LAMI-004

Enhancing lipid recovery from *Chlorella sorokiniana* through pretreatment and solvent optimization

M. Tsami, K. Fragkis, E. P. Kalogianni

Oils and Interfaces Engineering Group, Department of Food Science and Technology, International Hellenic University, Thessaloniki, Greece

https://www.oilinterfaces.eu/

*Email: mariatsami99@ihu.gr, elekalo@ihu.gr

Introduction

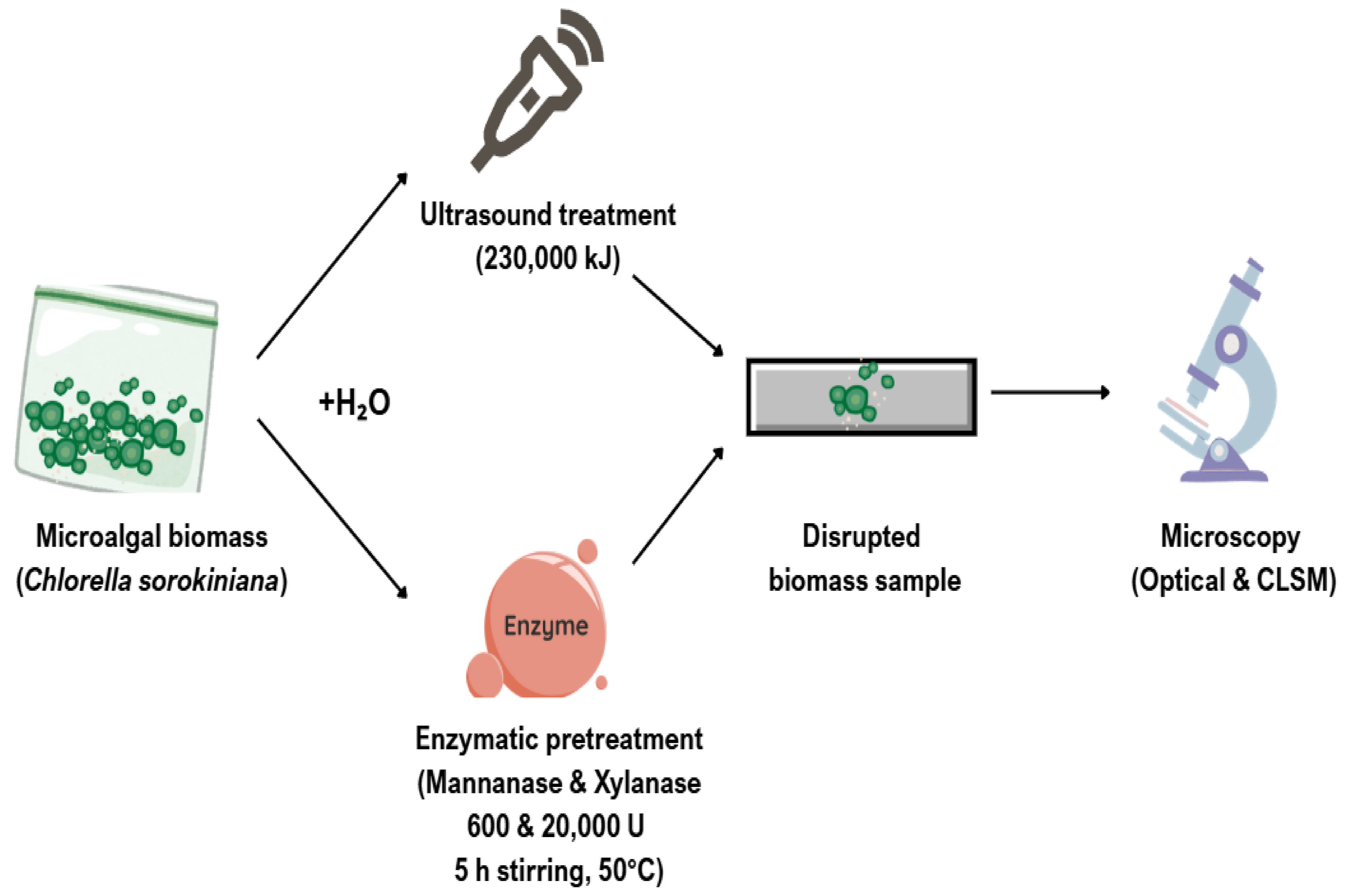
Chlorella sorokiniana is a lipid-rich microalga considered a promising feedstock for biodiesel production. Efficient lipid recovery requires overcoming the rigidity of its multilayered cell wall. **Mechanical pretreatments such as ultrasound** are commonly used to enhance cell disruption, while the application of **specific enzymes, including endo-β-1,4-mannanase and endo-β-1,4-xylanase**, represents a modern and energy-efficient alternative. The **choice of solvent** also plays a crucial role in the extraction process, as different solvents can significantly influence lipid recovery efficiency. Different cell disruption methods and solvents were tested to optimize lipid recovery for sustainable biodiesel production

