Bioenerg. Res. (2014) 7:1002–1013
DOI 10.1007/s12155-014-9440-x

Microalgae Versus Land Crops as Feedstock for Biodiesel: Productivity, Quality, and Standard Compliance

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Published online: 19 March 2014

Abstract Despite certain environmental advantages over fossil diesel, land crop-derived biodiesels may not satisfy the increasing worldwide demand for transportation fuels. As an abundant photosynthesizer, algae could be an adequate surrogate for biodiesel production. Nevertheless, high production costs, scarce selected species, and inaccurate assumptions about production yields represent industrial uncertainties. In this study, a reliable approach to analyzing algal biodiesel production has been developed based on species-to-species variations in oil productivity and quality. This approach compares biodiesels from Chlorophyta strains with land crop feedstock according to (i) potential yields, (ii) oil quality, and (iii) compliance with biodiesel quality standards. Algal yields were assessed by (i) extrapolating the strain-specific laboratory results to commercial-scale growth systems; (ii) converting volumetric to areal biomass productivity; and (iii) estimating oil yields for each strain, as the product of their projected areal biomass productivity for each growth system, and the oil percentage in biomass as determined in the laboratory. Biodiesel fuel properties were estimated by using fatty acid methyl ester profile predictive models. The Chlorophyta strains in this study provided annual oil yields that were generally higher than those of land crops by one order of magnitude. Six strains yielding more than 40 mg oil l^{-1} day^{-1} were identified as adequate for sustaining biodiesel production. Trebouxiophyceae algae were the most productive. Critical biodiesel parameters from both feedstock types suggest that most microalgae-derived biodiesels meet international fuel quality standards with better values than those of land crops. Because some of the highly productive feedstock does not simultaneously meet all the standards for a high quality biodiesel, optimization solutions are discussed.

Keywords Microalgae oil yields · Microalgae-based biodiesel · Biodiesel productivity · Biodiesel fuel quality · Biodiesel feedstock selection

Abbreviations

ANP	Brazilian National Agency for Petroleum, Natural Gas and Biofuels
ASTM	American Society for Testing and Materials
CFPP	Cold filter plugging point
CN	Cetane number
DU	Degree of unsaturation
FA	Fatty acids
FAME	Fatty acid methyl esters
GC-FID	Gas chromatograph with a flame ionization detector
GHG	Greenhouse gases
IV	Iodine value

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L_c	Total lipid content
LCSF	Long-chain saturated factor
L_p	Volumetric lipid productivity
MJ	Megajoule
MUFA	Monounsaturated fatty acids
OD	Optical density
ORP	Open raceway pond
OS	Oxidative stability
PBR	Photobioreactor
P_v	Volumetric biomass productivity
P_a	Areal biomass productivity
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
SV	Saponification value
TAG	Triglycerides

Introduction

Biodiesel is currently used to replace part of the diesel consumed by the transport sector. It provides environmental gains because of its higher degree of biodegradability and lower toxic emissions [1]. The consumption of a 20 % biodiesel/diesel blend, for instance, can reduce CO and CO_2 net emissions by 78 and 16 %, respectively [2]. This characteristic could help to offset the increasing global demand for diesel. In recent years, biodiesel production has been increasing in many countries, and based on previous estimates, Gouveia and Oliveira [3] projected that annual worldwide production may reach 168 billion liters by 2016, representing an approximate market value of US $139.6 billion.

One negative aspect of biodiesel is that it is predominantly produced from land crops. It has been suggested that such crops cannot realistically support additional increases in production [3, 4]. The requirement for large swaths of arable land and fertilizers would offset the expected benefits [4, 5]. Thus, the success of using biodiesel as an alternative to diesel depends on actions that lead to the following: (i) conserving sensitive ecosystems and biodiversity; (ii) avoiding resource competition with food production, such as soil and water; (iii) prospecting new feedstock; (iv) developing technological expertise for processing multi-feedstock sources at lower costs; (v) using high production yield systems in association with industrial waste as sources of nutrients; and (vi) selecting and modeling biodiesel production processes to meet regulatory standards. Algal-based biodiesel production systems have been suggested because these systems address all of these issues and also alleviate greenhouse gas emissions more effectively [4, 6] than land crop-derived systems. Microalgae are promoted as having rapid growth and higher lipid yields compared with oilseed crops [3, 4, 6]. The complete energy conversion of microalgae biomass into biodiesel is also more advantageous than the use of land crop sources as feedstock [6, 7]. Nevertheless, a robust, large-scale, algal-based biofuel industry is still not economically feasible. Although new production strategies are being developed, cost-effective methods of harvesting and dewatering algal biomass still represent economic bottlenecks [3, 4]. Additionally, sound knowledge of algal productivity for biodiesel production is limited to a few species [1, 7]. Scaling up from average experimental yields to commercial production projections from diverse microalgae strains and diverse growth locations has led to erroneous estimates [8]. Strain-specific data on growth rates, daily biomass, and oil productivity are important for planning commercial production [4, 6]. Consequently, this paper estimates the potential for using Chlorophyta in large-scale production systems based on extrapolations from small-scale laboratory results by focusing on strain-specific data regarding biomass and oil productivity. The scaling limitation can be more critical if oil yields are used as the only comparative criterion for biodiesel production. Thus, a comprehensive and comparative system considering both oil yield and quality must be established to evaluate algal biodiesel production.

Most of the available data on biodiesel quality comes from land crop feedstock [9–11]. Triacylglycerols (TAG), which range from 11 to 80 % (*w*/*w*) in algal oil [2, 14], are the main components for biodiesel production [9, 15]. Biodiesel quality is defined by the fatty acids that compose the TAG molecules [10, 12, 13]; however, literature comparing algal oil quality is scarce [12, 13]. Different algal species have different fatty acid compositions, which leads to biodiesels of differing fuel quality. The main parameters that determine biodiesel fuel quality are the cetane number (CN), which estimates the ignition delay and combustion performance; the iodine value (IV), which represents the total unsaturation within a mixture of fatty acid methyl esters (FAME); and the saponification value (SV), which is a measure of the average molecular weight of all the FA present in the oil [9–11]. The cold filter plugging point (CFPP) specifies the temperature at which biodiesel will clog filters and fuel lines [11]. The CFPP is based on the long-chain saturation factor (LCSF), which represents the impacts of FA chain saturation and length on the fuel cold flow properties [9–11]. Biodiesels with higher CFPP values are more likely to clog filters and fuel lines at low temperatures than biodiesels with lower CFPP values. The oxidation stability (OS) estimates the biodiesel's susceptibility to deterioration and is primarily related to the double-bond content of the component FAME molecules. The higher the polyunsaturated methyl ester content, the higher the biodiesel oxidation potential [11]. This factor can be predicted on the basis of the degree of unsaturation (DU) in the FAME chains.

