nevertheless, the proper identification of *Dunaliella* strains still imposes a main concern, demanding a concerted community effort to resolve it. New strains are often incorrectly identified as *D. salina* [149], for instance, the strain CCAP 19/30, which is now known as *D. tertiolecta*. The correct identification is crucial to propose using *D. salina* to produce high-value compounds on an industrial scale.

Over the past decades, most studies aiming to enhance the production of bioproducts in *D. salina* have focused on cultivation conditions. These methods are known as biochemical engineering approaches and contribute to understanding microalgae's metabolic response and pathways toward different environments. This is demonstrated by the red cluster in Fig. ***14***. However, the same condition that boosts the intercellular accumulation of a compound can also impede cell growth; as a result, the overall productivity is jeopardized. A recent countermeasure to be employed is the genetic engineering approach. This approach comprises the overexpression of rate-limiting enzymes; overexpression of enzymes that enhance the accumulation of bioproducts; partial blockage of competing pathways; and a multi-gene transgenic approach [72]. The green cluster in Fig. ***14*** most likely represents the genetic engineering approach. The blue cluster associates exposure with toxic compounds that may be dose- and time-dependent and impact the cell.

There are many challenges regarding the use of microalgae. The appropriate and optimum method for yield and recovery of *D. salina* biomass for producing compounds of industrial relevance is currently in debate. The extraction process is one of the main restraints for the commercial production of beta-carotene, fuels, food, and feed and depends directly on the desired compound [150]. The lack of a cell wall makes *D.*

salina susceptible to integrity damage during the harvest process [151] which explains the focus on more feasible approaches to overcome this issue. Another constraint is the contamination risk of the culture by other microorganisms that can jeopardize the yield of bioproducts, especially in an outdoor environment. However, the ability of *D. salina* to cope with toxic compounds permits the use of disinfection products to eliminate invader microorganisms without ceasing the growth of the microalgae [152]. The cultivation medium is another constraint for *D. salina* cultivation, as its composition strongly influences the cell's biochemical composition and growth rate. It also introduces high costs during cultivation, which could be potentially offset by using suitable wastewater as a cultivation medium [153-155].

8. CONCLUSION

D. salina is an important species for generating compounds of industrial relevance. Its research has been getting a spotlight over the past century for either primary use, such as biofuel and feedstock, and secondary use, such as carotenoid, protein, and high-quality lipids yield. The research on the subject presents increasing trends, as there are controversies regarding methods for producing and recovering biomass and bioproducts and their corresponding metabolic pathways.

It is possible to conclude:

I. China is the biggest contributor to the research on *D. salina*; however, other countries have been increasingly developing and addressing the microalgae's studies.

II. Harvesting and extracting biomass and compounds remain challenging regarding costs and cell integrity.

III. Pretreatment before dewatering is recommended to lower the costs related to biomass recovery.

IV. The light regime must be addressed to enhance biomass and bioproduct quantity and quality.

V. Salinity affects glycerol, carotenoid, lipid, and protein content; thus, it must be addressed to enhance bioproduct production.

VI. *D. salina* extracts have pharmaceutical features, such as anti-inflammatory, anticarcinogenic, antibiofilm, and bacterial and immunoregulatory properties, which can be associated with the presence of volatile compounds, fatty acids, phenolics, flavonoids, and proteins.

VII. Regarding the biofuel potential, *D. salina* can be used for the generation of biodiesel, bioethanol, biohydrogen (through anaerobic acetone-butanol fermentation), and biogas (through fermentation into CH_4).

VIII. The production of different bioproducts can be combined to increase the profitability of algae-based industries.

IX. Biochemical and genetic engineering seems to drive the future of *D. salina*.

Declarations

Funding

This study was partially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by Brazilian National Council for Scientific and Technological Development (CNPq) – protocol 310228/2019-0.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contribution

GSC: Data curation, Investigation, Writing- Original draft, Reviewing and Editing. **RCSS**: Supervision, Conceptualization, Reviewing, and Editing. **TMR:** Supervision, Conceptualization, Reviewing, and Editing. **YS:** Reviewing and Editing. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors authorize the publication of this paper and its content.

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