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Technical note

Comparison of pretreatment methods for total lipids extraction from mixed microalgae

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ABSTRACT

Cell disruption can increase the extraction efficiency of total lipids from microalgae for further conversion to biodiesel. Four different pretreatment methods were tested on mixed cultures of microalgae harvested in a stabilization pond system treating sewage: ultrasonication (US), microwaving (MW), autoclave (AC) and electroflotation by alternating current (EFAC). The best results in terms of total lipid yield were: MW (33.7 ± 5.3%), followed by EFAC (24.8 ± 7.1%), AC (15.4 ± 2.3%), and US (13.3 ± 3.0%). However, when both efficiency and costs are considered, EFAC gave the best result and can be an excellent option for simultaneous microalgae harvesting and cell disruption.

1. Introduction

The depletion of oil reserves, the resulting increase in fossil fuel prices and the international awareness of the environmental impact of greenhouse gas emissions have contributed to worldwide interest in developing sustainable alternative energy sources to meet current and future demands [1–3]. Some of the promising alternative energy sources include biohydrogen, biodiesel, bioethanol and biomethane produced from various raw materials, including microalgae biomass [4–6]. Production of biodiesel from microalgae biomass presents some advantages: it can be produced year round (depending on climate and solar radiation); can grow at very high rates; can utilize of a wide variety of water sources (fresh, brackish, seawater and wastewater); and can be produced on marginal land, hence not competing for arable land used to produce food and the production of valuable co-products [6–9].

The lipid content of microalgal cells can vary from 2 to 77% depending on species and environmental/growth conditions [4,10,11]. Lipids extracted from microalgae may be converted into biodiesel with low energy consumption [9] by transesterification, the most common method [12]. Biodiesel can be used in conventional diesel engines without modification and can be mixed with petroleum diesel in any proportion, making it the preferred final product from microalgae [9,13].

Cultivation of microalgae in closed and controlled systems (photobioreactors) usually presents high costs and may not be economically feasible [14,15]. Microalgae cultivation in open systems such as waste stabilization ponds can be achieved at very low costs by using CO_2, water and nutrients readily available in sewerage [15]. Waste stabilization ponds can be a cheap option for microalgal biomass and biodiesel production [9,15]. However, their applicability depends on the local climate and land availability. In Brazil, a country with a tropical climate and vast land availability at a low cost, these systems are widely used [16]. For example, in the state of Ceará, located in the Northeast of Brazil, near the equator line, there are approximately 85 waste stabilization pond systems, which correspond to more than 80% of the sewage treatment systems in operation in the state. However, biomass separation and reuse are not widely used in pond systems and microalgae are often discharged directly into water bodies, representing potential hazards to the environment and to human health [17]. In order to effectively couple pollution control with biodiesel production from microalgae it is necessary to separate the biomass from the treated

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sewage and subsequently carry out biomass drying, cell wall disruption, lipid extraction and transesterification [18]. Biomass recovery has often been achieved by coagulation/flocculation, filtration, dissolved air flotation and centrifugation, and all these processes often present very high costs [2,19].

Oil and other intracellular products can be difficult to extract from wet biomass. Dewatering of microalgae is commonly performed to increase the shelf life of biomass feedstock in order to produce biofuels and to enhance the range of possible solvents. Some drying methods used include spray drying, drum drying, freeze-drying and sun drying [7].

Cell disruption can be used to enhance the release of lipids from algae and improving the access of the extracting solvent to fatty acids [4]. Ultrasonication, microwave, bead mill and autoclave pretreatments are the commonly used methods to promote vegetable cell disruption and have been tested in pure cultures of microalgae cells [20–23]. There are no reports in the literature of using electrolytic processes to promote microalgae cell disruption.

This work aims to compare microwave, ultrasonication and autoclave methods on the disruption of microalgae cells harvested from waste stabilization ponds as well as to propose an electroflotation by alternating current as a methodology which combine harvesting and cell disruption steps on this biomass.

2. Materials and methods

2.1. Microalgae harvesting and identification

Microalgae biomass was harvested from a stabilization pond system treating sewage composed of a mechanically aerated facultative pond, followed by a secondary facultative pond and two maturation ponds in series. The ponds were located in the city of Fortaleza, state of Ceará, Brazil. Samples were collected in the last maturation pond, near the outlet, using a plankton nylon net with a 20 μm opening. Although some microalgae have smaller sizes than the openings used, the filtered volume enables the collection of many species at random, allowing the study of phytoplankton diversity.

