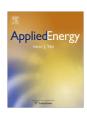
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Applied Energy

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



Advances and perspectives in using microalgae to produce biodiesel

Helena M. Amaro ^a, A. Catarina Guedes ^b, F. Xavier Malcata ^{c,d,*}

- a CIMAR/CIIMAR Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas no. 289, P-4050-123 Porto, Portugal
- b CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal
- ^c ISMAI Instituto Superior da Maia, Avenida Carlos Oliveira Campos, Castelo da Maia, P-4475-690 Avioso S. Pedro, Portugal
- d Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Avenida da República, P-2780-157 Oeiras, Portugal

ARTICLE INFO

Article history: Received 23 October 2010 Accepted 4 December 2010 Available online 12 January 2011

Keywords:
Biofuels
Microalgae
Biodiesel
Process engineering
Genetic engineering

ABSTRACT

Carbon-neutral renewable liquid biofuels are needed to displace petroleum-derived transport fuels in the near future - which contribute to global warming and are of a limited availability. A promising alternative is conveyed by microalgae, the oil content of which may exceed 80% (w/w_{DW}) – as compared with 5% of the best agricultural oil crops. However, current implementation of microalga-based systems has been economically constrained by their still poor volumetric efficiencies – which lead to excessively high costs, as compared with petrofuel prices. Technological improvements of such processes are thus critical - and this will require a multiple approach, both on the biocatalyst and bioreactor levels. Several bottlenecks indeed exist at present that preclude the full industrial exploitation of microalgal cells: the number of species that have been subjected to successful genetic transformation is scarce, which hampers a global understanding (and thus a rational design) of novel blue-biotechnological processes; the mechanisms that control regulation of gene expression are not fully elucidated, as required before effective bioprocesses based on microalgae can be scaled-up; and new molecular biology tools are needed to standardize genetic modifications in microalgae - including efficient nuclear transformation, availability of promoter or selectable marker genes, and stable expression of transgenes. On the other hand, a number of pending technological issues are also present: the relatively low microalga intrinsic lipid productivity; the maximum cell concentration attainable; the efficiency of harvest and sequential recovery of bulk lipids; and the possibility of by-product upgrade. This review briefly covers the state of the art regarding microalgae toward production of biofuels, both from the point of view of the microalgal cell itself and of the supporting bioreactor; and discusses, in a critical manner, current limitations and promising perspectives in this field.

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1. Introduction

The world has been confronted in recent decades with an energy crisis, associated with irreversible depletion of traditional sources of fossil fuels; their use as major form of energy is indeed unsustainable, further to accumulation of greenhouse gases in the atmosphere that bring about global warming. With the urgent need to reduce carbon emissions, and the dwindling reserves of crude oil, liquid fuels derived from plant material (also termed biofuels) appear to be an attractive alternative source of energy. Compared with other forms of renewable energy (e.g. wind, tidal and solar), biofuels allow energy to be chemically stored, and can also be used in existing engines and transportation infrastructures after blending to various degrees with petroleum diesel [1]. This biodie-

E-mail address: fmalcata@ismai.pt (F.X. Malcata).

sel is in essence a set of monoalkyl esters of long-chain fatty acids – and at present is derived chiefly from the acylglycerols of plant oils. Besides renewable, biodiesel is also non-toxic and biodegradable [2].

Currently, the most widely available form of biodiesel comes from such oil crops as palm, oilseed rape and soybean. However, several concerns have been raised about sustainability of this mode of production: to produce 2,500 billion liters of biodiesel from oilseed rape (i.e. the current demand of petroleum diesel in the whole UK), 17.5 Mha would be required for plantation – i.e. more than half the land area of UK itself! Moreover, the overall savings in energy and greenhouse gas emissions if the lifecycle of biofuel is considered as a whole are typically below what is normally anticipated; e.g. for biodiesel from oilseed rape or soya [3], a lifecycle assessment indicates that ca. 50% of the energy contained in the fuel will be spent in biodiesel processing itself [4].

This is why a renewed interest has arisen in recent years towards producing biodiesel from microalgae [19]. Microalgae clearly present a few advantages: they have much higher biomass

^{*} Corresponding author at: ISMAI – Instituto Superior da Maia, Avenida Carlos Oliveira Campos, Castelo da Maia, P-4475-690 Avioso S. Pedro, Portugal. Tel.: +351 968 017 411; fax: +351 229 825 331.

productivities than land plants (doubling times may be as short as 3.5 h), some species can accumulate up to 20-50% (w/w_{DW}) triacylglycerols, while no high quality agricultural land is required to grow the biomass – and even no land at all, if offshore farming is considered [1,4]. In spite of their growth in aqueous media, microalgae need lower rates of water renewal than terrestrial crops need as irrigation water, so the load on freshwater sources is strongly reduced – and microalgae may actually be cultivated in brackish water (thus avoiding herbicide or pesticide application, and permitting use as nutrients of NH $_4^+$, NO $_3^-$ and PO $_3^+$ that contaminate effluents from agrofood processing), requiring only sunlight and a few simple and non-expensive nutrients (including those associated with local, specific environments) [5,6], and on non-arable land (thus minimizing environmental impacts or otherwise) [1,7].

