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New procedure for extraction of algal lipids from wet biomass: A green clean and scalable process

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HIGHLIGHTS

- A new extraction procedure of lipids from wet microalgae.
- *Nannochloropsis oculata* and *Dunaliella salina* were chosen as a microalgae.
- Influence of operating parameters on the extraction yields will be studied.
- Utilization of terpenes bio-solvents recognized as environmentally safer.

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ABSTRACT

A new procedure, called Simultaneous Distillation and Extraction Process (SDEP), for lipid extraction from wet microalgae (*Nannochloropsis oculata* and *Dunaliella salina*) was reported. This method does not require a pre-drying of the biomass and employs alternative solvents such as d-limonene, α-pinene and p-cymene. This procedure has been compared with Soxhlet extraction (Sox) and Bligh & Dyer method (B&D). For *N. oculata*, results showed that SDEP-cymene provided similar lipid yields to B&D (21.45% and 23.78%), while SDEP-limonene and pinene provided lower yields (18.73% and 18.75% respectively). For *D. salina*, SDEP-pinene provided the maximum lipid yield (3.29%) compared to the other solvents, which is quite close to B&D result (4.03%). No significant differences in terms of distribution of lipid classes and fatty acid composition have been obtained for different techniques. Evaluation of energy consumption indicates a substantial saving in the extraction cost by SDEP compared to the conventional extraction technique, Soxhlet.

1. Introduction

Petroleum reserves depletion and global climate change have strongly encouraged the development of fuel production from various feedstocks such as vegetable oils, waste cooking oils, animal fat and microalgae. Among these options, microalgae have been recognized as potential good sources for biofuel production because they synthesize and accumulate large quantities of neutral lipids (20–50% dry weight of biomass) and grow at high rates (Demirbas, 2008). In addition, microalgae can grow on non-arable, nutrient-poor land that cannot support conventional agriculture (Singh and Gu, 2010). A recent life-cycle assessment (LCA) of biofuel production from microalgae feedstocks mentioned that drying and *n*-hexane extraction accounted for up to 90% of the total process energy (Lardon et al., 2009). The extraction of crude oil is usually performed with petroleum solvents such as conventional *n*-hexane, chloroform and methanol, with techniques are highly energy-consumption and environmentally damaging (Halim et al., 2012). Other extraction processes such as supercritical CO_2, expelling, microwave-ultrasonic assisted extraction have also been reported (Cheng et al., 2011). Many processes have been investigated and reported for converting directly wet algae to crude biodiesel or biocrude (Anastasakis and Ross, 2011; Biller and Ross, 2011; Biller et al., 2011; Patil et al., 2011). But this biocrude differs from biodiesel that we want to extract as it is composed primarily of hydrocarbons in contrast to biodiesel which is composed of lipids in particular FAME after transesterification.

However, various conventional methods are available for oil extraction, but they generally require long extraction times, petroleum-based solvents, dried biomass with water content no more than 10% and high energy inputs. Upon harvesting, typical microalgal concentrations in cultures range from about 0.1–1.0% (w/v) (Cooney et al., 2009). This means that as much as 1000 times the

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amount of water per unit weight of microalgae must be removed before attempting oil extraction. The microalgae paste obtained from centrifugation (dewatering step) contains as much as *ca.* 80% water content. Therefore energy consumption for drying microalgae is dramatically high. A lipid extraction step that eliminates biomass drying and petroleum solvent use could lead to significant energy and cost savings.

Here we propose a new procedure of lipid extraction from microalgae, such as *Nannochloropsis oculata* (*N. oculata*) and *Dunaliella salina* (*D. salina*), that does not require drying of the harvested microalgal biomass and employs bio-solvents recognized as environmentally safer (Virot et al., 2008). Terpenes are natural solvents existing both in the citrus fruits and in many other plants, with extraordinary technical and chemical properties. Mamidipally and Liu recently demonstrated that the industrial extraction of oil from rice bran was possible by using terpene such as d-limonene instead of the regular *n*-hexane (Mamidipally and Liu, 2004; Liu and Mamidipally, 2005).

Extracted lipids obtained using this new procedure, conventional Soxhlet with *n*-hexane and Bligh & Dyer method have been compared in term of total lipid content, lipid classes distribution and fatty acid composition.

