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An overview on biofuel and biochemical production by photosynthetic microorganisms with understanding of the metabolism and by metabolic engineering together with efficient cultivation and downstream processing

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Abstract

Biofuel and biochemical production by photosynthetic microorganisms such as cyanobacteria and algae is attractive to improve energy security and to reduce CO_2 emission, contributing to the environmental problems such as global warming. Although biofuel production by photosynthetic microorganisms is called as the third generation biofuels, and significant innovation is necessary for the feasibility in practice, these fuels are attractive due to renewable and potentially carbon neutral resources. Moreover, photosynthetic microorganisms are attractive since they can grow on non-arable land and utilize saline and wastewater streams. Highly versatile and genetically tractable photosynthetic microorganisms need to capture solar energy and convert atmospheric and waste CO_2 to high-energy chemical products. Understanding of the metabolism and the efficient metabolic engineering of the photosynthetic organisms together with cultivation and separation processes as well as increased CO_2 assimilation enables the enhancement of the feasibility of biofuel and biochemical production.

Keywords: Microalgae; Cyanobacteria; Biofuels; Metabolic engineering; CO_2 fixation; Metabolic regulation

Background

International Panel on Climate Change (IPCC) keeps warning the global society on global warming caused by green-house gases such as CO_2 based on the accumulating data and the reliable prediction model. IPCC asks world societies to make decisions to invest for the reduction of CO_2 emissions mostly caused by human activities. This may be also considered from the point of view of future cost caused by the severe climate change due to global warming. Namely, the global warming may cause serious local climate change as well as the rise in the sea level, which give severe damage to the societies worldwide. In fact, we have often experienced disastrous climate change year by year, and it seems to be more and more severe.

The global carbon cycle has been perturbed by emissions from the combustion of fossil fuels and by changes in land use and land intensity. These perturbations have led to cumulative anthrogenic CO_2 emissions of 570 ± 70 petagrams carbon since 1750 to 2012 [1]. Seventy percent of these emissions originated from the combustion of fossil fuels [1].

According to the data of International Energy Agency (OECD, 2011), total energy consumption in the world increased more than 78% over the last three decades. Major usage of fossil fuels causes serious environmental problems worldwide, and much attention has been focused on reducing their usage by alternative clean fuels. Namely, due to the global warming problem caused by the increased use of fossil fuels together with limited amount of fossil fuels and the fluctuating cost caused by unstable political disturbances, alternative renewable

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energy sources have recently been paid much attention [2]. In fact, at the present staggering rate of consumption, the world fossil oil reserves will be exhausted in less than 50 years [3]. Carbon neutral biofuels are needed to replace the petroleum oil which causes global warming caused by the emission of green house gases. Currently, the world consumes about 15 terawatts of energy per year, and only 7.8% of this is derived from renewable energy sources [4]. Moreover, in comparison with other forms of renewable energy such as wind, tidal, and solar energy, liquid biofuels allow solar energy to be stored and also to be used directly in existing engines and transport infrastructure [5].

Annually, about $5{,}500 \times 10^{21}$ J of solar energy reaches the Earth's atmosphere [6]. Photosynthetic organisms including higher plants, microalgae, and cyanobacteria play the crucial roles of capturing solar energy and storing it as chemical energy [7]. The amount of solar energy currently captured by arable crops is limited by arable land area (about 3.9% of the Earth's surface area), fresh water (about 1% of global water), nutrient supply, and solar energy-to-biomass conversion efficiency [8-10]. Terrestrial plants capture 121.7×10^9 metric tons of carbon from the atmosphere each year [11] using solar light and CO_2 as the energy and carbon sources. Photosynthesized carbon is then chemically converted to a variety of chemical compounds, and it is attractive to use photosynthetic organisms as green factories for producing carbohydrates, liquid fuels, and pharmaceutical drugs as well as food and feed, thus contributing to the balancing of the atmospheric carbon [12].

