trans algae

Wastewater grown green microalgae – fatty acids characterization and quantification

The extraction method and pre-treatment effect

Sandra Lage
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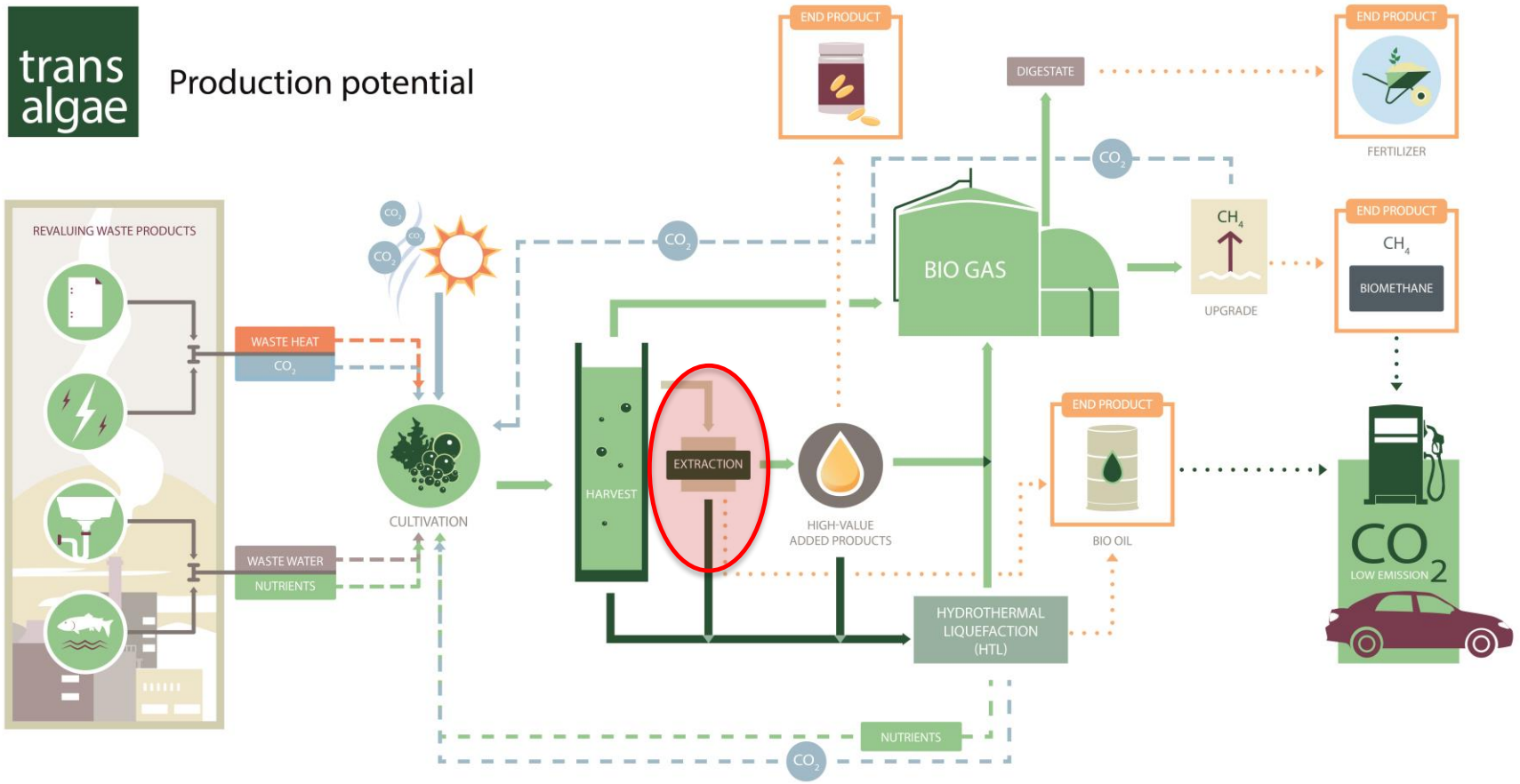
Production potential



