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ORIGINAL RESEARCH ARTICLE

Lipid content and fatty acid composition of Mediterranean macro-algae as dynamic factors for biodiesel production☆

Dahlia M. El Maghraby, Eman M. Fakhry*

Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

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Summary Using the total lipid contents and fatty acid profiles, the marine macro-algae *Jania rubens* (Rhodophyceae), *Ulva linza* (Chlorophyceae) and *Padina pavonica* (Phaeophyceae) were evaluated for biodiesel production during the spring, summer and autumn. Seawater parameters such as pH, salinity and temperature were measured. The total lipid content varied from 1.56% (*J. rubens*) to 4.14% (*U. linza*) of dry weight, with the highest values occurring in spring. The fatty acid methyl ester profiles were analysed using gas chromatography. The highest percentage of total fatty acids was recorded in *P. pavonica*, with 6.2% in autumn, whereas the lowest was in *J. rubens*, with 68.6% in summer. The relative amount of saturated to unsaturated fatty acids was significantly higher in *P. pavonica* than in the other macro-algae. Seasonal variations in pH, salinity and temperature had no significant effect on the total lipid and fatty acid contents. Principal component analysis grouped brown and green algae together, whereas red alga grouped out. Furthermore, methyl ester profiles indicate that brown and green seaweeds are preferred, followed by red seaweeds, which appears to have little potential for oil-based products. Therefore, these seaweeds are not targets for biodiesel production.

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* Corresponding author at: Department of Botany and Microbiology, Faculty of Science, Alexandria University, 21511 Alexandria, Egypt. Tel.: +20 1220338104; fax: +20 1006231928.
E-mail address: emfakhr@hotmail.com (E.M. Fakhry).
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1. Introduction

Energy is the most essential requirement for human survival. The complete dependence of mankind on fossil fuels may cause a major shortage in the future. Biofuels made from bio-products reduce the need for petroleum oil and offer considerable benefits for sustainability and reduce pollutant and greenhouse gas emissions (Hansen et al., 2009). Of the biofuels, biodiesel is highly promising. The main

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advantages of using biodiesel are that it is renewable, non-toxic, and biodegradable and can be used without modifying existing engines because it possesses similar properties to diesel fuel and produces less harmful gas emissions, such as sulphur oxide (Agarwal, 2007; Hansen et al., 2009). Biodiesel reduces net carbon dioxide emissions by 78% on a lifecycle basis compared to conventional diesel fuel (Gunvachai et al., 2007). Biodiesel consists of fatty acid methyl esters prepared from triglycerides by transesterification with methanol (Gerpen, 2005). During transesterification, the glycerides in fats or oils react with an alcohol in the presence of a catalyst (Banerjee and Chakraborty, 2009; Enweremadu and Mbarawa, 2009; Zabeti et al., 2009) and are converted into monoesters, yielding free glycerol as a by-product.

Biodiesel can be produced from different feedstocks. Each originating oil or fat is characterised by a different fatty acid composition, and the final ester properties differ significantly based on the feedstock, alcohol used in the esterification and the exact chemical process followed (Knothe, 2005). Recently, much research has focused on the production of biodiesel from non-edible sources, such as *Jatropha* and algae (Komninos and Rakopoulos, 2012; Pinzi et al., 2009). There has been increased interest in the marine production of biofuels derived from macro-algae (seaweed) and microalgae (single cell plants) (Singh and Cu, 2010; Williams and Laurens, 2010). Biodiesels derived from micro- and macro-algae have become known as one of the most encouraged unusual sources of lipids for use in biodiesel production because they are renewable in nature, can be produced on a large scale and are environmentally friendly (Carvalho et al., 2011). Macro-algae are multicellular, macroscopic algae, which are abundant in coastal environments, primarily in near-shore coastal waters with suitable substrates for attachment (Murphy et al., 2013). Seaweed is one of the best growing plants worldwide. It does not require irrigation or fertilisers, and it does not require arable land. A previous study reported that seaweed species have total lipid contents of less than 5% dry weight. By contrast, there are many species with total lipid contents greater than 10% dry weight, and these are interesting candidates for oil-based products (Gosch et al., 2012). Because fossil fuel prices are likely to increase and because macro-algal production costs will likely decrease as production is expanded, it is prudent to develop methods to obtain significant quantities of biofuel from marine biomass to meet European energy needs and climate change targets (Hughes et al., 2012).

The objective of this study was to assess the potential of *Jania rubens* (Rhodophyceae), *Ulva linza* (Chlorophyceae) and *Padina pavonica* (Phaeophyceae) that inhabit the Abu Qir Bay coast, Alexandria, Egypt, for biodiesel production. The quantification of total lipid content and identification of fatty acid profiles for these species was performed. The total lipid content in relation to the fatty acid content for the macro-algae during different seasons was estimated. Additionally, the variation in the fatty acid profiles of these species between and within seasons was determined to identify the most favourable conditions to produce seaweeds with high lipid contents and optimal fatty acid profiles.

2. Material and methods

2.1. Area of study and sampling

Seaweed species belonging to different classes, including *J. rubens* (Rhodophyceae), *U. linza* (Chlorophyceae) and *P. pavonica* (Phaeophyceae), were collected seasonally through the spring, summer and autumn from Abu Qir Bay. Winter showed a quantitative reduction in algal flora. The samples were identified based on the morphological features using the herbarium and the identification scheme of the late Prof. A. H. Nasr (Botany Department, Faculty of Science, Alexandria University). Abu Qir Bay is a semi-circular bay along the Egyptian Mediterranean seashore, approximately 30 km east of Alexandria, with an average water depth of 11 m and an area of approximately 360 km^2. This bay is characterised by the presence of abundant rocks with several petite and fine holes that are excellent domains for the attachment of algae.

Algal thalli were placed separately in plastic bags, stored in an icebox and transported to the laboratory. They were washed thoroughly with tap water to remove any impurities. The water was drained off, and the algae were spread on filter paper to remove the excess water. The weighed samples were dried until they reached a constant weight. These shade-dried samples were ground into a fine powder. The original weight decreased approximately 10 times. Therefore, 1 kg wet seaweed will weigh 100 g (10 to 1 wet to dry ratio).

2.2. Water measurements

During three successive seasons, namely spring, summer and autumn, seawater samples were collected using clean glass bottles for the field measurements. The pH of the water samples was measured using a digital pocket pH metre (FG-211). The salinity was measured with an inductive salinometer (model MI-150). The water temperature was measured with a mercury thermometer graduated to 0.1°C.

2.3. Lipid extraction and fatty acid analysis

The seaweed samples were analysed in triplicate for their proximate lipid content using the Bligh and Dyer (1959) method. Samples were homogenised with a 1:2 mixture of chloroform and methanol and incubated in the dark overnight. The residues were extracted 2—3 times with a small amount of chloroform and methanol. The chloroform layer was removed with a separating funnel and then vaporised in an evaporator. The lipid content was calculated by weighing the residues and was expressed as a percentage of dry weight. The fatty acids were converted to methyl esters using the method of Christie (1998). The samples were esterified in 1% sulphuric acid in absolute methanol and extracted with hexane to separate the layers. The hexane layer was washed with water containing potassium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated using a rotary evaporator. The fatty acid methyl esters (FAMEs) were analysed on a Shimadzu gas-liquid chromatographer equipped with a flame ionisation detector with a packing column with Hp-5 material. The carrier gas was nitrogen, and the short speed was 5 mm/min.