Aim

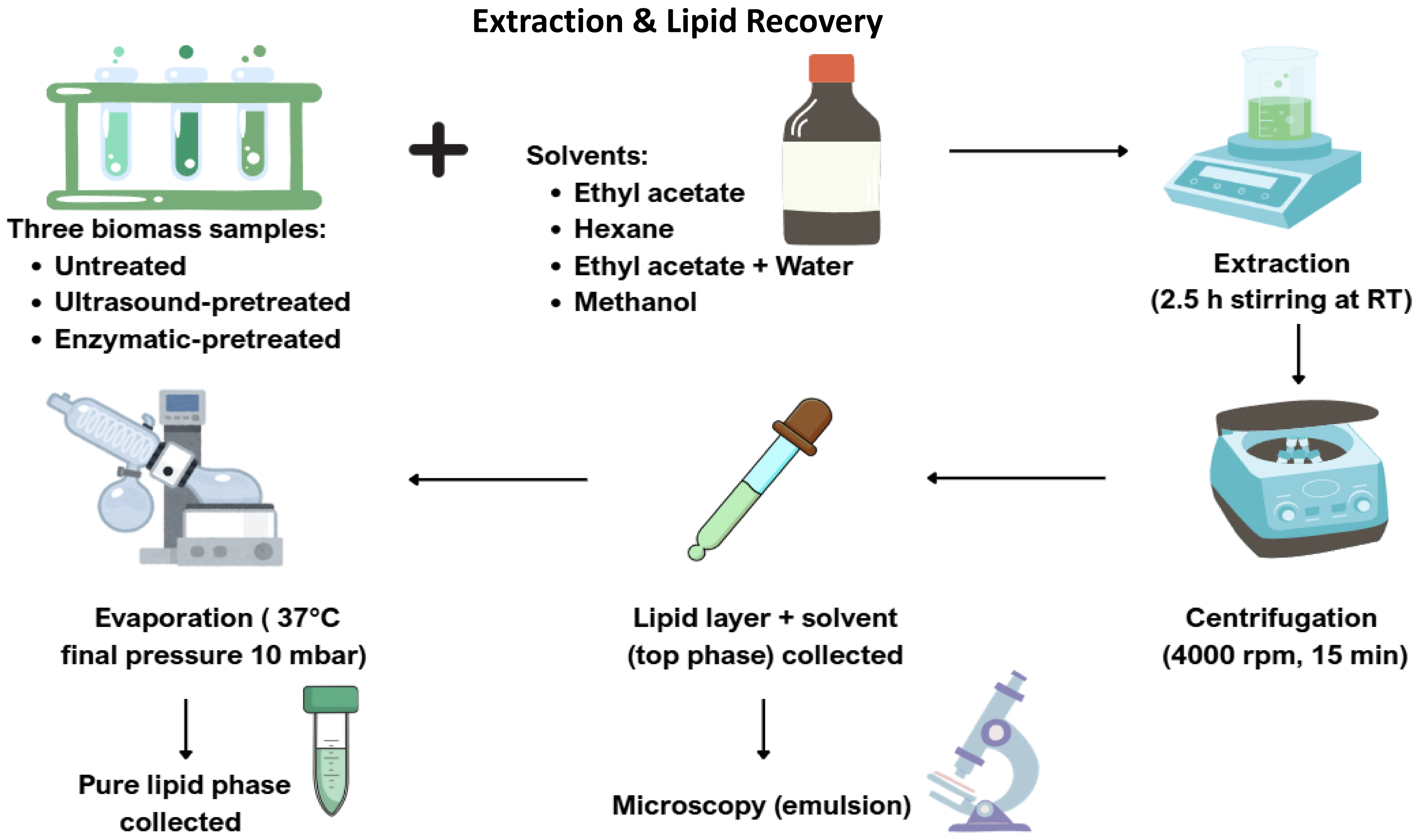
The aim of this study was to enhance lipid recovery from *C. sorokiniana* by evaluating the effectiveness of different pretreatment strategies and optimizing solvent selection and extraction parameters