PROJECT COURSE

REVALUATION OF WASTE PRODUCTS | CULTIVATION | HARVEST | TRANSFORMATION | SYSTEM ANALYSIS

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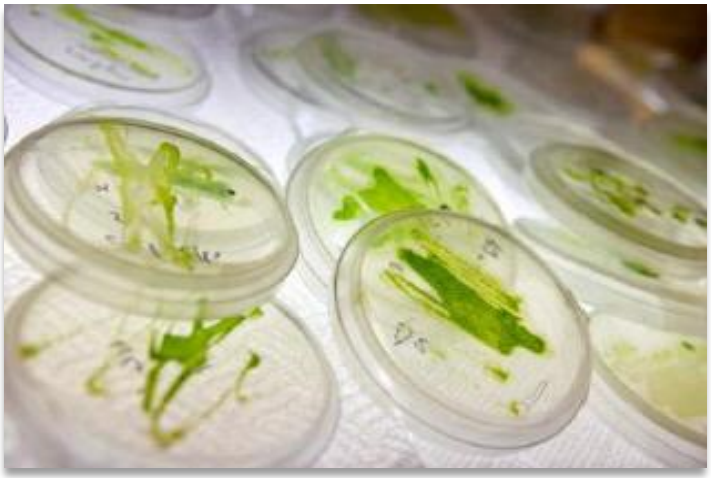
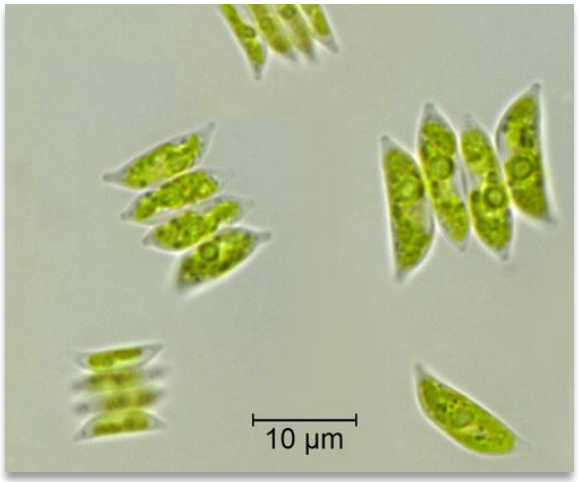
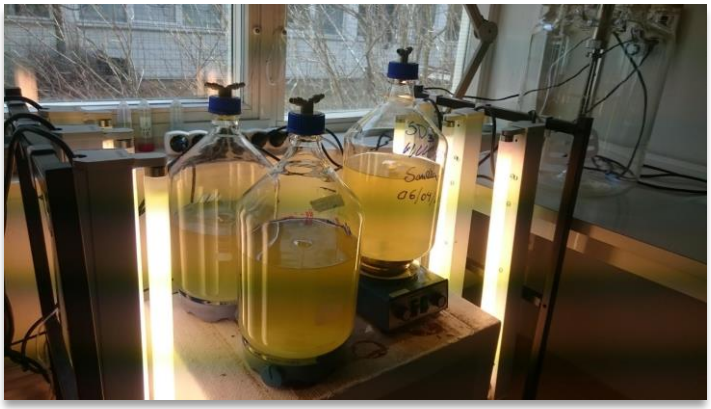
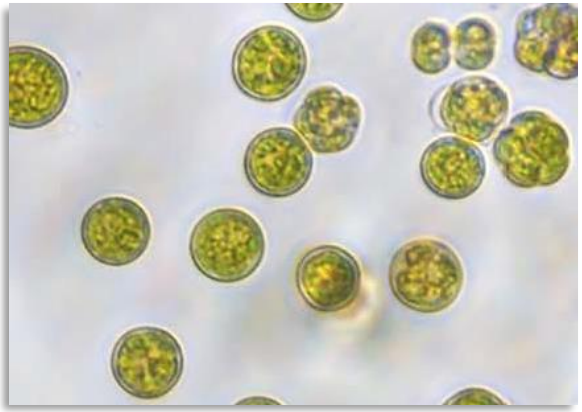
Microalgae cultivation





Scenedesmus dimorphus





***Coelastrella* sp.**

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How to extract lipids from microalgae?