Samples were placed in a sterile glass container and fixed with *Transeau* solution (6 parts of water, 3 parts of 95% ethanol and 1 part of formaldehyde) in a 1:1 ratio (effluent:*Transeau*).

Identification of the dominant genus was carried out in quintuplicate with a trinocular optical microscope (L-1000T, Bioval, Brazil) and taxonomic identification guides [24,25].

The *Transeau* solution was used only to preserve the morphology of microalgal specimens and these samples were used exclusively in the microalgae identification assays.

2.2. Pretreatment methods

Biomass was harvested from the maturation pond and concentrated to 4 $g \cdot L^{-1}$ using a 20-μm nylon plankton net. Drying was carried out by lyophilization (Liotop, L202, Brazil).

To perform the pretreatments in the modified Bligh and Dyer method, experiments were divided into two blocks, according to Fig. 1. According to Halim et al. (2012) [4], the microalgae cell disruption process step may take place before or after biomass drying, as some methods require a certain amount of water in the biomass to be successful, while others are more efficient with dry biomass. For the group of dried samples, lyophilization was

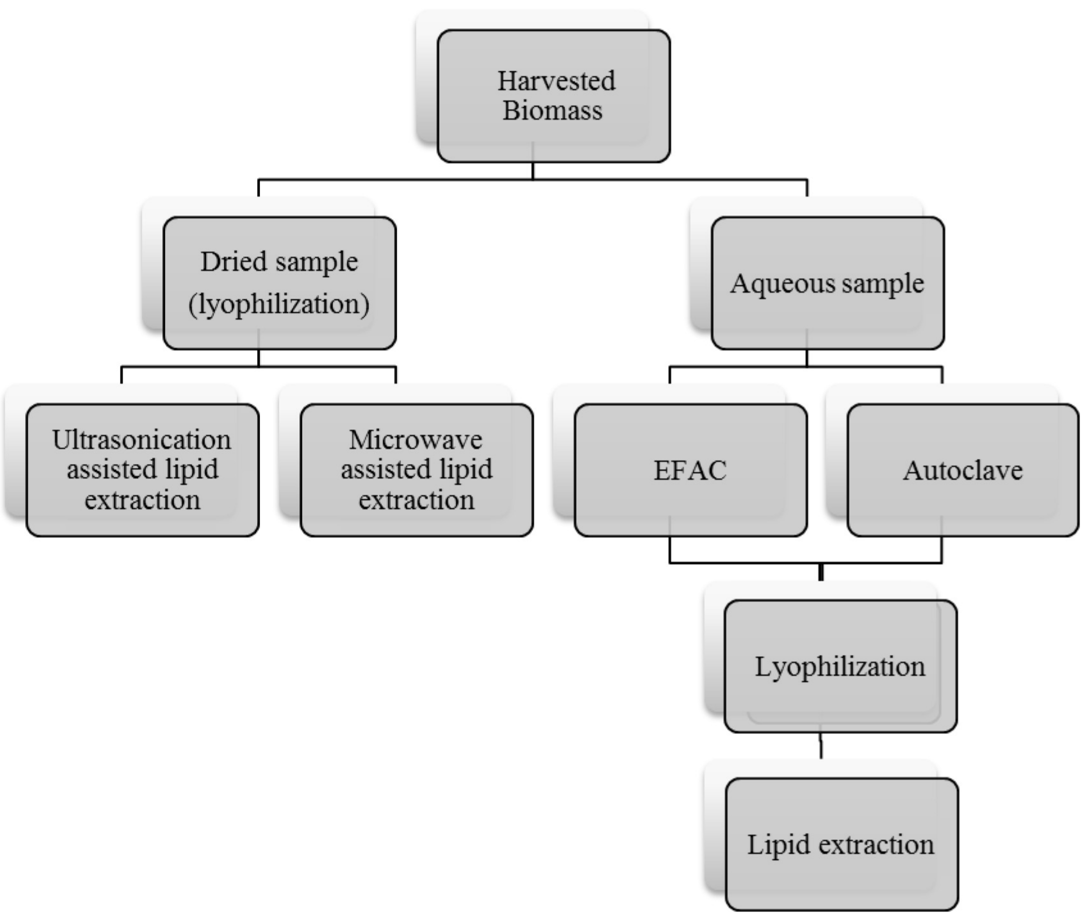

Fig. 1. Experimental flow diagram.