

Supercritical Fluid Application Notes

SFE542: Supercritical Extraction of Lipids from Microalgae for Biodiesel Synthesis

Introduction

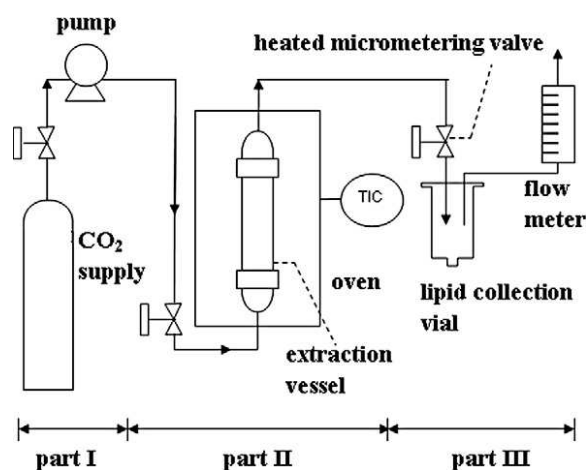
The rapid depletion of fossil fuels has increased interest in finding alternate energy supplies that are renewable. Fatty acid methyl esters (FAME) or biodiesel is a prime candidate to replace fossil fuels. Commercial production of biodiesel usually involves alkaline-catalyzed transesterification of triglycerides found in soybeans and rapeseed. Unfortunately, these crops are a source for food throughout the world and therefore are not sustainable as a fuel source.

Microalgae are a promising alternative to food crops as a source for biodiesel lipids. They are photosynthetic organisms that can accumulate up to 50% of dry cell weight as lipids. In addition, many microalgae grow in brackish water or seawater, eliminating the need for fresh water.

Supercritical carbon dioxide (SCCO₂) extraction is a green technology that can replace the use of traditional organic solvents for lipid extraction. The benefits of using SCCO₂ extraction include solvating power that is tunable, low toxicity, favorable mass transfer equilibrium, and the production of solvent-free extracts.

Equipment

Applied Separations Supercritical Extraction Equipment including the Helix, SFE 2, SFE 4, or Basic model.



Materials

Liquid CO₂ cylinder

Methylation catalysts -(10 wt% H₂SO₄ in methanol and 25 wt% KCH₃O in methanol)

n-hexane, isopropanol, and methanol were analytical grade.

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The microalgal strain used for this study was a *Chlorococcum* sp. and was cultivated in outdoor bag bioreactors. The species had a high growth rate, survived well in outdoor conditions and displayed wide temperature tolerances over the period of the study.

Method

Sample Preparation

Dewatering

Dewater the microalgae culture via centrifugation and rinse the microalgal paste with deionized water to remove residual salts.

Dry, Grind and Mix

The microalgal paste was dried at 85 °C in an oven for 16 h. A ring mill was then used to grind the dried biomass into powder. Microalgal powder (20 g) was mixed with inert diatomaceous earth) at specific ratios (powder: d.e. = 2:1 w/w). The resulting homogeneous mixture was tightly packed into an extraction vessel.

Supercritical CO₂ Extraction Conditions

Sample: 20 g

Pressure: 500 bar

Temperature: 60 °C

Valve temperature: 120 °C

CO₂ Flow Rate: 0.4 L/min (gas)

Collection: 60 mL pre-weighed vial

Dynamic time: 80-120 minutes

Fatty Acid Methylation

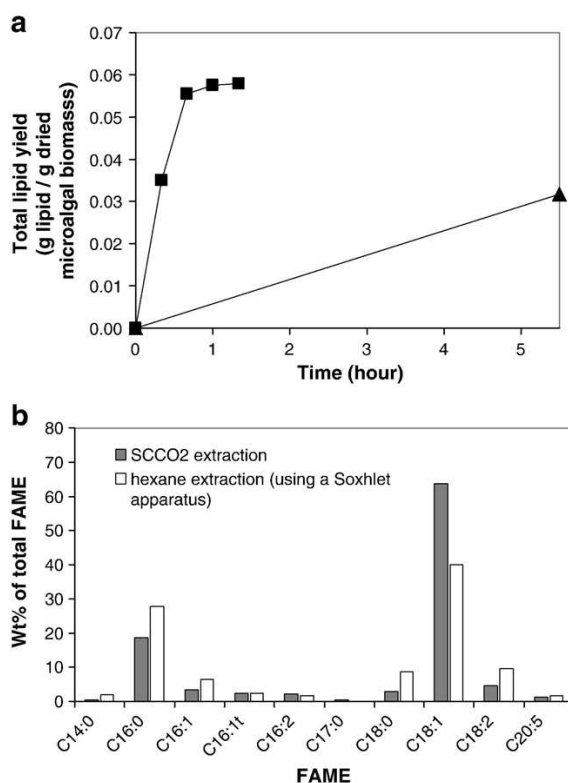
Acid Catalysis

Redissolve the lipid extract in 20 mL of hexane. Add 20 mls of methanol and 1ml of 10 wt% H₂SO₄ in methanol to each vial. Transfer the mixture into a flask, heat to 50 °C, and moderately agitate for 2 h. Replenish evaporated methanol as required.

Alkaline Catalysis

25 wt% KCH₃O in methanol was added dropwise to the gently stirred reaction mixture until a pH value of 13 was attained. Heat the mixture to 55 °C and moderately agitate for 2 h. Replenish the evaporated methanol as needed. Evaporate the mixture in an oven @ (60 °C) to obtain the dried post-methylated lipid extract. Finally, the lipid was re-dissolved in 20 mL of hexane for FAME analysis.

Results



(a) Comparison of total lipid yield between dynamic SCCO₂ extraction and dynamic hexane extraction. ■ SCCO₂ extraction, ▲ Hexane extraction (using a Soxhlet apparatus).
(b) FAME composition of the crude lipids.



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Conclusion

The yield of the SCCO₂ lipid extraction of microalgae was found to decrease with temperature and to increase with pressure. There was no significant difference in the FAME compositions of lipid extracts obtained under different SCCO₂ extraction parameters. Soxhlet extraction using hexane was found to be significantly less efficient than SCCO₂ extraction.

Supercritical carbon dioxide extraction is a promising green technology that can potentially be used for large-scale microalgal lipid extraction. It is rapid, non-toxic, has high selectivity towards acylglycerols, and produces solvent-free lipids.

References

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- Halim R, Gladman B, Danquah MK, Webley PA. Oil extraction from microalgae for biodiesel production. *Bioresource technology*. 2011 Jan 1;102(1):178-85.
- Hogan P, Otero P, Murray P, Saha SK. Effect of biomass pre-treatment on supercritical CO₂ extraction of lipids from marine diatom *Amphora* sp. and its biomass evaluation as bioethanol feedstock. *Heliyon*. 2021 Jan 1;7(1):e05995.