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ORIGINAL PAPER

Microalgae as a raw material for biofuels production

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Abstract Biofuels demand is unquestionable in order to reduce gaseous emissions (fossil CO_2, nitrogen and sulfur oxides) and their purported greenhouse, climatic changes and global warming effects, to face the frequent oil supply crises, as a way to help non-fossil fuel producer countries to reduce energy dependence, contributing to security of supply, promoting environmental sustainability and meeting the EU target of at least of 10% biofuels in the transport sector by 2020. Biodiesel is usually produced from oleaginous crops, such as rapeseed, soybean, sunflower and palm. However, the use of microalgae can be a suitable alternative feedstock for next generation biofuels because certain species contain high amounts of oil, which could be extracted, processed and refined into transportation fuels, using currently available technology; they have fast growth rate, permit the use of non-arable land and non-potable water, use far less water and do not displace food crops cultures; their production is not seasonal and they can be harvested daily. The screening of microalgae (*Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis* sp., *Neochloris oleabundans*, *Scenedesmus obliquus* and *Dunaliella tertiolecta*) was done in order to choose the best one(s), in terms of quantity and quality as oil source for biofuel production. *Neochloris oleabundans* (fresh water microalga) and *Nannochloropsis* sp. (marine microalga) proved to be suitable as raw materials for biofuel production, due to their high oil content (29.0 and 28.7%, respectively). Both microalgae, when grown under nitrogen shortage, show a great increase (~50%) in oil quantity. If the purpose is to produce biodiesel only from one species, *Scenedesmus obliquus* presents the most adequate fatty acid profile, namely in terms of linolenic and other polyunsaturated fatty acids. However, the microalgae *Neochloris oleabundans*, *Nannochloropsis* sp. and *Dunaliella tertiolecta* can also be used if associated with other microalgal oils and/or vegetable oils.

Keywords *Neochloris oleoabundans* · *Scenedesmus obliquus* · *Nannochloropsis* sp. · *Dunaliella tertiolecta* · Lipids · Biofuels · Biodiesel

Introduction

Finding sufficient supplies of clean energy for the future is one of society's most daunting challenges and is intimately linked with global stability, economic prosperity, and quality of life.

Fuels represent around 70% of the total global energy requirements, particularly in transportation, manufacturing and domestic heating. Electricity only accounts at present for 30% of global energy consumption.

In the European Union (EU), the transport sector is responsible for almost one quarter of greenhouse gas emissions [15] and it is, therefore, essential to find ways of reducing emissions. Vehicles must be cleaner and more fuel efficient and the use of biofuels can also play an important role in avoiding the excessive dependence on fossil fuels and ensuring security of supply, in promoting environmental sustainability and meeting the target of at least of 10% by 2020 for biofuels in the transport sector. Biodiesel fuel has received considerable attention in recent years, as it is made from non-toxic, biodegradable and renewable

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resources, and provides environmental benefits, since its use leads to a decrease in the harmful emissions of carbon monoxide, hydrocarbons and particulate matter and to the elimination of SO_x emissions, with a consequent decrease in the greenhouse effect, in line with the Kyoto Protocol agreement. Biodiesel is usually produced from oleaginous crops, such as rapeseed, soybean, sunflower and from palm, through a chemical transesterification process of their oils with short chain alcohols, mainly methanol [1, 2, 19, 30].

However, the use of microalgae can be a suitable alternative because algae are the most efficient biological producer of oil on the planet and a versatile biomass source and may soon be one of the Earth's most important renewable fuel crops [6], due to the higher photosynthetic efficiency, higher biomass productivities, a faster growth rate than higher plants (which is also important in the screening step), highest CO_2 fixation and O_2 production, growing in liquid medium which can be handled easily, can be grown in variable climates and non-arable land including marginal areas unsuitable for agricultural purposes (e.g. desert and seashore lands), in non-potable water or even as a waste treatment purpose, use far less water than traditional crops and do not displace food crop cultures; their production is not seasonal and can be harvested daily [6–8].

As a matter of fact, average biodiesel production yield from microalgae can be 10 to 20 times higher than the yield obtained from oleaginous seeds and/or vegetable oils [7, 34] (Table 1).

Some microalgae have high oil content (Table 2) and can be induced to produce higher concentration of lipids (e.g. low nitrogen media, Fe^{3+}concentration and light intensity) [18, 21, 28, 32, 35].

