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Biofuels from microalgae: Lipid extraction and methane production from the residual biomass in a biorefinery approach

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HIGHLIGHTS

- More lipids are extracted by $SCCO_2$ than using conventional extraction methods.
- $SCCO_2$ extracts most of neutral lipids for biodiesel production.
- $SCCO_2$ allows valorisation of the resulting microalgal biomass.
- The highest methane yield was obtained after lipid extraction by $SCCO_2$.
- $SCCO_2$ enhances microalgal biodegradability to increase methane production.

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ABSTRACT

Renewable fuels and energy are of major concern worldwide and new raw materials and processes for its generation are being investigated. Among these raw materials, algae are a promising source of lipids and energy. Thus, in this work four different algae have been used for lipid extraction and biogas generation. Lipids were obtained by supercritical CO_2 extraction ($SCCO_2$), while anaerobic digestion of the lipid-exhausted algae biomass was used for biogas production. The extracted oil composition was analyzed (saturated, monounsaturated and polyunsaturated fatty acids) and quantified. The highest lipid yields were obtained from *Tetraselmis* sp. (11%) and *Scenedesmus almeriensis* (10%), while the highest methane production from the lipid-exhausted algae biomass corresponded to *Tetraselmis* sp. (236 mL CH_4/g VS_{added}).

1. Introduction

The search for sustainable and renewable fuels is becoming increasingly important as a direct result of climate change and rising fossil-fuel prices (Gravilescu and Chisti, 2005). In this context, liquid biofuels are expected to contribute significantly to diminish greenhouse gas emissions and fossil fuels dependence in a near future. Currently, commercial production of biodiesel involves alkaline-catalyzed transesterification of triglycerides from first generation biofuels, like oleaginous food crops mainly rapeseed in Europe and soybean in the USA (Brennan and Owende, 2010). However, their impacts in transport sector will remain limited due to competition with food and fiber production for the use of arable land, regionally constrained market structures, lack of well managed agricultural practices in emerging economies, high water and fertilizer requirements, and a need for conservation of biodiversity (Chisti, 2007).

Microalgae are considered to be one of the most promising alternative sources for biodiesel (Brennan and Owende, 2010) due to the potential high oil yields that can be obtained from them, which is about 16–70 times the oil that can be obtained from coconut, sunflower and palm (Amin, 2009). Many different species like *Chlamydomonas reinhardtii*, *Botryococcus braunii*, *Chlorella* sp., *Nannochloropsis* sp., among others, may be considered as a suitable source of lipids due to their ability to accumulate over 60% DW (dry weight) of lipids; estimating an annual biodiesel production for *Nannochloropsis* sp. between 23,000 and 34,000 L/ha (Scott et al., 2010). Microalgae are also promising due to their high growth and photosynthetic rates, enabling microalgae to capture carbon faster than terrestrial crops, and to accumulate high percentage of lipids in their biomass (Rodolfi et al., 2008). They can also be cultivated on non-arable lands, in saline water mediums

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and in agroindustrial wastewaters (Riaño et al., 2012). Moreover, they do not need herbicides or pesticides for their growth (Rodolfi et al., 2008).

Different techniques have been used to extract high value compounds from microalgae. The most important methods described in literature to extract lipids from microalgae are microwave assisted extraction, Kochert method, Soxhlet extraction, soxtec extraction, accelerated extraction and ultrasonic extraction (Balasubramanian et al., 2011; Kochert, 1978; Mendes et al., 2006). The main disadvantages of the above mentioned methodologies include high-energy inputs, the requirement of high operational temperatures and the use of organic solvents such as *n*-hexane, methanol–chloroform, that are flammable reagents and present low selectivity. An alternative method to avoid the use of toxic solvents is the use of supercritical carbon dioxide ($SCCO_2$). The $SCCO_2$ extraction technology is well known, and it is considered as a green process (Crampon et al., 2013) since CO_2 is a Generally Recognized As Safe (GRAS) solvent and not flammable. One of the main advantages of $SCCO_2$ is its high selectivity for non polar lipids such as triglycerides. In addition, it does not solubilize phospholipids, which results very useful for biodiesel applications as it avoids degumming operations (Crampon et al., 2013). Furthermore, after depressurization, CO_2 becomes gaseous and is then spontaneously separated from the extracted phase and residue, which are completely free of toxic solvent traces. This enables a direct valorization of both extracts and residues without any additional processing. In this manner, CO_2 can safely be recycled, which represents an economic and environmental benefit. Another advantage is that $SCCO_2$ does not require toxic solvents enabling a subsequent valorization of resulting microalgal biomass, for instance through anaerobic digestion. On the other hand, microalgal lipid extraction by Kochert or Soxhlet method requires toxic solvents as methanol and chloroform, inhibiting anaerobic digestion.

