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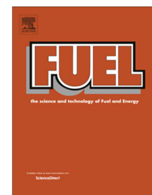

Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production

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HIGHLIGHTS

- Microwave and sonication techniques are compared for efficient lipid extraction.
- Qualitative lipid variation caused by cell disruption and drying methods is studied.
- Energy consumption calculated for drying and lipid extraction processes.
- Lipid quality has been assessed for its suitability for biodiesel production.
- Sun drying with efficient microwave extraction could be possible processing step.

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ABSTRACT

Downstream processing of microalgal biomass presents a significant challenge to large scale biodiesel production. *Scenedesmus* sp. which is known to be a potential feedstock for biodiesel production was cultivated in an open circular pond. The biomass productivity peaked at day 21 with a yield of 1.16 g L^{-1}. Biomass was harvested by gravitational settling followed by centrifugation. Harvested biomass was dried using the freeze drying, oven drying and sun drying processes followed by lipid extraction which yielded 29.65%, 28.63% and 28.33% lipid g^{-1} DCW (dry cell weight) respectively. Lipids were extracted from microalgal biomass dried by selected drying techniques using microwave and sonication for cell disruption in the presence of mixture of chloroform and ethanol (1:1). Microwave assisted extraction of sun dried biomass yielded 28.33% lipid g^{-1} DCW, as compared to 18.9% lipid g^{-1} DCW achieved by sonication assisted extraction. The saponification and acid values of the lipid obtained from *Scenedesmus* sp. dried by selected drying techniques showed high saponification and acid value indicating presence of high free fatty acid content. Effect of different drying and cell disruption technique on fatty acid profile of lipids extracted from *Scenedesmus* sp. biomass was also studied. These values indicate promising potential of the oil produced for conversion into biodiesel.

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1. Introduction

Microalgae have emerged as promising feedstocks for biodiesel production. Microalgal biodiesel has several advantages over crop based biodiesel such as high potential for biomass production with minimal land and freshwater requirement; no arable land requirement, no food security concerns; a higher carbon-dioxide sequestration potential; wastewater utilization during cultivation; and the production of value-added products from de-oiled microalgal biomass. The production of a sustainable and economically viable biodiesel from microalgae is however still a challenge [1–6].

Biodiesel production from microalgae is a multi-step process including cultivation, harvesting and dewatering of microalgal biomass, extraction of lipids from biomass, and conversion of lipids to biodiesel. Photoautotrophic cultivation of microalgae is widely accepted as a more economically viable method for large scale microalgal biomass production. Microalgae essentially require light, carbon dioxide, inorganic nutrients, and water for their growth [7]. Microalgae are either cultivated in open raceway ponds or in photo-bioreactors for a high biomass and lipid production. The choice of technique for cultivation and production of microalgal biomass with high quantity of lipids depends upon microalgal strain selection, culture conditions, land area availability, natural light and production scale [2].

Harvesting and dewatering of microalgal biomass are crucial steps in commercial production of microalgae. The harvesting

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method is dictated largely by the microalgal strain and the cultivation procedure. The common harvesting methods currently employed include flocculation, filtration, flotation and centrifugation or any combination of these methods [8]. Harvesting is followed by drying of the wet biomass. Drying of harvested biomass is necessary to increase the viability of biomass for lipid extraction. Drying methods may include natural sun drying or using advanced techniques like freeze drying, drum drying, oven-drying, spray-drying and fluidized bed-drying. Despite sun drying being amongst the slower methods, it is cost and energy effective as compared to other techniques. Freeze drying is widely used for dewatering of microalgal biomass. Freeze drying is a gentle process in which all the cell constituents are preserved without rupturing the cell wall [8–10]. Extraction of lipids from microalgae is carried out by using either physical (mechanical expeller) or chemical methods (solvent extraction) or by using a combination of these methods. Cell disruption followed by solvent extraction is a widely-used method for oil extraction. Disruption techniques like autoclave, bead-beating, sonication, microwave and osmotic shock are usually coupled with solvent extraction for improved lipid yield. Conventional soxhlet extraction is a time consuming process taking several hours for complete extraction of lipids. The extracted lipids are then converted to biodiesel via transesterification [8,11–13]. Due to the vast diversity of microalgae, investigating suitable harvesting, drying, cell disruption, and extraction techniques for a particular microalga becomes necessary. Development of effective and economical drying, cell disruption and extraction techniques are required to address the challenge of scaling up of the biodiesel production process from pilot to industrial scale.

The present research compares drying and cell disruption techniques for effective extraction of lipids from fresh water *Scenedesmus* sp. grown in an open circular pond. The effect of drying and cell disruption techniques on lipid quality has also been assessed for its suitability for biodiesel production. The conversion of microalgal lipids to fatty acid methyl esters (FAME) was carried out using acid catalyzed transesterification reaction.