Cultivation methods are known to alter fatty acid composition significantly in microalgae oils [10, 13]. Nevertheless, because of their shorter reproductive cycle relative to land

crops, algal oil yield and quality can be more easily modified and optimized [12, 13]. Therefore, biodiesel quality estimates based on FA microalgae profiles, growth conditions, and extraction techniques [13] could facilitate process optimization and reduce production costs. As discussed above, it is not only important to define the algal productivity potential but also to estimate the final biodiesel quality [10, 12, 13]. The interaction of these two aspects is the focus of this research paper. The objectives are to compare the productivity and quality of biodiesels generated from land crops and microalgae cultivated in a variety of systems and to provide a useful tool for production planning.

Methods

Microalgae Growth Kinetics and Productivity

Seven local Chlorophyceae microalgae strains and three species of Trebouxiophyceae that were identified and maintained by LABIOMAR (IBL-Microalgae Collection) at the Federal University of Bahia, Brazil, were tested in this research project, namely *Ankistrodesmus falcatus* (IBL-C113), *Ankistrodesmus fusiformis* (IBL-C111), *Chlamydomonas* sp. (IBL-C108), *Chlamydocapsa bacillus* (IBL-C103), *Coelastrum microporum* (IBL-C119), *Desmodesmus brasiliensis* (IBL-C106), *Scenedesmus obliquus* (IBL-C110), *Chlorella vulgaris* (IBL-C105), *Botryococcus braunii* (IBL-C117), and *Botryococcus terribilis* (IBL-C115). The trials were carried out in triplicate in Erlenmeyer flasks containing 600 ml of standardized medium (modified CHU 13), which was a nutrient-replete medium [16], and a 10 % volume of algal inoculums in the exponential growth phase was added. The flasks were kept under constant temperature and agitation (25±2 °C and 90 rpm, respectively); the aeration rate was 0.50 vvm (volume gas per volume broth per minute) of atmospheric air enriched with 2 % CO_2. Cells were incubated at a neutral pH range (6.8±0.8), and light (170 μE m^{-2} s^{-1}) was provided at a photoperiod of 12:12 h light and dark cycles. Growth was monitored every 48 h by using a hemocytometer cell counter and optical density (OD) measurements. A Helios Epsilon UNICAM spectrophotometer was used to determine the OD at 680 nm. The cells per milliliter and/or OD_{680} measurements from the triplicate cultures were plotted against time and used to estimate growth kinetics. For all the strains, the standard deviations in triplicate culture data were within an acceptable range (below 5 % of the mean). The growth kinetics were monitored through growth curves and adjusted to the Boltzmann sigmoid model using Origin version 7 software (Origin Lab Data Analysis and Graphing Software), which can also test model validity ($p \geq 0.05$).

Kinetic parameters such as the specific growth rate [μ] and volumetric biomass productivity (g l^{-1} day^{-1}) were calculated based on the exponential growth phase, according to Nascimento et al. [12]. The cultures were aborted at the stationary growth phase just after an evident decline in growth, which indicated that reproduction was being inhibited by a limiting factor, such as nitrogen. This decline happened at different cultivation times according to the strain-specific growth rates (Table 1). As reported in the literature, neutral lipid production increases [17] until the end of the stationary growth phase and net biomass growth is zero. The bulk of these neutral lipids are generally triacylglycerols (TAG), which can represent up to 80 % (*w*/*w*) of the algal oil during the stationary growth phase [14].

Cells were harvested from the culture samples using a centrifuge at 4 °C for 5 min at 5,000*g* (Sorvall Ultracentrifuge, Evolution RC); the supernatant was discarded and the pellets were washed with distilled water and freeze-dried, and the gravimetrically measured biomass dry weight was used to determine the biomass concentration (g l^{-1}); the lipid content (L_c) was determined as the percentage of lipids in dry biomass after oil extraction by the chloroform/methanol approach, as previously reported [12]. Lipid productivity (mg l^{-1} day^{-1}) was calculated by multiplying the lipid content value by the volumetric biomass productivity (P_v) determined for each microalgae species. P_v values (g l^{-1} day^{-1}) obtained in the laboratory were calculated as the product of the specific growth rate (μ) and biomass concentration (g l^{-1}), and they were then converted and reported on the basis of the surface area (P_a) in tons per hectare per year (Table 1) by using Eq. 1 [17].

$$P_v = P_a/D \times 1,000 \quad (1)$$

To express differences among species regarding the kinetic growth response to the same experimental conditions with this equation, the biomass concentration (X_{max}) for each strain (Table 1) was used instead of an average value for the microalgae taxonomic group, as previously stated [17]. In lab experiments, a depth (D) of 0.10 m was used for all algal reactors, which had the same medium volume, reactor geometry, and light trajectory. The areal biomass results (g m^{-2} day^{-1}) were then converted to tons per hectare per year for 90 % of the 365 days in a year, with an assumed reduction of 10 % for bioreactor maintenance and cleaning [6].

A comparison between algal and land crop feedstock productivity was assessed by three different approaches. In the first approach (laboratory production), the areal biomass productivity (tons ha^{-1} $year^{-1}$) was calculated for ten Chlorophyta species by using the kinetic parameters as described above. The second and third approaches cross-checked these laboratory data with previously obtained data by using the commercial-scale photobioreactor (PBR) and open raceway pond (ORP) growth systems, and the projected biomass productivity was estimated by considering 48 and 25 g m^{-2} day^{-1}

Table 1 Land crop and microalgae production yields

Microalgae	Exponential growth phase time[a] (days)	Specific growth rate[a] [μ] (day^{-1})	Biomass concentration[a] (X_{max}) (g l^{-1})	Volumetric biomass productivity[a,b] (g l^{-1} day^{-1}±SD)	Lipid content[a] (% dwt±SD)	Lipid productivity[a] (mg l^{-1} day^{-1})	LAB biomass yield[c] (tons ha^{-1} $year^{-1}$)
C. vulgaris	2.0, r^2=0.9943	0.529	1.040	0.55±0.018a	28.1±2.3	154.5	180.7
A. falcatus	1.8, r^2=0.9995	0.568	0.598	0.34±0.010b	16.5±0.4	56.1	111.7
C. bacillus	1.8, r^2=0.9986	0.554	0.577	0.32±0.014b	13.5±0.7	43.3	105.1
B. braunii	11.8, r^2=0.9974	0.144	1.740	0.25±0.012c	45.0±3.6	112.4	82.1
Chlamydomonas sp.	2.7, r^2=0.9928	0.299	0.802	0.24±0.009c	15.1±1.0	36.1	78.8
A. fusiformis	1.6, r^2=0.9998	0.391	0.613	0.24±0.006c	20.7±2.1	49.6	78.8
B. terribilis	12.0, r^2=0.9976	0.129	1.550	0.20±0.010d	49.0±1.5	98.0	65.7
S. obliquus	3.8, r^2=0.9989	0.208	0.769	0.16±0.002e	16.7±1.4	26.8	52.6
D. brasiliensis	1.7, r^2=0.9995	0.280	0.464	0.13±0.004f	18.0±0.4	23.4	42.7
C. microporum	5.9, r^2=0.9959	0.134	0.821	0.11±0.003f	20.5±1.0	22.6	36.1

