Dunaliella salina Biorefinary: An Evaluation of the State-of-the-Art

Stephanie de Melo Santana[1*], Ana Lucia Barbosa de Souza[1], Fernando Luiz Pellegrini Pessoa[1]
[1]SENAI CIMATEC University Center; Salvador, Bahia, Brazil

Biorefinery is an important concept that can be applied to improve the industrial processes, making them more sustainable, and reducing waste, and costs of operation. Algal biomass is too onerous to be exploited, and only a few microalgae have products that are feasible to commercialize. Combining this concept and other ones, such as intensification and integration procedures, can also help reduce the costs and become more attractive to industries. *Dunaliella salina* is one of the most critical microalgae studied nowadays. Currently, its process exploits only 10% of its compounds in beta-carotene production. Studies show that different compounds can be exploited and the processes can be combined in a biorefinery approach.
Keywords: *Dunaliella salina* **Biorefinery. Beta-Carotene Production. Microalgal. Biorefinery.**

Introduction

Microalga is a large group of photosynthesizing microorganisms that along with some cyanobacteria have been studied to produce a wide range of compounds. They are considered cell factories because their biomass might be used for various industrial applications as a sustainable raw material. They are also critical players in the future of the blue-bioeconomy, which is about living aquatic resources [1,2]. Some of them have been studied for decades and were the first commercial cultures, mainly in the food and feed industries, like *Arthrospira platensis* (Spirulina), *Chlorella genus*, *Dunaliella salina*, and *Haematococcus pluvialis*. Nowadays, they are also studied and used in the bioenergy, pharmaceutical, and cosmetic industries. Some compounds are lipids or oils such as biofuels, pigments (carotenoids), proteins, polyunsaturated fatty acids (PUFAs), and carbohydrates [1,3].

As the world faces challenges like global climate changes, water scarcity, and increasing global population, there is a need for sustainable production chains. Microalgal biomass has been widely studied to be a sustainable alternative in industry, and one of the main topics of the current research is the bioenergy industry. It is very promising as a source of bioenergy, because it doesn't compete with food supplies, doesn't require large amounts of land or freshwater to be cultivated, and doesn't require complex treatment methods as the lignocellulosic-enriched biomass does for producing biofuels, for example [1,3,4]. Despite it, the algal biomass production is onerous, and some steps of the downstream processing can be energy-intensive, which are barriers to an industrial scale [1-3].

Techno-economic-analysis works indicate that it is necessary to make multiple products in a single cycle to reduce the costs, which is a biorefinery concept. It can also be a way to valorize the other components of the microalgal biomass and reduce waste. Sustainability can be obtained by combining the biorefinery concept with integration and intensification of the processes, technical-economic evaluation, and life-cycle assessment [1-4].

The existing commercial cultures are feasible because they are focused on some bioactive food compounds, like single-cell protein (dried biomass) supplements and carotenoids (main beta-carotene from *Dunaliella salina* and astaxanthin from *Haematococcus pluvialis*). Jacob-Lopes and colleagues (2019) discuss them in a review of bioactive food compounds from microalgae and say that they are less competitive but are economically attractive because they are more effective than synthetic molecules. The natural

Received on 26 February 2022; revised 25 April 2022.
Address for correspondence: Stephanie de Melo Santana. Av. Orlando Gomes, 1845 - Piatã, Salvador - BA- Brazil. Zipcode: 41650-010. E-mail: sms.teca@gmail.com.
DOI 10.34178/jbth.v5i2.205.

J Bioeng. Tech. Health 2022;5(2):96-99.

sources are better absorbed by the human body, and some of these compounds are hard to synthesize, like the isomers that comprise the mixture of the natural form of beta-carotene [5,6].

Even though this microalga can produce many compounds, the existing industrial processes are made for specific applications. A biorefinery approach should also produce other compounds. Thus, this work aims to investigate the state-of-the-art biorefinery concept applied to *Dunaliella salina*. Among the microorganisms cited, it can be highlighted as a potential raw material to be explored in a biorefinery, for being a marine microalga that is highly resistant to different stress conditions and is rich in carotenoids (high-value components) and also in oil content for biofuels (medium/low-value) [1,5,7].