The advantages of using photosynthetic microorganisms include the photosynthetic efficiency, location on non-arable land (about 25% of the Earth's surface), and the use of saline and wastewater source [7], where less than 1% of the available solar energy flux is converted into chemical energy by photosynthesis [13], and much effort has been focused on the enhancement of photosynthetic carbon fixation.

The so-called first generation biofuels have been produced from corn starch and sugarcane. However, this causes the problem of the so-called 'food and energy issues' as the production scale increases. The second generation biofuels production from lignocellulosic biomass has thus been paid recent attention. However, it requires energy-intensive pretreatment for the degradation of lignocellulosic biomass [14]. The third generation biofuel production from photosynthetic organisms such as cyanobacteria and algae has been also attracted some attention, but the cell growth rate is quite low, and thus the productivity of the metabolites is significantly low [15].

Although the biofuel and biochemical production by photosynthetic organisms has a big hurdle to overcome, it is still highly attractive due to CO_2 fixation with sunlight (and water) from environmental protection point of view, and thus contributing to the global warming problem as well. A variety of host organisms such as bacteria, fungi, and microalgae may be considered for the production of biofuels and biochemicals from CO_2 with sunlight. Although photosynthetic organisms offer the ability to produce biofuels and biochemicals directly from CO_2 and sunlight, significant innovation is inevitable for the process development in relation to large-scale cultivation, harvesting, and product separation, since the production rate is significantly low.

The commonly used photosynthetic organisms for biofuel and biochemical production are algae and cyanobacteria [16,17]. Microalgae are photosynthetic eukaryotic organisms with size ranging from 1 to 100 μm, while cyanobacteria are prokaryotic organisms with size ranging from 1 to 10 μm. Cyanobacteria gave rise to the chloroplasts of eukaryotic algae and also land plants, and they share many features such as the ability to drive photosynthetic water photolysis and thereby contribute to the production of both atmospheric oxygen and reduced organic carbon [7].

Microalgae are unicellular photosynthetic microorganisms that can convert solar energy to chemical energy with efficiency of 10 to 50 times greater than terrestrial plants [18]. Algae have far higher cell growth rates than plants and, therefore, have much smaller footprints for land required for producing energy [19-21]. Many microalgae are rich in oil especially under nitrogen-starved condition, which can be converted to biodiesel using existing technology. The productivity of these photosynthetic microorganisms in converting CO_2 into carbon-rich lipids, only a step or two away from biodiesel, significantly exceeds that of agricultural oleaginous crops, without competing for arable land [22]. They require aquatic environments that may vary from freshwater to seawater. Not only do these organisms fix CO_2, but they also have the potential to be used for the production of inexpensive bulk chemicals, because the major inputs to the system (light and CO_2) are essentially free [23]. Microalgae cells contain approximately 50% of carbon, in which 1.8 kg of CO_2 is fixed by producing 1 kg of microalgae biomass [19].

Recent studies have reported that *Chlorella* sp., *Scenedesmus* sp., and *Botryococcus braunii* are among the microalgae strains that have shown promising result to bio-mitigate O_2 emission with typical CO_2 consumption rate of 200 to 1,300 mg/L/day [24-28]. Successful commercial utilization of microalgae has been established in low-volume, high-value derivatives such as nutritional supplements, antioxidants, cosmetics, natural dyes, and polyunsaturated fatty acids (PUFA) [29].

In the case of plants, it is frequently found that metabolite pools exist in more than one location or that the subcellular location of one or more reactions is uncertain

[30]. Entire sections of metabolic pathways like glycolysis are duplicated between organelles, particularly the plastid and cytosol, with both being potentially active and carrying flux [31]. The simplest way is to examine metabolites which are formed in only one of the compartments [32-36]. Another method involves the fraction of cellular material prior to metabolite analysis [37]. Unfortunately, even with the supplemental information provided by analyzing compartment-specific metabolites, it may still be difficult to statistically distinguish different configurations of the metabolic map [38].