Microalgae	ORP biomass yield[c] (tons ha^{-1} $year^{-1}$)	PBR biomass yield[c] (tons ha^{-1} $year^{-1}$)	LAB oil yield[d] (m^3 ha^{-1} $year^{-1}$)	ORP oil yield[d] (m^3 ha^{-1} $year^{-1}$)	PBR oil yield[d] (m^3 ha^{-1} $year^{-1}$)	Land crops feedstock	Oil yield (m^3 ha^{-1} $year^{-1}$)
C. vulgaris	82.0	158.0	50.8	23.0	44.4	Peanut [18]	1.1
A. falcatus	50.7	97.7	18.4	8.4	16.1	Rapeseed [22]	1.2
C. bacillus	47.7	91.9	14.2	6.4	12.4	Sunflower [15]	0.9
B. braunii	37.3	71.8	37.0	16.8	32.3	Cottonseed [23]	0.3
Chlamydomonas sp.	35.8	68.9	11.9	5.4	10.4	Palm [15]	6.0
A. fusiformis	35.8	68.9	16.3	7.4	14.3	Soybean [15]	0.4
B. terribilis	29.8	57.5	32.2	14.6	28.1	Coconut [15]	2.7
S. obliquus	23.9	46.0	8.8	4.0	7.7	Olive [19]	1.2
D. brasiliensis	19.4	37.3	7.7	3.5	6.7	Corn [21]	0.2
C. microporum	16.4	31.6	7.4	3.4	6.5	Grape seed [20]	1.1

[a] Laboratory data. In the first column, r^2 indicates how the growth curves fit the Boltzmann sigmoid model (Origin Lab Data Analysis and Graphing Software®)

[b] Means having different lowercase letters (a–f) are significantly different (p 0.05) by Tukey-Kramer post test

[c] Projections from laboratory to commercial-scale production

[d] Calculated as the product of lipid content (% in biomass) and the projected biomass yields for the diverse growth systems

as reference values, respectively, which were calculated according to the PBR geometry and ORP depth [6]. For each species and growth system, the oil yields were the resulting products of the calculated biomass productivity and their respective lab-determined oil contents. These results were then compared with land crops that were reported in the literature [18–24].

Microalgae Fatty Acid Profiles and Biodiesel Fuel Property Estimates

The approaches used for lipid extraction and transesterification into FAME are described by Nascimento et al. [12]. To determine the FA profile of each strain (Table 2), the analyses were applied to the transesterified oil, which was extracted from biomass in mixed triplicate samples. Lipid extraction and transesterification were performed by the methanol/chloroform method and BF3 catalyst, respectively. FAME separation was performed by using a gas chromatograph (Varian 3800) equipped with a flame ionization detector (GC-FID) and an Elite-WAX fused silica capillary column (30 m×0.32 mm× 0.25 mm). The potential biodiesel quality was estimated by using individual FAME profiling for each algal strain, as previously described [12, 25–27]. The estimates were based on correlative models involving empirical equations, the accuracy and predictive capacity of which have been tested in reference to vegetable and microalgae oils, demonstrating that the molecular structure of fatty acids directly affects the quality of the resulting biodiesel [25–27].

The CN estimate model (Eq. 2) involved two independent variables, namely the chain length and degree of unsaturation of each component ester [26]. The chain length was expressed by the saponification (SV) that was inversely related to the esters' molecular weight, using Eq. 3, and the degree of unsaturation was expressed by the iodine value (IV) by using Eq. 4 [26].

$$\mathrm{CN} = 46.3 + (5.458/\mathrm{SV}) - (0.225 \times \mathrm{IV}) \quad (2)$$

$$\mathrm{SV} = \sum(560 \times N)/M \quad (3)$$

$$\mathrm{IV} = \sum(254 \times D \times N)/M \quad (4)$$

where D=double bonds, M=molecular mass, and N=percentage of each FAME.

The degree of unsaturation (DU) was calculated using Eq. 5 by considering the amount of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) present in the algal oil in weight percentage [27].

$$\mathrm{DU} = \mathrm{MUFA} + (2 \times \mathrm{PUFA}) \quad (5)$$

The cold filter plugging point (CFPP) was calculated by using Eq. 6 in correlation with the LCSF, as estimated by using Eq. 7. The LCSF influences the CFPP by weighing up the values of the longer FA chains (weight percentages) to reproduce their impacts on fuel cold flow properties [25, 27].

$$\mathrm{CFPP} = (3.1417 \times \mathrm{LCSF}) - 16.477 \quad (6)$$

$$\mathrm{LCSF} = (0.1 \times \mathrm{C16}) + (0.5 \times \mathrm{C18}) + (1 \times \mathrm{C20}) + (1.5 \times \mathrm{C22}) + (2 \times \mathrm{C24}) \quad (7)$$

The estimated properties for each microalgae-based biodiesel were presented as the average of the products for the calculated FAME values and their percentage in the mixture [12, 25–27]. Further details are described by Nascimento et al. [12]. The fuel properties (CN, SV, IV, and CFPP) of the algal biodiesels were compared with biodiesels from diverse seed crops [9, 25–27].

Statistical Analysis: Integration of Biodiesel Productivity and Fuel Quality for Comparing Microalgae and Oilseed Crops

A new approach was used to graphically represent algal distinct characteristics and their respective potential for oil production. Both biodiesel quality and productivity were analyzed using this new approach (Fig. 1). PCA I delineated the relationship between the feedstock and fatty acid contents of saturated fatty acids (SFA), MUFA, and PUFA. The plot indicated the saturation/unsaturation balance of each feedstock. PCA II related the feedstock to fuel quality parameters such as the cetane number (CN), iodine value (IV), saponification value (SV), cold filter plugging point (CFPP), and long-chain saturated factor (LCSF). A matrix was constructed on the basis of these results [25–27]. The mutual influences of CFPP, CN, IV, LCSF, and SV were represented by the scores of the first axis that indicate fuel quality. Algal and land crop oil yields were plotted against PCA II's first axis, which made it possible to compare the primary variables for both feedstock and algae (Fig. 2).