OPEN ACCESS Freely available online

PLOS ONE

A Single-Step Method for Rapid Extraction of Total Lipids from Green Microalgae

Martin Axelsson, Francesco Gentili*

Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden

Gravimetric analysis

Gas Chromatography

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PLOS ONE

A Single-Step Method for Rapid Extraction of Total Lipids from Green Microalgae

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Purification step

Gas Chromatography

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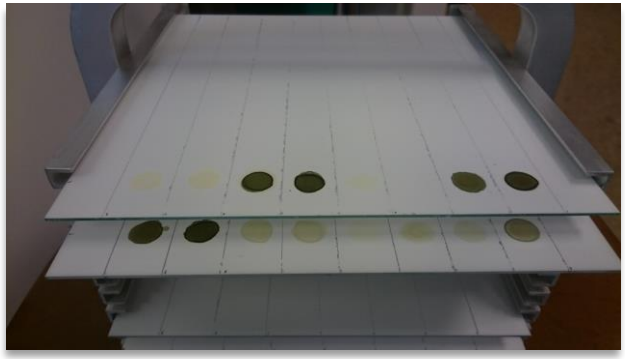
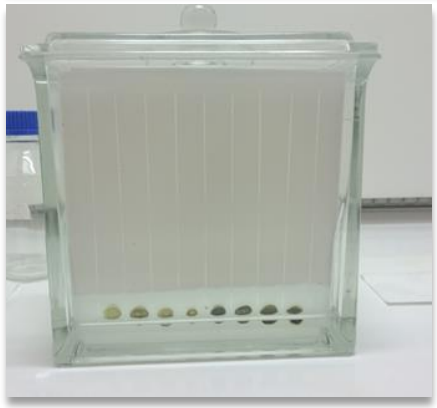
How to purify total lipid extracts for Gas chromatography (GC) analysis?

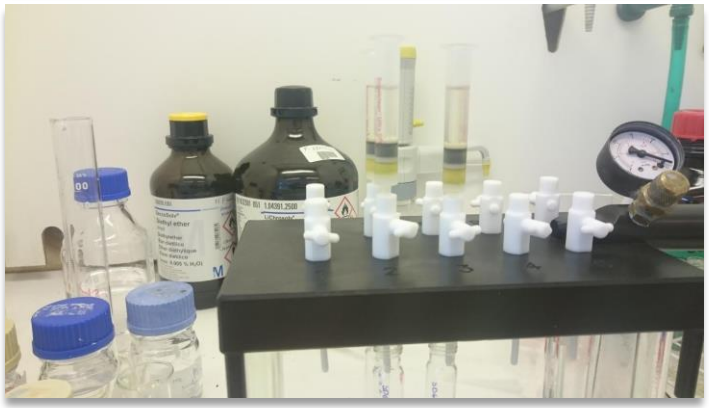
Thin-layer chromatography (TLC)





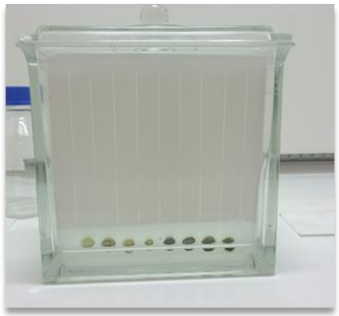
Transmethylation

Solid phase extraction (SPE)