2. Materials and methods

2.1. Chemicals and reagents

A mixed FAME standard comprising of 37 components was obtained from Sigma–Aldrich, USA. All organic solvents (chloroform, ethanol, methanol, iso-propanol, dichloromethane, hexane and toluene) and other chemicals purchased from Sigma–Aldrich were of analytical grade.

2.2. Cultivation of microalgae for production of biomass

A strain of *Scenedesmus* sp. isolated from the Durban region, Kwa-Zulu Natal, South Africa was used for the present study. *Scenedesmus* sp. was grown under natural light (400–1200 μmol m^{-2} s^{-1}) and water temperatures ranging from 18 to 27 °C in an open circular pond of 8000 L capacity for biomass production. BG11 medium [14,15] was used as a nutrient medium to culture the microalgae. Mixing and aeration of the suspended algal biomass was accomplished by submersible pumps having flow rate of 110 L min^{-1}. Lipid accumulation in *Scenedesmus* sp. cells was monitored using Nile Red staining [2,16]. The biomass yield of *Scenedesmus* sp. was monitored by gravimetric analysis [17]. Dewatering is required to obtain thick biomass slurry. Harvesting of biomass was initially done on day 21 by gravitational settling to remove the bulk amount of water followed by centrifugation to obtain thick slurry.

2.3. Drying techniques

The harvested wet microalgal biomass was dried using three different drying techniques (sun drying, oven drying, and freeze drying). Thick slurry of wet biomass obtained from 20 L microalgal culture after gravitational settling followed by centrifugation was used for different drying techniques. Microalgal biomass was sun dried on a drying bed lined with white plastic of 1500 μm thickness at ambient temperature (25–30 °C) for 72 h [18]. For freeze drying, wet biomass was frozen overnight at −84 °C and lyophilized using freeze dryer (Mini lyotrap, LTE scientific Ltd., United Kingdom) [15]. Oven drying was carried out using a hot air oven for 12 h at 60 °C [19]. The dried microalgal biomass was crushed in a mortar and pestle and the dry microalgal powder was stored in desiccator to avoid moisture absorption. The harvesting and drying techniques were repeated to accumulate substantial dried biomass for further experiments. Energy consumption of process is calculated using following equation:

$$E\ (\mathrm{kW}) = P \times t/1000 \quad (1)$$

where P is the power (W) consumption of all the instruments used, t the operating time (h) of instruments.

2.4. Cell-disruption and extraction of lipids

Cell disruption and extraction of *Scenedesmus* sp. biomass was carried out by slight modification in methods used by Lee et al. [15] for microwave extraction and Kumari et al. [20] for sonication extraction. The dried biomass (2 g) was added to a 40 ml mixture of chloroform and ethanol (1:1, v/v) and subjected to cell disruption by microwave (Milestone S.R.L., Italy, output power 1200 W) at 100 °C for 10 min at 1000 W. Mixture was centrifuged to separate solvent mixture and cell debris. The mixture was vacuum filtered followed by distillation of solvent in rotary evaporator at 70 °C. The amount of crude lipid was quantified gravimetrically and the lipid yield (%) was calculated. For sonication assisted extraction, microalgal biomass (2 g) was mixed in solvent mixture (20 ml) and the oil was extracted using sonicator (Misonix XL-2000-010, output power 100 W, output frequency 22.5 kHz) in 50 ml tubes at 15 kHz for 2 min. The solvent mixture was centrifuged to separate solvent mixture with lipids and residual biomass in the form of a pellet. Residual biomass was again mixed with solvent mixture (20 ml) and subjected to sonication followed by centrifugation. Solvent mixture was pooled together and filtered through a vacuum filter. The solvent was removed in a rotary evaporator at 70 °C to obtain the microalgal oil. The crude microalgal lipid was measured gravimetrically and the lipid yield (%) was quantified. The lipid yields obtained from biomass dried by different drying processes using the two techniques viz. microwave and sonication were compared. The energy consumption of the microwave and sonication extraction process was calculated by using Eq. (1).

2.5. Lipid qualitative analysis and conversion to biodiesel

The conversion of microalgal lipids to fatty acid methyl esters (FAME) was carried out by acid catalyzed transesterification reaction [21]. The lipid quality was assessed by determination of saponification value and acid value using ASTM methods D5558-95, Reapproved 2011; and D664-07 respectively. The potentiometric titration for determination of acid and saponification value was carried out by using an automatic titrator (TIM 855 Titration manager, Radiometer analytical, Titralab, France). The extracted lipids were then subjected to simultaneous esterification and transesterification using sulfuric acid as catalyst and methanol as acyl acceptor for its conversion to biodiesel. The reaction conditions were

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