DUNALIELLA SALINA BIOREFINERY: AN EVALUATION OF THE STATE-OF-THE-ART

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

Keywords: Dunaliella salina Biorefinery; beta-carotene production, microalgal biorefinery

BIORREFINARIA DE DUNALIELLA SALINA: UMA AVALIAÇÃO DO ESTADO-DA-ARTE

Resumo: Biorrefinaria é um importante conceito que pode ser aplicado na melhoria de processos industriais para que sejam mais sustentáveis, reduzindo os resíduos e tenham menos custos operacionais. O processamento de biomassa de microalga é muito oneroso e, até agora, somente algumas microalgas têm produtos que são viáveis para comercialização. Combinar esse conceito a outros, como intensificação e integração de processos pode ajudar a reduzir custos e torná-lo mais atraente para ser aplicado na indústria. Dunaliella salina é uma das microalgas mais importantes estudadas atualmente e seu processo explora apenas 10% de seus compostos na produção de beta-caroteno. Estudos mostram que existem outros compostos que podem ser explorados em outros processos numa abordagem de biorrefinaria.

Palavras-chave: Biorrefinaria de Dunaliella salina, produção de beta-caroteno, biorrefinaria microalgal

1. INTRODUCTION

Microalga is a large group of photosynthesizing microorganisms that along with some cyanobacteria have been studied for the production of a wide range of compounds. They are considered cell factories because of it and their biomass can be exploited for various industrial applications as a sustainable raw material. They are also considered important players for the future of blue-bioeconomy, which is about the living aquatic resources. [1,2] Some of them can be highlighted as species that 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 considered in the bioenergy, pharmaceutical and cosmetic industries. Some of their compounds are lipids or oils as biofuels, pigments (carotenoids), proteins, polyunsaturated fatty acids (PUFAs), and carbohydrates. [1,3]

As the world faces challenges like the global climate changes, water scarcity and increasing global population, there is a need for sustainable chains of production. 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 for 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 do 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 for an industrial scale. [1,2,3]

Techno-economic analysis works indicate that it is needed 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. [1,2,3,4]

The existing commercial cultures are feasible because they are focused in some bioactive food compounds, like single-cell protein (dried biomass) supplements and carotenoids (mainly beta-carotene from Dunaliella salina and astaxanthin from Haematococcus pluvialis). Jacob-Lopes et al (2019) discuss about them in a review about bioactive food compounds from microalgae and say that they are less competitive, but are economically attractive because they are more effective than the synthetic molecules. The natural sources are better absorbed by human body and some of these compounds are hard to synthesize, like the isomers that comprises the mixture of the natural form of beta-carotene. [5,6]

Even though the cited microalgae have potential to produce a diverse variety of compounds, the existing industrial processes are made for specific applications. A biorefinery approach should be used to exploit the potential to also produce other compounds. Thus, this work aims to investigate the state of the art of the biorefinery concept applied to Dunaliella salina. Between the microorganisms that were 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]

2. METHODOLOGY

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 the articles made between 2019 and 2021 by relevance order. The studied articles 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 in order to understand how much these species are being explored in research, as shown in Table 1.

Table 1. Quantity of scientific reviews

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

It was 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 that were considered the most relevant ones, some references were also consulted for better understanding and other 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 bibliographic research consisted in finding information about the processes involved, the compounds of interest, characteristics of the microalgae and biorefinery perspectives.

3. RESULTS AND DISCUSSION

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

3.1. 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 in processes that transform 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 was valued at USD 439 million with a forecasted growth of 3,8% CAGR (compound annual growth rate) in 2017 and it was expected to continue to growth. In Ribeiro's (2011) work is shown that the global market size of beta-carotene was 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 in the 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. In Table 2, the conditions for cultivation of Dunaliella salina for beta-carotene are shown: [7, 8,10]

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

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

The main nutrients that are limited are Nitrogen and Phosphorus. Pourkarimi (2020) presents that although nitrogen depletion can increase beta-carotene

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production, its long-time limitation can lead to the high rate of the cell's death. Salinity can also be affected, by reducing cell's growth. It is shown a two-stage strategy in the article, that in the first stage, the cells grow in a nutrient rich environment and 18% NaCl and than they are moved to an environment poor of nutrients and 27% 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 is used to extract it. [10]

3.2. 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 biofuels production by transesterification. Pirwitz (2016) shows that carbohydrates can be converted into glucose by hydrothermal liquefaction (HTL) and be successfully used as carbon source by Chlorella vulgaris, Escherichia coli and Saccharomyces cerevisiae. [8,12]

3.3. 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 of the production, 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 to valorize the other compounds. [3,4,9] Dunaliella salina is a very promising raw material, already has a scaled up process in the industry with a product with high commercial interest and there are studies that show that the unused biomass can also be exploited.

4. CONCLUSION

A biorefinery approach is indicated to reduce 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 goes to waste, but also has important compounds that can be exploited. Integrating the main process with the processes that can obtain these other compounds should be done. Some of these processes are the HTL conversion of glycerol, the transesterification of the fatty acids, and other processes of energy conversion with the remaining biomass. Defining the routes, intensification and optimization should also be applied and that would result in circular and sustainable economy.

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