In the present article, we focus on the typical photosynthetic microorganisms such as algae and cyanobacteria and attempted to make a review on the metabolic regulation, metabolic engineering, and process development with efficient operation for the production of biofuels and chemicals to understand the current status and expect future perspectives.

Candidate photosynthetic microorganisms for biofuel production

Perhaps the most comprehensive evaluation of algal species has been orchestrated by the US Department of Energy's Aquatic Species Program (ASP) to develop microalgae as a source of biodiesel [29]. Over 3,000 strains of microalgae have been isolated from ponds and seas. Cellular oil content varies with growth phases [39]. The cells of the chlorophyte microalga *Parietochloris incise* synthesize almost twice as many triacylglycerols (TAGs) in the stationary phase than in the exponential growth phase [40]. Although microalgae have a high level of biodiversity, only a few species can be subjected to genetic manipulation [41]. The algae with the best developed genetic toolbox are the unicellular green microalgae *Chlamydomonas reinhardtii* [42]. It is a well-established model organism for the study of various cellular processes such as photosynthesis, flagella, starch metabolism, and photobiological production of hydrogen [42]. Like many other algal species, *C. reinhardtii* can accumulate significant amount of oil when subjected to unfavorable environmental conditions [43-47]. *C. reinhardtii* has proven to be a useful model organism to study the improvement of biodiesel production by microalgae [48,49]. It is unicellular and stays as haploid during most of its life cycle [42], thus it is particularly useful in the context of a forward genetic approach, because the mutant phenotype can be observed during the first generation and does not need to reach a diploid homozygous stage [50]. The freshwater green microalgae *P incise* enhances not only its production of TAG under nitrogen starvation but also the production of arachidonic acid, a valuable nutraceutical [51]. Green algae including *Spirogyra* sp. and *Chlorococum* sp. have been shown to accumulate high levels of polysaccharides both in their complex cell walls and as starch [4]. This starch can be used for bioethanol production. Bioethanol production from algae shows significant potential due to their low percentage of lignin and hemicelluloses as compared to other lignocellulosic plants [52]. Microalgae and cyanobacteria are also able to directly produce biohydrogen through photofermentation in an anaerobic process involving oxidation of ferredoxin by the hydrogenase enzyme [53].

Many species of macroalgae are known to have high levels of carbohydrate, although in many cases these carbohydrates consist of galactose [54]. Recent research has shown that the red algae *Gelidium amansii* and the brown algae *Laminaria japonica* are both a potential biomass source for biohydrogen production through anaerobic fermentation [54,55]. Recently, microalgae have also been paid attention from the point of view of biogas production in the anaerobic fermentation [4].

Metabolism of photosynthetic microorganisms

Oxygenic photosynthesis is the process by which plants, algae, and cyanobacteria convert sunlight and CO_2 into chemical energy and biomass. The algal photosynthesis is at least able to convert approximately 5% to 7% of incident light energy to biomass, where a systems-based approach to understand the stresses and efficiencies associated with light energy harvesting, CO_2 fixation, and carbon partitioning is necessary to make headway toward improving photosynthetic yields [56].

The cell growth conditions are roughly classified as *autotrophic* condition for the case of using only CO_2 under light condition, *mixotrohphic* condition for the case of using both carbohydrate and CO_2 under light condition, and *heterotrphic* condition for the case of using carbohydrate under dark condition. Although autotrophic condition is preferred from the environmental protection point of view using only CO_2 as a carbon source, the cell growth rate is significantly low, and thus the productivity of the metabolic products is low.

The atmospheric CO_2 is fixed either by C_3 photosynthesis where the three carbon molecule such as 3-phosphogrycerate (3PG) is used as the product of ribulose 1,5-bisphosphate carboxylase (RubisCO) reaction or by C_4 photosynthesis where four carbon molecule such as oxaloacetate (OAA) is used as the product of phosphoenol pyruvate (PEP) carboxylase (Ppc) reaction followed by the decarboxylation at malic enzyme (Mez) from malate yielding pyruvate. The C_4 photosynthesis may be created by evolution from ancestral C_3 photosynthesis during a global decline in atmospheric CO_2 level [57]. The C_4 pathway will have higher efficiency than the C_3 pathway in CO_2 fixing with which they consume water and nitrogen [58].

