Algal Research 7 (2015) 33–44

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Characterization of five fresh water microalgae with potential for biodiesel production

Ruby Valdez-Ojeda [a,*], Muriel González-Muñoz [a], Roberto Us-Vázquez [a], José Narváez-Zapata [c], Juan Carlos Chavarria-Hernandez [a], Silvia López-Adrián [d], Felipe Barahona-Pérez [a], Tanit Toledano-Thompson [a], Gloria Garduño-Solórzano [e], Rosa María Escobedo-Gracia Medrano [b]

[a] Unidad de Energía Renovable, Centro de Investigación Científica de Yucatán A.C., Mérida, Yucatán, Mexico
[b] Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán A.C., Mérida, Yucatán, Mexico
[c] Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, Mexico
[d] Facultad de Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, Mérida, Yucatán, Mexico
[e] Facultad de Estudios Superiores Iztacala, Universidad Autónoma de Mexico, Tlalnepantla, Edo. de México, Mexico

ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 21 October 2014
Accepted 24 November 2014
Available online 10 December 2014

Keywords:
Scenedesmus
Desmodesmus
ITS-2
Fatty acids
Biodiesel properties
CBC analysis

ABSTRACT

Microalgae biodiesel feedstocks have been investigated by numerous research groups to overcome dependence on fossil fuels. This study describes a detailed characterization of five freshwater microalgae strains of the family Scenedesmaceae, based on cell wall ultrastructure, ITS-2 sequence and secondary structure analysis, as well as the estimation of biodiesel properties from fatty acids produced before and after N-limitation. Characterization permitted the reclassification of SCRE-1 and SCRE-2 strains into the subgenus *Scenedesmus*; DSRE-1 and DSRE-2 strains into the subgenus *Desmodesmus*; and CORE-1 strain into the genus *Coelastrum*. Transesterification of fatty acids of the five strains was performed before and after N-limitation, and seven important biodiesel quality parameters were predicted by applying selected correlations. These parameters were then compared to the corresponding specifications in the American and European biodiesel standards. N-limitation promoted higher yields of biomass (up to 3.5 times) and lipids (up to 4.1 times) in all strains under study. Moreover, it was found that SCRE-2 was the only biodiesel that met all estimated parameters of the more stringent European standard before N-limitation. This was also true for DSRE-1 and DSRE-2 biodiesels after limitation, with the exception of their oxidative stability parameter, whose values met the limit of the American but not the European standard.

1. Introduction

Microalgae (eukaryotes and prokaryotes) have attracted significant attention as renewable energy feedstocks due to their rapid biomass production and great potential to synthesize fatty acids suitable for biodiesel [1,2] compared to oleaginous plants [3]. The oil content of microalgae is usually between 10 and 50% of their dry cell weight, although some microalgae species can reach as high as 80% under certain conditions [4,5].

Species selection is one of the biggest challenges in biofuel production based on microalgae [6–8]. This selection is based principally on the fatty acid profile, which determines the main biodiesel properties, and thus the biodiesel quality. Some of the most important biodiesel properties include density, cetane number, kinematic viscosity, higher heating value, iodine value, cold flow plugging point, and oxidative stability. The density (ρ) of biodiesel is strongly influenced by temperature, and some quality standards have established limits for this property at 15 °C. The cetane number (CN) is a dimensionless descriptor related to the ignition quality of a fuel in a diesel engine. Generally, a higher CN value indicates a better ignition quality of the fuel and vice versa [9]. Kinematic viscosity (ν), on the other hand, affects the atomization of a fuel upon injection into the combustion chamber, and thereby ultimately the formation of engine deposits. The higher the viscosity, the greater the tendency of the fuel to cause such problems [10]. The iodine value (IV) is a measure of total unsaturation of an oil or fat. Biodiesels with high IV are easily oxidized in contact with air [11]. Gross heat of combustion (HG) or higher heating value (HHV) is another fuel property indicating the suitability of fatty compounds as diesel fuel. The presence of oxygen in the ester molecules decreases the heating value of biodiesel by 10 to 13% compared to that of diesel fuel. The HHV of petroleum diesels is around 45 MJ/kg, while that of biodiesels is around 40 MJ/kg. The cold filter plugging point (CFPP) is an estimate of the lowest temperature at which a fuel will give trouble-free flow in a given fuel system. Saturated fatty compounds have significantly higher melting points than unsaturated ones, and in a mixture they crystallize at higher temperature than unsaturated ones [11]. Crystals grow rapidly at low temperature and agglomerate, clogging filters partially or totally.

