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Claudios scientific interests are applied research in marine and environmental science, biotechnology, bioenergy and marine microalgal production.

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# DINOFLAGELLATES AS FEEDSTOCK FOR BIODIESEL PRODUCTION

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## 1. Introduction

The biotechnological use of microalgae biomass for biofuel production has been developing rapidly over the last few years (Chisti, 2007; Hu et al., 2008). Most of the known microalgae already used for biodiesel production are freshwater microalgae from the chlorophycean group. Target species for biomass production have traditionally been those with a known growth cycle, fast cell growth and those that usually were cultivated for other aims, as a protein source such as *Tetraselmis suecica*, *Spirulina platensis* or those for aquaculture activities such as *Isochrysis galbana, Nannochloropsis oculata* (Rodolfi et al., 2008; Chu et al., 2009) and others that produce special metabolites such as *Haematococcus pluvialis* (Grewe and Griehl, 2008) or *Scenedesmus almeriensis* (Sánchez et al., 2008), which are widely used in industry in the synthesis of pigments and as a food additives. Microalgae are composed, at the cellular level, of varying percentages of lipids, proteins, and carbohydrates. Lipids, especially the polar glycolipids (phospholipids) fraction, function as a structural component of microalgal cell membranes, but the lipids also modulate cellular activity and energy storage. In fact, one of the main biological functions of neutral lipids (triacylglycerol, TAG) is to provide energy for immediate and delayed metabolic requirements (Geider and La Roche, 2002). The values of oil concentration in microalgae vary between 20 and 50% of their total biomass (Chisti, 2007). Accordingly, some microalgae have the potential to synthesize 30 times more oil per hectare than terrestrial plants (Sheehan et al., 2006), and this encourages the use for biofuel production (Hu et al., 2008).

Nowadays, most of the microalgae used for biodiesel production are mainly "green microalgae". These species use principally freshwater as a liquid growth medium, and therefore, strains can be cultivated in countries where this strategic resource is found in abundance. Taking into account that freshwater element would be a problematic resource in the near future (Griffiths and Harrison, 2009;

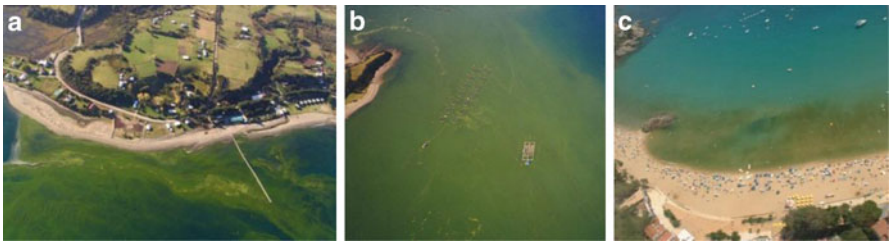


**Figure 1.** Aerial photograph of phytoplankton blooms. (**a**) and (**b**) *Gymnodinium* cf. *chlorophorum,* south Chile, 42° S, 71° W (photograph courtesy of Plancton Andino Ltda.). (**c**) *Alexandrium taylori*, La Fosca, Catalonia, Spain, 41.5° N, 03° E (Photograph courtesy of PBL group, ICM-CSIC).

Grobbelaar, 2010), it is highly recommended exploring marine strains to significantly reduce the water footprints (Yang et al., 2011). The use of local or "autochthonous" marine microalgae from the same growth location is needed, since this strategy allows the use of the same environmental parameters when cultured in enclosed systems (Morweiser et al., 2010). The use of local marine microalgae avoids the introduction of exotic organisms and any possible ecological problems.

## 2. Dinoflagellates and Raphidophytes Microalgal Groups

Strains with different growth strategies adapted to live in seawater and with high fatty acids content as neutral lipids or triacylglycerols (TAG) are found naturally in two groups of microalgae, the dinoflagellates and raphidophytes. Most of the dinoflagellates and raphidophytes can grow and produce large blooms under natural conditions. These blooms can cover from up to a few hundred metres (Fig. 1c) to hundreds of kilometres (Fig. 1a, b) and reach cell abundances of million cells per litre. Bloom-forming species have a cosmopolitan distribution, and they can easily be isolated from their environment; this characteristic makes them strategic marine strains because they can grow under local natural conditions in coastal countries around the world. Usually, these microalgae form patches in the upper layers at the surface of the sea, or proliferate in zones where abiotic conditions in the water column such as temperature, density, salinity or nutrient concentration are adequate to trigger rapid growth.

Certain species of dinoflagellates and raphidophytes have been studied for decades from an ecological and physiological point of view (Anderson, 1989; Smayda, 1997) because they are associated in some cases with toxic and noxious events such as massive mortalities of different marine organisms, from small crustaceans (e.g. copepods) to large mammals (e.g. sea lions). The anthropic interest in these noxious organisms has allowed for an extensive development in their ecological research. However, their use for energetic aims is poorly known. In fact, in spite of their negative effect on the environment, these microalgae group could be an interesting and strategic group that can produce natural oils.

It is well established that dinoflagellates tend to have lower growth rates compared to other microalgae taxa with similar cell size (Tang, 1996) and they reflect the lower photosynthetic capacity per unit of biomass of dinoflagellates (Chan, 1980; Tang, 1995). It appears that the low growth rate of dinoflagellates is compensated by their high size and biovolume when these groups are compared with the green algae groups in terms of biomass concentration or cell carbon content (Tang, 1995).

Dinoflagellates and raphidophytes have a huge range of size varying from 8 to 10 μm up to macroscopic size of >0.5 mm. Their biovolume is significantly different from other algae classes. Table 1 shows the range of biovolume for dinoflagellates and raphidophytes compared to other microalgae classes. Dinoflagellates and raphidophytes show biovolume values from 400 up to 88.000 $\mu m^3$ (Smayda, 1997; Stolte and Garcés, 2006; Olenina et al., 2006) which are several orders of magnitude higher, even, in the carbon content than the green algae (Tang, 1995). This characteristic could be useful in terms of lipid storage, using the hypothesis that they can accumulate more carbon per cell and they can transform it in lipids. Contrarily, cell density in autotrophic cultures of dinoflagellates and raphidophytes achieves concentration of $10^5$*$mL^{-1}$ (in enclosed system or photobioreactor PBR), two orders of magnitude lower than the green algae group ($10^7$*$mL^{-1}$) in a similar production system. Although this difference exists in cell abundance, the equilibrium between cell abundance and biovolume leads to a final biomass production (in terms of grams of dry weight per litre) quite similar to both groups of algae. This can be explained due to the differences in biovolumes when comparing green algae against dinoflagellate and raphidophyte classes (Table 1).

