

Microalgae Cultures: Environmental Tool and Bioenergy

José C. M. Pires^{1,2,*}  and Ana L. Gonçalves^{1,2,†} 

¹ LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

² ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

* Correspondence: jcpires@fe.up.pt; Tel.: +351-22-508-2262; Fax: +351-22-508-1449

† The authors contributed equally to this work.

1. Introduction

Microalgae have been intensively studied for CO₂ capture, nutrient removal from wastewater, and biofuel production applications. Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms presenting a unicellular or simple multicellular structure, which allows them to grow in various environmental conditions, even harsh ones. Photosynthesis is a key process for microalgal growth, whereby they convert solar radiation and CO₂ (absorbed from the atmosphere or large emission sources) into organic matter and energy. Therefore, microalgal cultivation can play an important role in climate change mitigation, reducing the concentration of a major greenhouse in the atmosphere. Besides CO₂ sequestration, microalgae can also be used in wastewater bioremediation. They can grow in low-quality waters and use the water's contaminants (mainly nitrogen and phosphorus species, which are macronutrients for microalgal growth) as nutrient sources. Moreover, the produced biomass, very rich in a wide variety of interest compounds, can be used as raw material for diverse applications, such as the production of nutraceuticals, human food and animal feed, pharmaceuticals, cosmetics, fine chemicals, bioenergy, and biofertilizers. Regarding bioenergy production, the fatty acids produced by microalgae can be extracted and used for biodiesel production, and products, such as proteins and residual biomass, can be fermented to produce ethanol or methane. The wide range of applications, together with the high growth rates described for these microorganisms, have contributed to a growing interest in microalgal biomass production in recent decades.

This Special Issue presents some recent research studies concerning the environmental applications of microalgae and their potential for biofuels production, focusing on the main challenges for their large-scale application. Since microalgal culturing can address different environmental and non-environmental issues, the achievements of integrating multiple microalgal applications are also considered in this Special Issue.

2. Contributions to This Special Issue

Regarding the environmental applications of microalgae, Figler et al. [1] evaluated the growth and nutrient removal capacity of green alga *Coelastrum morus* under different nitrogen and phosphorus concentrations and different N:P ratios. Since the composition of wastewater varies daily and seasonally, it is essential to understand the effect of the N:P ratio and salinity on microalgal growth and remediation potential. This study demonstrated that low N:P values, achieved with high nitrate and phosphate concentrations, were not favorable for microalgal growth. However, this microalga can be considered a halotolerant species, growing with NaCl concentrations up to 1000 mg L⁻¹. The results also showed that the favorable N:P ratio is about 5 or higher. Kujawska et al. [2] optimized biomass and docosahexaenoic acid (DHA) production with *Schizochytrium* sp., using waste glycerol from biodiesel production as an organic carbon source. Microalgal growth and DHA production were promoted in the following conditions: (i) crude glycerin concentration



Citation: Pires, J.C.M.; Gonçalves, A.L. Microalgae Cultures: Environmental Tool and Bioenergy. *Energies* **2022**, *15*, 5809. <https://doi.org/10.3390/en15165809>

Received: 2 August 2022

Accepted: 9 August 2022

Published: 10 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

in the medium around 150 g dm^{-3} for both; (ii) process temperatures of 27 and 26 °C, respectively; (iii) oxygen concentration in the reactor equaling 50 and 30%, respectively; and (iv) peptone concentration of 9.99 and 2.21 g dm^{-3} , respectively. To improve the viability of the process, the authors proposed a two-step cultivation system, in which the first step presents the favorable conditions for microalgal growth and the second presents the conditions that promote DHA accumulation in cells.