Since CO_2 fixation is attractive from the environmental protection point of view, several strategies have been considered for the efficient carbon fixation for the cell

synthesis [59], where carbon fixation can be enhanced by amplifying carboxysome expression [60] or heterologous expression of RubisCO gene *rbcLS* [61]. The CO_2 fixation can be also enhanced by a hybrid RubisCO, which contains both plant and microalgae subunits [62].

The green organisms such as plants and algae gain energy via aerobic respiration, and the metabolism changes depending on the oxygen availability. The green organisms are exposed to a variety of oxygen availability in the environment that may vary from fully aerobic state (normoxia) to oxygen deficiency (hypoxia) or the anaerobic condition (anoxia). In the context of recent climate change, excess rainfall and frequent flooding may cause the green cells subjected to hypoxia or anoxia condition [63]. Oxygen is the final acceptor of electrons in the mitochondrial oxidative phosphorylation to generate ATP, while under hypoxia and anoxia conditions, the small amount of ATP is generated through glycolysis, and NAD(P)H must be reoxidized by the fermentative pathways. Under oxygen-limiting condition, the glycolysis flux is accelerated by the so-called 'Pasteur effect', and plant metabolism uses pyruvate to direct towards ethanolic and lactic fermentations [64]. In almost all plant, a rapid activation of lactate dehydrogenase (LDH) has been observed under oxygen limitation [63]. Lactate production causes damage to the cell by lowering cytoplasmic pH, and thus lactate production is transient eventually replaced by ethanolic fermentation. The α-ketoglutaric acid (αKG) in the TCA cycle can be oxidized with the incorporation of NH_4^+ and NADH to form glutamate (Glu), which is then decarboxylated to ɤ-amino butyric acid (GABA) by glutamate decarboxylase (GDC), where some protons are utilized in GDC reaction and stabilizes the cytosolic pH [63].

Metabolism of algae

It is critical to properly understand the metabolism in response to culture environment, where the systems biology approaches including metabolite profiling [65] and integration of different levels of information such as metabolites, fluxes, transcript, and protein abundance [66] are useful.

Consider the metabolism of photosynthetic microorganisms [67]. The light energy is incorporated into the cell, where light quanta absorbed by pigments drive the photosynthetic electron transport, where NADPH instead of NADH is used to generate ATP at the respiratory chain. The primal pathway for CO_2 fixation is the Calvin-Benson-Bassham (CBB) cycle, where the first step is catalyzed by RubisCO (Figure 1). This enzyme is also an oxygenase, which can react with O_2 and lead to a different pathway called photorespiration. Algae have the photorespiration pathway, and photosynthesis is inhibited by high O_2 concentration. Photosynthesis reactions such as light reactions, CBB cycle, and starch synthesis occur in chloroplasts. Algae and plant cells have subcellular compartments such as chloroplast, mitochondria, and cytoplasm. After the export of GAP from the chloroplast to cytoplasm, the carbon flow is divided into the sugar synthesis pathway or the glycolytic pathway to form pyruvate. Sugars such as sucrose are the major storage products in the cytoplasm of plant cells. In plant cells, replenishment of carbon to maintain the operation of the TCA cycle is achieved by anaplerotic reactions involving CO_2 fixation by PEP carboxylase (Ppc). The pentose phosphate (PP) pathway operates in the cytoplasm, where CBB cycle is functioning in the chloroplast.