Results and Discussion

Microalgae Oil Yields

The oil yield ($m^3\ ha^{-1}\ year^{-1}$) has been used as the primary parameter to compare biodiesel production for land crops and algae [1–4, 15, 17]. Microalgae biodiesel production has been widely reported to outperform conventional land-based oilseed crops. However, many of these reports on algal biodiesel applied a linear scaling of average laboratory results, and most analyses do not consider species-to-species

Table 2 Fatty acid composition (wt%) of land crop and microalgae oils

Fatty acids	Names	Peanut[a]	Palm[a]	Olive[a]	Rapeseed[a]	Soybean[a]	Sunflower[a]	Grape seed[a]	Corn[a]	Coconut[b]	Cottonseed[b]
C12	Lauric	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	47.5	0.0
C14	Myristic	0.1	0.7	0.0	0.0	0.0	0.0	0.1	0.0	20.6	0.0
C16	Palmitic	8.0	36.7	11.6	4.9	11.3	6.2	6.9	6.5	9.9	28.7
C18	Stearic	1.8	6.6	3.1	1.6	3.6	3.7	4.0	1.4	3.0	0.9
C18:1	Oleic	53.3	46.1	75.0	33.0	24.9	25.2	19.0	65.6	7.2	13.0
C18:2w6	Linoleic	28.4	8.6	7.8	20.4	53.0	63.1	69.1	25.2	1.6	57.4
C18:3w6	Linolenic	0.3	0.3	0.6	7.9	6.1	0.2	0.3	0.1	0.6	0.0
C18:4	Octadecatetraenoic	0.0.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C20:1	Gadoleic	2.4	0.2	0.0	9.3	0.3	0.2	0.0	0.1	0.0	0.0
C22:1	Erucic	0.0	0.0	0.0	23.0	0.3	0.1	0.0	0.1	0.0	0.0
Others	–	5.7	0.7	1.9	0.0	0.5	1.3	0.0	1.0	9.6	0.0
Fatty acids	Names	*C. vulgaris*	*Chlamydomonas* sp.	*S. obliquus*	*A. falcatus*	*A. fusiformis*	*C. bacillus*	*C. microporum*	*D. brasiliensis*	*B. terribilis*	*B. braunii*
C4	Butyric	0.0	4.9	1.2	1.5	1.7	0.9	0.6	0.4	0.0	0.0
C14	Myristic	0.6	2.5	1.1	1.7	2.9	2.6	0.8	0.7	0.0	0.7
C16	Palmitic	40.3	50.8	52.1	30.2	26.9	24.5	25.7	27.6	35.2	7.2
C16:1	Palmitoleic	3.2	0.3	0.0	0.5	0.8	1.2	1.0	0.0	0.0	0.0
C18	Stearic	8.0	11.5	7.5	2.7	2.1	3.2	2.9	3.3	3.1	1.6
C18:1c	Oleic	29.3	7.8	21.5	24.1	18.8	7.1	44.2	42.4	39.7	76.3
C18:1 t	Octadecenoic	0.6	5.9	0.0	0.7	1.0	11.1	1.1	0.0	0.0	0.9
C18:2w6	Linoleic	8.5	3.9	4.6	2.0	12.2	13.5	8.6	12.0	5.0	5.2
C18:3w6	Linolenic	0.0	0.8	2.8	0.4	0.2	4.5	11.1	1.0	0.0	0.0
C18:3w3	α-Linolenic	1.6	1.9	0.0	26.5	26.3	21.5	0.0	8.5	7.2	5.3
C20:1w9	Gadoleic	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0
Others	–	7.9	9.7	9.2	9.7	7.1	9.9	4.0	4.1	7.0	2.8

[a] [25]

[b] [23]

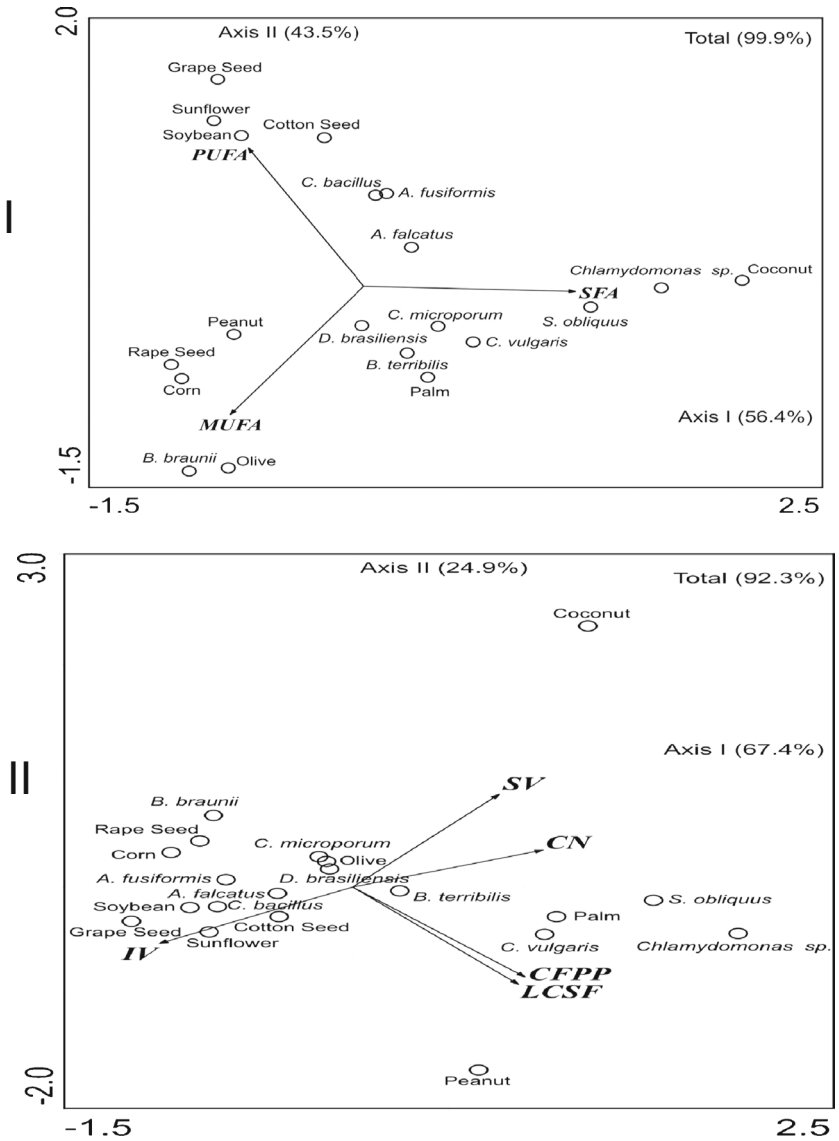

Fig. 1 PCA ordination diagrams associated with fatty acid composition from vegetable and microalgae oils (PCA I) and biodiesel quality parameters (PCA II). A 99.9 % portion of the variation was explained by PCA I analysis. The first and second axes in the PCA II plot explained 67.4 and 24.9 % of the results, respectively

variations [6, 28–31]. This error has resulted in incorrect numbers that sometimes surpass theoretical maximum productivities [8, 31, 32]. To avoid overestimates and diminish the uncertainties of algal biodiesel production, laboratory-scale experimental productivity was cross-checked with commercial-scale cultivation [6], taking into account microalgae-specific productivity results.

Table 1 presents the oil yields of microalgae and land crop feedstock [18–24]. It should be emphasized that a higher lipid concentration may be obtained in the biomass when microalgae are subjected to stressful conditions, such as nutrient depletion [17, 33]; this normally happens during the stationary growth phase at the expense of reduced biomass production, which may ultimately reduce the net lipid productivity for most microalgae species [34]. Nonetheless, the algal strains used here showed lipid contents varying from 13 to 49 % dwt (Table 1), which were generally similar to the data previously reported for Chlorophyta [17, 33–36].

