ARTICLES: BIOCATALYSTS AND BIOREACTOR DESIGN

Biofuels from Microalgae

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Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that grow rapidly due to their simple structure. They can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner. Microalgae have been investigated for the production of a number of different biofuels including biodiesel, bio-oil, bio-syngas, and bio-hydrogen. The production of these biofuels can be coupled with flue gas CO_2 mitigation, wastewater treatment, and the production of high-value chemicals. Microalgal farming can also be carried out with seawater using marine microalgal species as the producers. Developments in microalgal cultivation and downstream processing (e.g., harvesting, drying, and thermochemical processing) are expected to further enhance the costeffectiveness of the biofuel from microalgae strategy.

Introduction

A variety of biomasses from different sources, including forestry, agricultural, and aquatic sources have been investigated as the feedstock for the production of different biofuels including biodiesel, bio-ethanol, bio-hydrogen, bio-oil, and bio-gas. Techno-economic assessments indicated that cost-effectiveness of biofuel production was achievable.[1] However, burning fuels derived from existing biomass has an environmental impact similar to the combustion of fossil fuels in terms of its impact to the carbon cycle (carbon balance), i.e., conversion of fixed carbon into CO_2. In addition, depletion of certain existing biomasses (e.g., wood) without appropriate compensation (e.g., replanting) may result in massive biomass deficit, resulting in serious environmental problems (e.g., deforestation).

Conventional terrestrial plants are not very efficient in capturing solar energy. It was estimated that switchgrass, the fastest-growing terrestrial crop, can convert solar energy to biomass energy at a yearly rate of no more than 1 W/m^2, less than 0.5% of the solar energy received at a typical mid-latitude region (200–300 W/m^2).[2,3] On the other hand, studies have shown the photosynthetic efficiency of microalge could well be in the range of 10–20% or higher.[4,5] Furthermore, recent studies showed that the extra N_2O entering the atmosphere as a result of using nitrogen fertilizers to produce crops for biofuels, when calculated in "CO_2-equivalent" global warming terms and compared with the quasi-cooling effect of "saving" emissions of fossil fuel derived CO_2, could contribute as much or more to global warming by N_2O emissions than cooling by fossil fuel savings.[6] These concerns may be addressed by using fastgrowing microalgal species for biofuel production.

For this review, we define microalgae as all unicellular and simple multicellular photosynthetic micro-organisms, be they prokaryotes (cyanobacteria) or eukaryotes (also called microalgae in a more narrow sense). They have high growth rates and photosynthetic efficiencies due to their simple structures. It is estimated that the biomass productivity of microalgae could be 50 times more than that of switchgrass, which is the fastest growing terrestrial plant.[7,8]

Biofuel production using microalgal farming offers the following advantages:

(1) The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources without causing potential biomass deficit.

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(2) Microalgal cultivation consumes less water than land crops.

(3) The tolerance of microalgae to high CO_2 content in gas streams allows high-efficiency CO_2 mitigation.

(4) Nitrous oxide release could be minimized when microalgae are used for biofuel production.

(5) Microalgal farming could be potentially more costeffective than conventional farming.

On the other hand, one of the major disadvantages of microalgae for biofuel production is the low biomass concentration in the microalgal culture due to the limit of light penetration, which in combination with the small size of algal cells makes the harvest of algal biomasses relatively costly. The large water content of harvested algal biomass also means its drying would be an energy-consuming process. The higher capital costs of and the rather intensive care required by a microalgal farming facility compared to a conventional agricultural farm is another factor that impedes the commercial implementation of the biofuels from microalgae strategy. Nevertheless, these problems are expected to be overcome or minimized by technology development. Given the vast potential of microalgae as the most efficient primary producers of biomass, there is little doubt that they will eventually become one of the most important alternative energy sources.

Biofuels from Microalgae

Algae was initially examined as a potential replacement fuel source for fossil fuels in the 1970s amidst the gas scare,[9] but prohibitive production costs and limitations discouraged the commercial development of algae-based fuel production. Subsequent studies, continued through the 1980s and heightened in the last 15 years, illustrate that research developments are enabling the commercial potential of microalgae to shift from aquaculture, fine chemicals, and health food[10] to fuel production.