Materials & Methods

Pretreatment strategies: Ultrasound & Enzymes



Extraction & Lipid Recovery



Results

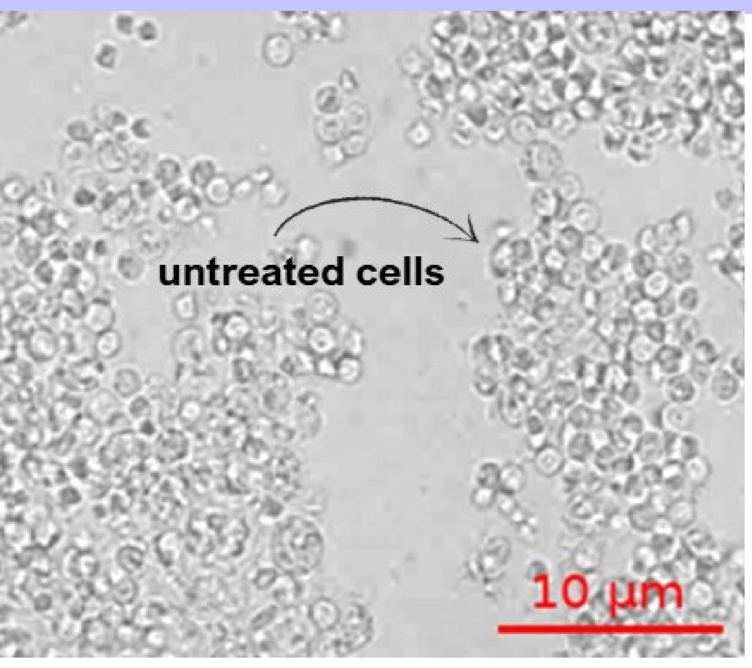


Figure 1: Optical microscopy image of Untreated *Chlorella sorokiniana* cells.

- ✓ Ultrasound greatly increases lipid recovery.
- ✓ Enzymes show moderate improvement.
- ✓ Pretreatment boosts extraction efficiency.
- ✓ Cell wall disruption enhances extraction.

