

A Magnetic Approach to Sustainable *Chlorella vulgaris* Harvesting: Methods and Applications

Maia C.^{1,2,*}, Loureiro J.A.^{1,2,3}, Soares O.S.G.P.^{2,4}, Pereira M.C.^{1,2}, Pires J.C.M.^{1,2}

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

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

³Department of Mechanical Engineering, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

⁴LSRE-LCM - Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials, Faculty of Engineering, University of Porto, Rua Dr Roberto Frias, 4200-465 Porto, Portugal

*corresponding author:

e-mail: up201704692@up.pt

Abstract Microalgae are unicellular microorganisms with many applications; however, their high harvesting costs compromise their large-scale use. Magnetic separation has been explored as a potential solution for overcoming harvesting challenges. In addition to promoting microalgal harvesting, the magnetic capacity of particles allows their recovery, regeneration, and reuse, lowering process costs and promoting a circular economy. This study explored the impact that production conditions have on the properties of magnetic particles and, consequently, on the harvesting of *Chlorella vulgaris*. By using the base NH_4OH to produce magnetite, it was possible to reduce the particle size and increase their magnetic capacity, essential parameters for a successful harvesting. In addition, by increasing the stirring time and the temperature (80 °C), the particle size was further reduced. As a result, microalgal harvesting was increased by optimising magnetite production.

Keywords: *Chlorella vulgaris*, Circular economy, Magnetic particles, Microalgal harvesting, Resource recovery.

1. Introduction

Microalgae are a diverse group of unicellular photosynthetic organisms that have attracted increasing attention for their potential applications in biofuel production, wastewater treatment, and as a source of high-value compounds for the food, pharmaceutical, and cosmetic industries (Gerulová et al., 2022). Despite the enormous potential, the high cost of harvesting techniques, which can represent 20–30% of total production costs, hinders their large-scale application. Harvesting microalgae is challenging because of their small size, low density, negative surface charge, and diluted cultures (Chan et al., 2023). To overcome the harvesting limitation, alternative techniques such as magnetic separation have been explored.

Magnetic nanoparticles (MNPs), including magnetic iron oxides like magnetite (Fe_3O_4), have been employed in a variety of fields like biomedicine and environmental remediation because of their unique characteristics, which include high surface area and magnetic and sorption properties (Pereira et al., 2017). When applied for microalgal harvesting, these particles interact and aggregate the biomass, enabling their separation from the growth medium using an external magnetic field. This technique reduces the overall costs by allowing the medium and the MNPs to be reused after biomass separation. Consequently, due to its simplicity, high efficiency, affordability, and sustainability, magnetic separation is the most promising approach to address the challenges of traditional harvesting methods. Magnetic particles can be produced by several methods, with coprecipitation being the most widely used. Grain size and magnetic properties can be adjusted by changing the reaction conditions during production. As a result, this procedure must be optimised so that the particles produced can effectively harvest microalgae (Chan et al., 2023; Gerulová et al., 2022; Zhu et al., 2024).

This study aims to assess the effects of magnetic particle properties on the microalga *C. vulgaris* harvesting process. To this end, magnetite particles were produced using the coprecipitation method, and the impact of procedural variables such as temperature, stirring duration, and inert environment on particle properties were examined.

2. Materials and Methods

2.1. Synthesis of magnetic particles

Magnetic particles were synthesised by coprecipitation of iron chlorides in a ratio greater than $2 \text{ Fe}^{3+}:1 \text{ Fe}^{2+}$, in a base (NH_4OH or NaOH). As indicated in Table 1, different bases and solvents were employed in

accordance with procedures outlined in the literature. After 30 min of constant stirring at room temperature, the precipitation process was completed, and a neodymium magnet was used to gather the particles. After that, distilled water was used to wash and store the particles. Method 3 was also performed under different conditions to assess the impact of temperature (20 or 80 °C), stirring time (from 15 to 60 min), and atmospheric composition (oxygen-free environment and normal atmosphere). Coulter LS 230 was used to analyse the size distribution of the generated particles (3 runs per sample).

Table 1. Variations in solvent and base composition for magnetite synthesis

Method	Solvent	Base	Reference
1	2 M HCl	1.5 M NaOH	(Pereira et al., 2017)
2	H ₂ O	1 M NaOH	(Yazdani & Seddigh, 2016)
3	H ₂ O	NH ₄ OH (25 wt.%)	(Gerulová et al., 2022)

2.2. Harvesting Assays

Microalga *C. vulgaris* was inoculated into a modified OECD culture medium at 400 mg L⁻¹. All experiments were conducted 24 hours after inoculation at room temperature, by adding a specific amount of the particles to 10 mL of the microalgal suspension. The flask containing the mixture was shaken for 1.5 min at 240 rpm and then placed on a magnet (Nd45) for 3 min to separate the magnetic particles coated with microalgae.