Open raceway pond systems (ORP) and closed cultivation systems (PBR) are two widely used algae cultivation reactor configurations. The oil yields reported for ORP growth systems fell within a range from 12 m^3 ha^{-1} $year^{-1}$ [4] to 31.1 m^3 ha^{-1} $year^{-1}$ or to 51.9 m^3 ha^{-1} $year^{-1}$ (for 20 or 50 % lipid in dry biomass, respectively), and in PBR growth systems, yields may reach 58.7 or 136.9 m^3 ha^{-1} $year^{-1}$, with 30 or 70 % lipids, respectively, in dry biomass [6]. According to the data from the present paper, ten Chlorophyta strains produced oils ranging from 7.4 to 50.8 m^3 ha^{-1} $year^{-1}$ under laboratory conditions (Table 1). Nevertheless, these same strains showed oil yields (Table 1) ranging from 3.4 to 23.0 m^3 ha^{-1} $year^{-1}$ (ORP) and from 6.5 to 44.4 m^3 ha^{-1} $year^{-1}$ (PBR). These large production ranges for ORP and PBR indicate that oil productivity is dependent on the diversity of the metabolic characteristics

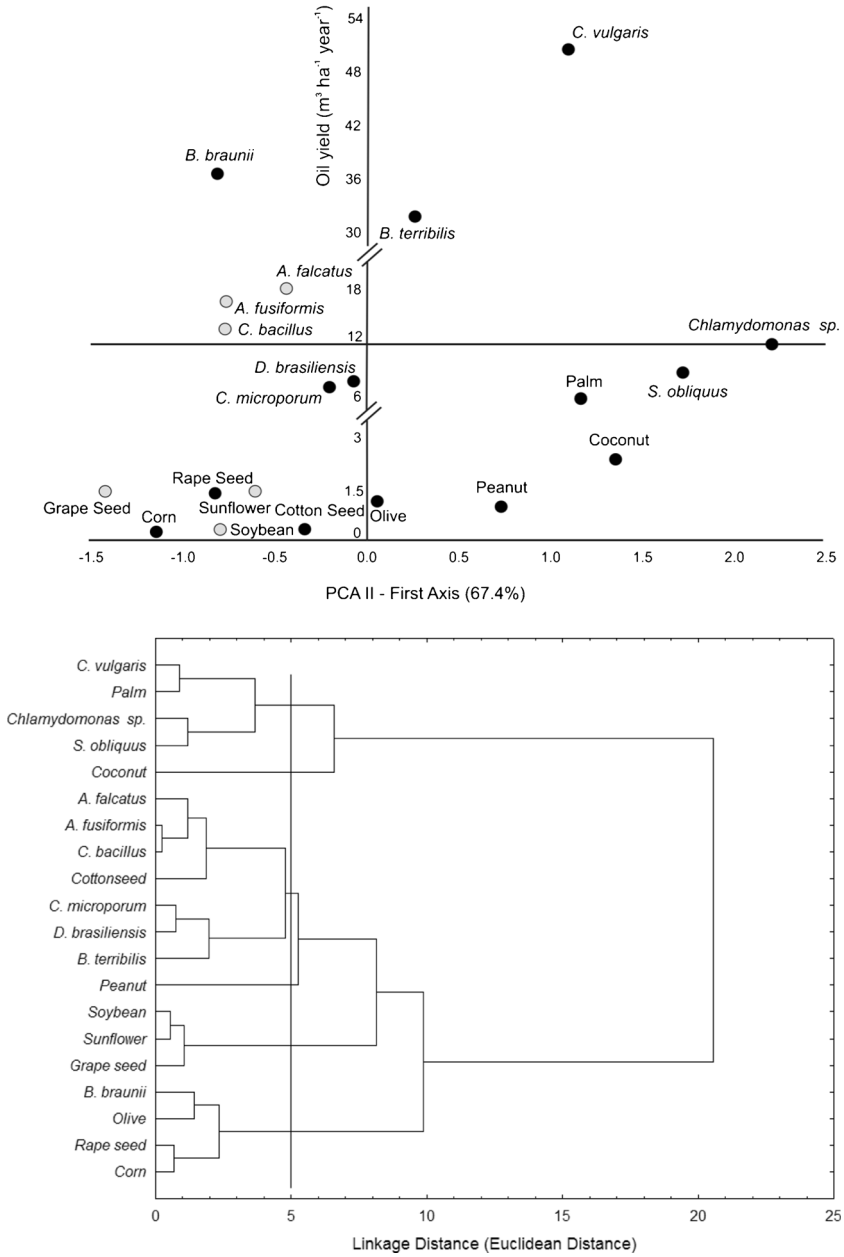

Fig. 2 Cluster and multivariate analysis for comparing land crops and microalgae as feedstock for biodiesel production. The horizontal line crossing the graph is the required minimum oil productivity (12 m^3 ha^{-1} $year^{-1}$). This analysis combines biodiesel quality indices (axis 1 of PCA II) with feedstock productivity. *Dark circles* represent the feedstock that meet the European CN standard, and the *gray circles* indicate those that do not

from tested algal strains, which stresses the need for strain selection in commercial production planning.

The highest oil yields observed in the present work came from Trebouxiophyceae strains, such as *C. vulgaris* and *Botryococcus* sp. (Table 1). *C. vulgaris* also showed the highest volumetric lipid productivity (155 mg l^{-1} day^{-1}), primarily because of its comparatively higher biomass production and to a lesser degree to its lipid content (28.1 % dwt). In the case of *B. terribilis* and *B. braunii*, however, the highest lipid contents in the biomass (49 and 45 % dwt, respectively) were the primary contributing factors to the high oil yields. Among the tested algal species, *C. microporum* and *D. brasiliensis* were the strains with the lowest biomass productivity and oil yields (Table 1).

Comparing Feedstock Oil Yields

When compared with land crops, the oil yields of *Chlamydomonas* sp., *S. obliquus*, *C. microporum*, and *D. brasiliensis* were barely lower than that of palm when cultivated in open raceway systems (Table 1). Nevertheless, most of the algal strains in this study showed higher oil yields than land crops in all growth systems. *C. vulgaris* yields were 23.0 and 44.4 m^3 ha^{-1} $year^{-1}$ for ORP and PBR growth systems, respectively. Assuming the weight of algal oil is 880 k m^{-3} [31], those values were 3.3 and 6.5 times higher than the palm oil yield and 101 and 195 times higher than the corn oil yield (Table 1). A comparison of algae and land crop

oil yield variations may demonstrate the superiority of algal oil over land crop production. However, this comparison may be distorted if it does not account for differences in oilseed crop productivity.