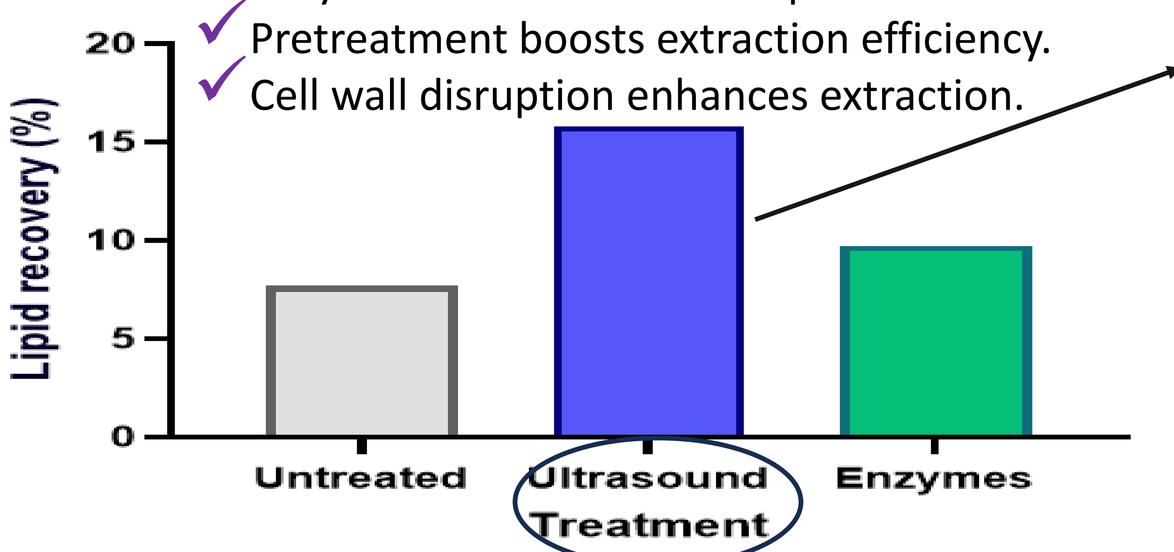


Figure 2: Effect of pretreatment on lipid recovery using ethyl acetate extraction

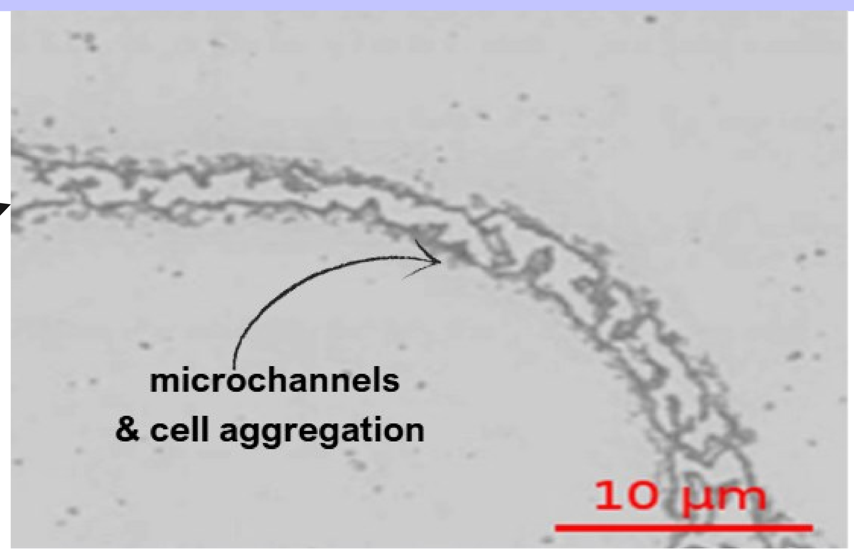


Figure 3: Optical microscopy image of *Chlorella sorokiniana* cells after ultrasound treatment, illustrating cell aggregation and the formation of microchannels.

Figure 4: Optical microscopy image of *Chlorella sorokiniana* cells after Enzymatic treatment