Make it Simpler, Make it Better

Extraction, Purification and Transmethylation of Fatty acids in a Single-Step Method



or

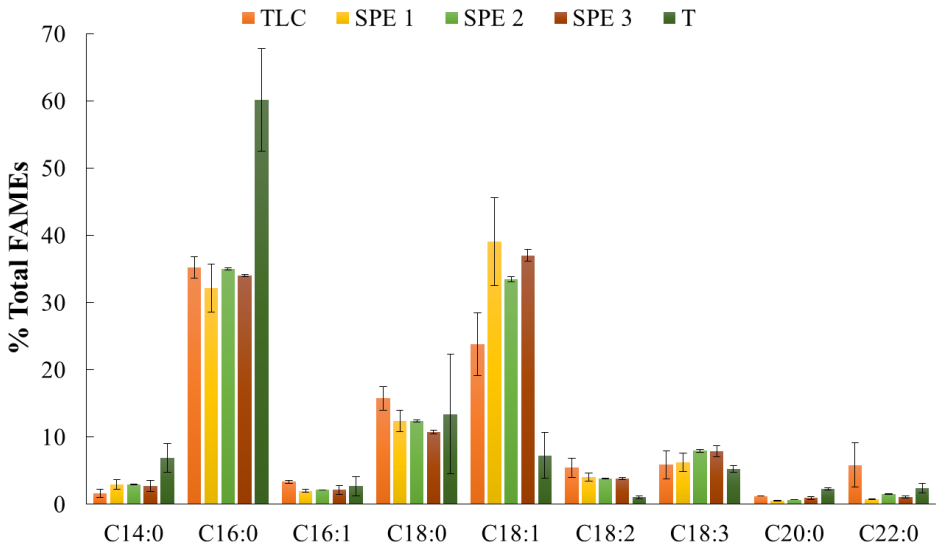
or

Nothing

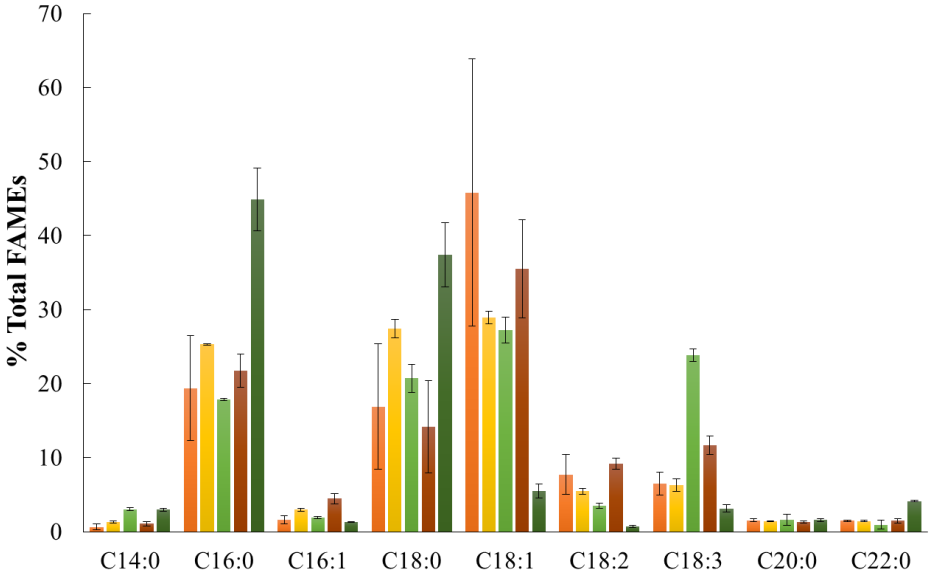
Transmethylation

FAMEs characterization
The extraction method effect

Scenedesmus dimorphus



***Coelastrella* sp.**



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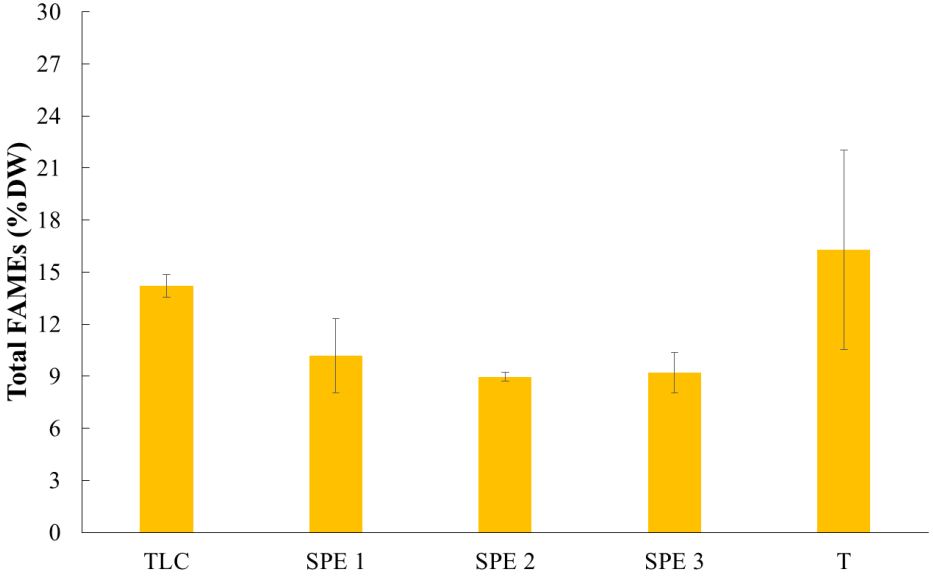
