



Microalgae bioengineering: From CO₂ fixation to biofuel production

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ABSTRACT

The recognised deficiencies in sustainable development and the extensive environmental deterioration and global warming concerns caused by anthropological CO₂ emissions are major issues facing the world today. Massive reduction in atmospheric CO₂ concentration, through the development of processes that utilize CO₂ or minimise CO₂ emissions, is critical to ensure environmental sustainability. One of the major contributors to anthropological CO₂ emission is the combustion of petroleum fuels in vehicular engines for transportation. Biofuel, as an alternative to petroleum transport fuels, has become a partial substitute for fossil fuel. The use of microalgae for biofuel production has gained enormous research interests in recent years, primarily due to the ability to photosynthetically convert CO₂ (a biology-inspired process engineering route) into potential biofuel biomass, as well as food, feed stocks, and high value biochemicals. In this review, the CO₂ fixation ability of microalgae in comparison to other plant species and genetic engineering methods of improving microalgae photosynthetic rate, have been discussed. Advances in bioprocess technologies for microalgal biomass creation and biodiesel production are also described and other important matters are discussed.

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Table 1Comparing CO₂ capturing capacity and biodiesel productivity of microalgae and higher plants.

Plants	CO ₂ capture capacity (%) ^a	Productivity (g/m ² d)	Productivity of the theoretical max (%)	Lipid content of biomass (%)	Biodiesel productivity (g/m ² d) ^b
Theoretical [8]	100	77	100	–	N/A
Green microalgae [9]	26–52	20–40	27–54	30–45	6–12
<i>Chlorella</i> [10,11]	22–26	17–20	22–27	40–60	5.4–6
<i>Miscanthus giganteus</i> [8,12,13]	10–22	8–17	11–22	N/A	2.4–5.4
Oilseed rape [14]	1–2	0.8–1.6 (of seed)	1.0–1.2 (of seed)	40–44 (of seed)	0.24–0.48
Soya [15]	N/A	0.65 (of seed)	0.8 (of seed)	20 (of seed)	0.2
Jatropha [16]	N/A	2.2 (of seed)	2.8 (of seed)	30 (of seed)	0.66

^a In the theoretical view, to produce 77 g biomass, 3.6 mol (158.4 g) of CO₂ is captured by autophyte [8].^b It is assumed that 30% of biomass is lipid [17].

1. Introduction

In recent years, due to increasing awareness of global energy crisis in conjunction with escalating concerns regarding sustainability and the environment, interest in biofuels and its production has fast risen [1]. The increase in atmospheric CO₂ concentration, which is the main component of greenhouse gas emissions, poses great challenges to worldwide pro-environment and sustainability. Current available technologies for CO₂ capture include physicochemical adsorption, injection into deep oceans or geological formations, and enhanced biological fixation. Physicochemical adsorption process is difficult to control, and the adsorbent materials are typically non-renewable and expensive. Abiotic methods, such as direct injection of CO₂ into the deep ocean, geological strata, old coal mines, oil wells or saline aquifers, as well as mineral carbonation of CO₂, present significant challenges of high space requirements and potential leakage with time [2]. One of the most environmentally sustainable ways to reduce greenhouse gas emissions associated with energy production is to generate energy from reduced-carbon-emission sources. With the progress of research and development into new energy forms, biofuel is thought of as an effective and practical alternative transport fuel that may, in the future, play a significant role in the reduction of transportation related CO₂ emissions.

CO₂ biological fixation is a long-term environmental sustainable technology. CO₂ in the atmosphere and flue gas is converted into biomass by autotrophs, whilst nutrient utilization and energy feedstock production is achieved in a sustained fashion [3]. Different kinds of energy compounds such as oil, ethanol, and bio-hydrogen can be generated from higher plants, photosynthetic bacteria and microalgae.

Compared to other plant feedstock, microalgae have a number of advantages in CO₂ capture and bio-oil generation. These include (i) high photosynthetic conversion efficiencies, (ii) rapid biomass production rates, (iii) the capacity to produce a wide variety of biofuel feedstock, (iv) ability to thrive in diverse ecosystems, (v) distinguished environmental bioremediation such as CO₂ fixation from the atmosphere or flue gas, and water purification [4,5], (vi) non competitiveness for land with crops and (vii) non competitiveness with the food market. Unlike plants, unicellular microalgae do not partition large amounts of biomass into supportive structures such as stems and roots that are energetically expensive to produce and often difficult to harvest and process for biofuel production. Additionally, microalgae have carbon concentrating mechanisms that suppress photorespiration [6,7]. The CO₂ capturing capacity and oil productivity of different kinds of plants are summarized by the authors in Table 1. The process merit of CO₂ fixation by microalgae cultivation is that the biomass produced can be converted efficiently into biofuels for energy production directly.

Microalgal biodiesel production system involves the following process steps: cultivation, harvesting, dewatering, extraction, and transesterification [18]. To achieve high oil yields and CO₂ fixa-

tion capacity during cultivation, the key process considerations are the choice of microalgal strain, cultivation conditions, and the cultivation system (photobioreactors or open ponds). Different technologies are available for harvesting, dewatering, extraction, and transesterification. However, high efficiency, energy saving and low CO₂ emission technologies are the optimum targets for full-scale industrial application of microalgae biotechnology.

2. Microalgae genetic engineering

In recent years, new biotechnological approaches relating to genome perturbation of microalgal cells to endow them with different properties are rapidly increasing. However, the full potential of genetic engineering of some microalgal species, particularly diploid diatoms, can be fully realized only if conventional breeding methods become firmly established, thereby allowing useful mutations to be easily combined [19]. Significant advances in microalgal genomics have been achieved during the last decade [19–21]. Expressed sequence tag databases have been established; nuclear, mitochondrial, and chloroplast genomes of several microalgae strains have been sequenced. Historically, the green algae *Chlamydomonas reinhardtii* has been the focus of molecular and genetic physiological research. Therefore, most of the tools developed for the expression of transgenes and gene knockdown are specific for this kind of species. Current genetic engineering pursuits are towards microalgae that are of greater interest in industrial applications and environmental conservation [19]. To improve microalgae biomass or lipid production and CO₂ capturing efficiency, several approaches have been developed.

2.1. Genetic engineering of microalgae to enhance carbohydrate and protein storage

Starch is the basic energy storing biochemical of plants including microalgae. Adenosine diphosphate-glucose pyrophosphorylase (AGPase) and 3-phosphoglyceric acid (3-PGA) are the rate-limiting molecules of starch synthesis [19,22,23]. Much has been reported on the catalytic and allosteric properties of AGPases in crop plants to increase starch production [24–26]. Most cellular AGPases are far from the pyrenoid and this could result in 3-PGA inactivation, some AGPases, such as Mos(1-198)/SH2 AGPase, have activity even without an activator [27]. In some microalgal species, starch production is improved when Mos(1-198)/SH2 AGPase or other AGPases is overexpressed. Although, the precise mechanism of starch catabolism in most microalgae is largely unknown, some studies have reported decreasing starch degradation in microalgal cells [28,29]. In *A. thaliana*, α -amylase is thought to participate in starch degradation. Interestingly, starch is degraded even when all amylases are knocked out, indicating phosphorolytic mechanism for starch degradation [30,31]. Catabolism of starch provides stock and intermediate of lipid and protein synthesis, and this is sometimes the key rate-limiting step of lipid and protein synthesis [31].

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