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## Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition



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#### ABSTRACT

Renewable energy sources e.g. biofuels, are the focus of this century. Economically and environmental friendly production of such energies are the challenges that limit their usages. Microalgae is one of the most promising renewable feedstocks. However, economical production of microalgae lipid in large scales is conditioned by increasing the lipid content of potential strains without losing their growth rate or by enhancing both simultaneously. Major effort and advances in this area can be made through the environmental stresses. However, such stresses not only affect the lipid content and species growth (biomass productivity) but also lipid composition. This study provides a comprehensive review on lipid enhancement strategies through environmental stresses and the synergistic or antagonistic effects of those parameters on biomass productivity and the lipid composition. This study contains two main parts. In the first part, the cellular structure, taxonomic groups, lipid accumulation and lipid compositions of the most potential species for lipid production are investigated. In the second part, the effects of nitrogen deprivation, phosphorus deprivation, salinity stress, carbon source, metal ions, pH, temperature as the most important and applicable environmental parameters on lipid content, biomass productivity/growth rate and lipid composition are investigated.

#### 1. Introduction

The world population in 2050 is estimated to be 1.5 times the current population. Never before the necessity and challenge for sustainable production methods for food and energy was larger than in this century. On the other hand, about 85% of the current national energy needs are met by combustion of fossil fuels i.e oil, natural gas and coal-finite resources that increases concerns about the depletion of fossil fuel reserves and environmental pollution caused by carbon emissions. Therefore, the national energy strategy is changing towards using and getting more benefit from renewable and sustainable sources of energy to achieve energy security in an environmentally friendly manner. Biochar, biodiesel, bioethanol, biohydrogen are a few promising renewable energy sources, most of which are mainly produced from plant residues. As an example, more than 95% of current commercial production of biodiesel (Fatty Acid Alkyl Ester) is produced from edible vegetable oils at the cost of destruction of vital soil resources, deforestation, consumption of large amount of freshwater and

much of the arable land. The increasing price of vegetable oil and the crisis of food versus fuel are the other problems associated with biofuel generation from plants, trees and oil crops. Accordingly, demand for other possible sources of biofuel has significantly increased. There is a wide range of raw materials for biofuel production. Based on their biomass feedstock, biofuels and biofuel production are classified into four different generations. First-generation biofuels are produced from mostly edible oil seeds, food crops, and animal fats. The main products of this generation include biodiesel, bioethanol, biobutanol. In contrast, second-generation biofuels use lower-value biomass residues such as Nonedible oilseeds, waste cooking oil and lignocellulosic feedstock materials (e.g. forest residues, sugarcane bagasse, cereal straw). In addition to the previous biofuels, syngas is also considered as the product of this generation. The third and fourth generations of biofuels are mainly produced from algae and other algae/microbes respectively. These feedstocks have potential to produce biodiesel, bioethanol, biobutanol, syngas as well as biohydrogen and methane [1]. Microalgaebased oil and biomass have several superiorities over terrestrial

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#### oleaginous crops [2].

- Algae grow quickly. They double every few hours and can be harvested daily. Generally, each unit area of land used for microalgae plant produces 10 times more oil compared to that of a typical terrestrial oleaginous crop.
- Algae use sunlight, consume carbon dioxide and release oxygen (O<sub>2</sub>)
  as they grow. Generally, they are able to photosynthesize up to 2 kg
  of carbon dioxide per kg of biomass produced. It has been reported
  that microalgae generate approximately half of the atmospheric
  oxygen.
- Different from terrestrial oleaginous crops, microalgae do not require soil fertility and freshwater. They can grow in saline water medium and thus do not compete with other terrestrial crops [2].
- They can live and grow in wastewater and purify wastes while producing a biomass and oil suitable for biofuel production at the same time.
- The plant growth and the energy of terrestrial oleaginous crop depend on seasonal conditions, solar radiation and all other weather-based changes while algae tolerate extreme weather conditions.
- The photo-conversion efficiency of terrestrial crops versus incoming solar radiation for terrestrial crops is generally below 1% in temperate climates while the value can increase up to 5% for microalgae biomass.

The oil production efficiency of microalgae versus traditional vegetable oil crops was compared in term of land use in Table 1. As observed, although the oil content of microalgae is strain-dependent and similar to the oil content of terrestrial seeds, there is a significant difference in the overall biomass productivity and resulting oil yield. Besides, microalgae containing 30% oil by weight of dry biomass could vield almost 10 times more oil than palm, which is the most efficient vegetable oil crop. The value significantly increases for those strains that contain higher amount of oil. It is far in excess of what can be generated from palm, soybean and corn, which are currently the global sources of vegetable oil, respectively. Although microalgae-based oil and biomass yields are strain-dependent, significant economical and noneconomical advantages can be reached by using microalgae as biofuel feedstock. However, microalgae oil is estimated to be 3-4 times more expensive than plant oil [3]. Generally, the oil production from microalgae includes three steps: (1) microalgae cultivation (in open ponds or photobioreactors; (2) harvesting/concentration; and (3) oil extraction. Cultivation and harvesting steps are the limiting keys imposing 40% and 20-30% of the cost and energy in microalgal biofuel production, respectively [4,5]. Identifying an optimal balance among these stages is essential to reduce the environmental impacts and costs of the overall process. In this study, different potential organisms are reviewed in terms of biomass and lipid productivity/composition at first. Then, environmental factors e.g. nitrogen deprivation, phosphorus deprivation, NaCl stress, carbon, pH, temperature, and Fe(III) ion, as the most important and applicable strategies to enhance microalgae lipid content are investigated. The main objective of this study is to provide a comprehensive reference on the synergistic or antagonistic effects of environmental stresses on growth rate (biomass production), lipid productivity, lipid fatty acids compositions assisting the researchers to better understand the reactions of microalgae to the environmental factors. (Fig. 1)