Of all the pigments, chlorophyll takes a major fraction. δ-aminolevulinic acid (δ-ALA) is the key chlorophyll precursor molecule. The classical succinate-glycine pathway is the condensation of glycine and succinyl-CoA catalyzed by δ-ALA synthetase. In addition, glutamate and αKG are incorporated into δ-ALA much more efficiently than are glycine and succinate in many green cells. Although most of the fatty acid synthesis occurs in the chloroplast, the source of acetyl-coenzyme. A (AcCoA) derives from its synthesis in the mitochondria. The fatty acid composition of the lipids of *Chlorella* cells varies considerably, particularly for the α-linolenic acid (C18:3) content [67].

Under autotrophic condition, significant ATP is formed from mitochondrial oxidative phosphorylation. The CBB cycle is the main ATP sink in the autotrophic culture. The ATP yield decreases in the following order: heterotroph > mixotroph > autotroph [67].

Metabolism of cyanobacteria

Cyanobacteria are commonly used as model systems for the metabolism of higher plants. Cyanobacteria possess certain promising properties such as (1) large amounts of lipids, commonly present in thylakoid membranes, (2) higher photosynthetic levels and the cell growth rates compared to algae and higher plants, (3) easy growth with basic nutritional requirements such as air ($CO_2 + N_2$), water, and mineral salts with light [68].

The central metabolic network in *Synechocystis* is shown in Figure 2, which includes those of the glycolysis, PP pathway, CBB cycle, part of TCA cycle, and the C1 metabolism. Cyanobacteria have an incomplete TCA cycle lacking αketoglutarate dehydrogenase (KGDH) [69,70]. The enzymes, isocitrate lyase (Icl) and malate synthase (MS), which form the glyoxylate pathway, function in cyanobacteria [71]. The Mez and PEP synthase (Pps) are responsible for the gluconeogenetic steps, where the PEP carboxy kinase (Pck) is absent in *Synechocystis*. The PP pathway operates for glucose catabolism mainly in the heterotrophic conditions, while the CBB cycle is active under mixotrophic and autotrophic conditions.

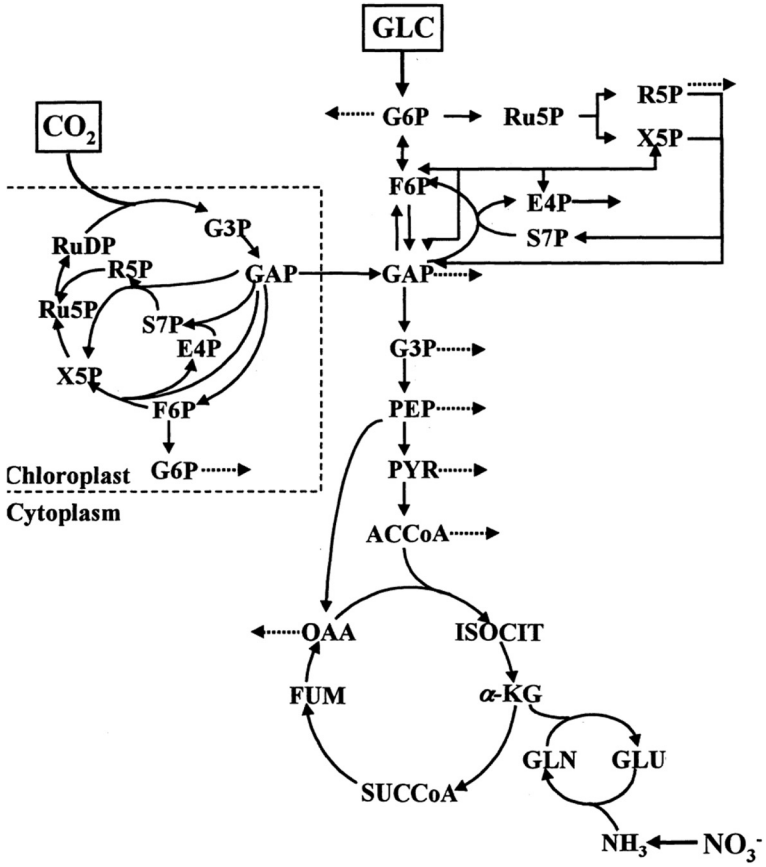

Figure 1 Metabolic pathways of photosynthetic microorganism (adapted from [67]).