Material and Methods

For an initial comprehension, Science Direct was the scientific database chosen to explore the review articles about microalgae biorefinery. The keyword searched was "microalgae-based biorefinery", sorting articles between 2019 and 2021 by relevance order. The articles chosen were the ones that presented the current highlighted species, discussion about the different routes that a microalgal biorefinery can have, the different products that can be obtained, the bottlenecks, and techno-economic assessments. Then, other keywords were explored with the same filters to understand how much these species are being explored by researchers (Table 1).

We decided to investigate the *Dunaliella salina* potential in a biorefinery for being able to tolerate different stress conditions, having a consolidated commercial production of a high-value compound, and its potential to produce various other products and energy. Scholar Google was also used, sorting articles between 2016 and 2021 by relevance order. From the articles considered the most relevant, some references were consulted for better understanding, and different keywords were chosen to find information using the Scholar Google database to show all works between 2016 and 2021 by relevance order. The other keywords were "beta-carotene production", "*Dunaliella salina* products", and "biorefinery of *Dunaliella salina*". The literature research consisted in finding information about the processes involved, the compounds of interest, characteristics of the microalgae, and biorefinery perspectives.

Table 1. Quantity of scientific reviews.

Keywords	Quantity
Arthrospira platensis biorefinery	264
Chlorella genus biorefinery	982
Dunaliella salina biorefinery	298
Haematococcus pluvialis biorefinery	230
Spirulina platensis biorefinery	512

Results and Discussion

Dunaliella salina is a unicellular flagellated green microalga that occurs in hypersaline environments [1,8]. It can produce carotenoids to protect the cell from harsh conditions, such as high salinity, high light intensity, alkaline environment, lack of nutrients, and/or extreme temperatures, resulting in an orange-ochre color [6,8].

Carotenoids are colored lipid-soluble pigments naturally found in plants, macro and microalgae, bacteria, and fungi, presenting yellow, orange, and red coloration [1,8,9]. The principal carotenoid accumulated in *D. salina* cell is beta-carotene, which can constitute up to 10% of its dry mass [6,8,9].

Microalgal Beta-Carotene Commercial Production

Carotenoids are known as Vitamin A precursors (pro-vitamin A) and are largely used in industry as food and feed colorant. Synthetic beta-carotene is mainly produced by Roche and BASF that transforms beta-ionone into beta-carotene. Most of the beta-carotene produced worldwide is synthetic, and the main source of its natural form is the microalga *Dunaliella salina* [1,8,10].

Remahnji's (2021) work shows information about the global market size of beta-carotene from Polaris Market Research's report, in which it is valued at USD 439 million with a forecasted growth of 3,8% CAGR (compound annual growth rate) in 2017 with an expectation to continue to grow. Ribeiro's (2011) work showed that the global market size of beta-carotene is valued at circa USD 245 million. The 10-Year Bibliometric Review, comprising works between 2009 and 2019, confirms that it's a market that is still growing [6,10,11].

The basic process steps consist of microalgae cultivation, harvesting, dewatering, and extraction. *Dunaliella salina* is industrially cultivated in shallow open tanks in Israel and Australia. Warm and arid environments are ideal for it. We presented the conditions for the cultivation of *Dunaliella salina* for beta-carotene in Table 2 [7, 8,10].

The central nutrients that are limited are Nitrogen and Phosphorus. Pourkarimi (2020) presents that although nitrogen depletion can increase beta-carotene production, its long-time limitation can lead to a high rate of cell death. Salinity can also be affected by reducing cell growth. The article showed a two-stage strategy: in the first stage, the cells grow in a nutrient-rich environment (NaCl 18%) and then transfer to a poor medium in nutrients with 27% of NaCl (high salinity) to improve the carotenoids production by the cells [7,8,10]. Even though natural and synthetic forms of beta-carotene have the same structure, the natural form comprises several other carotenoids, including isomers as 9-cis-beta-carotene, and only 10% of the *Dunaliella salina* biomass extracts it [10].