Chisti (2007) evidenced that many different high added value products must be obtained from microalgae (ω-3 and ω-6 fatty acids, pigments, antioxidants, biofuels) to achieve economically feasibility, and therefore it is possible apply the biorefinery concept to the complete exploitation of microalgal biomass. In the present study the concept of total valorization of microalgae to obtain fatty acids (FFA) using $SCCO_2$ and biogas through anaerobic digestion has been considered. The effect of microwave pre-treatment previous to $SCCO_2$ extraction has also been evaluated, as well as lipid extraction by Kochert and Soxhlet methods.

2. Methods

2.1. Microalgae

Microalgal biomass was obtained in lyophilized form from the Food Innovation and Sustainability Center (Almería, Spain). *Isochrysis* T-ISO and *Tetraselmis* sp. were cultured according to Fábregas et al. (1984). *Nannochloropsis gaditana* and *Scenedesmus almeriensis* were cultured according to González-López et al. (2010) and Sánchez et al. (2008), respectively. Lyophilized samples were ground and sieved before the experimental runs, obtaining a particle size distribution lower than 500 μm. The biomass was stored at 4 °C for further use.

2.2. Extraction technologies

2.2.1. Kochert method

Lipids were extracted from the lyophilized biomass using methanol–chloroform 1:2 (v/v) as solvent, following the method proposed by Kochert (1978). Once the extraction was completed, the mass extracted was quantified by gravimetric analysis at 45 °C. Experiments were carried out in duplicate and results were expressed as average values.

2.2.2. Soxhlet method

Solvent extraction was carried out by traditional Soxhlet apparatus using methanol–chloroform 2:1 (v/v) as solvent (Cheung et al., 1998). The extraction temperature was kept at 105 °C for 18 h and the extract was separated from the solvent by a rotatory evaporator (Inlabo Rotatory Evaporator EVI 68 with water bath EVI 90; Padova, Italy) at 41 ± 0.1 °C. Experiments were carried out in duplicate and results were expressed as average values.

2.2.3. Supercritical fluid extraction

Supercritical extraction tests were performed using laboratory scale equipment developed by Solana et al. (2014). The diagram of the process is shown in Fig. 1. The experiment involved several steps. Firstly, the stainless steel extraction cell (16) was filled with 0.5 g of lyophilized microalgae powder. Then, CO_2 was pumped through the extraction cell at a pressure of 30 MPa (controlled by two pressure gauges (6, 14)) and temperature of 45 °C, controlled by a thermo-resistance placed around the extraction cell. Temperature was measured in the internal flow before and after the cell (15, 17). Ethanol was used as co-solvent, pumped by an intelligent pump (Jasco PU-1580) and mixed with CO_2 before the extraction cell. After extraction, the mixture of the solvent, co-solvent and extract was expanded by a valve inserted in a water bath at 40 °C, avoiding CO_2 freezing caused by sudden pressure reduction (18). Extract samples were collected every 15 min in ethanol and they were finally separated from the ethanol by a rotatory evaporator.

The experiments of $SCCO_2$ from *Isochrysis* T-ISO, *N. gaditana*, *S. almeriensis* and *Tetraselmis* sp. were carried out at 30 MPa and 45 ± 2 °C for 90 min, with a constant CO_2 flow rate of 0.4 ± 0.05 kg/h, measured by a flow meter after depressurization. As 5% of ethanol was added as a co-solvent the critical temperature of the mixture increased to 43 °C (Mendes et al., 2006).

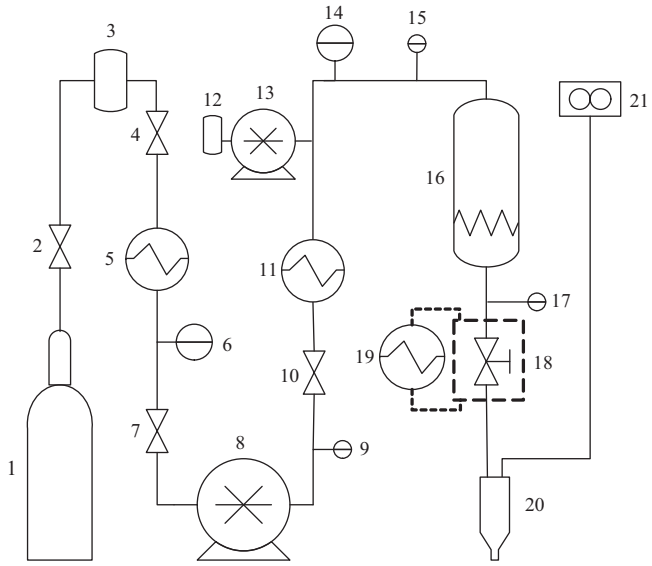

Fig. 1. Schematic diagram of supercritical extraction equipment. 1. CO_2 tank; 2, 4, 7, 10. Valves; 3. CO_2 container; 5. Cooler; 6, 14. Pressure gauges; 8. High pressure pump; 9, 15, 17. Temperature indicators; 11, 19. Heater; 12. Co-solvent container; 13. Co-solvent pump; 16. Extraction cell; 18. Depressurization valve immersed in a water bath; 20. Collector; 21. Flow meter.