The metabolic flux analysis of *Synechocystis* cultivated under heterotrophic and mixotrophic conditions has been made based on ^{13}C-metabolic flux analysis (^{13}C-MFA) [72,73], and the metabolic regulation analysis has also been made with integration of different levels of information [74]. In the heterotrophic cultivation of *Synechocystis*, more than 90% of glucose is channeled through the PP pathway (Figure 3) [72,73]. The high flux through the oxidative PP pathway yields a large amount of NADPH, as well as biosynthetic precursors such as ribose 5-phospate (R5P) and erythrose 4-phosphate (E4P). In the mixotrophic culture, CO_2 is fixed through the CBB cycle. The conventional ^{13}C-MFA is based on the steady state and thus limited to heterotrophic and mixotrophic conditions [72,73,75], while ^{13}C-MFA for autotrophic condition can be made by the isotopically nonstationary metabolic flux analysis (MFA) [76] with transient measurements of isotope incorporation following a step change from unlabeled to labeled CO_2 (Figure 3) [77].

Since cyanobacteria have negligible photorespiration and produce little or no glycolate during photosynthesis, it is unlikely that serine is synthesized, as in higher plants, from glycine by the glycolate pathway. Serine is synthesized directly from 3PG through a phosphorylated route in cyanobacteria [78].

Under both heterotrophic and mixotrophic conditions, the relative flux through Ppc is high. The reaction catalyzed by Ppc contributes to about 25% of the assimilated CO_2 under mixotrophic condition [72,73], indicating that Ppc is important for the fixation of CO_2 in cyanobacterial cells [79], where cyanobacterial cells fix significant amounts of carbon as C4 acids under light conditions. Considering that Mez in cyanobacteria is NADP-linked [80], it is more likely that Ppc and Mez serve as a device to fix a large amount of CO_2 as C4 acids and then release CO_2 and produce NADPH by the decarboxylation of malate. This Ppc and Mez pathways can effectively bypass the Pyk reaction, where its activity is repressed under light condition [77]. This is similar to the carbon metabolism in C4 plants, for which CO_2 and NADPH generated by Mez are utilized by the CBB cycle. In fact, although the major pathway of CO_2 fixation in the CBB cycle is similar to that in C3 plants, cyanobacteria have many of the physiological characteristics of C4 plants.

In the mixotrophic culture, copious amounts of reducing power are required in the CBB cycle to fix CO_2 to