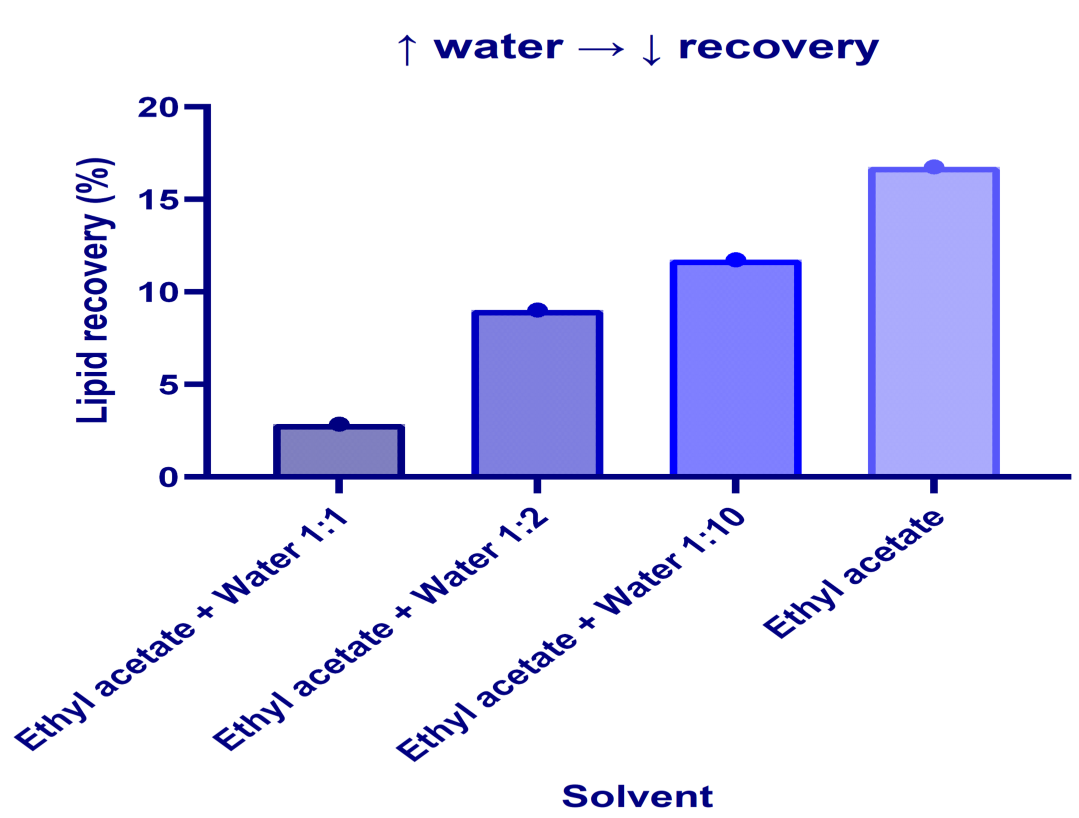
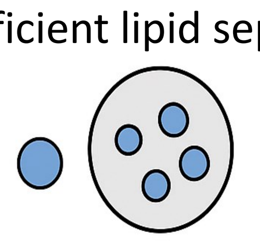


Figure 5: Effect of water-to-ethyl acetate ratio on lipid extraction efficiency

- ✓ Water presence reduces lipid recovery.
- ✓ Higher water ratios lead to lower extraction efficiency.
- ✓ Pure ethyl acetate gives the highest recovery.
- ✓ Emulsion formation hinders efficient lipid separation.