The ability of algae to fix CO_2 can also be an interesting method of removing gases from power plants, and thus can be used to reduce greenhouse gases with a higher production microalgal biomass and consequently higher biodiesel yield [22, 39]. Algal biomass production systems can be easily adapted to various levels of operational and technological skills; some microalgae have also a convenient fatty acids profile and an unsaponifiable fraction allowing a biodiesel production with high oxidation stability [11, 16, 24, 25]. The physical and fuel properties of biodiesel from microalgal oil in general (e.g. density, viscosity, acid value, heating value, etc.), are comparable to those of fuel diesel [23, 27].

Table 1 Comparison of some sources of biodiesel [7]

Crop	Oil yield (L ha^{-1})
Corn	172
Soybean	446
Canola	1,190
Jatropha	1,892
Coconut	2,689
Palm	5,950
Microalgae[a]	136,900
Microalgae[b]	58,700

[a] 70% oil (by wt) in biomass

[b] 30% oil (by wt) in biomass

Table 2 Lipid content of some microalgae (% dry matter) (adapted from [3, 18, 21, 23, 26, 33, 35, 38])

Species	Lipids
Scenedesmus obliquus	11–22/35–55
Scenedesmus dimorphus	6–7/16–40
Chlorella vulgaris	14–40/56
Chlorella emersonii	63
Chlorella protothecoides	23/55
Chlorella sorokiana	22
Chlorella minutissima	57
Dunaliella bioculata	8
Dunaliella salina	14–20
Neochloris oleoabundans	35–65
Spirulina maxima	4–9

Key technical challenges include identifying the strains with the highest growth rates and oil content with adequate composition, which were the aim of this work. Oil extraction procedure was selected and the fatty acid profile analyzed for all microalgae tested. The oil was also characterized in terms of its iodine value, a parameter that must be considered if biodiesel production is the purpose.

Materials and methods

Microalgae production

The microalgae used in this study were *Chlorella vulgaris* (INETI 58), *Spirulina maxima* (LB 2342), *Nannochloropsis* sp., *Neochloris oleabundans* (UTEX # 1185, USA), *Scenedesmus obliquus* (FCTU Coimbra) and *Dunaliella tertiolecta* (IPIMAR). The microalgae were cultivated in appropriate growth medium [37]. All the microalgae tested were initially grown in airlift bioreactors and then in polyethylene bags with bubbling air under low lighting conditions (150 μE m^{-2} s^{-1}), at the optimal temperature for each microalga (indoors), and finally in outdoor raceways agitated by paddle wheels, during 4 months (May–August). For *N. oleabundans* and *Nannochloropsis* sp. growth was also performed under N-starvation during 5 days, after removing culture medium (initially $NaNO_3 = 0.25$ g L^{-1} and $KNO_3 = 0.2$ g L^{-1} as N-sources for *N. oleabundans* and *Nannochloropsis*, respectively) by centrifugation and re-inoculating it in N-deficient medium. Microalgal biomass harvesting was processed without flocculation by simply removing agitation and concentrating by centrifugation

(Beckman Avanti, J-25I (small volumes) and Alfa-Laval LAPX202 (big volumes)) and freeze-dried.

Growth evaluation

Growth parameters such as optical density (OD) (540 nm) (Hitachi U-2000) and ash free dry weight (AFDW) (Whatman GF/C 45 μm) were measured three times a week.

Oil extraction

Oil extraction from microalgal biomass was performed in a Soxhlet apparatus using *n*-hexane as solvent with sample pre-treatment (propanol) after cell disruption by sonication during 20 min. These conditions were established after selection from a wide range of procedures and by comparison with the results obtained with the Bligh and Dyer extraction method [5].

Oil characterization

Fatty acid composition

To determine the fatty acid composition of each raw material, oil samples (~150 mg) (in duplicate) were chemically derivatized using the borum trifluoride method described in the EN ISO 5509 [12]. The organic phase obtained was analyzed by gaseous chromatography using a CP-3800 GC (Varian, USA) equipped with 30 m DB-WAX (J&W, Agilent) capillary column (0.25 mm of internal diameter and 0.25 μm of film thickness). Injector (split 1:100) and detector (flame ionization) temperatures were kept constant at 250°C. The oven temperature program started at 180°C for 5 min, increased at 4°C min^{-1} until 220°C, and kept constant at this temperature for 25 min. Carrier gas, He, was kept at a constant rate of 1 mL min^{-1}. Fatty acid composition was calculated as percentage of the total fatty acids present in the sample, determined from the peak areas.