The large microalgae oil yield variations may also help to explain some of the inaccurate estimates regarding the superior productivity of microalgae, which are sometimes reported to be of several orders of magnitude [8, 31]. Some figures are near the theoretical limit of maximum photosynthetic efficiency (oil yields of 354 m^3 oil ha^{-1} $year^{-1}$), which is clearly unachievable [32]. However, current algal oil production data have led to more accurate estimates. For regions with high solar radiation, a maximum daily algal biomass production of 100 g m^{-2} day^{-1} at 10 % solar energy conversion has been claimed [32–35]. These conditions would generate approximately 324 tons ha^{-1} $year^{-1}$ of biomass (assuming production for 360×0.90 days in a year), which could result in 97.2 m^3 oil ha^{-1} $year^{-1}$ (considering 30 % oil in biomass dwt). This amount has never been achieved on a long-term basis [32]. Nevertheless, a solar energy conversion efficiency of up to 5 % has been reported by some authors [4, 14], which represents about half of the oil yields projected above (48.6 m^3 oil ha^{-1} $year^{-1}$) based on 30 % oil in the biomass. However, other authors reported a higher biomass oil content of approximately 40 %, leading to a production of 53.5 m^3 oil ha^{-1} $year^{-1}$ [37]. Algal species selection and technological development may make it possible to improve oil production above these levels [2, 4, 6, 36–39], up to an absolute ceiling of 94 to 155 m^3 oil ha^{-1} $year^{-1}$ [40]. However, these levels are still less than half of the theoretical maximum oil yield [32], estimated at 354 m^3 ha^{-1} $year^{-1}$, which are considered by many to be unattainable.

In the present paper (Table 1), maximum oil yields for *C. vulgaris* (23.0, 44.4, and 50.8 m^3 oil ha^{-1} $year^{-1}$) growing in different systems are very similar to those suggested [32] as the best possible values (40.7 to 53.2 m^3 ha^{-1} $year^{-1}$). These values, which were estimated according to location and biological variations under real environmental conditions, may only be attainable under conditions that do not limit photosynthetic efficiency [32].

Comparing Biodiesel Quality from Diverse Feedstock

Oil quality is another essential factor for the success of the algal-based biodiesel industry [12, 13]. Several countries have established biodiesel quality standards and guidelines to regulate biodiesel production, for example, EN14214 in Europe, ASTM D6751-10 in the USA, RANP/2008 in Brazil, and similar guidelines for South Africa and Australia [13]. Parameters such as the cetane number, saponification value, iodine value, cold filter plugging point, and oxidation stability (CN, SV, IV, CFPP, and OS) are used to evaluate biodiesel quality in terms of ignition readiness, combustion performance, fuel-line plugging temperature, and resistance to oxidative damage during storage (Fig. 1). These parameters can be estimated by analyzing the molecular characteristics of biodiesels. In other words, biodiesel quality is determined by the product of a quantitative/qualitative balance among the total fatty acid compositions and not on any particular fatty acid in the oil. The ratios (Table 3 and Fig. 1) of SFA, MUFA, and PUFA contents reportedly have a major impact on the biodiesel quality [9, 12, 13]. Biodiesels with long-chain fatty acids and a low degree of unsaturation (DU) tend to have a good ignition quality (higher CN values), but they do not exhibit good flow performance at low temperatures (CFPP) [9–13], and biodiesels with good CFPP can be achieved by short-chain fatty acids and high DU [9, 13]. Therefore, it is clear that a balanced fatty acid distribution is critical to improving biodiesel quality.

In the present study, palmitic (C16:0) and oleic (C18:1) acids were the primary fatty acids in most Chlorophyta oils (Table 2), and the stearic acid contents (C18:0) were generally low (<3.3 %) except in *Chlamydomonas* sp., *C. vulgaris*, and *S. obliquus* (11.54, 8.01, and 7.5 %, respectively). MUFAs such as oleic (C18:1) and linoleic (C18:2) acids were the predominant fatty acids in most land crop oils (Table 3). In addition, among all the feedstock tested in this study, *A. fusiformis*, *C. bacillus*, *A. falcatus*, soybeans, sunflowers, and grape seeds contained significant amounts of PUFA (Table 2). Biodiesels generated from these stocks would not comply with the European standard minimum CN of 51. However, these biodiesels would meet the American and Brazilian standards with minimum CNs of 47 and 45, respectively [12, 13]. All the other feedstock would meet the European CN standard (Table 3 and Fig. 1). Table 3 also indicated that most studied feedstocks except coconut, *Chlamydomonas sp.*, and *S. obliquus* had DUs higher than 50. High DUs tend to decrease the ignition quality and increase susceptibility to oxidation [9, 10]. To improve biodiesel quality, the DU in oils must be limited. European standards for biodiesel stipulate that the linoleic ester (C18:2w6) and polyunsaturated ester (≥4 double bonds) content must be less than 12 % (mol/mol) and 1 % (mol/mol), respectively [10, 13]. In fact, most of the land crop biodiesels failed to satisfy this criterion (Table 2), and only *A. fusiformis* and *C. bacillus* biodiesels, with 12.2 and 13.3 % linoleic esters, respectively, were close to meeting this criterion.

Biodiesels with more SFAs have high melting points, and they could crystallize at normal engine temperatures [11]. This parameter is defined as a poor CFPP property [10]. Despite the fact that these characteristics provide good ignition properties, they decrease the flow and enhance the chances of plugging filters and fuel lines. With the exception of biodiesels from *Chlamydomonas* sp., *S. obliquus*, and *C. vulgaris*, which have the highest levels of saturated long-chain FAME, the estimated CFPP values for the algal

Table 3 Microalgae and land crop biodiesel quality. Vegetable oil data were obtained from the literature [23, 25]

Land crops and microalgae	SFA (% wt)	MUFA (% wt)	PUFA (% wt)	DU	LCSF	CN[a]	SV (mg KOH g^{-1})	IV[b] (gI^2 100 g^{-1})	CFPP (°C)
Peanut[c]	15.6	55.7	28.7	113.1	10.7	53	195	97	17
Palm[c]	44.7	46.4	8.9	64.2	7.7	61	209	57	10
Olive[c]	15.7	76.0	8.4	92.7	4.2	57	196	84	−6
Rapeseed[c]	6.5	65.3	28.3	121.9	1.3	55	190	109	−10
Soybean[c]	15.3	25.6	59.1	143.8	3.4	49	195	128	−5
Sunflower[c]	11.1	25.6	63.3	152.2	4.2	50	193	132	−3
Grape seed[c]	11.3	19.1	69.4	157.8	3.0	48	187	138	−6
Corn[c]	8.0	66.4	25.3	117.0	1.5	53	183	101	−12
Coconut[d]	90.0	6.0	2.0	10.0	2.3	63	255	12	−6
Cottonseed[d]	26.0	18.0	52.0	122.0	4.7	51	192	90	−2
Chlamydomonas sp.	78.6	14.0	6.7	28.1	10.8	65	220	27	17.4
S. obliquus	66.7	21.5	7.4	36.6	8.9	64	216	35	11.6
C. vulgaris	49.6	36.3	10.1	56.5	8.0	62	199	53	8.6
A. falcatus	41.4	28.4	30.2	88.8	4.4	50	202	101	−2.7
A. fusiformis	37.3	22.4	40.2	102.9	3.7	48	200	113	−4.7
C. bacillus	35.7	23.6	40.7	105.1	3.9	48	197	114	−4.1
C. microporum	45.9	38.0	16.1	70.2	4.0	53	206	88	−3.8
D. brasiliensis	34.5	44.1	21.4	86.8	4.4	53	205	87	−2.6
B. terribilis	39.0	42.5	12.2	58.2	5.1	59	193	67	−0.5
B. braunii	9.8	79.6	10.5	100.7	1.5	53	197	95	−11.7