Emulsion formation hindered separation

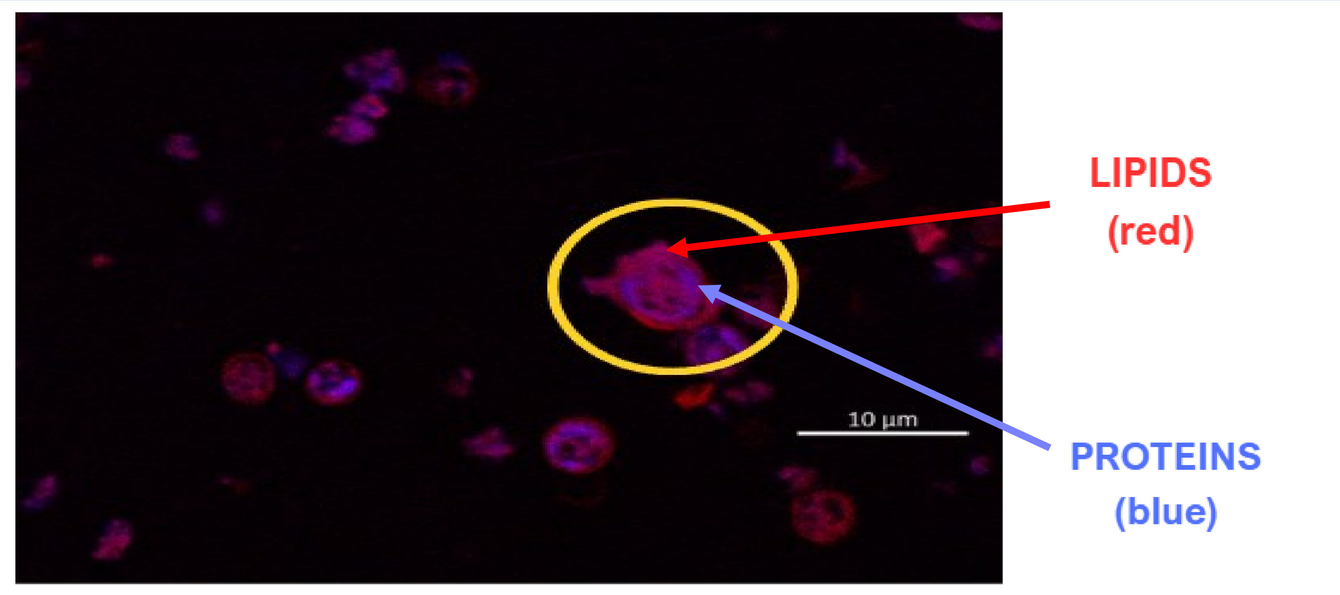


Figure 6: Confocal Laser Microscopy Image of lipid emulsion stained with Nile Red (lipids) and Nile Blue (proteins)

- **Microscopic image of the emulsion formed during ethyl acetate extraction after water addition, stained with Nile Red (lipids) and Nile Blue (proteins)**. Lipids (red) are trapped within the emulsion, while proteins (blue) are surrounded by lipids, illustrating how emulsion formation can hinder lipid recovery.