3. Results and Discussion

It can be observed in Figure 1 that methods 1 and 2 generate particles with similar size distribution, although the particles from method 2 have a much lower magnetic capacity. Method 3 produces the smallest particles.

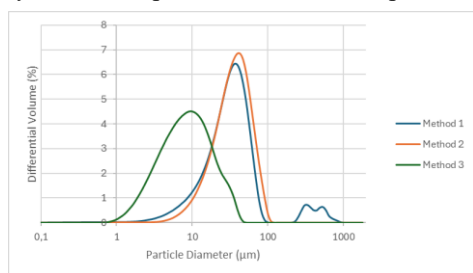


Figure 1. Size distribution of the produced magnetic particles

By changing the process variables to method 3, it was concluded that adopting an oxygen-free atmosphere had no impact on the particle size. On the other hand, by increasing the temperature and the stirring time, the particle size was reduced by 2 orders of magnitude, to values lower than 3 µm (data not shown). When applied to *C. vulgaris*, these particles with different characteristics demonstrated the ability to interact and enable efficient microalgal harvesting. Promising results indicated that high microalgal harvesting efficiencies can be achieved by optimising magnetite production.

4. Conclusions

This study showed that microalgal harvesting efficiency is greatly increased by optimising magnetite production. Particle size and magnetic characteristics were directly impacted by the base chosen, reaction temperature, and stirring time. Using NH₄OH and performing the production at 80 °C for 60 min reduces particle size, leading to better *C. vulgaris* harvesting. These findings reinforce magnetic separation as a sustainable and economically viable solution to microalgal harvesting by producing particles capable of promoting harvesting and being recycled and reused.

Acknowledgements

This work was financially supported by: (i) project PTDC/BTA-BTA/2902/2021 – PhotoBioValue – Light effect on photobioreactor design for microalgae cultivation: enhancement of photosynthetic efficiency and biomass value, with DOI 10.54499/PTDC/BTA-BTA/2902/2021 (<https://doi.org/10.54499/PTDC/BTA-BTA/2902/2021>), funded by national funds through FCT/MCTES (PIDDAC); and (ii) LEPABE, UIDB/00511/2020 (DOI: 10.54499/UIDB/00511/2020) and UIDP/00511/2020 (DOI: 10.54499/UIDP/00511/2020), ALiCE, LA/P/0045/2020 (DOI: 10.54499/LA/P/0045/2020) and LSRE-LCM, UIDB/50020/2020 (DOI: 10.54499/UIDB/50020/2020) and UIDP/50020/2020 (DOI: 10.54499/UIDP/50020/2020), funded by national funds through FCT/MCTES (PIDDAC). Carolina Maia thanks FCT for financial support via the FCT-funded PhD Research Scholarship 2023.04096.BD (DOI: 10.54499/2023.04096.BD).

References

- Chan, K.-S., Leung, S.-K., Wong, S. S.-Y., Chan, S.-S., Suen, D. W.-S., Tsang, C.-W., & Chan, C.-Y. (2023). Development of an energy-efficient rapid microalgal cell-harvesting method using synthesized magnetic nanocomposites. *Water*, 15(3), 545.
- Gerulová, K., Kucmanová, A., Sanny, Z., Garaiová, Z., Seiler, E., Čaplovičová, M., Čaplovič, L., & Palcut, M. (2022). Fe₃O₄-PEI nanocomposites for magnetic harvesting of *Chlorella vulgaris*, *Chlorella ellipsoidea*, *Microcystis aeruginosa*, and *Auxenochlorella protothecoides*. *Nanomaterials*, 12(11), 1786.
- Pereira, L., Dias, P., Soares, O., Ramalho, P., Pereira, M., & Alves, M. (2017). Synthesis, characterization and application of magnetic carbon materials as electron shuttles for the biological and chemical reduction of the azo dye Acid Orange 10. *Applied Catalysis B: Environmental*, 212, 175-184.
- Yazdani, F., & Seddigh, M. (2016). Magnetite nanoparticles synthesized by co-precipitation method: The effects of various iron anions on specifications. *Materials chemistry and physics*, 184, 318-323.
- Zhu, J., Wakisaka, M., Omura, T., Yang, Z., Yin, Y., & Fang, W. (2024). Advances in industrial harvesting techniques for edible microalgae: Recent insights into sustainable, efficient methods and future directions. *Journal of Cleaner Production*, 436, 140626.