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Supercritical carbon dioxide treatment of the microalgae *Nannochloropsis oculata* for the production of fatty acid methyl esters

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ABSTRACT

The aim of this work was to evaluate the potential of supercritical carbon dioxide (CO_2) to extract fatty acid methyl esters (FAME) from the microalgae *Nannochloropsis oculata* (*N. oculata*) at low temperatures (37 and 55 °C) and pressures (5.9 and 7.6 megapascals (MPa)). A qualitative gas chromatography (GC) analysis showed that the individual FAMEs extracted varied depending on the co-solvent (methanol or hexane) used with supercritical CO_2. Using hexane, FAME compounds produced were similar to those extracted with soxhlet extraction alone while longer chain FAME were produced when methanol was the co-solvent. The effects of pressure and temperature variations were shown to be of statistical significance. The chromatograms produced in this work demonstrate that altering one of these parameters (co-solvent, temperature, pressure) can produce different compounds owing to the tunability of the technique.

1. Introduction

In previously published work supercritical methanol [1] and CO_2 [2] have been used to extract and transesterify fatty acids to biodiesel in a single step using high temperatures and pressures. The aim of this work was to use supercritical CO_2 at low temperatures and pressures to convert microalgal oils to biodiesel in a single step, thus saving time and money. Milder conditions have been found to be less destructive to natural substrates such as algae [3]. Lower temperatures and pressures showed the production of long chain FAMEs which are of interest owing to their potential health benefits and other uses.

1.1. The composition and uses of microalgae

Microalgae are rich sources of MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) which are of interest in biodiesel production as well as human and animal health. Examples of MUFA and PUFA structures can be seen in Fig. 1. MUFAs are fatty acid chains containing one unsaturated C–C bond i.e. double or triple bonds while PUFAs have more than one unsaturated C–C bond. Both compounds are of interest in the production of biodiesel [4]. There are a range of criteria which are required to identify a reliable substrate for biodiesel production including iodine value, heating value, and lubricity but a high MUFA and low PUFA composition is thought to be preferable [5]. However, sunflower oil has high PUFA content and can produce biodiesel which meets the requirements of the European legislation [6]. Of the total fats produced by *Nannochloropsis oculata* between 35% and 46% are MUFAs and between 8% and 22% are PUFAs [7].

Nannochloropsis is a marine species in the Eustigmatophyceae class of microalgae [9] which has previously been studied as a source of biodiesel production [1,10–14]. Under optimal growth conditions *N. oculata* has a lipid content above 50% [15] making it a substrate of interest in biodiesel production. Large amounts of microalgal biomass can be produced in a small space [16]. Biomass can double daily [17] and is high in the valuable fatty acid, EPA (eicosapentaenoic acid) when compared to other microalgae [18].

Abbreviations: µm, micrometres; *N. oculata*, *Nannochloropsis oculata*; ANOVA, analysis of variance; C, carbon; CO_2, carbon dioxide; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FID, flame ionization detector; g, grammes; GC, gas chromatography; He, helium; ml, millilitre; MPa, megapascals; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; rpm, revs per minute; SCF, supercritical fluids; sp., species (singular); TS, total solids.

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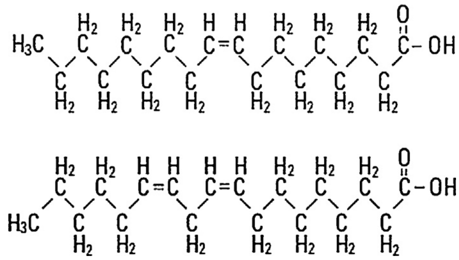

Fig. 1. Example of MUFA (oleic acid) and PUFA structure (linoleic acid) [8].

1.2. The biodiesel production process

Biodiesel has been defined as the conversion of renewable animal and vegetable long chain fatty acids to FAMEs [19]. Transesterification reduces the viscosity of the fats so that it can be used in vehicle engines [20]. When compared to petroleum based diesel, the emissions from biodiesel are lower in pollutants such as unburned hydrocarbons, carbon monoxide and particulate matter [21,22].

Before the biodiesel refinement process begins, the fatty acids must be separated from the other components of the substrate (in this case, the microalga). A number of techniques have been used to achieve this, among them microwave assisted extraction and ultrasound assisted extraction [23] but soxhlet extraction has traditionally been used and that is the technique referenced in this work. The soxhlet extraction procedure involves using a solvent such as hexane or petroleum ether to dissolve the oils of interest [24].