## 3. Strains Growth in Microalgae

In terms of the growth rate, the green algae usually has a duplication time of hours during the exponential phase, and their entire growth curve in batch cultures takes just a few days (between 6 and 20 depending on the species and the culture strategy used). Most of the studies reviewed for dinoflagellates and raphidophytes show growth rates from natural populations where they develop blooms, reaching occasionally fast growth (Stolte and Garcés, 2006). This natural behaviour in the environment is not comparable in controlled culture conditions. In most of the species, it is necessary to determine the abiotic parameters as temperature, salinity, turbulence, quantity and quality of light and sufficient nutrient concentration that regulate growth in controlled culture conditions. In outdoor conditions where the advantage is the natural source of energy provided from the sun, these parameters are almost unknown for dinoflagellates and raphidophytes. Every microalgae species has their own specific requirement in terms of abiotic parameters, culture performance or turbulence regime. The key to a successful microalgal biomass production will be to characterize, test and improve those parameters that affect the growth of autotrophic microalgal cells.

**Table 1.** Biotic parameters of different dinoflagellate and raphidophyte species compared with those that usually are used for biomass production.

| Class | Species | Growth rate div*day$^{-1}$ ± SD | Biovolume ($\mu m^3$ ± SD) | Biomass productivity (g*L$^{-1}$*day$^{-1}$) | Lipid content (% DW) | References |
|---|---|---|---|---|---|---|
| Dinophyceae | *Alexandrium andersoni* | 0.10 | – | – | – | (3) |
| Dinophyceae | *Alexandrium catenella* | 0.22 ± 0.02 | – | – | – | (2) |
| Dinophyceae | *Alexandrium insuetum* | 0.40 ± 0.33 | – | – | – | (2) |
| Dinophyceae | *Alexandrium insuetum* | 0.06 | – | – | – | (3) |
| Dinophyceae | *Alexandrium minutum* | 0.87 ± 0.14 | – | – | – | (2) |
| Dinophyceae | *Alexandrium minutum* | 0.18 | 3,260 ± 917 | 0.40 ± 0.1 | 23 | (3) |
| Dinophyceae | *Alexandrium tamarense* | 1.03 ± 0.13 | 16,485 | – | – | (1) |
| Dinophyceae | *Alexandrium tamarense* | 0.23 | 17,684 | – | – | (2) |
| Dinophyceae | *Alexandrium taylorii* | 0.31 ± 0.26 | – | – | – | (2) |
| Dinophyceae | *Amphidinium carterae* | 2.89 ± 0.08 | 432 | – | – | (1) |
| Dinophyceae | *Ceratium furca* | 0.44 ± 0.22 | 50,000 ± 20,000 | – | – | (2) |
| Dinophyceae | *Ceratium tripos* | 0.17 | 88,420 ± 74,540 | – | – | (2) |
| Dinophyceae | *Dinophysis acuminata* | 0.36 ± 0.22 | 16,211 ± 10,037 | – | – | (2) (4) |
| Dinophyceae | *Dinophysis acuta* | 0.38 ± 0.04 | 63,150 ± 33,936 | – | – | (2) (4) |
| Dinophyceae | *Dinophysis norvegica* | 0.36 ± 0.23 | 41,945 ± 26,075 | – | – | (2) (4) |
| Dinophyceae | *Dinophysis tripos* | 0.21 | 37,680 | – | – | (2) (4) |
| Dinophyceae | *Gymnodinium* spp. | 0.20 ± 0.11 | 65,765 ± 52,758 | – | 13 | (2) (4) |
| Dinophyceae | *Glenodinium halli* | 2.19 ± 0.11 | – | – | – | (1) |
| Dinophyceae | *Gymnodinium resplendens* | 1.53 | 8,146 | – | – | (1) (4) |
| Dinophyceae | *Gymnodinium nelsoni* | 1.43 ± 0.17 | – | – | – | (1) |
| Dinophyceae | *Heterocapsa triquetra* | 0.08 ± 0.06 | 2,295 ± 1,405 | – | – | (2) |
| Dinophyceae | *Heterocapsa triquetra* | 1.34 ± 0.22 | 419 ± 213 | – | – | (1) |
| Dinophyceae | *Heterocapsa pygmaea* | 2.12 ± 0.30 | – | – | – | (1) |
| Dinophyceae | *Karlodinium* sp. | 0.60 ± 0.20 | – | – | – | (2) |
| Dinophyceae | *Karlodinium veneficum* | 0.39 ± 0.02 | 494 ± 61 | 0.21 ± 0.07 | 27 | (3) (5) |
| Dinophyceae | *Lingulodinium polyedra* | 0.86 | 28,157 ± 12,719 | – | – | (1) |
| Dinophyceae | *Prorocentrum minimum* | 0.21 | 1,240 ± 351 | – | – | (2) (4) |
| Dinophyceae | *Prorocentrum triestinum* | 0.14 | – | – | – | (2) |
| Dinophyceae | *Prorocentrum minimum* | 2.37 ± 0.12 | 1,240 ± 351 | – | – | (1) (4) |
| Dinophyceae | *Prorocentrum redfieldii* | 2.17 ± 0.22 | – | – | – | (1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Dinophyceae | *Prorocentrum micans* | 2.04 ± 0.10 | 20,292 ± 10,379 | – | – | (1) (4) |
| Dinophyceae | *Scrippsiella trochoidea* | 1.25 ± 0.22 | 5,505 ± 3,873 | – | 16 | (1) |
| Dinophyceae | *Scrippsiella trochoidea* | 0.11 | 5,505 ± 3,873 | – | 16 | (3) |
| Raphidophyceae | *Heterosigma akashiwo* | 0.17 | 1,020 ± 249 | 0.30 ± 0.04 | 24 | (3) |
| Eustigmatophyceae | *Nannochloropsis* sp. | 0.36 ± 0.06 | 14 | 0.19 ± 0.02 | 33 | (6) (7) |
| Prymnesiophyceae | *Isochrysis* sp. | 0.22 ± 0.03 | 69 | 0.16 ± 0.02 | 29 | (7) |
| Prasinophyceae | *Tetraselmis suecica* | 0.23 ± 0.01 | – | 0.30 ± 0.02 | 10 | (3) (6) |
| Chlorophyceae | *Chlorella vulgaris* | 0.13 ± 0.01[a] | – | 0.18 ± 0.02 | 19 | (7) (8) |
| Cryptophyceae | *Rhodomonas* sp. | 0.28 ± 0.03 | 112 ± 38 | – | 13 | (4) (5) (6) |

Key to references = (1) Smayda (1997), (2) Stolte and Garcés (2006), (3) Fuentes-Grünewald et al. (2009), (4) Olenina et al. (2006), (5) Calbet et al. (2011), (6) Huerlimann et al. (2010), (7) Rodolfi et al. (2008), (8) Converti et al. (2009).

[a]Average of growth rates in cultures under different temperatures.