Finally the biomass resulting after oil extraction can be processed into ethanol or methane to be used also as biofuels, as well as into livestock feed – or simply used as organic fertilizer, owing to its high N:P ratio; or merely burned for energy cogeneration (electricity and heat) [1,7]. Alternatively, a wide range of fine chemicals or bulk products – e.g. polyunsaturated fatty acids, natural dyes and antioxidants, may also be extracted from spent biomass, depending on the species at stake [8]. Furthermore, 1 kg of dry algal biomass requires ca. 1.83 kg of CO₂, which can readily be obtained from industrial flue gases via bio-fixation [9].

2. Choice of wild microalga species

Many microalgal species can be induced to accumulate substantial contents of lipids [10]; although average lipid contents vary between 1% and 70%, some species may reach 90% (w/w_{DW}) under certain conditions [9,11,12] – see Table 1.

Several differences are apparent in Table 1 among the various species listed, and even within the same genus. The oil content in microalgae reaches 75% (w/w_{DW}) in *Botryococcus braunii*, but is associated with a low productivity – and much of it is secreted into

the cell wall [13]. The most common microalgae (viz. *Chlorella*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum* and *Porphyridium* spp.) possess oil levels between 20% and 50%, along with interesting productivities; *Chlorella* appears in particular to be a good option for biodiesel production. One can also conclude on greater lipid productivities of marine microalgae – which make them more prone to mass production, coupled with realisation that a high salinity prevents extensive contamination, while allowing sea water to be directly used instead of depleting fresh water resources.

Besides high productivities, selection of the most adequate species for biodiesel production should take other factors into account - e.g. the ability to uptake available nutrients or grow under specific environmental conditions. The fatty acid profile of the microalgal cell is also relevant, because the heating power of the resulting biodiesel hinges upon that composition; most of said moieties are saturated and unsaturated fatty acids containing 12–22 carbon atoms, often of the ω 3 and ω 6 types. Thomas et al. [14] analyzed the fatty acid compositions of seven fresh water microalga species; all of them could synthesize C14:0, C16:0, C18:1, C18:2 and C18:3 fatty acids - whereas the relative contents of other fatty acid residues were species-specific, e.g. C16:4 and C18:4 in Ankistrodesmus sp., C18:4 and C22:6 in Isochrysis sp., C16:2, C16:3 and C20:5 in Nannochloris sp., and C16:2, C16:3 and C20:5 in Nitzschia sp. Note that different nutritional and processing factors, cultivation conditions and growth phases will likely affect the fatty acid composition of microalgae: e.g. nitrogen deficiency and salt stress induced accumulation of C18:1 in all species, and of C20:5 to a lesser extent in B. braunii [7,14]. In what concerns CO₂ assimilation, the final destination also varies between species; e.g. at 10% (v/v) CO₂, the biomass productivity and C-fixation ability of Scenedesmus sp. is particularly high, whereas B. braunii is more suitable for biodiesel production due to its high proportion of oleic acid [15].

A multicriterion-based strategy ought thus to be considered when selecting a specific wild microalga strain, including a balance

Table 1Lipid content and productivity of various marine and freshwater microalga species [7].

	Microalga species	Lipid content (%, w/w _{DW})	Lipid productivity (mg L^{-1} d ⁻¹)
Fresh water	Botryococcus sp.	25.0-75.0	-
	Chaetoceros muelleri	33.6	21.8
	Chaetoceros calcitrans	14.6-16.4/39.8	17.6
	Chlorella emersonii	25.0-63.0	10.3-50.0
	Chlorella protothecoides	14.6-57.8	1214
	Chlorella sorokiniana	19.0-22.0	44.7
	Chlorella vulgaris	5.0-58.0	11.2-40.0
	Chlorella sp.	10.0-48.0	42.1
	Chlorella pyrenoidosa	2.0	_
	Chlorella sp.	18.0-57.0	18.7
	Chlorococcum sp.	19.3	53.7
	Ellipsoidion sp.	27.4	47.3
	Haematococcus pluvialis	25.0	_
	Scenedesmus obliquus	11.0-55.0	_
	Scenedesmus quadricauda	1.9-18.4	35.1
	Scenedesmus sp.	19.6–21.1	40.8-53.9
Marine water	Dunaliella salina	6.0-25.0	116.0
	Dunaliella primolecta	23.1	_
	Dunaliella tertiolecta	16.7-71.0	_
	Dunaliella sp.	17.5-67.0	33.5
	Isochrysis galbana	7.0-40.0	_
	Isochrysis sp.	7.1-33	37.8
	Nannochloris sp.	20.0-56.0	60.9-76.5
	Nannochloropsis oculata	22.7-29.7	84.0-142.0
	Nannochloropsis sp.	12.0-53.0	60.9-76.5
	Neochloris oleoabundans	29.0-65.0	90.0-134.0
	Pavlova salina	30.9	49.4
	Pavlova lutheri	35.5	40.2
	Phaeodactylum tricornutum	18.0-57.0	44.8
	Spirulina platensis	4.0-16.6	-

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