Biodiesel

Biodiesel is produced by a mono-alcoholic trans-esterification process, in which triglycerides reacts with a mono-alcohol (most commonly methanol or ethanol) with the catalysis of alkali, acids, or enzymes.[11,12] It has combustion properties similar to those of diesel[13] and has been produced commercially or in backyard facilities to fuel vehicles. Significant technical advances have been achieved to optimize the trans-esterification process. For instance, Canadian researchers in the Department of Chemical Engineering at the University of Ottawa have developed a novel two-phase membrane reactor,[14] which exploits the immiscibility of canola oil in methanol to enable the separation of reaction products (biodiesel/glycerol) from the residual canola oil. The two-phase membrane reactor was particularly useful in removing unreacted canola oil from the product, yielding high purity biodiesel and shifting the reaction equilibrium to the product side. Nevertheless, one major challenge of biodiesel production is the high costs of feedstock. Currently, biodiesel production relies on animal fats and plant oils. This agricultural approach will eventually compete for land resource against food industry. For instance, it was estimated that to produce 5.54 Mtoe (million tons of oil equivalent) of biodiesel (1.72% of the 321 Mtoe estimated EU-25 (the 25 European Union countries) consumption for transportation fuels in 2003) would require 9.3 Mha of land for canola (rapeseed) and sunflower cultivation. This is equivalent to 150% of the current land used for these crops in EU-25, which is approximately 6.4 Mha.[15] On the other hand, some microalgal species could accumulate lipids to a significant portion of their biomass (30–50% on dry weight basis), serving as a promising alternative source of lipids for biodiesel production.[16–18] It was estimated by Sheehan and his co-workers that microalgal farming using 200,000 ha of land (less than 0.1% climately suitable lands in the U.S. or 3.2% of the land currently used for the cultivation of sunflower and rapeseed in EU-25) would allow the production of a quad (i.e., a quadrillion BTU) of fuel in the form of biodiesel.[19] To put this in perspective, one quad is approximately one-eighth of Canada's total energy consumption in 2004 (8543.3×10^{15} J).[20]

Yusuf Christi discussed the economics and quality constraints of biodiesel from microalgae in his recent review paper.[21] He pointed out that the cost of growing microalgae for biofuel production must be drastically reduced to compete *directly* with traditional energy sources. It is essential to consider the other roles algal cultures can play concurrently with biofuel production and the long term benefits this entails. It is interesting to notice that, even though the two major project sponsored by the U.S. government and the Japanese government concluded that algal oil was not economically feasible,[5] the private sector has moved forward in building commercial facilities to produce biodiesel using algal oils. It was reported that a privately funded US$20 million program has engineered, built, and successfully operated for several years a commercial-scale (2 ha) modular production system coupling photobioreactors with open ponds in a two-stage process to produce *Haematococcus pluvialis* for biodiesel production with a annual averaged rate of achieved microbial oil production equivalent to 420 GJ ha^{-1} yr^{-1}, which exceeds the most optimistic estimates of biofuel production from plantations of terrestrial "energy crops." The maximum production rate achieved was equivalent to 1014 GJ ha^{-1} yr^{-1}. It was claimed that a rate of 3200 GJ ha^{-1} yr^{-1} is feasible using *Chlorella* under conditions that prevail in the existing production system, a rate possible to replace reliance on current fossil fuel usage equivalent to about 300 EJ yr^{-1} and eliminate fossil fuel emissions of CO_2 of about 6.5 gigatons of carbon (GtC) per year using only 7.3% of the surplus arable land projected to be available by 2050.[5] This rather optimistic estimation was based on a photosynthetic efficiency of 20% and a biomass productivity of around 70 g m^{-2} Day^{-1} of *Chlorella*.[5] It was also expected that some algal biodiesel processes, such as the one being developed at the University of Utah, would be costcompetitive with regular diesel by 2009.[22] Wu[23] in China reported the production of biodiesel using *Chlorella protothecoides* at a scale of 11,000 L. However, they adopted a heterotrophic cultivation strategy, which does not necessarily fulfill the mandate of microalgal farming to convert solar energy to biofuel. Instead, organic carbons such as glucose are used for fuel production.