Among the target microalgae studied in the work of Fuentes-Grünewald et al. (2009), the growth of *Karlodinium veneficum* was the highest, 0.14 $day^{-1}$ in the exponential phase, corresponding to an abundance of $44 \times 10^6$ cells $L^{-1}$ at day 30 of culture. For *Heterosigma akashiwo*, maximum abundance was approximately $26 \times 10^6$ cells $L^{-1}$ at day 35 of culture, reflecting a growth rate of 0.10 $day^{-1}$ (1 division every 10 days). *K. veneficum* could reach growth rate of 0.47 $day^{-1}$ in photobioreactors. Among the species examined in the study, this raphidophyte was unique in that cell abundance was maintained for more than 6 months (data not shown). Moreover, the cells remained healthy without the addition of fresh medium. This was in contrast to the other cultures, which gradually decayed such that total cell lysis has occurred ~2 months after inoculation. The growth rate of dinoflagellates belonging to the genus *Alexandrium* differed depending on the species. The highest growth rate was that of *A. andersoni*, 0.10 $day^{-1}$ similar to that of the raphidophyte *H. akashiwo*, but the cell abundance of the former (maximum of $9 \times 10^6$ cells $L^{-1}$) was lower than that of the raphidophyte. The growth rates of *A. minutum* and *A. catenella* were two orders of magnitude slower (0.04 and 0.03 $day^{-1}$, respectively) than those of faster-growing microalgae. In terms of abundance, *A. minutum* reached a maximum of $2.6 \times 10^6$ cells $L^{-1}$ and *A. catenella* a maximum of $9.4 \times 10^5$ cells $L^{-1}$ at culture days 36 and 35, respectively. Among the Dinophyceae, *K. veneficum* showed the best performance in terms of growth, although the rate measured in this study was much lower than that of wild populations (Stolte and Garcés, 2006). Nonetheless, it was high enough to yield a large biomass in culture within a reasonable period of time. Further studies will be needed to determine whether the growth rate in culture can be improved, e.g. by isolating new strains and/or inoculating the cells in exponential phase before the maximum growth rate is established.

## 4. Lipids in the Target Microalgae

The approximate elemental compositions of eukaryotic photosynthesizing microalgae show different percentages of carbohydrates (5–45%), proteins (30–65%) and lipids (10–50%) in their cell mass (Geider and La Roche, 2002). This biochemical composition could change with the culture growth phase (culture age) or under environmental changes that cause cell stress. Lipids, the polar and the neutral fraction, are the source of oil in cells. The polar and the neutral fraction have different roles inside the cells, while the polar fraction is composed principally of phosphoglycerides which are use as structural component in cell membranes; neutral lipids serve as energy storage reserves (Geider and La Roche, 2002; Guschina and Harwood, 2006). With regard to lipid content, most of the algae usually utilized for biodiesel purposes (e.g. *Chlorella* species) show concentrations ranging from 22 to 38% of dry weight in standard conditions of growth (Chen and Yeh, 2011). Nevertheless, after a review of the literature, only a few studies presented results in lipid content of dinoflagellates and raphidophytes,

which varied from 13 to 27% of dry weight in standard growth conditions (Table 1). However, strictly for energetic purposes, there is a lack of data on lipid content, lipid and biomass productivity, TAG production in dinoflagellates and raphidophytes cultured in different systems, and conditions for long-term microalgal biomass production.

Contrary to the lack of quantitative data on total lipid content in dinoflagellates and raphidophytes, there are many studies on lipid profile of these groups primarily done as a tool to identify different genera or species, or as a biomarker in the trophic food chain in the ocean (Mansour et al., 1999; Leblond and Chapman, 2000; Reuss and Poulsen, 2002; Marshall et al., 2002; Fiorillo and Rossi, 2010). Data obtained from 62 dinoflagellate species and 11 raphidophyte species showed a long list of fatty acids presented in dinoflagellates and raphidophytes. These studies identified 58 different fatty acids, from short carbon chain as undecaenoic acid (C11:0) to long-chain carbon fatty acid as octacosaenoic acid (C28:0 (Table 2)). In general, the saturated fatty acids (SAFA) are those fatty acids that work as precursor for other monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (Guschina and Harwood, 2006). Short-chain saturated such

**Table 2.** List of fatty acids present in the lipid profile of dinoflagellates and raphidophytes.

| Fatty acid name | Nomenclature |
|---|---|
| Undecaenoic acid | C11:0 |
| Lauric acid | C12:0 |
| Tridecaenoic acid | C13:0 |
| Myristic acid | C14:0 |
| Myristoleic acid | C14:1 |
| | C14:2 |
| Pentadecaenoic acid | C15:0 |
| Cis-10-pentadecenoic acid | C15:1 |
| Palmitic acid | C16:0 |
| Palmitoleic acid | C16:1 |
| | C16:1 *n*5 |
| | C16:1 *n*7 |
| | C16:1 *n*13 |
| | C16:2 *n*4 |
| | C16:2 *n*6 |
| | C16:2 *n*7 |
| | C16:3 |
| | C16:3 *n*3 |
| | C16:4 *n*1 |
| | C16:4 *n*3 |
| Heptadecaenoic acid | C17:0 |
| Cis-10-heptadecaenoic acid | C17:1 |
| Stearic acid | C18:0 |
| Oleic acid | C18:1 *n*3 |
| Elaidic acid | C18:1 *n*7 |

(continued)

**Table 2.** (continued)

| Fatty acid name | Nomenclature |
|---|---|
| | C18:1 *n*9 |
| | C18:1 *n*13 |
| Linoleic acid | C18:2 *n*6 |
| Linolenic acid | C18:3 *n*3 |
| Gamma-linolenic acid | C18:3 *n*6 |
| | C18:4 *n*3 |
| | C18:5 *n*3 |
| Arachidic acid | C20:0 |
| Cis-11-eicosenoic acid | C20:1 |
| | C20:1 *n*9 |
| | C20:2 |
| Cis-11-14-eicosadienoic acid | C20:2 *n*6 |
| | C20:2 *n*9 |
| Cis-11,14,17-eicosatrienoic acid | C20:3 *n*3 |
| Cis-8,11,14,17-eicosatrienoic acid | C20:3 *n*6 |
| Arachidonic acid (AA) | C20:4 *n*3 |
| | C20:4 *n*6 |
| Cis-5,8,11,14,17-eicosapentaenoic (EPA) | C20:5 *n*3 |
| | C20:6 *n*3 |
| Heneicosaenoic acid | C21:0 |
| Behenic acid | C22:0 |
| Erucic acid | C22:1 *n*9 |
| | C22:1 *n*11 |
| Cis-13,16-docosadienoic acid | C22:2 |
| | C22:5 *n*2 |
| | C22:5 *n*3 |
| | C22:5 *n*6 |
| Cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) | C22:6 *n*3 |
| Tricosaenoic acid | C23:0 |
| Lignoceric acid | C24:0 |
| Nervonic acid | C24:1 *n*9 |
| | C28:8 *n*3 |
| Octacosaenoic acid | C28:y |

Data was obtained from 62 dinoflagellate species and 11 raphidophyte species from Fernández-Reiriz et al. (1989), Viso and Marty (1993), Mansour et al. (1999), Leblond and Chapman et al. (2000), Marshall et al. (2002), Leblond et al. (2006), Mooney et al. (2007), Usup et al. (2008), Xu et al. (2006), Giner et al. (2008), Chu et al. (2009), Dorantes-Aranda et al. (2009) and De Boer et al. (2009).

as lauric acid C12:0 or palmitic acid C16:0 are the initial step of the metabolic pathways of fatty acid synthesis and were found to be the main components of neutral lipids (Molina Grima et al., 1994, 1995); by contrast, the PUFA portion or those fatty acids with long-chain carbon are involved in the construction process of cells and work as structural lipids, mainly found in glycolipids and phospholipids fraction (Molina Grima et al., 1995), and their content in autotrophic cells are associated due to the state of growth in microalgae cultures.

