Renewable and Sustainable Energy Reviews 72 (2017) 154–162

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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

# Oleaginous microalgae Nannochloropsis as a new model for biofuel production: Review & analysis

Junying Liu[a,*,1], Yunmeng Song[a,1], Wen Qiu[b]

[a] The Engineering Research Center for Biomass Conversion, Ministry of Education, Nanchang University, Nanchang 330047, China
[b] State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

*Keywords:*
*Nannochloropsis*
Growth aspects
Harvesting
Lipid productivity
Genome
Transcript
Genetic engineering

ABSTRACT

*Nannochloropsis* is attracting renewed interest due to member species' potential application as sustainable feedstocks in biofuel production and high-value compounds. This genus is rapidly emerging as a model for research on algal biofuel production as it is predicted to have great potential in the bioenergy sector. The handful of known species in this genus has been heavily investigated for fuel purposes owing to their high lipid content, biomass production and highly efficient homologous recombination system. This review reveals advances in the aspects of *Nannochloropsis* growth, cultivation systems, harvesting and biorefinery technologies associated with lipid production (mainly neutral lipids) over the past few years. We also present recent advances in high-throughput techniques that generate highly accurate datasets and thus advanced improvement in lipid production. Here, we aim to provide useful information to further the development of *Nannochloropsis* as a cost-effective biofuel feedstock.

## 1. Introduction

Rapidly depleting fossil fuel supplies plus the contribution of these fuels to the accumulation of carbon dioxide in the atmosphere and oceans have strongly motivated research on biofuel production [1]. Therefore, renewable, carbon-neutral, environment-friendly fuels from biomass are necessary for sustainability, both environmental and economic. Biofuels based on agricultural crops or bioethanol are a potentially renewable and carbon-neutral alternative to petroleum. They have the key advantage of using the existing distribution networks and current engine technology. Unfortunately, biodiesel from crops and bioethanol cannot realistically satisfy even a small fraction of the existing demand for transport fuels. Moreover, crop-based sources can lead to land competition and biodiversity loss. Due to these disadvantages, low-cost biodiesel produced from feedstock is preferable, such as non-edible oils, used frying oils, animal fats, soap stocks and greases. Available quantities of waste oils and animal fats are not enough to meet current demand for biodiesel. Thus, the use of the first-generation biofuel, such as biodiesel from crops, as an alternative to fossil fuels, has been the subject of controversy and is likely unsustainable [2].

Microalgae offer an attractive alternative for generating biofuels such as ethanol, not least because of their lack of demand on agricultural lands. Compared with other biofuel crops, microalgae possess higher energy yields per hectare [1]. Furthermore, microalgae are able to accumulate lipids and have very high actual photosynthetic yield. About 3–8% of accessed solar energy is converted by microalgae to biomass, whereas observed yields for terrestrial plants average about 0.5% [3]. The high productivity, combined with both noncompetition with food crops and the potential bind industrial $CO_2$ as growth fuel, has motivated studies on microalgae as an alternative source of oil for biodiesel.

Despite quick growth of interest in microalgal biodiesel production, this field is still in its infancy. A large investment in research and development, conducive policy and strategy are needed for all stages of the biofuels value chain, from raw material production to delivery and endpoint consumption. A wide range of photosynthetic algae have been proposed as potential feedstocks in the production of biodiesel for bioenergy application [4]. Several strains of *Nannochloropsis* are emerging as models for algal lipid production and successful genetic manipulation based on their genomes has been successfully demonstrated [5]. Here, we review the current thinking on large-scale algal biodiesel processes. We also provide a brief review about the biology and lipid metabolism of algae in general terms, as well as the strategies applied to increase lipid content of *Nannochloropsis*, which is known to have high-biomass and lipid productivities and to be tolerant of wide-ranging conditions [6]. In the past 30 years, research on micro-

* Corresponding author.
*E-mail address:* liuhailinshef@hotmail.com (J. Liu).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.rser.2016.12.120
Received 6 March 2015; Received in revised form 23 May 2016; Accepted 25 December 2016
1364-0321/ 

algae has increased exponentially, particularly since 2011 [7]. Hitherto, current review focuses on the optimization of key parameters for lipid productivity through modeling [7]. Our following review covers the latest information about varying environmental conditions, and photobioreactor design. We also provide a review of harvesting and lipid extraction, and also the current processing algal residues since these provide the means to control the overall production cost. The last section of this review focuses on the lipid composition and lipid metabolism analyzed through genome sequencing and metabolic network.

## 2. Biological potential of oleaginous microalga genus *Nannochloropsis*

More than 10 microalgal genomes have been sequenced and annotated to various extents, of which *Chlamydomonas reinhardtii* is the most studied over the recent decades [5]. Although *Chlamydomonas* currently provides the best model for microalgal lipid research, it is not likely to become a candidate for biofuel production owing to its naturally lower lipid accumulation potential in wild-type strains [5]. Therefore, other microalgal genera with greater lipid production potential have recently been the target for lipid biosynthesis research. Over the past four years, several species of *Nannochloropsis* have emerged as models for algal lipid production [7,8]. Strong efforts are underway to sequence *Nannochloropsis* genomes, which will facilitate the genetic manipulation developments for *Nannochloropsis* strains including *N. oceanica* strains IMET1 and CCMP531, *N. salina* CCMP537, *N. gaditana* CCMP526, *N. oculata* CCMP525 and *N. granulata* CCMP529. Naturally high rates of homologous recombination can create a more attractive model of algal organism through reverse genetics and targeted gene knockouts [9].

