Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–7, 2017

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Potential of macroalgae for biodiesel production: Screening and evaluation studies

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Received 31 May 2017; accepted 17 August 2017
Available online xxx

Nowadays, biofuel production is a fast expanding industry and is facing a growing dilemma about a feedstock source capable of keeping up with demand. Recently, macroalgae have been attracting a wide attention as a source for biofuel. In the present study, ten macroalgae were collected and screened as biodiesel feedstocks. As a result of their high biomass production and relatively high lipid content, *Ulva lactuca*, *Padina boryana* and *Ulva intestinalis* showed the highest significant lipids and fatty acid methyl esters (FAMEs) areal productivities among the studied species. Saturated fatty acids (SAFs) showed insignificant differences in the selected species, with noticeably significant higher polyunsaturated fatty acids (PUFAs) content in *U. lactuca* by 4.2 and 3 times, with respect to *P. boryana* and *U. intestinalis*, respectively. The recorded increase in PUFAs was attributed to higher content of C16:4*n*-3, C18:3*n*-3 and C18:4*n*-3. By lipid fractionation, *P. boryana* showed significant higher concentration of neutral lipids (37.7 mg g^{-1} CDW, representing 46.7% of total fatty acids) in comparison to *U. lactuca* and *U. intestinalis*, which showed 16% and 17% lower neutral lipid fractions, respectively. In addition, biodiesel characteristics of the studied macroalgae complied with that of international standards. Furthermore, oil-free residual biomass can be readily converted into fermentable sugars or biogas due to its high carbohydrates content, which adds to the economics of macroalgae as biofuel feedstock. In conclusion, the present study confirmed that macroalgae represent an attractive alternative renewable feedstock for biodiesel and other biofuels.

[**Key words:** Biodiesel production; Wild macroalgae; Seaweeds harvest; Energy conversion; Fatty acid methyl esters]

Continuous growth of human population and industrialization increased the energy demands all over the world. The current consumption of petroleum is at least 105 times faster than nature can create. If the development of the world energy consumption continues, the world will be threatened with an energy crisis, as the worldwide fossil oil reserves will be exhausted in shorter than 30 years (1). In addition to the fact of limited oil resources, extensive use of fossil fuel contributes to increase the atmospheric CO_2 (2). Currently, one-fifth of the global CO_2 emission is due to transportation and trucking (3). Therefore, the green replacement of fossil based energy is the trending strategy that has gained much attention from governments and research sectors all over the world. Biomass-based fuels, or bioenergy, such as bioethanol, biobutanol, biogas and biodiesel, are considered sustainable, renewable and environment friendly alternatives.

Nowadays, the two most abundant and feasible biofuels for large-scale production are bioethanol from corn or sugarcane and biodiesel from oil crops such as soybean, sunflower or palm oils (4). These food crops are widely used as biofuel feedstocks because of well-established farming practices and simple, cheap processes for release of starches, sugars, or oils (5). However, expansion of food crops use for biofuel production has many ethical issues. One possible alternative that has been a focus of extensive research is the use of terrestrial non-food lignocellulosic biomass such as agricultural residues, sawdust, or energy grasses as raw materials for second generation biofuels production (6,7). However, their use is limited due to the high cost of lignin degradation (7,8). Therefore, algae-based bioenergy progressed recklessly in the midst of renewable energy research for countering these issues.

Marine algae have been cited as one of the best non-edible feedstocks for renewable energy applications compared to crops, such as soybean, rapeseed and oil palm. The priority of macroalgae in bioenergy production is due to; (i) higher photon conversion efficiency which enable them to rapidly synthesize biomass (9). Therefore, production yields of algae per unit area are significantly higher than those for terrestrial biomass. (ii) As marine algae have a higher photosynthetic rate, they may have greater potential for CO_2 remediation (10). (iii) Does not necessarily require arable land for growth (11). (iv) Marine algae lack lignin, which is essential for structural support of terrestrial plants (6), and thus can be depolymerized relatively easily as compared to lignocellulosic biomass. (v) Require much less land areas compared to conventional crops (1). Moreover, marine macroalgae have additional preference that they don't compete for freshwater. On the other hand, macroalgae possess plant-like characteristics, making its harvesting more easily

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1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2017.08.020

compared to microalgae. According to a Life Cycle Assessment by Aitken et al. (12), macroalgae can generate a net energy of 11,000 MJ t^{-1} dry algae compared to 9500 MJ t^{-1} relevant to microalgae. Nevertheless, research on bioenergy production from macroalgae is at infancy for economically efficient technological solutions.