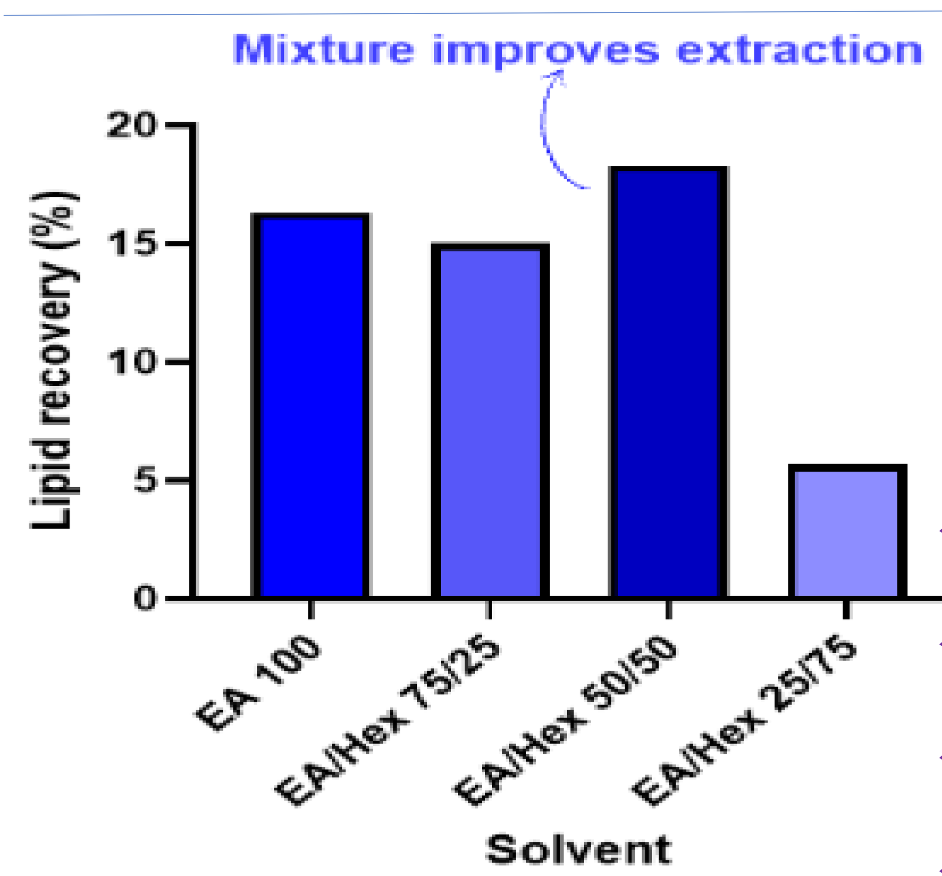


Figure 7: Effect of Solvent composition on extraction recovery

- EA : Ethyl acetate
- Hex: Hexane
- Met : Methanol

- ✓ Methanol shows the highest extraction recovery.
- ✓ High Hexane content significantly lowers recovery.
- ✓ Solvent composition clearly affects extraction recovery.
- ✓ Optimal recovery achieved with a balanced EA/Hex mixture.

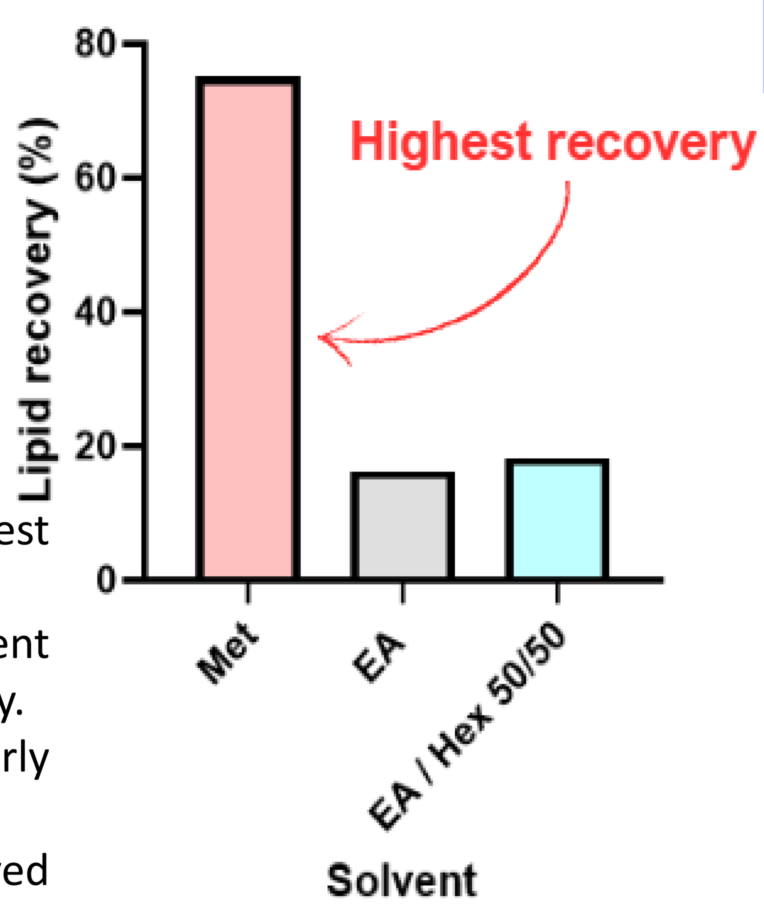


Figure 8: Effect of Solvent on extraction recovery

Conclusion

Ultrasound pretreatment markedly enhanced lipid recovery, while enzymes showed promising improvement. Methanol and ethyl acetate gave the highest yields, whereas water reduced efficiency due to emulsion formation. Further optimization of enzymatic methods and greener solvents could enable scalable, sustainable biodiesel production.

Acknowledgments

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor CINEA can be held responsible for them.

FUELPHORIA

Funded by the European Union