2. Methods

2.1. Strain, culture and harvesting conditions

N. oculata and *D. salina* were obtained by Greensea Company (Meze, France). *N. oculata* was incubated in tubular reactor at ambient temperature under deficiency conditions to obtain a high rate of lipids in the biomass. *D. salina* was grown in photo-bioreactor at ambient temperature with good sunniness under favourable conditions. For both, harvesting is being performed by centrifugation, resulting in a 20% dry weight paste that is directly frozen (−25 °C).

2.2. Extraction methods for total lipids

2.2.1. Bligh and Dyer method (B&D)

Total lipids content of both microalgae was determined using a modified B&D method (Bligh and Dyer, 1959) which is already described in Adam et al. (2012).

2.2.2. Soxhlet method (Sox)

Lipids were isolated from microalgae by means of Soxhlet extraction (Soxhlet, 1879). Lipids were extracted from 10 g of dry microalgae for 8 h using 300 mL of *n*-hexane. After the extraction, solvent was eliminated with a vacuum rotary evaporator. Extractions were performed in triplicate and the mean values were reported. Lipid extracts were dried under a stream of N_2 and re-suspended in solvent for HP-TLC or GC–FID analysis.

2.2.3. SDEP method

For SDEP extraction, 12 ± 0.5 g of 20% dry weight microalgae paste were placed in a 500 mL round-bottomed flask. 100 mL of terpene solvent (p-cymene, d-limonene or α-pinene) was added in order to immerse the wet microalgae sample. The round-bottomed flask was surmounted by a modified Dean stark receiver with a 3-way valve and fitted with a condenser (Fig. 1). At the beginning of the experiment (step 1), the electrical heating was maximized until collection of the first droplets of microalgae water in the modified Dean stark receiver with a 3-way valve. Then the heating was adapted until most of the water had been distilled and it was continued to allow lipid extraction step (step 2) with terpene solvent. The extraction was performed for 30 min. Then, the terpene elimination took place (step 3 and 4) and for that water was re-introduced by adjusting the 3-way valve to form a binary water-terpene mixture. To eliminate d-limonene from the distillation flask, we used the property that terpenes are traditionally extracted from their matrix by using a technique called hydrodistillation thus inducing the use of an azeotropic distillation to below the boiling point of terpene under the boiling point of the water (boiling point of the azeotrope: 97.4 °C). Terpene solvent was recovered from the water layer by phase separation in the modified Dean stark receiver and the extracted lipids were recovered from the water layer by phase separation in the distillation round-bottomed flask. Thus, the SDEP procedure was allowed elimination of microalgae water, extraction of lipids and elimination of terpene solvent in a single "in situ" step. Terpene solvent was recuperated at 100% and purity levels show that it can be recycled for other uses, including other SDEP processes. Lipid extracts were analysed by GC-FID (against an external calibration with the pure solvent) and did not contain contamination by solvent (less than 0.01% of solvent in lipid extract). Extractions were performed in triplicate and the mean values were reported. Lipid

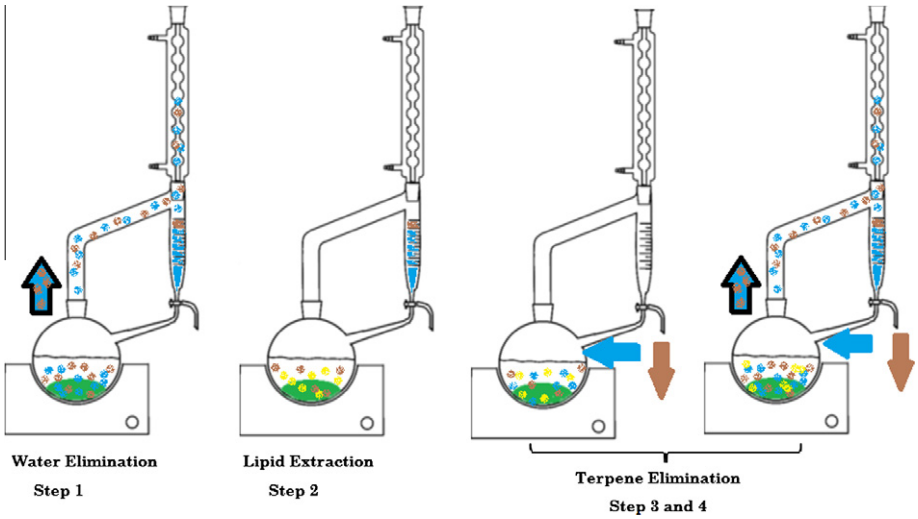

Fig. 1. Simultaneous Distillation Extraction Process (SDEP) (blue: water biomass, brown: terpene solvent, yellow: lipids, green: microalgae). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)