Reports indicate that the principal fatty acid portion presented in the dinoflagellates and raphidophytes reviewed is the SAFA fraction, and the main contributors to this fraction were palmitic acid C16:0 and stearic acids C18:0. Other fatty acids identified corresponded to the MUFA fraction; the main contributor to this fraction was the oleic acid C18:1*n*3. It was also observed in the PUFA fraction that the principal fatty acids with a high economical value was for eicosapentaenoic acid or EPA (C20:5*n*3) and docosahexaenoic acid or DHA (C22:6*n*3). The SAFA fraction and especially the palmitic acid (C16:0) and the stearic acid (C18:0) were present in 89% of the reviewed fatty acid profile of dinoflagellate and raphidophyte species, with an average of 18.4% of C16:0 and 6.7% of C18:0. Interestingly, a significant amount of the PUFA, docosahexaenoic acid (DHA) C22:6*n*3, a high-value fatty acid molecule, was found in the 40% of species reviewed, and the average DHA concentration was around of 8.3% of the lipid profile. The polyunsaturated fatty acids C20:5*n*3 or eicosapentaenoic acid (EPA) was present in 48% of the reviewed species with an average of 2.1% of the lipid profile, and the monounsaturated oleic acid (C18:1*n*3) was present in 14% of the species with an average of lipid concentration of 0.6 %. This and other important and essential fatty acids that cannot be synthesized by the human body but are vital for normal metabolism were found in the reviewed dinoflagellates and raphidophytes and are shown in Fig. 2.

A summary of the fatty acid profile of the dinoflagellates and raphidophytes reviewed is shown in Fig. 3. The graph shows specifically the percentage of SAFA, MUFA and PUFA per species. Most of the dinoflagellates have a SAFA concentration of 35% up to 60%. The most abundant fatty acids expressed by dinoflagellates during their growth were those of the C16:0, C18:0 (Fig. 2). The second major group of fatty acids was the MUFA portion, with an average of 20% for all dinoflagellate species reviewed. PUFA portion is high but only in some species. High values of PUFA are not useful for biodiesel production, but some can be valuable as a by-product such as C22:6*n*3 or C20:5*n*3 (Chi et al., 2009). Comparison of the fatty acid profile of the dinoflagellate and raphidophyte strains and terrestrial plants that are commonly used as oil feedstock (palm oil, soybean oil, sunflower oil, olive oil) for biodiesel production shows that most of the dinoflagellate species and some species of raphidophytes have a close fatty acid profile to palm oil (Fig. 3). Based on their fatty acids, the results of comparison among other oil feedstock allow us to infer that these groups of algae have an interesting lipid profile for biodiesel production (Fig. 3).

The fatty acid profile is one of the main characteristics to take into account when we are screening feedstock for biodiesel purposes because, depending on the composition of fatty acids, the biofuel obtained contains different qualities. The requirements of a specific lipid profile for biodiesel purposes have been analysed in the last few years, and according to the American Society for Testing and Materials (ASTM) D6751, (ASTM D6751–08 (2008), to obtain a biodiesel, the raw material has to comply different characteristics such as the cetane number, fatty acid profile and oxidation stability among others. The main desirable

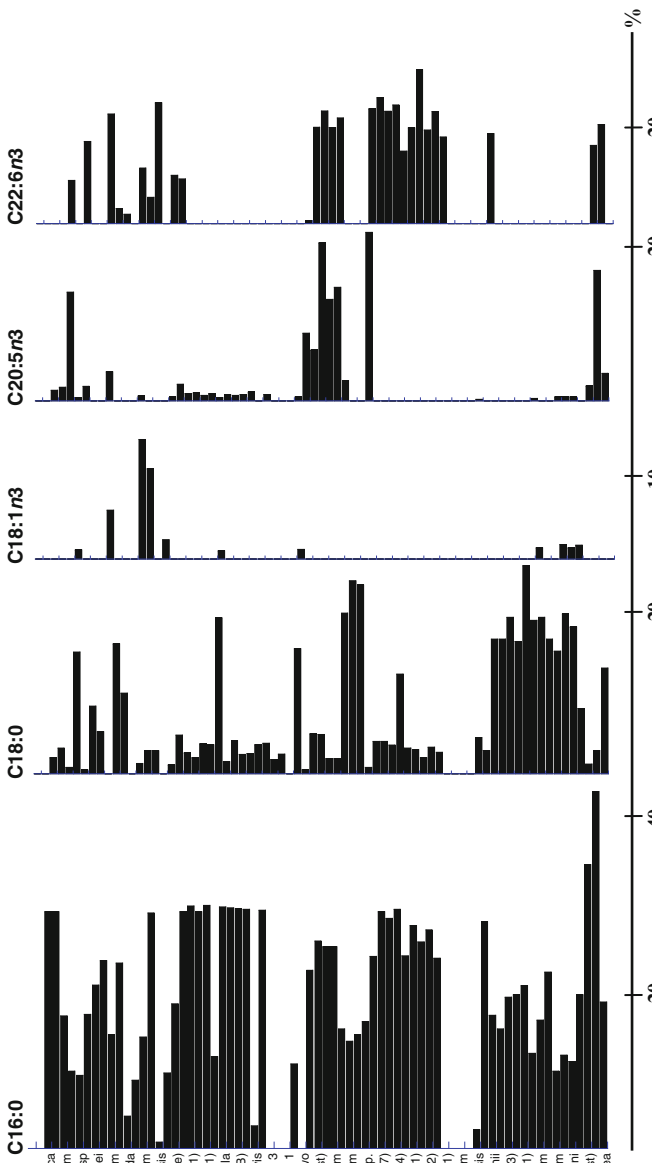


**Figure 2.** Principal fatty acids found in dinoflagellate and raphidophyte species reviewed. Data was obtained from 62 dinoflagellate and 11 raphidophyte species as in Table 2. C16:0 palmitic acid, C18:0 stearic acid, C20:5*n*3 eicosapentaenoic acid, C18:1*n*3 oleic acid, C22:6*n*3 docosahexaenoic acid (Fernandez-Reiriz et al., 1989; Viso and Marty, 1993; Mansour et al., 1999; Leblond and Chapman et al., 2000; Marshall et al., 2002; Leblond et al., 2006; Mooney et al., 2007; Usup et al., 2008; Xu et al., 2006; Giner et al., 2008; Chu et al., 2009; Dorantes-Aranda et al., 2009; De Boer et al., 2009).