In terms of bioenergy production, this Special Issue covers several process aspects, from the optimization of culture conditions to biomass processing. Mishra et al. [3] developed and tested an accessible microfluidic platform to reduce the experimental effort of the screening process, optimizing the UV-C dosage to induce random mutations for biomass production and lipid accumulation in the microalga *Botryococcus braunii*. Biomass production and lipid accumulation in the microalga exposed to the optimal UV-C dosage were 137 and 149% higher than those determined in the wild-type *B. braunii*. Guerra et al. [4] tested different operation regimes (batch, continuous, and semi-continuous) during the spring/summer seasons in 2.6 m^3 tubular photobioreactors to select the most suitable one for the production of the oleaginous microalga *Nannochloropsis oceanica*. Results obtained revealed that *N. oceanica* grown using the semi-continuous and continuous operation regimes enabled a 1.5-fold increase in the biomass's volumetric productivity compared to that cultivated in batch. The lipid productivity was 1.7-fold higher under semi-continuous cultivation than that obtained in the batch operation regime. On the other hand, the semi-continuous and continuous operation regimes spent nearly double the amount of water compared to that of the batch regime. Interestingly, the biochemical profile of produced biomass using the different operation regimes was not affected regarding the contents of proteins, lipids, and fatty acids. Moura et al. [5] investigated *Dunaliella tertiolecta* growth, pigment production, and bioenergetic parameters in different culture media to select the best cultivation conditions to overproduce such added-value compounds. The authors concluded that the composition of the culture medium has different effects on pigments production, growth kinetics, and energy content, which must be considered for any application of the produced microalgal biomass. Zuccaro et al. [6] investigated the mutualistic interactions between the oleaginous yeast *Lipomyces starkeyi* and the green microalga *Chloroidium saccharophilum* in mixed cultures to exploit possible synergistic effects in terms of lipid content and productivity. The mixed cultures overperformed the individual ones, which were also tested in this study in different cultivation media.

Zorn et al. [7] investigated the consortium between oleaginous filamentous fungal species *Mucor circinelloides* and microalga *Chlorella vulgaris* to promote biomass harvesting and to evaluate lipid production through four different inoculation strategies. Using a mature fungal mycelium with high microalgal cell concentration, biomass samples with up to 79% of the dry weight as algae and recovery rates greater than 97% were achieved. A synergistic effect on the lipid accumulation was observed, increasing four-fold when compared to the axenic control. Furuhashi et al. [8] evaluated the increase in the hydrocarbon recovery rate and the colony size of *B. braunii* when cultivated in a brackish medium. Using this medium resulted in a significant reduction in energy consumption (about 56%) for microalgal harvesting through filtration and a 12% reduction in the input energy for hydrocarbon recovery. In addition, the energy profit ratio was 2.92, which demonstrates the viability of growing this microalga in brackish medium for biofuel production. Zhang et al. [9] applied an effective and easily controlled cell-wall-disruption method based on the electro-Fenton reaction to enhance lipid extraction from the wet biomass of *Nannochloropsis oceanica* IMET1. The neutral lipid extraction yield increased from 40% to 87.5%, corresponding to 12.2% and 26.7% dry cell weights, respectively. The tested procedure may provide an economic and efficient method of lipid extraction from a wet biomass.

Sukacova et al. [10] evaluated the energetic efficiency of biomass and lipids production by *Chlorella pyrenoidosa* in multi-tubular, helical-tubular, and flat-panel airlift pilot-scale photobioreactors to ascertain the sustainability of microalgae-based biofuels. The main en-

energy consumption was relative to the constant light supply to the flat-panel photobioreactor and the culture circulation in the helical-tubular photobioreactor. Tubular photobioreactor presented the lowest energy requirements and the highest temperature sensitivity, with lower biomass productivities in warm periods. For the sustainable production of microalgae, a hybrid system is recommended. Caldwell et al. [11] demonstrated the ability of microalgae and cyanobacteria immobilization to intensify microalgal biotechnology and bioprocessing. Mass-transfer limitations in these systems were also discussed.

3. Conclusions

Microalgae have undeniable potential in several environmental applications. In wastewater treatment, future research should focus on the relationship between microalgae and microorganisms present in these effluents. Potential beneficial consortia can promote an increase in biomass production, effective effluent treatment, and under certain conditions, an increase in lipid accumulation (a precursor for biofuels production). Regarding this topic, several variables can influence lipid accumulation (e.g., microalgal species and strain, light conditions, culture medium composition, operation mode, etc.). Accordingly, the use of rapid and effective screening methods should be encouraged. The harvesting step is also a concern in terms of biofuels production and biomass valorization in general, as the current procedures still represent a significant fraction of the total production costs. Therefore, research should concentrate on the development of cost-effective harvesting methods and the modulation of cultivation conditions towards the achievement of high-density cultures to facilitate the recovery process. Co-cultivation with other microorganisms can also be advantageous for this purpose, facilitating the sedimentation of microalgal biomass. Finally, the use of hybrid cultivation systems has been gaining increasing interest in recent years. Using this approach, high-density cell cultures can be achieved because operational conditions are adapted to promote biomass production in the first stage of the cultivation step. Then, there is a second stage, where the operational conditions are shifted towards the accumulation of compounds of interest (lipids, in the case of biofuels production). These systems can improve microalgal biomass production and the accumulation of target compounds in the same operation, thus facilitating the harvesting process and contributing to an overall increase in compounds of interest's productivity.