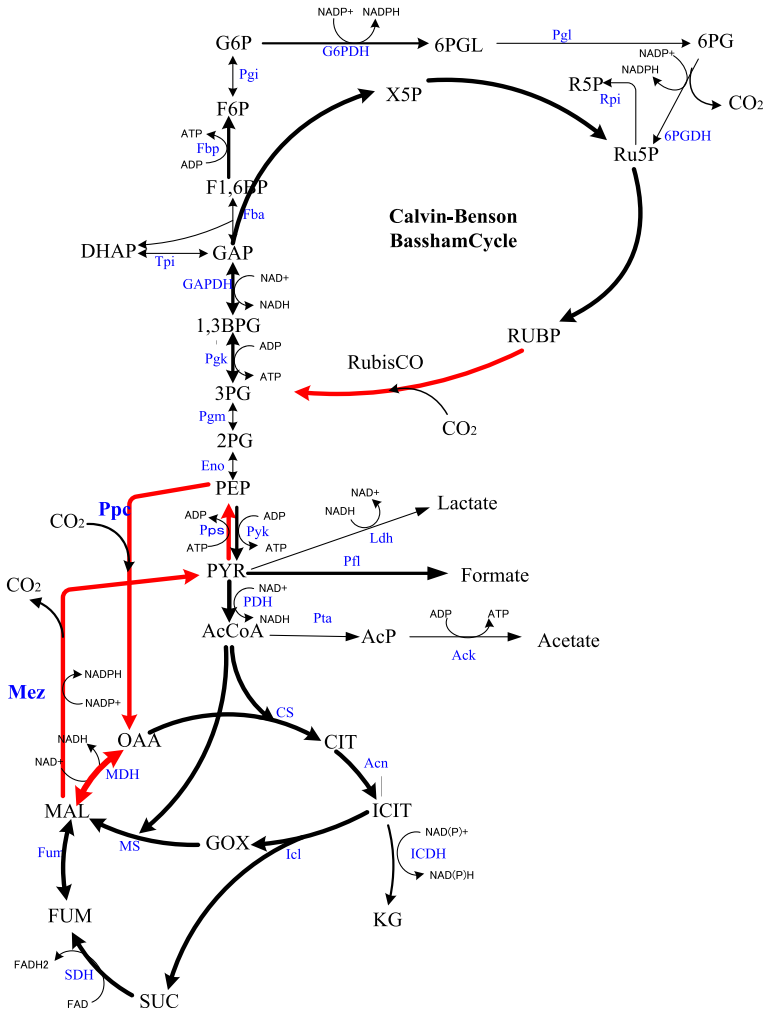

Figure 2 Metabolic pathways of cyanobacteria.

carbohydrates. Hence, there has to be a supply of NADPH in large amounts to fulfill the biosynthetic demands. The PP pathway in the heterotrophic culture and the photosynthetic electron transport in the mixotrophic culture accounts for a major fraction of NADPH production. Moreover, cyanobacteria utilize NADPH as an electron donor of the respiratory electron transport chain. Therefore, the excess NADPH is reoxidized during respiration to provide energy. The CBB cycle is the main ATP sink in the mixotrophic culture.

Catabolic regulation and carbon storage regulation under nitrogen limitation

Like many bacteria on earth, photosynthetic organisms are found in diverse ecological habitats, where the organisms are exposed to periods of severe nutrient starvation. In particular, cyanobacteria are found in a wide range of ecological habitats including oceans and lakes [81]. They also survive in deserts, polar regions, and hot springs, where the nutrient starvation is much more severe.

Under unfavorable growth condition such as nitrogen starvation, TAG is typically produced in microalgae, where its fraction ranges from 20% to 60% (weight/dry weight) [82]. The efficient production of TAG in microalgae requires a thorough understanding of lipid metabolism and TAG accumulation [83]. It is important to analyze different levels of information to uncover the molecular mechanism underlying the increased TAG accumulation for microalgae such as *C. reinhardtii* and its starchless and cell wall-deficient mutant strains [84].

Cyanobacteria have sophisticated mechanisms to cope with nitrogen limitation, where the primary step is the capture of nitrogen-containing compounds with high affinity, where nitrate, nitrite, and ammonium are the typical nitrogen sources with a preference for ammonium [85]. Some strains can fix dinitrogen gas and may use also urea, cyanate, and amino acids as additional nitrogen