FAMEs quantification
The extraction method effect

Scenedesmus dimorphus



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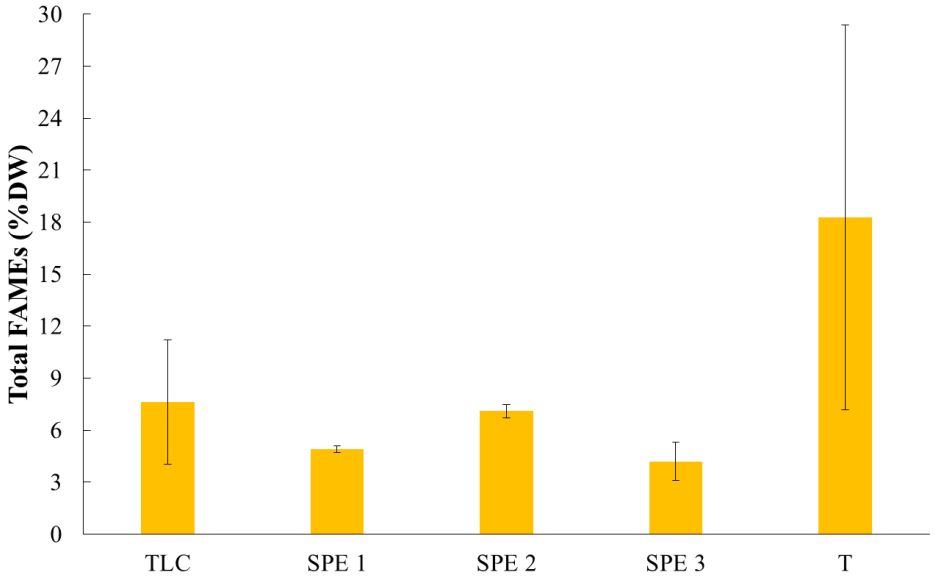

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Pre-Treatment of Green microalgae

Does it affect the quantity and/or quality of the lipids?

- Fresh boiled in Isopropanol
- Frozen at -20°C
- Freeze-Dry at -45°C
- Oven-Dry at 105°C

?