[a] EU Standards EN14214: CN minimum 51; US Standards ASTM D6751-10: CN minimum 47; BR-RANP/2008: CN minimum 45

[b] EU Standards EN14214: IV maximum 120

[c] [25]

[d] [24]

biodiesels in this study (−11.7 to −0.5 °C) were all acceptable (Table 3). Among the investigated land crop biodiesels, only palm and peanut, with their high saturation contents, have high CFPP values (10 and 17 °C, respectively). For biodiesels from other land crops, the CFPP values were within an acceptable range (−12 to −2 °C) [25]. However, when comparing biodiesels, those from *Chlamydomonas* sp., *S. obliquus*, and *C. vulgaris* and from palm and peanut generate biodiesels with the best OS, and they have the worst CPFF. According to Ramos et al. [25], biodiesels from olive, rapeseed, and corn have higher MUFA contents in their oils, and they are the ones that best satisfy all standards regarding CN and still have good CFPP values (Table 3).

It has been reported that a top quality biodiesel would be a product of a well-balanced mixture of monosaturated fatty acids C16:1 and C18:1 [10]. In addition, a well-balanced ratio (5:4:1) of the fatty acids C16:1, C18:1, and C14:0 would produce a biodiesel with good CFPP and high CN characteristics. None of the feedstock in this study provided a similar balance. Therefore, the results suggest that the oil chemical composition and fatty acid profiles are just as important as oil productivity for selecting biodiesel production feedstock.

Feedstock Selection for Biodiesel Production

By noting that higher oil yields could significantly reduce algal biodiesel production costs [38–40], the present study used the criterion [38] of a minimum volumetric lipid productivity of 40 mg l^{-1} day^{-1} (equivalent to approximately 12 m^3 ha^{-1} $year^{-1}$) to select the algal strains. Trebouxiophyceae (*Chlorella* and *Botryococcus* strains) were selected from all of the algal species studied in this project. These strains satisfied the oil yield criterion of 12 m^3 ha^{-1} $year^{-1}$ for both growth systems (open ponds and PBR), and *A. falcatus*, *A. fusiformis*, and *C. bacillus* only met this criterion when grown in PBR (Table 1). With the exception of *Chlamydomonas* sp., *S. obliquus*, *C. microporum*, and *D. brasiliensis* (cultivated in ORP), all of the strains studied in this project were more productive than any of the land crops (Table 1). The *C. vulgaris* and *Botryoccocus* spp. strains were the most productive, followed by *A. falcatus*, *A. fusiformis*, and *C. bacillus*, with yields above 12 m^3 ha^{-1} $year^{-1}$ (Fig. 2).

The results show that the biodiesels produced by all of the studied microalgae are in accordance with American and Brazilian quality standards (Table 3). *C. bacillus*, *A. falcatus*, and *A. fusiformis* (Fig. 2) produced biodiesels that are just

below the European standard requirement for CN. Biodiesels produced from soybeans, sunflowers, and grape seeds were also below the European standard [25]. All other feedstock in this paper met EU EN14214 fuel quality standards. If both the biodiesel yield and quality are considered, six algal species (*C. vulgaris*, *B. braunii*, *B. terribilis*, *A. falcatus*, *A. fusiformis*, and *C. bacillus*) could be recommended for production (Tables 1 and 3 and Fig. 2).

Many studies have concluded that *C. vulgaris* is one of the best algal species for biodiesel, primarily for its oil productivity [27]. When comparing *C. vulgaris* with other feedstock, this study took both productivity and biodiesel quality into account, and it identified the following factors to further support this conclusion: (i) high productivity, (ii) good ignition quality, and (iii) good oxidative stability. However, *C. vulgaris*-based biodiesel also had some disadvantages, such as low lubricity quality (an IV of 53 g I_2/100 g) and cold filter property (a CFPP of 8 °C) (Table 3), which limited the application of *C. vulgaris* biodiesel in cold climate regions. Conversely, the least productive of the studied microalgae, namely *D. brasiliensis* and *C. microporum*, had the best saturation/unsaturation ratios, a factor that could lead to a high quality biodiesel with balanced fuel properties (Table 3 and Fig. 2). With regard to land crops, soybeans, sunflowers, and grape seeds did not meet the CN standard, but these crops showed good cold filter properties (Fig. 2 and Table 3). Rapeseed and corn satisfied the EU EN14214 CN criterion with good CFPP values of −10 and −12 °C, respectively (Table 3). Olive and cottonseed biodiesels had good saturation/unsaturation ratios similar to those of *D. brasiliensis* and *C. microporum* (Table 3 and Fig. 2).

Yoo et al. [41] have already suggested that *Botryococcus braunii*, in conjunction with *C. vulgaris* and *Scenedesmus* sp., were the most appropriate species for biodiesel production on the basis of their productivity and CN value. However, significant differences have been reported in the composition between species of the same taxonomic group, which could lead to variations in biodiesel quality. The oils from the *Botryococcus* strains in this study were mostly made of oleic acid (Table 2). *B. braunii* produced 76.3 % oleic acid, which is almost double that of *B. terribilis.* This difference in composition leads to an imbalance in the saturation/unsaturation ratios and further reduces biodiesel quality. Oleic acid helps to balance the ignition quality and cold flow properties [10, 13]. Biodiesel with a high oleic acid content does not normally cause polymerization during combustion. However, the long carbon chains of oleic acid increase the CFPP value and may cause the formation of agglomerates. In addition, a higher oleic acid content increases unsaturation, which may decrease its oxidation stability.

Concluding Remarks

These results showed that most of the studied microalgae can provide higher biodiesel production yields when compared with land oil crops. The data also demonstrated that oil productivity is not the only factor used in selecting strains for commercial biodiesel production. Biodiesel quality, as defined by several standards, should also be taken into account for this purpose. The algal biodiesels have better saturation/unsaturation ratios than land crop-derived biodiesels, which leads to better biodiesel quality. Although a top quality algal biodiesel may not be easily achievable, oil yields can be significantly improved by growing strategies. Thus, although most land crop feedstock is near its maximum production levels, microalgae biodiesels still have great potential to be improved and make a major contribution to the next generation of biofuel production.

Acknowledgments This work was financially supported by the National Council for Scientific and Technological Development (CNPQ)/ Science and Technology Ministry, Brazil (Processes 574712/2008-9 and 551134/2010-0).