Iodine value

Oils from microalgae were characterized in terms of iodine value according to the European Standard EN 14111 [13].

Results and discussion

Microalgal biomass maximum concentration reached by all microalgae ranged between 2 g L^{-1} (*Neochloris oleabundans* and *Scenedesmus obliquus* in polyethylene bags) and 3.6 g L^{-1} (*Dunnaliella* in polyethylene bags) (Table 3) according to other authors [17, 29, 36]. Average concentration and productivities were similar for all microalgae tested ranging from 1.0–2.6 to 0.1–0.2 g L^{-1} day^{-1}, respectively.

The results of extraction methods, from a previous study, indicated that the best procedure is Soxhlet with *n*-hexane as a solvent. In terms of pre-treatment, propanol has a positive effect on oil extraction (results not shown). For microalgal cell disruption, the ultrasonic method is more efficient than vortex and homogeneizer.

The tested microalgae strains revealed similar average, maximum biomass concentration and productivities (Table 3) and it can be seen that *Nannochloropsis* sp. and *Neochloris oleabundans* are the strains with the highest oil content, in agreement with literature [7, 29, 35].

Fatty acid profile was determined for all microalgae and the results are presented in Table 4. All microalgal lipids are mainly composed of unsaturated fatty acids (50–65%) and a significant percentage of palmitic acid (C16:0) was also present (17–40%). Among the unsaturated fatty acids special attention should be taken to the linolenic (C18:3) and polyunsaturated (≥4 double bonds) contents, due to the EN 14214 [14] that specifies a limit of 12 and 1%, respectively, for a quality biodiesel. As can be seen from Table 4, only the oils extracted from *S. obliquus* and *Nannochloropsis* sp. present linolenic acid contents within specifications. The oil of *S. obliquus* also has a lower polyunsaturated fatty

Table 3 Microalgal biomass average concentration, biomass maximum concentration, productivities and microalgal biomass oil content

	Average biomass concentration (g L^{-1})	Maximum biomass concentration (g L^{-1})	Productivities (g L^{-1} day^{-1})	Oil content (%) (AFDW)
Sp	2.0	3.1	0.21	4.1
Cv	1.5	3.0	0.18	5.1
Sc	0.9	2.0	0.09	17.7
Dt	2.6	3.6	0.12	16.7
Nanno	1.6	2.5	0.09	28.7
Neo	1.5	2.0	0.09	29.0

Sp, *Spirulina maxima*; Cv, *Chlorella vulgaris*; Sc, *Scenedesmus obliquus*; Dt, *Dunaliella tertiolecta;* Nanno, *Nannochloropsis* sp.; Neo, *Neochloris oleabundans*

Table 4 Main fatty acids present in *Spirulina maxima* (Sp), *Chlorella vulgaris* (Cv), *Scenedesmus obliquus* (Sc), *Dunaliella tertiolecta* (Dt), *Nannochloropsis* sp. (Nanno) and *Neochloris oleabundans* (Neo) oil extracts

Fatty acid	Sp (% w w^{-1})	Cv (% w w^{-1})	Sc (% w w^{-1})	Dt (% w w^{-1})	Nanno (% w w^{-1})	Neo (% w w^{-1})
14:0	0.34	3.07	1.48	0.47	7.16	0.43
16:0	40.16	25.07	21.78	17.70	23.35	19.35
16:1	9.19	5.25	5.95	0.88	26.87	1.85
16:2	n.d.	n.d.	3.96	3.03	0.39	1.74
16:3	0.42	1.27	0.68	1.24	0.48	0.96
16:4	0.16	4.06	0.43	10.56	n.d.	7.24
18:0	1.18	0.63	0.45	n.d.	0.45	0.98
18:1	5.43	12.64	17.93	4.87	13.20	20.29
18:2	17.89	7.19	21.74	12.37	1.21	12.99
18:3	18.32	19.05	3.76	30.19	n.d.	17.43
18:4	0.08	n.d.	0.21	n.d.	n.d.	2.10
20:0	0.06	0.09	n.d.	n.d.	n.d.	n.d.
20:1	n.d.	0.93	n.d.	n.d.	n.d.	n.d.
20:2	0.48	n.d.	n.d.	n.d.	n.d.	n.d.
20:3	n.d.	0.83	n.d.	n.d.	n.d.	n.d.
20:4	n.d.	0.23	n.d.	n.d.	2.74	n.d.
20:5	n.d.	0.46	n.d.	n.d.	14.31	n.d.
Saturated	41.74	28.56	23.71	18.17	30.96	20.76
Unsaturated	51.97	51.91	54.66	63.14	59.20	64.60