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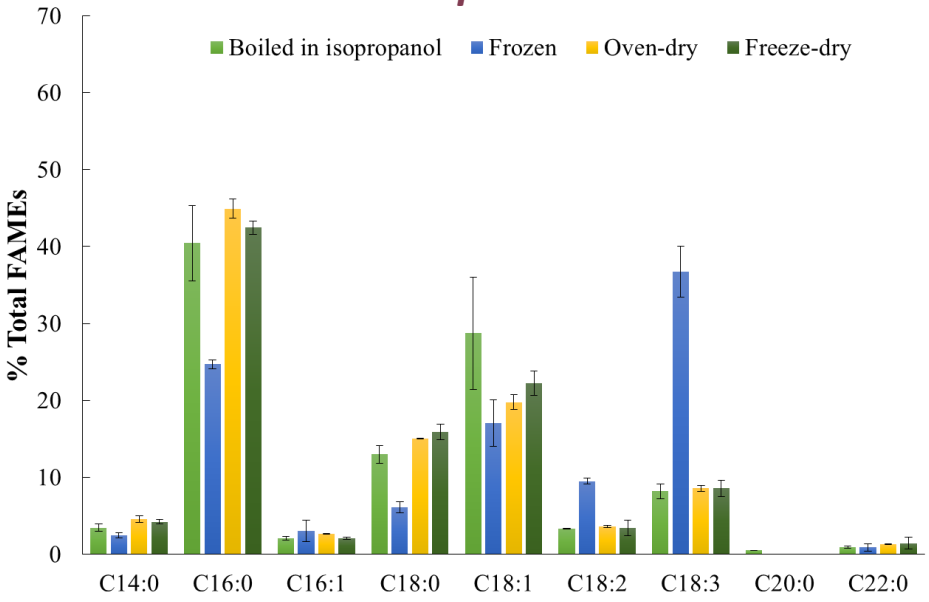
FAMEs characterization
The pre-treatment effect

Scenedesmus dimorphus



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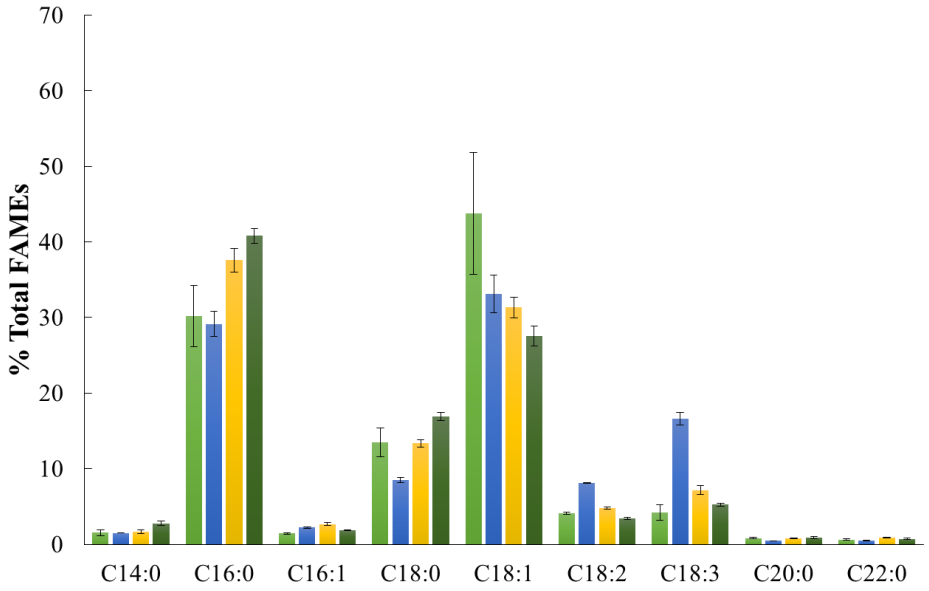

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FAMEs quantification
The pre-treatment effect

