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PRODUCTION OF BIOPRODUCTS FOR FOOD APPLICATION FROM MICROALGAE OF THE GENUS *DUNALIELLA* (CHLOROPHYTA)

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Production of bioproducts for food application from microalgae of the genus *Dunaliella* (Chlorophyta)

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RESUMO

O uso de microalgas para produzir bioprodutos que poderiam ser utilizados para alimentos, ajuda na resolução de um dos problemas mais significativos associados ao aumento da população: a produção insustentável de alimentos. Neste sentido, espécies do gênero *Dunaliella*, que são microalgas unicelulares flageladas halófilas pertencentes ao filo Chlorophyta, podem produzir bioprodutos com relevância industrial de alta qualidade e em grandes quantidades, tais como proteínas, glicerol e pigmentos. Entretanto, o alto custo associado ao meio de cultivo dificulta a produção destas espécies em larga escala, limitando a produção à escala laboratorial, destacando que a melhoria da produtividade pode mitigar os custos e impactos ambientais associados ao meio de cultivo. Embora existam estudos sobre a otimização de cultivo para aumentar a produtividade, algumas lacunas permanecem, como, por exemplo, os impactos econômicos e ambientais associados ao cultivo destas espécies, que comprometem a aceitabilidade pelos consumidores. Neste contexto, esta pesquisa teve por objetivo (1) analisar e identificar o potencial de espécies do gênero *Dunaliella* para produzir bioprodutos com relevância industrial, e as condições e caminhos para seus acúmulos, (2) avaliar o mercado para estes bioprodutos, (3) melhorar a produtividade da biomassa e dos bioprodutos utilizando diferentes composições de meio cultivo artificial, (4) identificar o gargalo relacionado ao meio de cultivo artificial e como superar os aspectos econômicos, e (5) analisar e prever diferentes cenários para a produção de bioprodutos e os impactos ambientais associados ao meio artificial. Esta tese é composta por seis artigos (dois artigos de revisão, três artigos experimentais, e uma avaliação do ciclo de vida) para abordar os objetivos alvo. Os estudos realizados demonstraram que (1) a comercialização global da biomassa de espécies de *Dunaliella* é promissora, especialmente para aplicações alimentícias; (2) as espécies selecionadas podem produzir proteínas de alta qualidade, pigmentos e glicerol, dependendo da salinidade e biodisponibilidade de nutrientes, principalmente nitrogênio, que impactam diretamente na composição delas; (3) aumentar a salinidade apresentou benefícios quanto à produção de glicerol, enquanto que a limitação de nitrogênio foi fundamental para o acúmulo de beta-caroteno; (4) o cloreto de sódio e sulfato de magnésio são uns dos componentes mais caros e com grande dano ambiental (principalmente à saúde humana e mudanças climáticas) no meio de cultivo artificial; (5) a substituição do cloreto de sódio por sal marinho colaborou para a redução dos custos; (6) o aumento da produtividade de biomassa, glicerol e beta-caroteno mitigou os custos e

danos ambientais associados ao meio de cultivo artificial; e (7) o cultivo destas espécies para a geração de bioprodutos poder ser um vetor para a redução das emissões gás carbono, diminuindo a pegada de carbono e colaborando para a economia verde. Com a pesquisa desenvolvida podemos concluir que o cenário mais promissor para a produção de bioprodutos com relação aos impactos econômicos e ambientais depende principalmente da produtividade e da espécie utilizada: *Dunaliella tertiolecta* apresentou maior capacidade de adaptação e produção de biomassa e de glicerol em salinidades maiores, enquanto que *Dunaliella salina*, embora um pouco mais restrita em termos de meio de cultivo, apresentou grande potencial para a produção de beta-caroteno com alta qualidade em ambientes com baixa disponibilidade de nitrogênio.

Palavras-chave: Gênero *Dunaliella*; otimização de meio de cultivo artificial; bioprodutos; análise econômica e ambiental.