Bio-oil and bio-syngas

When biomass is processed under high temperature at the absence of oxygen, products are produced as three phases: the vapor phase, the liquid phase, and the solid phase. The liquid phase is a complex mixture called bio-oil. The compositions of bio-oils vary significantly with the types of feedstock and processing conditions. For instance, the bio-oils produced from a commercial wood biomass feed (Lignocel HBS 150-500) originated from beech wood contain mainly phenols, alcohols, and carbonyls with concentrations varying significantly with conditions of prolysis,[24] while the bio-oils obtained via fast pyrolysis of rice husk comprise mainly formic acid (7.69%), β-hydroxybutyric acid (2.31%), toluene

(5.00%), benzoic acid, 3-methyl (1.15%), 1,2-benzenedicarboxylic acid (1.22%), and other organic compounds.[25]

Extensive studies have been carried out on biomass conversion; several technologies such as an entrained flow reactor, circulating fluid bed gasifier,[26–28] vacuum pyrolysis,[29] and vortex reactor[28] have been demonstrated to be effective. The overall energy to biomass ratio of a well-controlled pyrolytic process could be as high as 95.5%.[30] These technologies can be classified into two categories: (1) pyrolysis, the primary product of which is pyrolytic liquids (bio-oils) and (2) gasification, with "syngas" as the primary product.[23,26] Canadian companies have shown world leadership in this field. Ensyn Corp (EC) (http://www.ensyn.com/), a private company based in Ottawa, Ontario, developed one of the most successful biomass conversion technologies, Rapid Thermal Processing (RTP). RTP is a patented, state-of-the-art process that transforms carbon-based feedstocks, either wood "biomass" or petroleum hydrocarbons, into more valuable chemical and fuel products. Plascoenergy Group, another private company based in Ottawa, Ontario, has a proprietary Plasco Conversion System (PCS) that converts carbonaceous materials such as municipal solid wastes, into an energy-rich fuel or "syngas" and a commercially useful inert solid or "slag" (http://www.plascoenergygroup.com/).

Bio-oils have been demonstrated to be suitable for power generation via both external combustion (e.g., steam cycles, organic Rankine cycles, and Stirling engines) and internal combustion (e.g., diesel engines and gas-turbine engines) or by co-firing with fossil diesel or natural gas.[31–33] Nevertheless, they have several undesirable features such as high oxygen content, low heat content, high viscosity at low temperature, and chemical instability[31–33] that impede their use as quality transportation fuels. To overcome this limitation, studies have been taken to upgrade the bio-oils to high quality fuels. For instance, production of high-grade transportation fuels from biomass has been demonstrated to be technically feasible by gasification and subsequent Fischer Tropsch synthesis.[23,34] Recent work by a group in China has demonstrated that hydrogen can be derived reliably by steam-reforming bio-oil.[35]

Most studies have so far focused on the use of conventional biomasses from forestry and agricultural sources.[30] It was estimated that in year 2000, the majority of biomass energy was produced from wood and wood wastes (64%), followed by municipal solid waste (MSW) (24%), agricultural waste (5%) and landfill gases (5%).[30,36] Recently, a few investigations have been carried out regarding the suitability of microalgal biomass for bio-oil production.[8,37,38] It was shown that, in general, microalgae bio-oils are of higher quality than biooil from wood.[8]

Bio-hydrogen

Hydrogen is an important fuel with wide applications in fuel cells, liquefaction of coal, and upgrading of heavy oils (e.g., bitumen). Hydrogen can be produced biologically by a variety of means, including the steamreformation of bio-oils,[35] dark and photo fermentation of organic materials,[39] and photolysis of water catalyzed by special microalgal species.[39,40]

Integrated Pollution Control and Biofuel Production using Microalgae

Microalgal farming and CO_2 mitigation

One of the key advantages of using microalgae for biofuel production lies in the ability of some microalgal species to tolerate high CO_2 content in feeding air streams,[41] allowing efficient capturing of CO_2 from high-CO_2 streams such as flue gases and flaring gases (CO_2 content 5–15%).[42] In comparison to terrestrial plants, which typical absorb CO_2 from the atmosphere containing only 0.03–0.06% CO_2, the benefit of microalgae is evident in terms of CO_2 mitigation. It was reported that using a outdoor cultivation of *Chlorella* sp. in a 55 m^2 culture area photobioreactor, flue gas containing 6–8% by volume of CO_2, 10–50% CO_2 mitigation (flue gas decarbonization) was achievable and the residual NO_2 and NO in the flue gas was found not to affect algal growth.[43] In such a facility, employment of appropriate flue/flaring gas pretreatment procedure and optimizing culture media is of critical importance.[44] A higher CO_2 mitigation rate between 50.1 ± 6.5% on cloudy days and 82.3 ± 12.5% on sunny days was reported by other researchers using different algal species.[45] Depending on the microalgal species and condition used in the facilities, algal biomass produced could be further processed for biodiesel, bio-oil, and bio-syngas production.