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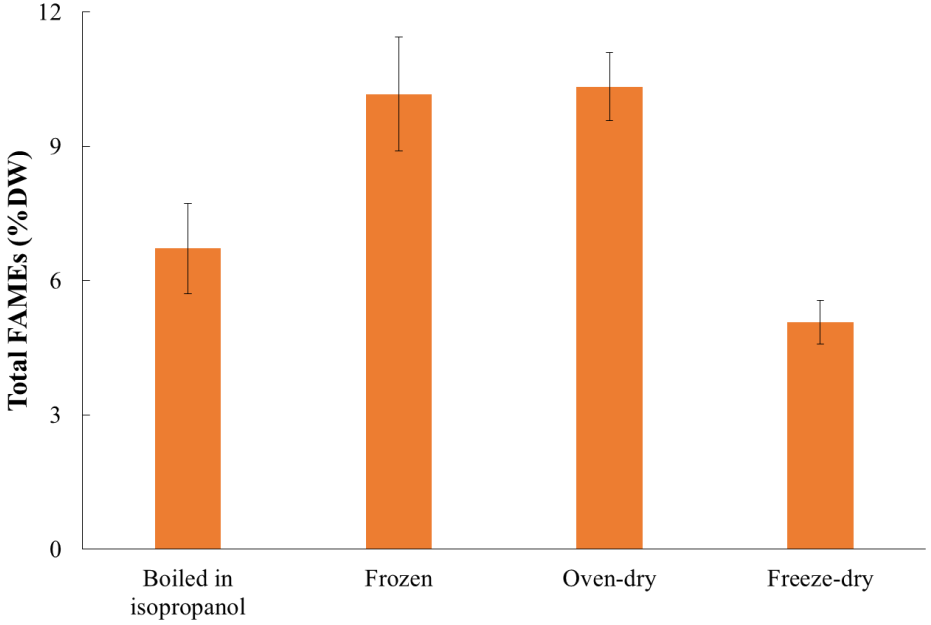

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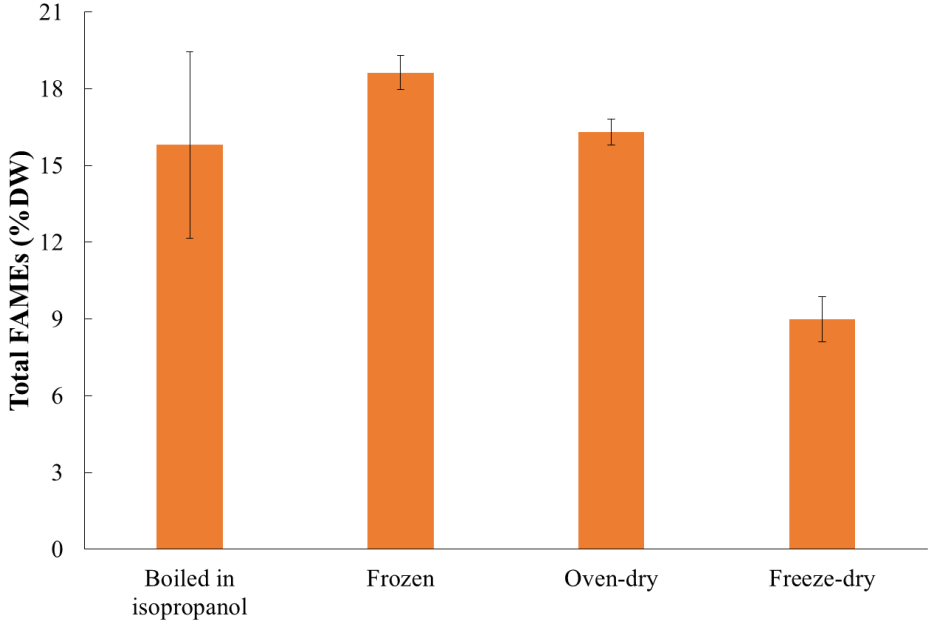

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Comparison of methods

-Determination of biodiesel yields in microalgae

Microalgae have the potential to rapidly accumulate lipids of high interest for the food and feed, cosmetics, pharmaceutical and energy (e.g. biodiesel) industries. However, current lipid extraction methods show efficiency limitation and until now, extraction protocols have not been fully optimized for specific lipid compounds. Thus, the present study evaluates the efficiency of several lipids extraction methods.

CRUDE LIPIDS EXTRACTION

Microalgae biomass has a high biodiesel potential. Neutral lipids (triglycerides (TAGs) and free fatty acids) are the present raw materials for biodiesel production through conversion into fatty acid methyl esters (FAMES). Microalgae biomass also contains polar lipids (glyceroglycolipids and phospholipids), proteins, carbohydrates, vitamins and pigments etc. Each microalgae species has characteristic TAGs profiles. Thus, an accurate quantification and characterization of TAGs are crucial for the selection of the species with the highest biodiesel potential.