ABSTRACT

Using microalgae to yield bioproducts that could be used for food addresses one of the most significant problems associated with population increase: unsustainable food production. In this sense, species of the genus *Dunaliella*, which are halophilic flagellated unicellular microalgae belonging to the phylum Chlorophyta, can produce high-quality and large amounts of bioproducts with industrial relevance, such as protein, glycerol, and pigments. However, the high-cost cultivation medium precludes large-scale of *Dunaliella* species for biomass production, limiting biomass production to a laboratory scale. Productivity improvement can mitigate the costs and environmental impacts associated with cultivation medium. Although there are studies on optimal conditions to increase productivity, some gaps remain, such as economic and environmental impacts associated with *Dunaliella* spp. cultivation, which jeopardize customers' acceptability. In this context, this research aims to (1) analyze and identify the potential of *Dunaliella* spp. to produce bioproducts with industrial relevance and the conditions and pathways for their accumulation, (2) assess the market for these bioproducts; (3) improve biomass and bioproduct productivity by using different cultivation medium composition; (4) identify the bottleneck in artificial cultivation medium and how to overcome the economic aspects; (5) analyze and predict different scenarios for bioproduct production and the environmental impacts associated with the artificial medium. This thesis comprises six articles (two review articles, three experimental articles, and a life cycle assessment) to address the target objectives. The conducted studies demonstrated that (1) the global marketing for *Dunaliella* spp. biomass is promising, especially for food applications; (2) *Dunaliella* spp. can produce high-quality protein, pigments, and glycerol depending on the species, salinity, and nutrient bioavailability (mainly nitrogen), which directly impact on *Dunaliella* spp. composition; (3) increasing salt concentration benefits glycerol yield, while nitrogen limitation was crucial to accumulate beta-carotene; (4) sodium chloride and magnesium sulfate are two of artificial medium's components more expensive and impactful (especially to human health and climate changes); (5) replacing sodium chloride with sea salt reduced the costs; (6) increasing biomass, glycerol, and beta-carotene productivity minimized the costs and environmental damage associated with artificial cultivation medium; and (7) *Dunaliella* spp. cultivation to yield bioproducts can be a vector to reduce carbon dioxide emissions, reducing carbon footprint and

collaborating to the green economy. It is possible to conclude with the research conducted that the most promising scenario, economically and environmentally wise, to yield bioproducts depends mainly on the productivity and species: *Dunaliella tertiolecta* presents better adaptation capacity and biomass and glycerol yield at higher salt concentrations, whereas *Dunaliella salina* shows great potential to produce high-quality beta-carotene under nitrogen-limited conditions, despite of being more restrict concerning the medium composition.

Keywords: genus *Dunaliella*; artificial medium optimization; bioproducts; economic and environmental assessment.

SUMMARY

INTRODUCTION

Over the past decades, population growth, fast urbanization, and rising consumption behavior have had a massive impact on the environment. Over the last 70 years, the population has triplicated and is expected to reach over 8.5 billion in 2030 (United Nations, 2021). It was estimated that around 2.4 billion people worldwide experience moderate or severe food insecurity (United Nations, 2021). Food demand is expected to increase by 50% in 2030 compared to 2012 (FAO, 2021). Pastures and meadows for grazing livestock account for two-thirds of the agricultural lands (FAO, 2020).

The current dietary pattern renders life on Earth unsustainable and hastens climate change. According to IPCC (2022), land use is close to collapse due to increasing pressure on these natural resources inducing climate changes. This makes necessary the search for more sustainable alternatives to increase food production without requiring more land and energy. To address these problems, all United Member States committed to striving for prosperity for people and the planet by setting 17 Sustainable Development Goals (SDGs) that need to be accomplished by 2030 (United Nations, 2022).

Biomass to produce food has been proposed for decades. Microalgae require less cultivation area than land plants; they produce high-value compounds that address the economic and environmental pillars. Among them, the species of the genus *Dunaliella*, which are halophilic flagellated unicellular microalgae belonging to the phylum Chlorophyta, are distinguished by the absence of a cell wall. They are commonly found in saline environments, such as saline lakes (Singh et al., 2017) and coastal marine waters (Dolapsakis et al., 2005), and are well-known for their ability to cope with extreme environments and to yield bioproducts with industrial relevance, such as carotenoids (Sui et al., 2019a), lipids (Chen et al., 2019), protein (Sui and Vlaeminck, 2020), and glycerol (Monte et al., 2020b) to respond to the increasing demand for alternative food, feed, and health supplement sources (Torres-Tiji et al., 2020).

The biomass's potential economic value depends on two main factors: the productivity of good quality bioproducts and the production costs. The research on the effect of light, nutrients bioavailability, temperature, and harvest on biomass quantity and quality has been extensively addressed already. The optimum conditions to produce substantial good-quality bioproducts by the genus *Dunaliella* is well documented and cover a wide array of combinations. Article 1 will enlighten this aspect. The biggest problem relates to the costs associated with the cultivation medium, which will be covered in Article 2 and addressed in Article 5. However, studies regarding the potential of growing *Dunaliella tertiolecta* in alternative cultivation mediums (Article 3), medium optimization to improve bioproduct yield (Articles 4 and 5), economic assessment (Article 5), and impact categorization of *Dunaliella tertiolecta* and *D. salina* cultivation (Article 6) still need further discussion.

This research aimed to identify the potential of *Dunaliella* spp. to yield bioproducts with industrial relevance in a food context using the best cost-benefit approach. With our results, we expect to improve *Dunaliella* spp. productivity (biomass, amino acids, carotenoids, and glycerol) by assessing different Modified Johnson's Medium compositions and predicting the best scenarios regarding the economic and environmental impacts.

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