* Corresponding author.
E-mail address: ruby.valdez@cicy.mx (R. Valdez-Ojeda).

http://dx.doi.org/10.1016/j.algal.2014.11.009
2211-9264/

Countries using UNE-EN 14214 can select one of the two options (moderate or arctic climate) for seasonal classes (summer and winter) and modify this specification based on national meteorological data. The cloud point (CP) is the related property in ASTM D6751 for which an analogous limit is not given, rather a report is required. CFPP values are approximately 4.5 °C lower than CP values [12]. Finally, the oxidative stability (OSI) of biodiesel is an important issue for storage purposes, as the presence of double bonds in fatty esters greatly facilitates oxidation of biodiesel. Autoxidation of unsaturated fatty compounds proceeds at different rates depending on the number and position of double bonds. Relative rates of oxidation are 1 for oleates (methyl, ethyl esters), 41 for linoleates, and 98 for linolenates [10]. The species formed during the oxidation process eventually cause the fuel to deteriorate. Two types of tests are currently used for determining the oxidation stability of biodiesel: the Rancimat test, specified in EN 14214; and the oil stability index (OSI), included in ASTM D6751 [11]. As stated by [10], the Rancimat method is nearly identical to the oil stability index (OSI) method.

Suitable fatty acid profiles are usually associated with specific microalgae subgenera, such as *Scenedesmus* and *Desmodesmus*. In this context, C16–C18 fatty acids were found to account for over 98% of the fatty acid composition of *Scenedesmus obliquus*, indicating that biodiesel from these cells could meet biodiesel standards. Biodiesel quality from *S. obliquus* complied with the ASTM D6751 standard in terms of cetane number (>58), oxidative stability, degree of unsaturation and iodine value (<69) [13]. In another study, the fatty acid profile of *S. abundans*, with C16–C18 accounting for 90.6% of total FAMEs, showed biodiesel properties such as cetane number (52.15), iodine value (94.06 g I_2/100 g) and saponification value (202.02 mg KOH/g) in accordance with the specifications of the Brazilian National Petroleum Agency (ANP255) and the European biodiesel standard (EN 14214) [14].

A detailed review of the literature on fatty acid profiles from a wide range of microalgae species indicates that most of them contain C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 fatty acids [8,15]. For example, Vidyashankar et al. worked with five different strains, including *Scenedesmus* sp. and *Coelastrum asteroidum*, using CO_2 supplementation from 0.03 to 2% (v/v) [16]. These authors reported that the most abundant fatty acid methyl esters (FAMEs) obtained for the five strains at all CO_2 concentrations were C16:0, C18:0, C18:1, C18:2 and C18:3. For *Scenedesmus* sp., the amount of C16:0 + C18:1 was approximately 70% at all CO_2 percentages (v/v) tested, while the combined concentration of C16:0 + C18:1 for *C. asteroidum* amounted to approximately 80%. Reports on fatty acid profiles for microalgae of the subgenera *Desmodesmus*, *Coelastrum* and *Scenedesmus* indicate that the sum of C14:0 + C16:0 + C18:0 + C18:1 + C18:2 fatty acid concentrations usually represents between 80 and 90% of the total fatty acid content [7,13,14,17,18].

Biomass productivity and oil content are also important for screening microalgae species. In the work of Musharraf et al., *Desmodesmus* sp. WC08 showed higher biomass productivity (370 mg l/d) and higher lipid productivity (115.73 mg/l/d) than seven other microalgae species [7]. The main fatty acids synthesized by this microalga (C16–C18) indicated that it is suitable for biodiesel production [19]. In comparison with other microalgae species, such as *Chlorella*, *Haematococcus*, *Ulothrix*, *Chlorococcum*, *Rivularia* and *Scytonema*, the species *Scenedesmus* registered the highest lipid content (27.4% w/w) [20]. Similarly, in comparison with *Coelastrum* and *Ankistrodesmus*, *Scenedesmus* showed a high lipid content of 17.83% w/w [16]. Based on those and other reports, numerous authors have selected species of this genus mainly due to their potential for producing biofuel [21–25].