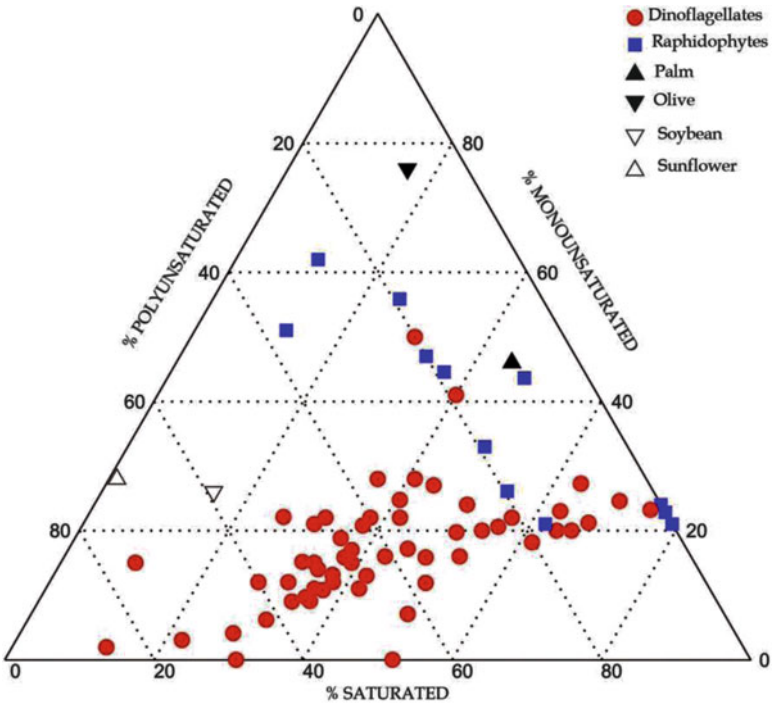


**Figure 3.** Fatty acid profile of dinoflagellates and raphidophytes, compared with the common oils used for biodiesel production. *Axis* shows the percentage of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. References as in Fig. 2.

characteristics of the biofuel obtained from vegetable oils are good oxidation stability (long-term storage) and a high-cetane number (good ignition capabilities). In a recent study, Sanford et al. (2010) compared and evaluated the characteristic of 36 different types of oil feedstock for biodiesel production, including microalgae.

The characteristic of the biodiesel analysed of algae oil meets the allowable limits, except flash point (>93°C) and oxidation stability (>3 h). For flash point, all the oils tested did not pass the test, and in the case of the oxidation stability, their results could be due to the fact that the fatty acid profile of the microalgae utilized in Sanford's work had a high percentage of monounsaturated fatty acids >70% with a consequent high oxidation stability, concluding that the fatty acid profile of the analysed algae was not adequate. But the author did not specify the algae strain used in their study, and the highest percentage presented (70%) probably corresponded to the "green" algae group, the most known group. Therefore, if the objective was to produce biodiesel from microalgae, a low percentage of MUFA and PUFA and high concentration of SAFA portion in the fatty acid profile are required and desirable, and this fatty acid profile is found in several species of dinoflagellates and raphidophytes (Fuentes-Grünewald et al., 2009).

### 4.1. Enhanced Lipid Production in Target Microalgae

In many studies on Chlorophyceae, Dinophyceae, Raphidophyceae and Eustigmatophyceae, it has been demonstrated that the fatty acid profile and lipid concentration on microalgae, specifically the neutral portion, increase in stationary phase parallel to a nutrient depletion in the culture (Mansour et al., 2003; Li et al., 2008; Liang et al., 2009; Widjaja et al., 2009; Fuentes-Grünewald et al., 2009). The growth curve of microalgae has three well-known steps: condition or lag phase, exponential phase and stationary phase. During the initial lag phase (a few hours or day depending on the species), microalgae cells adapt to culture conditions. The exponential phase is characterized for a high cell division and an increase in the growth rate and biomass production. When nutrient conditions decrease, microalgae cells stop growth and reach the stationary phase; after the stationary phase, the cultures decline and cells die. During the stationary phase and due to the nutrient depletion, the fatty acids in dinoflagellates increase as its showing for *K. veneficum* in Fig. 4.

When the objective of the microalgal biomass production is to produce biodiesel, it is necessary to determine the best harvest time. Also, it is desirable to determine the abiotic variable or a combination that allows us a high biomass production (in a reasonable period of time) and consequently a high lipid concentration in cells. Nowadays, the biochemical engineering using abiotic parameters such as $CO_2$, light, nutrient depletion, temperature or salinity to obtain lipids from autotrophic microalgae cell is mostly known only for freshwater green algae such as *Chlorella, Nannochloropsis, Neochloris* and *Isochrysis* (Flynn et al., 1993; Li et al., 2008; Converti et al., 2009). However, until now, there is no published information about the use of biochemical engineering strategy that allows an improvement in lipid production in dinoflagellates and raphidophytes.

The main objective in the recent work of Fuentes-Grünewald et al. (2012a) was to determine the lipidic percentage in which the target species (*A. minutum, H. akashiwo* and *K. veneficum*) store neutral lipids, varying abiotic parameters such as temperature (15–20–25°C), aeration and $NO_3$ concentration (880, 660, 440, 220 μM). The cultures were grown in L1 media, in 2-l Nalgene bottles with a 12:12 (L:D) photoperiod. Cell abundance was monitored by cell counting using inverted microscope, and by measurements, chlorophyll concentration (μg Chl a*$L^{-1}$) was used as a biomass indicator. Spectrofluorometry readings were obtained dying the cells with Nile red for neutral lipid analysis. The results were verified by lipid extraction for gas chromatography analysis and show an important increase (>200%) with respect to the initial TAG concentration in *A. minutum*, especially in the cultures maintained under higher temperatures >25°C, inorganic dissolved N concentration ≈330 μM and continuous aeration conditions. *A. minutum* cells submitted to the treatment and control cells are shown in Fig. 5. The final lipid production is influenced by the physiological state of the inoculated cells, and it also depends on the number of cells inoculated. To find

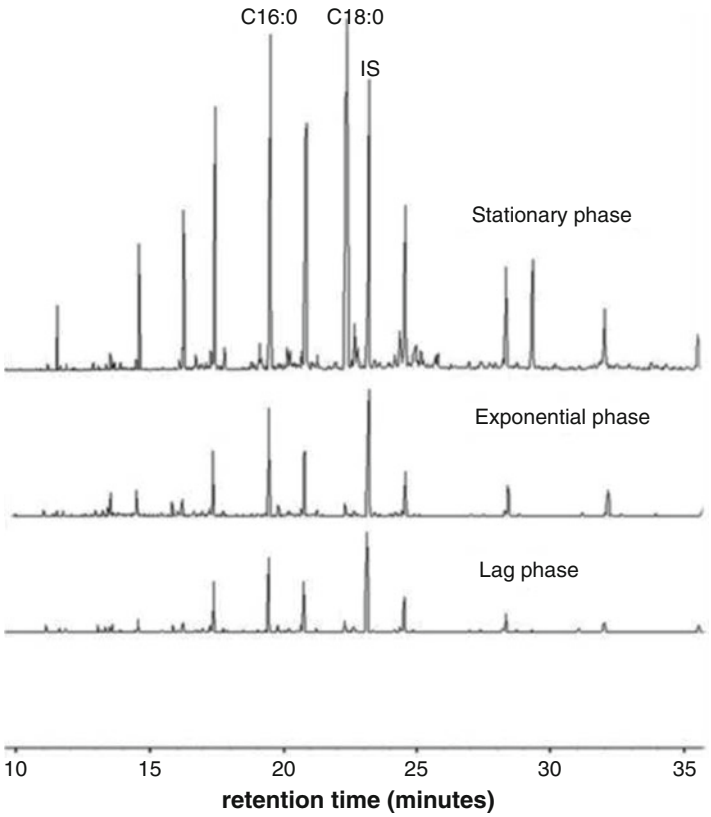


**Figure 4.** Chromatogram of different culture phases in *Karlodinium veneficum*; at the stationary phase, we observe the increase in their fatty acid profile (C16:0 palmitic acid; C18:0 stearic acid; IS internal standard).