Funding: This work was funded by: (i) LA/P/0045/2020 (ALiCE) and UIDB/00511/2020-UIDP/00511/2020 (LEPABE) funded by national funds through FCT/MCTES (PIDDAC); (ii) Project PIV4Algae (Ref. PTDC/BTA-BTA/31736/2017; POCI-01-0145-FEDER-031736), funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; (iii) Project PhotoBioValue (ref. PTDC/BTA-BTA/2902/2021), funded by FEDER funds through COMPETE2020—Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; and (iv) project “HyGreen&LowEmissions—Tackling Climate Change Impacts: the role of Green Hydrogen production, storage and use, together with low emissions energy systems”, with the reference NORTE-01-0145-FEDER-000077, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Figler, A.; Marton, K.; Márton, K.; Bacsi, I. Effects of Nutrient Content and Nitrogen to Phosphorous Ratio on the Growth, Nutrient Removal and Desalination Properties of the Green Alga *Coelastrum morus* on a Laboratory Scale. *Energies* **2021**, *14*, 2112. [[CrossRef](#)]
2. Kujawska, N.; Talbierz, S.; Debowski, M.; Kazimierowicz, J.; Zielinski, M. Optimizing Docosahexaenoic Acid (DHA) Production by *Schizochytrium* sp. Grown on Waste Glycerol. *Energies* **2021**, *14*, 1685. [[CrossRef](#)]
3. Mishra, S.; Liu, Y.J.; Chen, C.S.; Yao, D.J. An Easily Accessible Microfluidic Chip for High-Throughput Microalgae Screening for Biofuel Production. *Energies* **2021**, *14*, 1817. [[CrossRef](#)]
4. Guerra, I.; Pereira, H.; Costa, M.; Silva, J.T.; Santos, T.; Varela, J.; Mateus, M.; Silva, J. Operation Regimes: A Comparison Based on *Nannochloropsis oceanica* Biomass and Lipid Productivity. *Energies* **2021**, *14*, 1542. [[CrossRef](#)]
5. Moura, Y.A.S.; Viana-Marques, D.D.; Porto, A.L.F.; Bezerra, R.P.; Converti, A. Pigments Production, Growth Kinetics, and Bioenergetic Patterns in *Dunaliella tertiolecta* (Chlorophyta) in Response to Different Culture Media. *Energies* **2020**, *13*, 5347. [[CrossRef](#)]
6. Zuccaro, G.; del Mondo, A.; Pinto, G.; Pollio, A.; De Natale, A. Biorefinery-Based Approach to Exploit Mixed Cultures of *Lipomyces starkeyi* and *Chloroidium saccharophilum* for Single Cell Oil Production. *Energies* **2021**, *14*, 1340. [[CrossRef](#)]
7. Zorn, S.M.F.E.; Reis, C.E.R.; Silva, M.B.; Hu, B.; De Castro, H.F. Consortium Growth of Filamentous Fungi and Microalgae: Evaluation of Different Cultivation Strategies to Optimize Cell Harvesting and Lipid Accumulation. *Energies* **2020**, *13*, 3648. [[CrossRef](#)]
8. Furuhashi, K.; Hasegawa, F.; Yamauchi, M.; Kaizu, Y.; Imou, K. Improving the Energy Balance of Hydrocarbon Production Using an Inclined Solid–Liquid Separator with a Wedge-Wire Screen and Easy Hydrocarbon Recovery from *Botryococcus braunii*. *Energies* **2020**, *13*, 4139. [[CrossRef](#)]
9. Zhang, S.; Hou, Y.Y.; Liu, Z.Y.; Ji, X.; Wu, D.; Wang, W.J.; Zhang, D.Y.; Wang, W.Y.; Chen, S.L.; Chen, F.J. Electro-Fenton Based Technique to Enhance Cell Harvest and Lipid Extraction from Microalgae. *Energies* **2020**, *13*, 3813. [[CrossRef](#)]
10. Sukacova, K.; Losak, P.; Brummer, V.; Masa, V.; Vicha, D.; Zavrel, T. Perspective Design of Algae Photobioreactor for Greenhouses—A Comparative Study. *Energies* **2021**, *14*, 1338. [[CrossRef](#)]
11. Caldwell, G.S.; In-na, P.; Hart, R.; Sharp, E.; Stefanova, A.; Pickersgill, M.; Walker, M.; Unthank, M.; Perry, J.; Lee, J.G.M. Immobilising Microalgae and Cyanobacteria as Biocomposites: New Opportunities to Intensify Algae Biotechnology and Bioprocessing. *Energies* **2021**, *14*, 2566. [[CrossRef](#)]