Scenedesmus belongs to the subfamily Scenedesmoideae from the family Scenedesmaceae, which includes other subgenera such as *Desmodesmus* and *Acutodesmus*, among others. Different species and strains of *Scenedesmus* and *Desmodesmus* have been studied and tested under different conditions in relation to their fatty acid production: e.g., higher light intensity to induce neutral as opposed to polar lipid synthesis [26]; nitrogen and phosphorous limitation to increase fatty acid synthesis [25]; and CO_2 fixation and light to enhanced production of fatty acids [27,28]. However, only a few microalgae cultures have been thoroughly examined in terms of cell structure and genetic classification in order to facilitate the identification of microalgae species with suitable fatty acid profiles for biodiesel. The study carried out by Kaur et al. [22] included the characterization of strains of *Scenedesmus* and *Desmodesmus* species based on ultrastructure and genetic analysis, as well as fatty acid determination to screen them for suitable fatty acid composition according to the ATSM D6751 (US) and UNE-EN 14214 (EU) international biodiesel standards.

Biodiesel property estimation for microalgae screening can be performed with good accuracy by applying different correlations reported in the literature. The main reason for this is that biodiesel is a mixture of a limited number of compounds (usually less than 10) belonging to the same family. Thus, for certain properties like density or cetane number, an ideal mixing rule can be good enough to calculate the mixture properties based on those of the individual compounds [29]. This methodology is especially helpful when the required analytical equipment or large enough amounts of oils are not available, but it is possible to determine the fatty acid composition. The presence of impurities (compounds other than fatty acid alkyl esters) will surely affect property estimation, but they are usually present in amounts lower than 5% by weight [30,31], so the interaction parameter considered in non-ideal mixing rules, such as in the Grunberg–Nissan equation [32], can be neglected.

According to the above, many authors have estimated and reported biodiesel properties by using different correlations available in the literature [29,33–35]. For the properties to be calculated, it is necessary to identify the fatty acid alkyl esters present in the mixture and the percentage or fractional amount of each one. The triglyceride source from which the biodiesel was obtained (i.e., vegetable oil, animal fat, microalgae oil, etc.) does not matter. There are many reports in the literature in which biodiesel quality has been estimated theoretically with the use of correlations. The properties most commonly estimated include the cetane number, viscosity, higher heating value, density, and cold flow properties such as the cloud point or the cold filter plugging point. The reported works include studies on the quality of biodiesel from microalgal oil [7,13–15,17,31,36].

Among the microalgae species that have been studied, subgenera *Desmodesmus* and *Scenedesmus* [37] are two strains showing extreme plasticity. Classification based on metabolic capability and morphology using optical microscopy has been found to be insufficient for clearly distinguishing between them [38,39]. The internal transcribed spacer-2 (ITS-2) region has been found to be useful as a molecular marker for resolving the evolutionary relationships among these closely related green algae, such as the subgenera *Desmodesmus* and *Scenedesmus* [40,41].

The main aim of this work was to investigate the potential of five strains for use as a source of microalgal oil for biodiesel production. This was performed by analyzing their cell wall ultrastructure, ITS-2 secondary structure and fatty acid profile. In order to investigate the quality of biodiesel from the five strains, the following seven important biodiesel properties were predicted by using selected correlations taken from the literature: density at 15 °C, cetane number, kinematic viscosity at 40 °C, higher heating value, iodine value, cold flow plugging point, and oxidative stability.

2. Materials and methods

2.1. Microalgal strains

Five strains from the subfamily Scenedesmoideae were studied. All of them were acquired from a national or international culture collection. Strain SCX2 (*Scenedesmus* sp.), labeled DRES-1 in this study, and strain SCX1 (*S. obliquus*), labeled SCRE-1, were acquired from the