Microalgae crude lipids are usually extracted by a mix of organic solvents and quantified either by gravimetric methods or capillary gas chromatography-flame ionization detection (GC-FID), after the lipids derivatization (Transmethylation) to FAMES. Although, gravimetric analysis has been criticized of lipids overestimations since several compounds may be co-extracted with lipids due to their similar polarity, the interference of these compounds on the FAMES quantification with GC-FID has not yet been evaluated.

PURIFICATION METHODS

Alternatively to the direct analysis of crude lipid extracts with GC, a preliminary fractionation of the lipid classes can be performed by thin-layer chromatography (TLC) or solid-phase extraction (SPE) prior to GC analysis. Both techniques allow the separation of neutral lipids from the remaining lipid classes and other compounds present in the microalgae crude lipids extract.

Thus, in the present study the crude lipids of two green microalgae species, Scenedesmus dimorphus and Coelastrella sp. were extracted with a single-step method using a 2:1 choroform:methanol (v/v) solution and transmethylated. The effect of performing or not a purification step on the FAMES profile and content was evaluated. Additionally three different brands of SPE cartridges, i.e., SPE 1, SPE 2 and SPE 3, were tested, in order to identify any particular discrepancy.

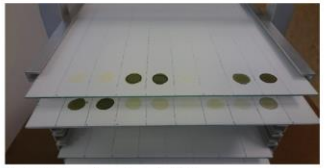

Fig. 1. Thin-layer chromatography (TLC).

Fig. 2. Solid-phase extraction (SPE).

DIRECT TRANSESTERIFICATION

Direct transesterification (DT), is a method of converting saponifiable lipids in situ directly to FAMES, combining extraction and transesterification into one step, and immediately quantifying FAMES by GC. In this study a DT method with combined basic and acidic catalysts was compared with the previous stated methods.

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Quantification and characterisation of fatty acid methyl esters in microalgae: Comparison of pretreatment and purification methods

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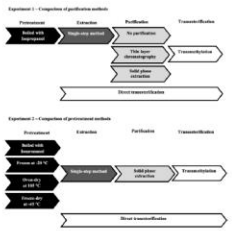
GRAPHICAL ABSTRACT



ARTICLE INFO

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Direct transesterification
Lipids
Solid-phase extraction
Thin-layer chromatography
Wastewater

ABSTRACT

A systematic qualitative and quantitative analysis of fatty acid methyl esters (FAMEs) is crucial for microalgae species selection for biodiesel production. The aim of this study is to identify the best method to assess microalgae FAMEs composition and content. A single-step method, was tested with and without purification steps—that is, separation of lipid classes by thin-layer chromatography (TLC) or solid-phase extraction (SPE). The efficiency of a direct transesterification method was also evaluated. Additionally, the yield of the FAMEs and the profiles of the microalgae samples with different pretreatments (boiled in isopropanol, freezing, oven-dried and freeze-dried) were compared. The application of a purification step after lipid extraction proved to be essential for an accurate FAMEs characterisation. The purification methods, which included TLC and SPE, provided superior results compared to not purifying the samples. Freeze-dried microalgae produced the lowest FAMEs yield. However, FAMEs profiles were generally equivalent among the pretreatments.

1. Introduction

Microalgae are currently being investigated worldwide as a promising sustainable and renewable energy source to meet future energy demands for liquid transportation fuels. In particular, the production of biodiesel coupled with wastewater treatment has been proposed as a cost-effective and feasible alternative to fossil fuels (Pulz and Gross, 2004). The harvest of microalgae biomass and the extraction of lipids

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http://biofuelregion.se/en/projekt/transalgae/infosheet/

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Conclusion

- **TLC and SPE purifications**, coupled with single-step crude lipids extraction and transmethylation, produced accurate, precise and reproducible results of **FAME yields and composition**.
- SPE is preferable due to **lower labor intensity.**
- Removal of a purification step strongly affected the **method reproducibility**.
- DT had a distinct FAME profile in comparison with the other methods.
- **Pretreatment did not** have a strong impact in FAME characterization.
- **Freeze-dried** microalgae produce the lowest FAME yields.

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