Table 2. Conditions for cultivation of *Dunaliella salina* for beta-carotene.

Factor	**Range**
Low concentration of nutrients	0.05–0.1 g/L
The concentration of NaCl	2-5M
Temperature	20-40°C
Light intensity 50-800μmol photons	m^{-2} s^{-1}
pH	6-9

Other Bioactive Compounds

Dunaliella salina biomass is also rich in other compounds, such as glycerol and fatty acids. Glycerol is a carbohydrate commonly used in food and pharmaceutical production, and fatty acids are lipids that can be exploited as nutritional supplements or as raw material for biofuel production by transesterification. Pirwitz (2016) shows that carbohydrates can be converted into glucose by hydrothermal liquefaction (HTL) and used as a carbon source by *Chlorella vulgaris*, E*scherichia coli*, and *Saccharomyces cerevisiae* [8,12].

Biorefinery Approach

Nowadays, a microalgal biorefinery that focuses on bioenergy is not feasible. Techno-economic assessments show that it is necessary to reduce the costs, focus on high-value products, or both [1,2,7]. A biorefinery approach aiming to produce different chemicals is a way to reduce the cost and also valorize the other compounds [3,4,9]. *Dunaliella salina* is a promising raw material that already has a scaled-up process in the industry with a product with high commercial interest, and some studies show that the unused biomass can be exploited.

Conclusion

A biorefinery approach reduces operational costs and valorizate the other components of microalgae biomass. *Dunaliella salina* is already exploited, but only 10% of its biomass is used in beta-carotene production. The unused biomass which goes to waste has critical compounds that should be exploited. Integrating the principal process with the processes that can obtain these other compounds should be done. These processes are the HTL conversion of glycerol, the transesterification of the fatty acids, and energy conversion with the remaining biomass. Defining the routes, intensification, and optimization should also be applied, resulting in a circular and sustainable economy.

Acknowledgments

We thank SENAI CIMATEC and the EIPQB (Engineering for Intensification of Chemical and Biochemical Processes) Group.

References

1. Jacobs E et al. Handbook of microalgae-based processes and products. London: Academic Press, 2020.
2. Gufini I et al. Current bottlenecks and challenges of the microalgal biorefinery. Trends Biotechnology 2019;37:242-252.
3. Bhattacharya M, Goswami S. Microalgae – A green multi-product biorefinery for future industrial prospects. Biocatalysis and Agricultural Biotechnology 2020;25(101580).
4. Wu W, Chang J-S. Integrated algal biorefineries from process systems engineering aspects: A review. Bioresource Technology 2020;291(121939).
5. Jacob-Lopes E et al. Bioactive food compounds from microalgae: An innovative framework on industrial biorefineries. Current Opinion in Food Science 2019;25:1-7.
6. Silva SC et al. Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis. Molecules 2020;25(3406).
7. Pourkarimi S et al. Factors affecting production of beta-carotene from *Dunaliella salina* microalgae. Biocatalysis and Agricultural Biotechnology 2020;29(101771).
8. Ben-Amotz A et al. The alga *Dunaliella*: biodiversity, physiology, genomics and biotechnology. Science Publishers, E.U.A., 2009.
9. Espada JJ et al. Environmental and techno-economic evaluation of β-carotene production from *Dunaliella salina*. A biorefinery approach. Biofuels, Bioproducts and Biorefining 2020;14(1):43-54.
10. Ribeiro BD, Barreto DW, Coelho MAZ. Technological aspects of β-carotene production. Food and Bioprocess Technology 2011;4:693-701.
11. Rehmanji M et al. Chapter 26 - microalgal cell factories, a platform for high-value-added biorenewables to improve the economics of the biorefinery. Microbial and Natural Macromolecules - Syntesis and Applications 2021:689-731.
12. Pirwitz K, Rihko-Struchmann L. Sundmacher K. Valorization of the aqueous phase obtained from hydrothermally treated *Dunaliella salina* remnant biomass. Bioresource Technology 2016;219:64-71.