*Nannochloropsis*, a picoplankton genus (3–4 µm in diameter) of the marine environment, is of spherical to slightly ovoid shape and non-flagellate [10]. The polysaccharide-walled cell contains a single chloroplast without pyrenoid, but with several bands of photosynthetic lamellae, each with three thylakoids per band [11]. *Nannochloropsis* species do not accumulate starch, but are of considerable interest collectively as a source of polyunsaturated fatty acids, and are used in the production of eicosapentaenoic acid for human disease-prevention and biodiesel [12]. Aside from these usages, the genus has also been recognized in human diet due to its nutritional value, which is an excellent source of proteins, carbohydrates and vitamins [13]. This alga genus is also well known as a source of valuable pigments produced in relatively high amounts, such as chlorophyll, zeaxanthin, canthaxanthin and astazanthin [14].

Genomic diversity and microalgal phylogenetics of *Nannochloropsis* are poorly understood. Sequences of various *Nannochloropsis* from NCBI were collected to generate a multiple sequence alignment using MEGA5.02 program. The alignment file was used to create a neighbor-joining polygenetic tree with 100 rounds of bootstrapping (Fig. 1). Among the published *Nannochloropsis* species, *N. gaditana* and *N. oculata* share a recent common ancestor and are clustered with two *N. oceanica* strains. Phylogenetic analyses using ITS and *rbc*L sequences more clearly separated those species published by NCBI, revealing differences in their genomic characteristics [15].

## 3. The effects of growth characteristics on biomass production and lipid metabolism

Growth characteristics of *Nannochloropsis* significantly affect the fatty acid profiles. These include non-biotic factors, such as light intensity, temperature, nutrient, $O_2$, $CO_2$, pH, salinity, and toxic chemicals, and biotic factors, which consists of pathogens (bacteria, fungi and viruses) and competition by other algae. In addition, operational factors alter growth, for instance, shear produced by mixing, dilution rate, depth, harvest frequency, and the addition of bicarbonate [16].

### 3.1. Nutrient sources

Various researchers have examined the above-described effects of cultivation factors on algal growth in considerable detail. The major obstacle to large-scale biodiesel production is the high cost of algae production [17]. F/2 is well-known as a lab-defined medium for *Nannochloropsis,* of which sodium nitrate is supplied as the major nitrogen source because it is much more stable than the other nitrogen sources (e.g. urea and ammonia) and is easier to control and analyze. However, the f/2 medium for *Nannochloropsis* is extremely expensive, which has discouraged the commercial development for biofuel [3,18]. Therefore, the cost associated with the medium is a major consideration for large-scale algal production [17]. One solution to the high cost of algal cultivation is to replace f/2 medium with a mixture of diammonium phosphate and ammonia or agricultural fertilizer containing the optimal nitrogen and phosphorus for algal cell growth [18,19]. Many researchers have focused on the development of a low-cost medium to achieve high quality *Nannochloropsis* biomass, as well as the searching for cheaper nitrogen and phosphorus sources [20]. According to our previous study, an optimal condition in an alternative fertilizer-based medium has been proposed with a nitrogen concentration of 11.3 mM, a phosphorus concentration of 0.16 mM and a micronutrient concentration of 30 µM [18]. From this alternative medium, we obtained a maximum biomass productivity of 0.51 g $L^{-1}$ $day^{-1}$ [18]. Additionally, the application of recycling medium helps to cut costs and prevent environmental contamination. Compared to other investigated sterilized methods including filtration, chlorination, heating and the addition of hydrogen peroxide, ozonation shows the best effect on lower bacterial load in recycling medium [21].

Moreover, the application of *N. salina* in biomass production and nutrient removal from anaerobically-digested municipal wastewater effluent also provides a cost-effective strategy for biofuel production in bench-scale batch reactors [22]. Regular medium refreshing with different ratio of wastewater influences the long-term algal growth and lipid accumulation. The maximum lipid content (64%) of *N. oceanica* DUT01 was obtained in nitrogen-depleted f/2 medium [24]. However, not enough municipal wastewater is available to support a large-scale biofuel industry without efficient water recycling and nutrient recovery [26]. Moreover, this result highlights the importance of high biomass accumulation for efficient lipid production, which agrees with previous work, where the highest lipid yield derived from the greatest biomass but not from high lipid content was demonstrated [25].

Nutrient source and ratio has a considerable impact on the fatty acid content and profile [23]. The production of *N. salina* biovolume and lipid accumulation increases when grown in combined nitrogen sources (ammonium, nitrate, urea)[24]. Although urea resulted in the highest maximum sustainable yield and led to the highest cell densities of *N. salina*, however, these positive characteristics were offset by a smaller cell size [25]. This finding is consistent with our previous study, in which algal cells had a larger cell diameter in f/2 medium (unpublished data). The different nitrogen sources used in algal growth culture might also cause contamination by invading organisms and/or increase the biomass of undesired organisms that could increase the possibility of an algal crash [25]. Although the optimal ratio of N:P appears to be 16:1, the N:P supply ratio can be raised to at least 32:1 without compromising the productivity of a maximum lipid content yield (52% of dry weight) and volumetric lipid concentration (233 mg $L^{-1}$) [26].

### 3.2. Water sources

Apart from medium nutrients, water source accounts for a large proportion of cost. The success of a microalgal biofuel industry depends upon its ability to fund a production process with a positive net energy ratio through high growth and lipid accumulation rates. The