trans algae INFOSHEET

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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 Coelastrel la 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.

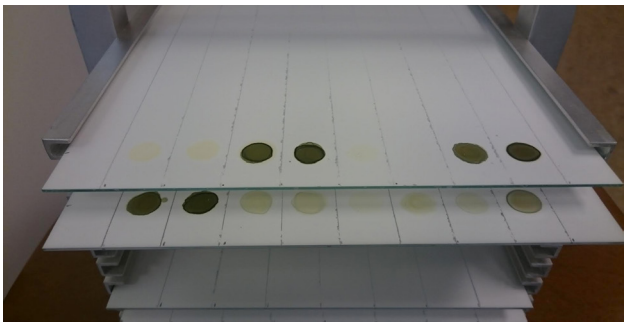

Fig. 1. Thin-layer chromatography (TLC).

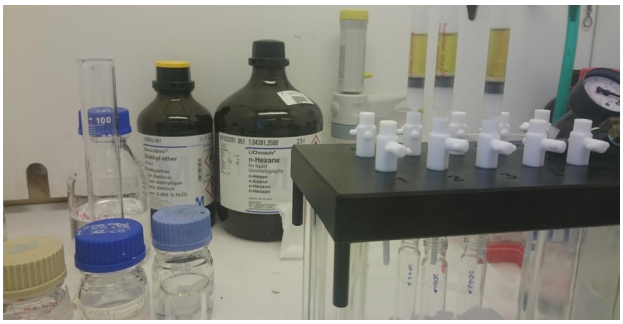

Fig. 2. Solid-phase extraction (SPE).

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.

FAMES CHARACTERIZATION

The FAMES composition of S. dimorphus and Coelastrella sp. was analogous on the samples extracted with the single-step method, transmetylated and either purified with TLC or any of the three types of SPE cartridges (Fig. 3). When no purification step was applied (T), the percentages of some FAMES types vary and had higher standard deviations, which can be attributed to the presence of interfering compounds compromising an accurate quantification. DT gave the more distinct profiles, suggesting that different extraction systems and solvent mixtures may generate different FAMES profiles.

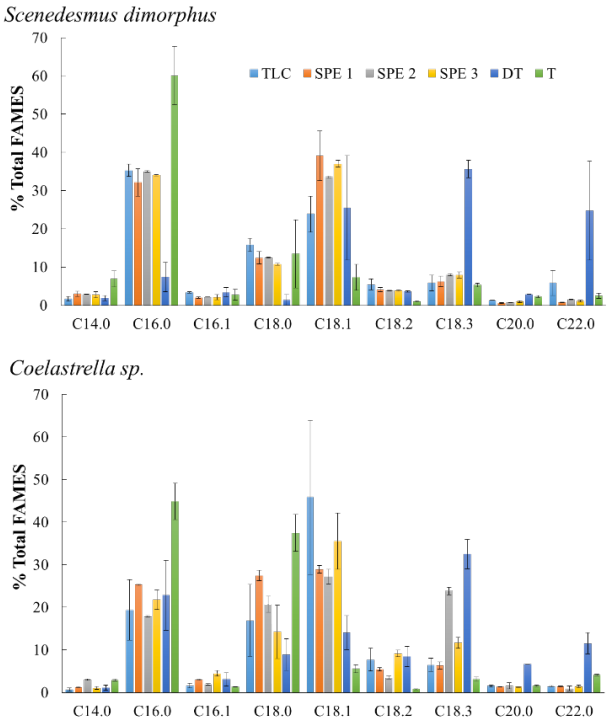

Fig. 3. Fatty acid methyl esters (FAMES) profiles.

FAMES QUANTIFICATION

The total FAMES concentration represents the biodiesel yield, which is dependent on several factors, including microalgae species, culture conditions, environmental factors and extraction methods. Total FAMES contents extracted in each species was analogous between the different methods. In exception of DT with the S. dimorphus samples, which extracted significantly less FAMES that the other methods (Fig. 4). Again, the high standard deviations of the method without purification (T) dissuades the use of this method for FAMES quantification.

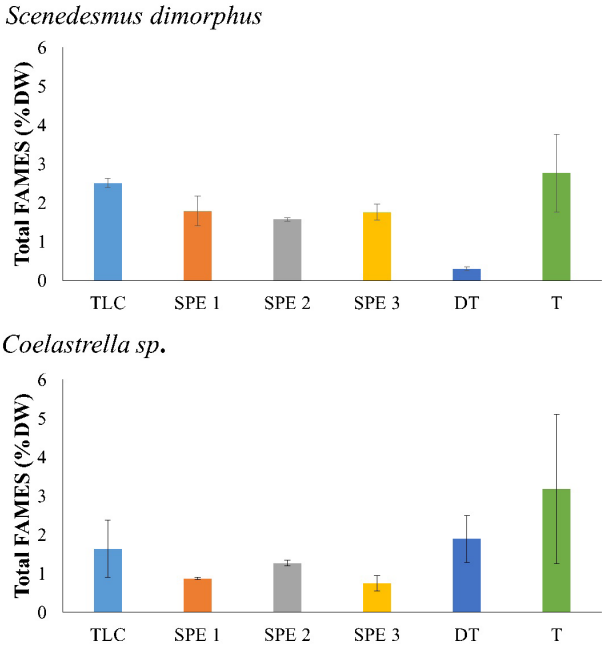

Fig. 4. Fatty acid methyl esters (FAMES) yields.

CONCLUSION

Both TLC and SPE purification coupled with single-step crude lipids extraction and transmethylation were proved to be the most accurate, precise and reproducible methods for FAMES quantification and characterization. With SPE samples can be purified easier and faster than with TLC. Thus, the method including SPE purification was selected for future FAMES analysis within the TransAlgae project.

The removal of a purification step strongly affected the method reproducibility. DT would not be the recommended methodology to determine if one microalgae species is more suitable than other for biodiesel production, because of the distinct FAMES profile obtained in comparison with the other methods. However, DT may be employed in total FAMES yields determination.

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