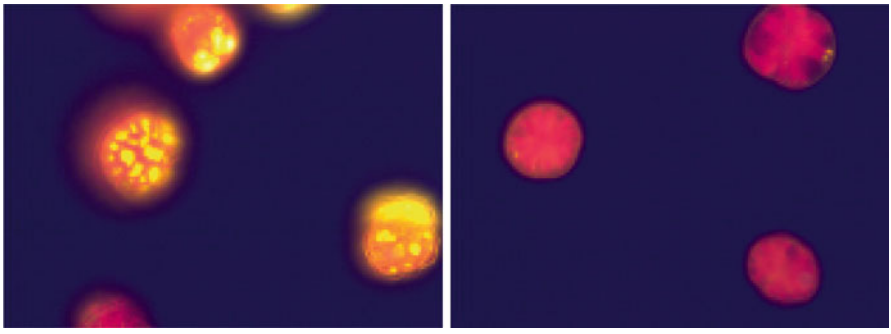

**Figure 5.** *Left photograph* normal condition (*Alexandrium minutum* cells), *right photograph* stress conditions. *Nile red* stain was used for TAG measurements 488/570 nm read by spectrofluorometer (Photograph from Fuentes-Grünewald et al., 2012a).

the balance between high biomass production and high lipid concentration will allow us to design culturing strategies for TAG production with energetic purposes (Fuentes-Grünewald et al., 2009).

## 5. Dinoflagellate Cultures: Indoor vs. Outdoor Conditions

Microalgae production used conventionally at outdoor conditions is largely more sustainable than indoor conditions in terms of energy. The main objective in the study of Fuentes-Grünewald et al. (2012b) was to evaluate and compare the growth of strains of dinoflagellate and raphidophyte species using bubble column photobioreactor at indoor and outdoor conditions (Barcelona location 41° 23′ 08.12 N–2° 11′ 45.84 E). To compare both conditions in terms of energy the biotic parameters in the target strains, it was quantified light, temperature, pH, cell yield, growth rate, biomass and lipid production. A bubble column vessel of Plexiglas with a working volume of 30 L in triplicate was used for both strategies. We tested the target strains: *A. minutum, K. veneficum* and *H. akashiwo.* A semicontinuous cultures at outdoor condition (using energy just for air pump) were established for 4 months, harvesting biomass and adding fresh medium depending on the growth rate of the species. Batch culture strategy was used at indoor conditions (using energy for air pump, light and temperature). At outdoor conditions, *H. akashiwo* cell yield was directly influenced by temperature, recording the highest growth rate (0.397 $day^{-1}$) and biomass (0.97 $g\,L^{-1}$ dry weight) when the range of temperature varied from 20 to 10°C (November–December). The three species decline their production in outdoor condition when a great amplitude of temperature >20°C was recorded or when the minimum temperature was near 0°C. At indoor condition, *H. akashiwo* shows a growth rate of 0.440 $day^{-1}$ and an average biomass production near to 1.17 $g\,L^{-1}$ of dry weight (Fuentes-Grünewald et al., 2012b).

## 6. Comparison of the Target Species Against the Commonest "Green Algae"

Despite different studies show that dinoflagellates have low growth rate compared with other microalgae taxa (Tang, 1995, 1996), the final biomass production of this group is higher in most of our strains compared with the green algae group (Table 3). This is due to the higher biovolume (total cell volume) of the species. Most of the strains of the chlorophycean group (*Chlorella, Neochloris, Chlamydomonas*), Prasinophyceae as *Tetraselmis* sp. or Prymnesiophyceae as *Isochrysis* spp., have small size and low biovolumes (Olenina et al., 2006). *Chlorella vulgaris* show an average of 13 $\mu m^3$, several orders smaller than our *Alexandrium minutum* strain with and an average biovolume of 2,856 $\mu m^3$. This can be an advantage for culturing the dinoflagellates in terms of biomass production, lipid accumulation and carbon storage, because, although growth rates and cell concentration usually are lower than green algae, the compared biomass productivity and lipid productivity are higher in *A. minutum* and *H. akashiwo* (Table 3) than the commonest freshwater phototrophic strains *Chlorella vulgaris* (Rodolfi et al., 2008; Gouveia and Oliveira, 2009).

**Table 3.** Comparison of growth rate, biomass productivity, lipid content and lipid productivity of different microalgae.

| Microalgae | Biomass productivity ($g*L^{-1}*day^{-1}$) | μ ($div*day^{-1}$) | Lipid content (% Biomass) | Lipid productivity ($mg*L^{-1}*day^{-1}$) | References |
|---|---|---|---|---|---|
| *Tetraselmis suecica* | 0.28 | – | 12.9 | 36.4 | Rodolfi et al. (2008) |
| *Tetraselmis* sp. | – | 0.19 | 10.1 | 22.7 | Huerlimann et al. (2010) |
| *Nannochloropsis* sp. | – | 0.41 | 32.7 | 20.0 | Huerlimann et al. (2010) |
| *Nannochloropsis* sp. | 0.21 | – | 29.6 | 61.0 | Rodolfi et al. (2008) |
| *Nannochloropsis* sp. | 0.09 | – | 28.7 | 25.8 | Gouveia and Oliveira (2009) |
| *Pavlova lutheri* | 0.14 | – | 35.5 | 50.2 | Rodolfi et al. (2008) |
| *Skeletonema costatum* | 0.08 | – | 21.1 | 17.4 | Rodolfi et al. (2008) |
| *Scenedesmus* sp. | 0.26 | – | 21.1 | 53.9 | Rodolfi et al. (2008) |
| *Scenedesmus obliquus* | 0.09 | – | 11–22/35–55 | 19.8/49.5 | Gouveia and Oliveira (2009) |
| *Chlorella vulgaris* | 0.20 | – | 18.4 | 36.9 | Rodolfi et al. (2008) |
| *Chlorella vulgaris* | 0.18 | – | 5.1 | 9.2 | Gouveia and Oliveira (2009) |
| *Isochrysis* sp. | – | 0.25 | 28.6 | 21.1 | Huerlimann et al. (2010) |
| *Alexandrium minutum* (in – outdoor) | 0.16–0.35 | 0.12–0.37 | 23–22 | 36.5–80.7 | Fuentes-Grünewald et al. (2012b) |
| *Karlodinium veneficum* (in – outdoor) | 0.15–0.22 | 0.13–0.25 | 27–12 | 40.3–26.7 | Fuentes-Grünewald et al. (2012b) |
| *Heterosigma akashiwo* (in – outdoor) | 0.20–0.25 | 0.18–0.28 | 24–23 | 48.8–56.1 | Fuentes-Grünewald et al. (2012b) |

In the work of Rodolfi et al. (2008), the strains were cultivated in 250-mL flasks and incubated at 25°C with continuous illumination in an orbital shaker with $CO_2$-enriched air. Gouveia and Oliveira (2009) used outdoor raceway ponds for cultures; the lipid content was expressed as % of wet weight. Huerlimann et al. (2010) used aerated batch cultures (10 L) at indoor conditions. In Fuentes-Grünewald et al. (2012b), it was used a photobioreactor (300 L) with injected air, at indoor–outdoor conditions.