There are many macroalgal species with total lipid contents greater than 10% dry weight, and these are interesting candidates for biodiesel production (13). Biodiesel is an excellent substitute for conventional diesel fuel because of being renewable, nontoxic and biodegradable. It consists of fatty acid methyl esters (FAMEs) produced by transesterification of lipids (14). Successful algal biotechnology mainly depends on choosing the right species with relevant properties such as biomass and fatty acid productivities (15). Therefore, selection of strains with high lipid productivity is the key characteristic for successful biodiesel production from macroalgae. In fact, the potential biological resources of marine environments at Saudi Arabia represented by the Red sea and the Arabian Gulf have not been adequately explored and harnessed for bioenergy applications. The objective of the present study was to assess the potential of macroalgae collected from Red sea coast at Jeddah, Saudi Arabia for biodiesel production in terms of lipid and FAMEs productivity. In addition, the characteristics of biodiesel produced from the selected species were compared to that of the international standards.

MATERIALS AND METHODS

Macroalgae collection and preparation Seaweeds were collected manually in March 2014 from different depths ranged between 0.3 and 1.0 m along the Red Sea at Jeddah Corniche, Saudi Arabia between 21°38′55.71″N, 39°6′2.72″E and 21°30′27.13″N, 39°9′44.39″E (Fig. 1). Macroalgae were collected by quadrate technique using 100 × 100 cm steel quadrate (16). Three quadrate samples were taken at each collection site. All algal populations growing within the quadrate were collected carefully and washed with seawater to remove epiphytes, sand and rock debris. After separation of each species, macroalgae were stored in plastic bags and transported to the laboratory under iced conditions. The samples were washed with freshwater to remove salts, and stored at 20 °C until extraction. The algal species were identified based on the schemes reported in the literature (17–19), and saved in the Phycology Laboratory, Biological Science Department, Science Faculty for Girls, King Abdulaziz University, Jeddah, Saudi Arabia. Seaweeds were air dried in the shade at room temperature on absorbent papers. Air dried samples were grounded in an electric mill and stored in stoppered bottles at room temperature. Biomass was determined for each species as gram cellular dry weight (CDW) per square meter (g m^{-2}).

Physicochemical analysis of water Water temperature, pH and salinity were measured in the field during sampling process. Water samples were collected from different collection sites and filtered using membrane filter of 0.45 μm pore size. The filtered seawater samples were kept frozen at −20 °C for later analysis. Water parameters, including dissolved oxygen (DO), biological oxygen demand (BOD), nitrate nitrogen NO_3–N, nitrite nitrogen NO_2–N, ammonia nitrogen NH_4–N and total phosphate (TP), were measured according to the standard methods for examination of water (20).

Estimation of total soluble proteins and carbohydrates A certain weight of dried cells was extracted using 1 N NaOH in a boiling water bath for 2 h as described by Payne and Stewart (21). Protein concentration was determined according to Bradford method (22) using bovine serum albumin as a standard reference. Total carbohydrates were quantitatively determined by the phenol sulfuric acid method described by Kochert (23) using glucose as a standard reference.

Lipid extraction and determination Total lipids were extracted according to Folch et al. (24) in chloroform:methanol, 2:1 in glass homogenizer, and washed with a 0.9% (w/v) NaCl solution in order to remove non-lipid components (pigments and lipoproteins). The pre-weighted glass vials containing the lipid extracts were dried under a stream of argon, then incubated at 80 °C for 30 min, cooled in a desiccator and weighted.

Fractionation of lipids Lipid fractionation of the promising seaweeds was done according to Fakas et al. (25). Macroalgal lipids (approximately 100 mg) were dissolved in 1 mL chloroform, and fractionated by using a glass column (20 mm × 120 mm) containing 1 g silicic acid previously activated by heating overnight at 80 °C. Successive applications of dichloromethane 100 mL, acetone 100 mL and methanol 100 mL, produced fractions containing neutral lipids, polar lipids and phospholipids, respectively. After evaporation of the respective solvent, lipid fractions were stored at −20 °C. Total fatty acids were measured in each fraction.

Fatty acid analysis Total fatty acids contained in lipid extract of the dried macroalgae samples were converted to FAMEs using one-step FAME method described by Komolafe et al. (26). Briefly, 3.3 mL of the methylating mixture composed of methanol:toluene:2,2-dimethoxypropane:sulfuric acid (39:20:5:2 *v*/*v*) was mixed with 1.7 mL of heptanes and then added to 0.2 g of the dried biomass

FIG. 1. Map showing the collection site of macroalgae at Jeddah corniche, Saudi Arabia.