Table 5 Microalgal iodine values

	Sc	Dt	Nanno	Neo
Iodine value	69	121	52	102

Sc, *Scenedesmus obliquus*; Dt, *Dunaliella tertiolecta;* Nanno, *Nannochloropsis* sp.; Neo, *Neochloris oleabundans*

acid content than the value referred by the European standard. However, all the analyzed microalgae oils may be used for good quality biodiesel if associated with other oils, or without restrictions as raw material for other biofuels production processes.

The oils obtained from the microalgae with higher oil content were characterized in terms of iodine value (Table 5). The obtained results meet the biodiesel quality specifications ($<120\ \mathrm{gI_2/100\ g}$) [13] which makes these microalgae oils competitive with some vegetable oils traditionally used for biodiesel production as soy or sunflower, that usually present iodine values higher than 120.

Neochloris oleabundans cultivated under nitrogen shortage, after 5 days of nitrogen starvation (results not shown), showed a fatty acid content increase of ~50% with no significant change in fatty acid profile indicating this is a high potential microalga for biofuel production purposes. These results are in agreement with other studies, see, e.g. Illman et al. [18], Liu et al. [21], Rudolfi et al. [28], Solovchenco et al. [32], and Tornabene et al.[35] that reported an increase of oil content as a response of stress conditions, such as nitrogen limitation, and high Fe^{3+} concentration and light intensity.

To reduce microalgal biomass overall production costs, the biomass cake remaining after oil has been extracted can be used as fertilizer or feed, can undergo anaerobic fermentation to obtain biogas and/or a pyrolysis process, or to extract high value chemical compounds (biorefinery concept) [7, 8, 10, 27]. Ran and Spada [27] suggest that to make plants accessible to small producers, such as agricultural farms, in the near future, could integrate this concept in order to obtain biofuels, electricity and feed for livestock.

The global biodiesel market is estimated to reach 37 billion gallons by 2016, growing at an average annual growth of 42%, being Europe the major biodiesel market for the next decade or so, closely followed by US market [31]. In order to meet these rapid expansion in biodiesel production capacity, observed not only in develop countries but also in developing countries such as China, Brasil, Argentina, Indonesia and Malaysia, other oil sources, especially non-edible oils, need to be explored [20]. Microalgae seems to be the only source of renewable biodiesel that has the potential to completely displace petroleum-derived transport fuels without the controversial argument "food for fuel" and to reach the 2003 Biofuels Directive target, achieving more than a 35% minimum greenhouse gas savings (this value represents the diminishing impact of oleaginous crops including the land use change) [7–9]. Some time around the end of 2009 or in early 2010 is when small, commercial-scale algae-based systems for biodiesel production are likely to start entering the mainstream [4]. US and EU may realize their visions to replace up to 20% of transports fuels by 2020 by using environmentally and economically sustainable biofuels from algae [4].

Conclusions

Microalgal biodiesel is technically feasible and to be economic competitive with petrodiesel, microalgal production, harvesting and extraction must be optimized, as well as improvements to algal biology through genetic and metabolic engineering. The use of the biorefinery concept and advances in photobioreactor engineering will further lower the cost of production.

From the microalgae tested in this work, *Neochloris oleabundans* and *Nannochloropsis* sp. proved to be suitable as raw materials for biofuels production, due to their high oil content (29.0 and 28.7%, respectively). They are fresh water and marine microalgae, respectively, which enlarge the environmental cultivation possibilities and do not compete with food crops.

Both microalgae, when grown under N-deficient culture medium, show a great increase in oil quantity (e.g. *Neochloris oleabundans* can reach 56%, results not shown).

If the purpose is to produce biodiesel from one algal species, *Scenedesmus obliquus* presents the most adequate fatty acid profile, namely in terms of linolenic and polyunsaturated fatty acids. However, *Neochloris oleabundans*, *Nannochloropsis* sp. and *Dunaliella tertiolecta* can also be used if associated with other microalgae oils and/or vegetable oils.

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