Microalgal farming using wastewater

In addition to the apparent benefit of combining microalgal biomass, and therefore biofuel, production and wastewater treatment, successful implementation of this strategy would allow the minimizing of the use of freshwater, another precious resource especially for dry or populous countries, for biofuel production. Extensive works have been conducted to explore the feasibility of using microalgae for wastewater treatment, especially for the removal of nitrogen and phosphorus from effluents,[46–50] which would otherwise result in eutriphication if dumped into lakes and rivers.[51] Ironically enough, it is algae in the lakes and rivers that cause this problem. It is simply a matter of allowing the consumption of nitrogen and phosphorus by microalgae in a controlled manner that benefits rather than deteriorates the environment. Levels of several contaminant heavy metals have also been shown to be reduced by the cultivation of microalgae, which is a subject discussed extensively by Muñoz and Guieysse.[52] A major concern associated with using wastewater for microalgae cultivation is contamination.[10,52] This can be managed by using appropriate pretreatment technologies to remove sediment and to deactivate (sterilize) the wastewater.[53]

Microalgal farming using marine microalgae

Freshwater is another natural resource besides variable land that may cap biofuel production. This concern is particular evident for populous countries such as China, India and dry coastal regions such as the Middle East. It is a novel idea to employ marine microalgae for CO_2 mitigation and biofuel production.

Extensive studies have been carried out for the cultivation of different marine microalgae using a variety of cultivation systems including both open ponds[54] and various types of closed photobioreactors.[54–56] A few examples of marine microalgal species that have been studied for microalgal farming include red marine alga *Porphyridium* sp.,[55] N-fixing cyanobacterium *Anabaena*,[54] macrophytic marine red alga *Agardhiella subulata*,[57] marine green alga *Dunaliella tertiolecta*,[56] and marine phytoplankter *Tetraselmis suecica*.[58] It is intriguing to notice that nitric oxide (NO) and carbon dioxide (CO_2) were reported to be simultaneously eliminated from a model flue gas using a marine phytoplankter, *Tetraselmis suecica*,[58] making it possible to combine biomass (biofuel) production with CO_2 and NO_x mitigation. It is expected

Table 1. Some High-Value Bioproducts Extracted from Microlage

Product group	Applications	Examples (producer)
Phycobiliproteins carotenoids	Pigments, cosmetics, pro vitamins, pigmentation	Phycocyanin (*Spirulina platensis*)[85] β carotene (*Dunaliella salina*)[86] astaxanthin and leutin (*Haematococcus pluvialis*)[78]
Polyunsaturated faty acids (PUFAs)	Food additive, nutraceutics	Eicosapentaenoic acid (EPA) (*Chlorella minutissima*) [87] docosahexaenoic acid (DHA) (*Schizochytrium* sp.)[88] Arachidonic acid (AA) (*Parietochlorisincise*)[89]
Vitamins	Nutrition	Biotin (*Euglena gracilis*)[90] α-tocopherol (Vitamin E) (*Euglena gracilis*[a])[91] ascorbic acid (Vitamin C) (*Prototheca moriformis*,[a] *Chlorella* spp.[a])[92,93]

[a] Heterotrophic growth.

that, with the selection of appropriate marine microalgal strains, costeffective production of different biofuels should be achievable using seawater as the medium. Indeed, the 2-ha demonstration facility reported by Huntley and Redalje,[5] which was discussed briefly in the introduction, utilized seawater as the medium and for temperature control.

Enhancement of Economic Feasibility of Biofuels from Microalgae

Biorefinery: The high-value coproduct strategy

The term biorefinery was coined to describe the production of a wide range of chemicals and biofuels from biomasses by the integration of bioprocessing and appropriate low environmental impact chemical technologies in a cost-effective and environmentally sustainable manner.[21] Examples include the twophase conversion reaction of fructose to 5-hydroxymethylfurfural,[59] fermentative production of ethanol from sugars derived from cellulose and semi-cellulose,[60] and bio-oils and/or biosyngas by the pyrolysis/gasification of woods or other types of biomasses.[61]