#### 2. Basic information about microalgae

#### 2.1. Microalgae cellular structure

Microalgae, named also as phytoplankton by biologists are very small single-cell plant-like organisms without leaves or roots. Green microalgae have a diameter between 1 and 50 micrometers, live in water systems such as streams, rivers, lakes and oceans and in fact are

Table 1
Comparison of microalgae oil potential with other biofuel feedstocks. Ref: [20.181–194].

Plant	Gal Oil/ Acre	Fat content in seed %
Oil Palm (Elaeis guineensi)	610	35.3
Macauba Palm(Acrocomia aculeate)	461	28.35
Pequi (Caryocar brasiliense)	383	45
Buriti Palm (Mauritia flexuosa)	335	19
Oiticica (Licania rigida)	307	80
Coconut (Cocos nucifera)	276	35.3
Avocado (Persea Americana)	270	8-32
Brazil Nut (Bertholletia excelsa)	245	66.9
Macadamia Nut (Macadamia terniflora)	230	71.6
Jatropha (Jatropha curcas)	194	28
Babassu Palm (Orbignya martiana)	188	60
Jojoba (Simmondsia chinensis)	186	48-56
Pecan (Carya illinoensis)	183	71.2
Bacuri (Platonia insignis)	146	13.5
Castor Bean (Ricinus communis)	145	48
Olive Tree (Olea europaea)	124	20
Rapeseed (Brassica napus), canola	122	30
Opium Poppy (Papaver somniferum)	119	45-50
Peanut (Ariachis hypogaea)	109	53-71
Sunflower (Helianthus annuus)	98	47.3
Tung Oil Tree (Aleurites fordii)	96	50-60
Rice (Oriza sativa L.)	85	10
Buffalo Gourd (Cucurbita foetidissima)	81	33
Safflower (Carthamus tinctorius)	80	30-40%
Crambe (Crambe abyssinica)	72	27.8-35.3
Sesame (Sesamum indicum)	72	49.1
Camelina (Camelina sativa)	60	40%
Mustard (Brassica alba)	59	35-46
Coriander (Coriandrum sativum)	55	13-20
Pumpkin Seed (Cucurbita pepo)	55	46.7
Euphorbia (Euphorbia lagascae)	54	48-52
Hazelnut (Corylus avellana)	49	50-70%
Linseed (Linum usitatissimum)	49	34%
Coffee (Coffea arabica)	47	10-20
Soybean (Glycine max)	46	17.7
Hemp (Cannabis sativa)	37	35
Cotton (Gossypium hirsutum)	33	40
Calendula (Calendula officinalis)	31	17-24%
Kenaf (Hibiscus cannabinus L.)	28	20%
Rubber Seed (Hevea brasiliensis)	26	24(seed) 40(kernel)
Lupine (Lupinus albus)	24	7.2–8.2%
Palm (Erythea salvadorensis)	23	20-45
Oat (Avena sativa)	22	3.1-11.6%
Cashew Nut (Anacardium occidentale)	18	41.7
Corn (Zea mays)	18	4
Microalgae (low oil content)	6275	30
Microalgae (medium oil content)	10,455	50
Microalgae (high oil content)	14,635	70

the first link in the oceanic food chain. These microorganisms convert carbon dioxide and water to oxygen and nutrient-rich biomass in the presence of sunlight through photosynthesis process. More than 50,000 microalga species are known to-date, which are categorized with respect to their ultrastructure, biochemical constituents, pigment composition and life cycle. Microalgae are classified into the microplankton (20–1000  $\mu m$ ), nanoplankton (2–100  $\mu m$ ), ultraplankton (0.5–15  $\mu m$ ) and the picoplancton (0.2–2  $\mu m$ ) with respect to their size.

Algae are made up of eukaryotic and prokaryatic cells, which are the cells with nuclei and organelles. However, most of the algae (some researchers say almost all) are eukaryotic. DNA in eukaryotic algae is localized within a minutely perforated nuclear membrane and their nuclei are similar to those of higher plants. All eukaryotic algae have intracellular organelle named as chloroplast, which contains photosynthetic lamellae with chlorophyll in which photosynthesis occurs. However, different types of algae have chloroplasts of different shapes with different combinations of chlorophyll molecules (Chlorophyll A, A

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