High biomass production is not just influenced by the growth rate but also is influenced for cell biovolume. The enhancement of growth rate combined with the high biovolume of the target strains, it was observed that one of the principal production parameter was affected the dry weight biomass production, reaching average values >$1.13 \pm 0.05$ g*$L^{-1}$ at indoor condition and $1.26 \pm 0.2$ g*$L^{-1}$ for outdoor condition in the same system production, involving a high biomass productivity for dinoflagellates and raphidophytes when were compared with microalgae from the green algae groups (Table 3).

The high biomass productivity of the target microalgae is obtained in a relatively low cell concentration, but this fact could be an advantage in the dewatering stage of the process production, because the energy consumption to extract the same water volume with a filtration technique is higher in those cultures with more cell concentration than those with less cell density. During the dewatering stage or extraction process, the morphology of dinoflagellates and raphidophytes can also be an advantage. The proposed strains *K. veneficum* and *H. akashiwo* are nude tecate that means with any type of cell wall, and *A. minutum* is a tecate with a cell wall composed of numerous plates that can be easily broken comparing with the typical two valves of the diatoms or other microalgae. The strong cell wall in some green microalgae used for biomass production could be an advantage in culture conditions, especially when the cultures are submitted to a higher mechanical or hydrodynamic forces, but can be a disadvantage in terms of cell disruption for lipid extraction, because it implies the use of physical methods (e.g. sonication) that require a high-energy consumption during the extraction process. In *K. veneficum* and *H. akashiwo,* the absence of cell wall implies an advantage in terms of energy during the extraction stage, because it might be easy to break the cell wall of the proposed strains and to perform the extraction process, consequently improving the energetic results of the whole process.

## 7. Conclusion

Several conclusions have been reached on the production of microalgal biomass of dinoflagellates and raphidophytes to be used as feedstock for biodiesel production reviewed in this chapter:

- Two species of Dinophyceae, *Karlodinium veneficum* and *Alexandrium minutum,* and one Raphidophyceae, *Heterosigma akashiwo*, were found to be a particular interest as a bioresource for biodiesel production based on their lipid content, their net growth rate, their high average in wet biomass and their short period of growth compared with terrestrial plants.
- Dinoflagellates and raphidophytes tested have a closed lipid profile to the commonest terrestrial oil (palm oil) used as feedstock for biodiesel production.
- An increase in the fatty acid content was observed during the transition from exponential phase to stationary phase in the dinoflagellates and raphidophyte tested; this increase was more evident in *K. veneficum* (97%).

- An increase in the TAG portion was observed when the target strains were submitted to a stress condition of growth using abiotic parameters, especially high-temperature and nitrogen depletion.
- There was a significant increase in fatty acid concentration by cells in the strains tested under treatment condition (25°C and 330 μM $NaNO_3$).
- No significant change in fatty acid profile was detected when the strains were submitted to stress condition of high-temperature and low-nitrogen concentration.
- *H. akashiwo, K. veneficum* and *A. minutum* can grow under natural environmental conditions (fluctuations of light, irradiance and temperature) in a bcPBR-enclosed systems for several months in the Mediterranean basin.
- The growth rate, biomass productivity and lipid productivity of dinoflagellate and raphidophyte species were directly affected by irradiance and temperature, reaching higher values of growth in outdoor condition than the indoor condition.
- Biomass productivity, lipid content and lipid productivity of *H. akashiwo* and *A. minutum* were higher to those obtained by the "green algae group" in similar culture conditions.
- No significant change in the fatty acid profile was recorded in those cultures at outdoor conditions, compared with those cultures at indoor conditions.

Finally, dinoflagellates and raphidophytes are widely distributed and readily isolated in many different countries. As shown here, they comprise several strategic species that can be used as a source of raw material for biofuels. An analysis of the biotic characteristics (growth rate, biomass, cell yield, lipid content) of several species of microalgae supports their use as feedstocks for biodiesel production. To make more viable industrial project of biodiesel production from dinoflagellates is mandatory to utilize all the cells as a biorefinery concept, using extraction of special metabolites (pharmaceutical, biomedicine), oil content (for biodiesel production) and the exceeding biomass (for anaerobic digestion to produce methane). More studies are highly recommended in other species of dinoflagellates and raphidophytes in order to obtain more and better knowledge of these organisms and use their natural capacity to form dense and large blooms in the environment and utilize these characteristics in controlled culture conditions in order to obtain biomass for biofuel purposes.

## 8. References

Anderson DM (1989) Toxic algal blooms and red tides: a global perspective. In: Okaichi T, Anderson DM, Nemoto T (eds) Red tides: biology, environmental science, and toxicology. Elsevier, New York, pp 11–16

ASTM D6751–08 (2008) Standard specification for biodiesel fuel blend stock (b100) for middle distillate fuels, Annual book of ASTM standards. ASTM Press, Philadelphia

Calbet A, Bertos M, Fuentes-Grünewald C, Alacid E, Figueroa R, Renom B, Garcés E (2011) Intraspecific variability in *Karlodinium veneficum*: growth rates, mixotrophy, and lipid composition. Harmful Algae 10:654–667

Chan A (1980) Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. II. Relationship between photosynthesis, growth and carbon/chlorophyll *a* ratio. J Phycol 16:428–432

Chen CY, Yeh KL (2011) Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 102(1):71–81

Chi ZY, Liu Y, Frear C, Chen SL (2009) Study of a two-stage growth of DHA-producing marine algae *Schizochytrium limacinum* SR21 with shifting dissolved oxygen level. Appl Microbiol Biotechnol 81(6):1141–1148

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Chu F-LE, Lund ED, Littreal PR, Ruck KE, Harvey E (2009) Species-specific differences in long-chain n-3 essential fatty acid, sterol, and steroidal ketone production in six heterotrophic protest species. Aquat Biol 6:159–172

Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M (2009) Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chem Eng Process 48:1146–1151

De Boer MK, Tyl MR, Fu M, Kulk G, Liebezeit G, Tomas CR, Lenzi A, Naar J, Vrieling EG, Van Rijssel M (2009) Haemolytic activity within the species *Fibrocapsa japonica* (Raphidophyceae). Harmful Algae 8:699–705

Dorantes-Aranda JJ, García-de la Parra LM, Alonso-Rodriguez R, Morquecho L (2009) Hemolytic activity and fatty acids composition in the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides* isolated from Bahía de La Paz, Gulf of California. Mar Pollut 58:1401–1405