References

1. Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sust Energ Rev 14:217–232
2. Sheehan J, Camobreco V, Duffield J, Graboski M, Shapouri H (1998) Life cycle inventory of biodiesel and petroleum diesel for use in an urban bus. Tech Rep NREL/SR-580-24089, US Department of Energy and US Department of Agriculture
3. Gouveia L, Oliveira AC (2009) Microalgae as raw material for biofuels production. J Ind Microbiol Biotechnol 36:269–274
4. Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg Res 1: 20–43
5. Altieri MA (2009) The ecological impacts of large-scale agro fuel monocultures' production systems in the Americas. B Sci Technol Soc 29(3):236–244
6. Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25: 294–306
7. Costa JAV, de Moraes MG (2011) The role of biochemical engineering in the production of biofuels from microalgae. Bioresour Technol 102:2–9
8. Quinn JC, Catton K, Wagner N, Bradley TH (2012) Current large scale US biofuel potential from microalgae cultivated in photobioreactors. Bioenerg Res 5:49–60
9. Knothe GH (2005) Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 86:1059–1070
10. Knothe GH (2008) "Designer" biodiesel: optimizing fatty ester composition to improve fuel properties. Energy Fuels 22:1358–1364
11. Meher LC, Vidya SD, Naik SN (2006) Technical aspects of biodiesel production by transesterification—a review. Renew Sust Energ Rev 10:248–268
12. Nascimento IA, Marques SSI, Cabanelas ITD, Pereira SA, Druzian JI, de Souza CO, Vich DV, de Carvalho GC, Nascimento MA (2013) Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty-acids profiles as selective criteria. Bioenerg Res 6:1–13

13. Stansell GR, Gray VM, Sym DS (2012) Microalgal fatty acid composition implication for biodiesel quality. J Appl Phycol 24(4):791–801. doi:10.1007/s10811-011-9696x
14. Zemke PE, Wood BD, Dye DJ (2010) Considerations for the maximum production rates of triacylglycerol from microalgae. Biomass Bioenerg 34:145–151
15. Demirbas A, Demirbas MF (2011) Importance of algae oil as a source of biodiesel. Energy Convers Manag 52:163–170
16. Dayananda C, Sarada R, Ushua RM, Shamala TR, Ravishankar GA (2007) Autotrophic cultivation of *Botryococcus braunii* for the production of hydrocarbons and exopolysaccharides in various media. Biomass Bioenerg 31:87–93
17. Griffiths M J, Dicks R G, Richardson C, Harrison S T L (2011) Advantages and challenges of microalgae as a source of oil for biodiesel. In Biodiesel—feedstocks and processing technologies, Stoytcheva M (Ed.), ISBN: 978-953-307-713-0, InTech, doi: 10.5772/30085. Available from: http://www.intechopen.com/books/biodiesel-feedstocks-and-processing-technologies/advantages-and-challenges-of-microalgae-as-a-source-of-oil-for-biodiesel. Accessed: March, 2013
18. Kaya C, Hamamci C, Baysal A, Akba O, Erdogan AS (2009) Methyl ester of peanut (*Arachis hypogea* L.) seed oil as a potential feedstock for biodiesel production. Renew Energy 34:1257–1260
19. Grattan SR, Berenguer MJ, Connell JH, Polito VS, Vossen PM (2006) Olive oil production as influenced by different quantities of applied water. Agric Water Manag 85:133–140
20. Baydar NG, Akkurt M (2001) Oil content and oil quality properties of some grape seeds. Turk J Agric For 25(3):163–168
21. El Boulifi N, Bouaid A, Martinez M, Aracil J (2010) Process optimization for biodiesel production from corn oil and its oxidative stability. Int J Chem Eng. doi:10.1155/2010/518070
22. Moaveni P, Ebrahimi A, Farahani HA (2010) Studying of oil yield variations in winter rapeseed (*Brassica napus*, L) cultivars under drought stress conditions. J Agr Biotechnol Sustain Dev 2(5):71–75
23. Demirbas A (2008) Studies on cottonseed oil biodiesel prepared in non-catalytic SCF conditions. Bioresour Technol 99:1125–1130
24. Demirbas A (2003) Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey. Energy Convers Manag 44:2093–2109
25. Ramos MJ, Fernández CM, Casas A, Rodriguez L, Pérez A (2009) Influence of fatty acids composition of raw materials on biodiesel properties. Bioresour Technol 100:261–268
26. Krisnangkura KA (1986) A simple method for estimation of cetane index of vegetable oil methyl esters. J Am Oil Chem Soc 63:552–553
27. Francisco EC, Neves DB, Jacob-Lopes E, Franco TT (2010) Microalgae as feedstock for biodiesel production: carbon dioxide sequestration, lipid production and biofuel quality. J Chem Technol Biotechnol 85:395–403
28. Chisti Y (2008) Response to Reijnders: do biofuels from microalgae beat biofuels from terrestrial plants? Trends Biotechnol 26(7): 351–352
29. Hirano A, Hon-Nami K, Kunito S, Hada M, Ogusshi Y (1998) Temperature effect on continuous gasification of microalgal biomass: theoretical yield of methanol production and its energy balance. Catal Today 45(1–4):399–404
30. Batan L, Quinn J, Wilson B, Bradley T (2010) Net energy and greenhouse gas emission evaluation of biodiesel derived from microalgae. Environ Sci Technol 44:7975–7980
31. Quinn J, de Winter L, Bradley T (2011) Microalgae bulk growth model with application to industrial scale systems. Bioresour Technol 102:5083–5092
32. Weyer KM, Bush DR, Darzins A, Willson BD (2010) Theoretical maximum algal oil production. Bioenerg Res 3:204–213
33. Rodolfi L, Zitelli GC, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR (2008) Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 102(1):100–112
34. Wang B, Li Y, Wu N, Lan CQ (2008) CO_2 biomitigation using microalgae. Appl Microbiol Biotechnol 79:707–718
35. Pienkos PT, Darzins A (2009) The promises and challenges of microalgal-derived biofuels. Biofuels Bioprod Bioref 3:431-440. John Wiley & Sons, Ltd
36. Metzger P, Largeau C (2005) *Botryococcus braunii* a rich source for hydrocarbons and related ether lipids. App Microbiol Biotechnol 6: 486–496
37. Benemann, J. (2009). Microalgae biofuels: a brief introduction. Available at http://advancedbiofuelsusa.info/wp-content/uploads/2009/03/microalgae-biofuels-an-introduction-july23-2009-benemann.pdf. Accessed March, 2013
38. Gong Y, Jiang M (2011) Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnol Lett 33:1269–1284
39. Huntley ME, Redalje DG (2007) CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitig Adapt Strateg Glob Chang 12(4):573–608
40. Stephens E, Ross I, King Z, Mussgnug J, Kruse O, Posten C, Borowitzka M, Hankamer B (2010) An economic and technical evaluation of microalgal biofuels. Nat Biotechnol 28(2):126–128
41. Yoo C, Jun S-Y, Lee J-Y, Ahn C-Y, H-M OH (2010) Selection of microalgae for lipid production under high levels carbon dioxide. Bioresour Technol 101:71–74