After the soxhlet extraction is completed the lipids are transesterified to biodiesel. Oils extracted from the substrate by soxhlet extraction consist of a glycerol backbone with 3 long chain fatty acids [25]. Glycerol is separated from the long chain fatty acids during transesterification by reacting the compound in excess alcohol and a catalyst. Water and free fatty acids residues have been shown to have a negative effect on FAME production. This effect is minimised by using an alkali catalysed reaction followed by an acid catalysed procedure [26,27].

McDaniel and Taylor [28] demonstrated the feasibility of the incorporation of the transesterification step into the supercritical extraction process. In recent times the use of supercritical methanol (260 °C and 8.3 MPa), [1,10] and supercritical ethanol (245–270 °C and 8.3–9.3 MPa) [29] to extract and transesterify fatty acids from *Nannochloropsis* species (sp.) in a single step has been achieved. Andrich et al. [30] found supercritical CO_2 was comparable to hexane soxhlet to extract bioactive lipids from *Nannochloropsis* sp. Supercritical CO_2 (40 °C, 40 MPa) was followed by transesterification to extract the polyunsaturated EPA from *Nannochloropsis*. Some of the conditions investigated in the production of biodiesel by microalgae to date are outlined in Table 1.

In this work supercritical CO_2 at low temperatures and pressures and using a co-solvent was used to identify if soxhlet and transesterification steps could be skipped completely to produce biodiesel directly.

1.3. The use of supercritical fluids with natural substrates

The use of various SCFs have been compared favourably to other extraction techniques with plant and algal samples. High temperatures and pressures have been used in some cases [31–37]. The use of co-solvents such as ethanol or methanol have been found to assist the extraction of non-polar lipids [35,38].

Cheung et al. [37] found the treatment of the seaweed *Sargassum hemiphyllum* with supercritical CO_2 gave a lipid yield equivalent to a methanol/chloroform soxhlet extraction and increased the proportion of EPA produced. When comparing soxhlet extraction and supercritical CO_2 Punín Crespo and Yusty [31] found extraction of aliphatic hydrocarbons from the brown seaweed, *Undaria pinnatifida* to be preferable using soxhlet extraction. Varying pressures were used to extract different components and different amounts of those components from the brown algae *Dilophus ligulatus* [32,33]. Supercritical CO_2 and thermochemical liquefaction are compared in the extraction of biodiesel from the green seaweed *Chaetomorpha linum* by Aresta et al. [39]. A higher amount of long chain fatty acids and polyunsaturated fatty acids were obtained from the SCF procedure when compared to the thermochemical liquefaction process. In the work of Halim et al. [40] decreasing pressure and increasing temperature resulted in increased lipid production, while Mendes et al. [41] found that a decrease in pressure was more effective. When using supercritical ethanol Levine et al. [42] found that higher temperatures were more productive.

Reverchon [3] suggests that lower temperatures (40–50 °C) and lower pressures (below 10.3 MPa) are more selective and less damaging for natural products. When extracting oil from ginger with supercritical CO_2, Roy et al. [43] observed that when higher pressures were used higher temperatures were preferable while at low pressures, lower temperatures were more effective. In this work relatively low temperatures and pressures were used to establish the effect of supercritical CO_2 on the extraction of FAME from *N. oculata* for use in biodiesel and human health products.

2. Materials and methods

2.1. Supercritical fluid microalgal study

Investigations were undertaken on the microalgae, *N. oculata* to evaluate the potential of using supercritical CO_2 to extract FAMEs for biodiesel and other applications.

The microalgae *N. oculata* used in this work was cultivated by researchers at Daithi O'Murchu Marine Research Station (DOMMRC) from stocks sourced from the Culture Collection of Algae and Protozoa (CCAP), based at the Scottish Association for Marine Science (SAMS) in Oban, Scotland. Cultures were grown in f/2 medium and after harvesting were freeze-dried for 24 h. The average total solids (TS) of the freeze dried samples was 34.9%.

It was then subjected to a range of SCF treatments followed by soxhlet extraction in some instances. These extraction procedures were compared to traditional soxhlet extraction. Samples were analysed by liquid injection GC and compared to a previously run carbon (C) standard containing all of the even FAMEs from C8

Table 1
Previously investigated supercritical fluids (SCF) conditions relating to biodiesel production from microalgae.

Species	SCF	Temp. (°C)	Pressure (MPa)	Reference
Botryococcus braunii	CO_2	40	30	Mendes et al. [41]
Chlorococcum sp.	CO_2	60	31	Halim et al. [40]
Scenedesmus dimorphus	CO_2	100	41	Soh and Zimmerman [2]
Nannochloropsis sp.	Methanol	260	8.3	Patil et al. [1]
Chlorella vulgaris	Water	250	*	Levine et al. [42]

* Pressure not reported but the pressure required for supercritical water is 22.1 MPa.