Fernández-Reiriz MJ, Perez-Camacho A, Ferreiro MJ, Blanco J, Planas M, Campos MJ, Labarta U (1989) Biomass production and variation in the biochemical profile (Total protein, carbohydrates, RNA, lipids and fatty acids) of seven marine microalgae. Aquaculture 83:17–37

Fiorillo I, Rossi S (2010) Biochemical features of a *Protoceratium reticulatum* red tide in Chipana Bay (Northern Chile) in summer conditions. Sci Mar 74(4):633–642

Flynn KJ, Garrido JL, Zapata M, Opik H, Hipkin CR (1993) Changes in carbon and nitrogen physiology during ammonium and nitrate nutrition and nitrogen starvation in *Isochrysis galbana*. Eur J Phycol 28:47–52

Fuentes-Grünewald C, Garcés E, Rossi S, Camp J (2009) Use of the dinoflagellate *Karlodinium veneficum* as a sustainable source of biodiesel production. J Ind Microbiol Biotechnol 36(9):1215–1224

Fuentes-Grünewald C, Garcés E, Alacid E, Sampedro N, Rossi S, Camp J (2012a) Improvement of lipid production in the marine strains *Heterosigma akashiwo* and *Alexandrium minutum* utilizing abiotic parameters. J Ind Microbiol Biotechnol 30:207–216

Fuentes-Grünewald C, Garcés E, Alacid E, Rossi S, Camp J (2012b) Biomass and lipid production of dinoflagellates and raphidophytes in indoor and outdoor photobioreactors. J Mar Biotechnol DOI: 10.1007/s10126-012-9450-7

Geider RJ, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17

Giner JL, Zhao H, Tomas C (2008) Sterols and fatty acids of three harmful algae previously assigned as *Chatonella*. Phytochemistry 69:2167–2171

Gouveia L, Oliveira AC (2009) Microalgae as a raw material for biofuels production. J Ind Microbiol Biotechnol 36(2):269–274

Grewe C, Griehl C (2008) Time and media-dependent secondary carotenoid accumulation in *Haematococcus pluviales*. J Biotechnol 3:1232–1244

Griffiths M, Harrison S (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21(5):493–507

Grobbelaar JU (2010) Microalgal biomass production: challenges and realities. Photosynth Res 106(1):135–144

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45(2):160–186

Hu Q, Jarvis SM, Ghirardi E, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54(4):621–639

Huerlimann R, De Ny R, Heimann K (2010) Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnol Bioeng 107(2): 245–257

Leblond JD, Chapman PJ (2000) Lipid class distribution of highly unsaturated long chain fatty acids in marine dinoflagellates. J Phycol 36:1103–1108

Leblond JD, Anderson B, Kofink D, Logares R, Rengefors K, Kremp AA (2006) Fatty acid sterol composition of two evolutionary closely related dinoflagellate morphospecies from cold Scandinavian brackish and freshwaters. Eur J Phycol 41(3):303–311

Li Y, Horsman M, Wang B, Wu N, Lan CQ (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl Microbiol Biotechnol 81:629–636

Liang YN, Sarkany N, Cui Y (2009) Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett 31:1043–1049

Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999) The fatty acids and sterol composition of five marine dinoflagellates. J Phycol 35:710–720

Mansour MP, Volkman JK, Blackburn SI (2003) The effect of growth phase on the lipid class, fatty acid and sterol composition in the marine dinoflagellate, Gymnodinium sp. in batch culture. Phytochemistry 63(2):145–153

Marshall JA, Nichols PD, Hallegraeff GM (2002) Chemotaxonomic surveys of sterols and fatty acids in six marine raphidophyte algae. J Appl Phycol 14:255–265

Molina Grima E, Garcia Camacho F, Sánchez Pérez JA, Acién Fernández FG, Fernández Sevilla JM, Valdés Sanz F (1994) Effect of dilution rate on eicosapentanoic acid productivity of *Phaeodactylum tricornutum* UTEX 640 in outdoor chemostat culture. Biotechnol Lett 16(10):1035–1040

Molina Grima E, Sanchéz Pérez JA, García Camacho F, García Sánchez JL, Fernández Sevilla JM (1995) Variation of fatty acid profile with solar cycle in outdoor chemostat culture of *Isochrysis galbana* ALII-4. J Appl Phycol 7:129–134

Mooney BD, Nichols PD, De Salas MF, Hallegraeff GM (2007) Lipid, fatty acid, and sterol composition of eight species of Kareniaceae (Dinophyta): chemotaxonomy and putative lipid phycotoxins. J Phycol 43:101–111

Morweiser M, Kruse O, Hankamer B, Posten C (2010) Developments and perspectives of photobioreactors for biofuel production. Appl Microbiol Biotechnol 87(4):1291–301

Olenina I, Hajdu S, Edler L, Andersson A, Wasmund N, Busch S, Göbel J, Gromisz S, Huseby S, Huttunen M, Jaanus A, Kokkonen P, Ledaine I, Niemkiewicz E (2006) Biovolumes and size-classes of phytoplankton in the Baltic Sea. HELCOM Baltic Sea environmental proceedings no. 106, 144. Copyright by the Baltic Marine Environment Protection Commission-Helsinki Commission-ISSN 0357-2994

Reuss N, Poulsen LK (2002) Evaluation of fatty acids as biomarkers for a natural plankton community: a field study of a spring bloom and a post-bloom period off West Greenland. Mar Biol 141:423–434

Rodolfi L, Chini ZG, Bassi N, Padovani G, Bionde 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 102:101–112

Sánchez JF, Fernández Sevilla JM, Acién FG, Cerón MC, Pérez PJ, Molina GE (2008) Biomass and lutein productivity of *Scenedesmus almeriensis*: influence of irradiance, dilution rate and temperature. Appl Microbiol Biotechnol 79:719–729

Sanford SD, White JM, Shah PS, Wee C, Valverde MA, Meier GR (2010) Feedstock and biodiesel characteristics report. Renewable Energy Group, Inc. www.regfuel.com

Sheehan J et al (2006) Are a biofuel sustainable? National Renewable Energy Laboratory, Golden

Smayda TJ (1997) Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol Oceanogr 42(5.2):1137–1153

Stolte W, Garcés E (2006) Ecological aspects of harmful algal in situ population growth rates: ecological studies. In: Graneli E, Turner JT (eds) Ecology of harmful algae. Springer, Heidelberg, pp 139–152

Tang EPY (1995) The allometry of algal growth rates. J Plankton Res 17(6):1325–1335

Tang EPY (1996) Why do dinoflagellates have lower growth rates? J Phycol 32:80–84

Usup G, Hamid SZ, Chiet PK, Wah CK, Ahmad A (2008) Marked differences in fatty acid profiles of some planktonic and benthic marine dinoflagellates from Malaysian waters. Phycologia 47(1): 105–111

Viso AC, Marty JC (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34(6):1521–1533

Widjaja A, Chien CC, Ju YH (2009) Study of increasing lipid production from fresh water microalgae *Chlorella vulgaris*. J Taiwan Inst Chem Eng 40:13–20

Xu H, Miao XL, Wu QY (2006) High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. J Biotechnol 126(4):499–507

Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y (2011) Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour Technol 102:159–165