Microalgae have the capacity of producing a vast array of high-value bioactive compounds that can be used as pharmaceutical compounds, health foods, and natural pigments.[62,63] Some well-studied examples include acetylic acids, β-carotene,[57,64] vitamin B,[65] ketocarotenoid astaxanthin,[66] polyunsaturated fatty acids,[67,68] and lutein[54,69] (see Table 1). The economical feasibility of microalgal biofuel production should be significantly enhanced by a high-value coproduct strategy, which would, conceptually, involve sequentially the cultivation of microalgae in a microalgal farming facility (CO_2 mitigation), extracting bioreactive products from harvested algal biomass, thermal processing (pyrolysis, liquefaction, or gasification), extracting high-value chemicals from the resulting liquid, vapor, and/or solid phases, and reforming/ upgrading biofuels for different applications. The employment of a high-value coproduct strategy through the integrated biorefinery approach is expected to significantly enhance the overall cost-effectiveness of microalgal biofuel production.

Design of advanced photobioreactors

The choice of cultivation systems is another key aspect that significantly affects the efficiency and cost-effectiveness of a microalgal biofuel production process. This topic has been discussed extensively by a few authors.[21,70–73] Carvalho[72] explained several closed systems in detail. Lee[70] discussed a few open systems and systematically compared them with closed systems over different geographical regions. Pulz[71] focused more on process parameters and suggested a number of open systems. Janssen et al.[74] offered useful conceptual diagrams for some of the discussed closed systems and described new systems to be examined, including the use of optical fiber to enhance lighting. Even though the open pond systems seem to be favored for commercial cultivation of microalgae at present due to their low capital costs, closed systems offer better control over contamination, mass transfer, and other cultivation conditions. The combination of the closed photobioreactor and open pond combines the benefits of the two and has been demonstrated to be effective at a 2-ha scale.[5]

Selection of cost-effective technologies for biomass harvesting and drying

Given the relatively low biomass concentration obtainable in microalgal cultivation systems due to the limit of light penetration (typically in the range of 1–5 g/L) and the small size of microalgal cells (typically in the range of 2–20 μm in diameter), costs and energy consumption for biomass harvesting are a significant concern needs to be addressed properly. Different technologies, including chemical flocculation,[75] biological flocculation,[76] filtration,[77] centrifugation,[78] and ultrasonic aggregation[79] have been investigated for microalgal biomass harvesting. In general, chemical and biological flocculation require only low operating costs; however, they have the disadvantage of requiring long processing period and having the risk of bioreactive product decomposition. On the other hand, filtration, centrifuge, and ultrasonic flocculation are more efficient but more costly. The selection of appropriate harvesting technology depends on the value of the target products, the biomass concentration, and the size of microalgal cells of interest.

Biomass drying before further lipid/bioproduct extraction and/ or thermochemical processing is another step that needs to be taken into consideration. Sun drying is probably the cheapest drying method that has been employed for the processing of microalgal biomass.[80,81] However, this method takes long drying time, requires large drying surface, and risks the loss of some bioreactive products. Low-pressure shelf drying is another low-cost drying technology that has been investigated.[81] It is nevertheless also of low efficiency. More efficient but more costly drying technologies having been investigated for drying microalgae include drum drying,[81] spray drying,[82,83] fluidized bed drying,[83] freeze drying,[80] and refractance window dehydration technology.[84] It is important to find the balance between the drying efficiency and cost-effectiveness to maximize the net energy output of the fuels from microalgae strategy.

Conclusions

Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that can grow rapidly due to their simple structure. They have been investigated for the production of different biofuels including biodiesel, bio-oil, bio-syngas, and bio-hydrogen. Microalgal biofuel production is potentially sustainable. It is possible to produce adequate microalgal biofuels to satisfy the fast growing energy demand within the restraints of land and water resources.

Microalgal farming can be coupled with flue gas CO_2 mitigation and wastewater treatment. It can also be carried out with seawater as the medium, given that marine microalgal species are adopted, providing a feasible alternative for biofuel production to populous and dry coastal regions.

Microalgae can produce a large variety of novel bioproducts with wide applications in medicine, food, and cosmetic industries. Combining microalgal farming and the production of biofuels using biorefinery strategy is expected to significantly enhance the overall cost-effectiveness of the biofuel from microalgae approach.

Technological developments, including advances in photobioreactor design, microalgal biomass harvesting, drying, and other downstream processing technologies are important areas that may lead to enhanced cost-effectiveness and therefore, effective commercial implementation of the biofuel from microalgae strategy.

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