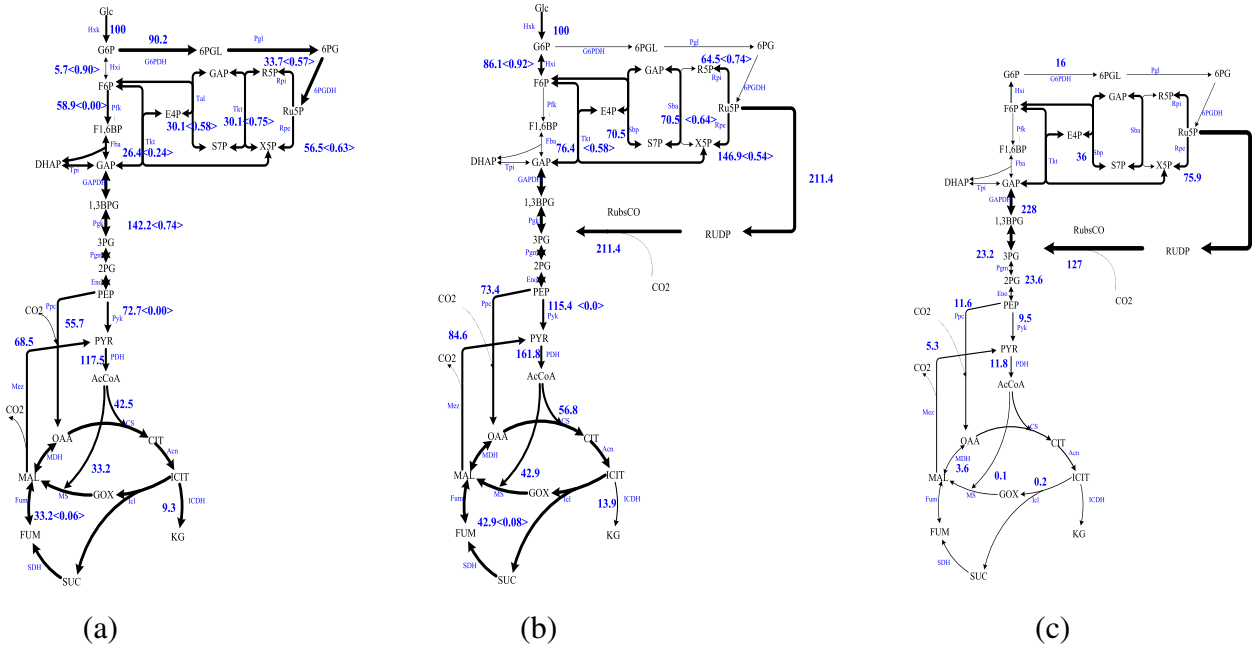

Figure 3 ^{13}**C-Metabolic flux distribution of *Synecocystis* sp.** PCC6803 cultivated under heterotrophic **(a)**, mixotrophic **(b)**, and **(c)** autotrophic conditions. The flux values were obtained from [73] for (a) and (b) and from [77] for (c).

sources [85-87]. Nitrogen compounds are eventually converted to ammonium and assimilated for biosynthesis via the glutamin synthetase (GS)-glutamine oxoglutarate aminotransferase or glutamate synthase (GOGAT) cycle, where glutamate dehydrogenase (GDH) pathway does not function, probably due to low affinity to ammonium.

For survival under nitrogen starvation, cyanobacteria accumulate reserve materials in the form of inclusions and granurs, where the induction for their accumulation is made upon high light or CO_2, nutrient starvation as well as addition of arginine or chloramphenicol [88]. Cyanophycin (multi-*l*-arginyl-poly-[L-aspartic acid]) is a nitrogen reserve and is a non-ribosomally synthesized peptide consisting of equi-molar quantities of alginine (Alg) and aspatic acid (Asp), where cyanobacteria may consume internal storage compounds such as cyanophycin as nitrogen source upon nitrogen starvation [88,89].

After cyanophycin is exhausted, cells degrade the phycobilisomes that are large protein-rich right-harvesting antennae attached to the outside of the thylakoid membranes and support the light-dependent reactions of photosynthesis [90], where it is composed of rod and core proteins to provide nitrogen, which leads to a color change of cells from blue-green to yellow-green, known as bleaching [90]. Upon availability of nitrogen source again, cyanophycin is immediately synthesized [89].

Nutrient balance is important for the cell growth, since proteins, nucleic acids, carbohydrates, lipids, and pigments must be supplied in a suitable ratio for the balanced growth. In eukaryotic microalgae, autophagy is induced by nitrogen starvation to degrade cytoplasmic components including plastids in the large vacuoles [91]. In cyanobacteria, a unique Nb1A-dependent mechanism is induced to degrade certain phycobiliproteins, where the non-bleaching phenotype gene, *nblA* plays an important role for the degradation of phycobiliprotein [92,93]. The phycobilisome has a role in nitrogen storage as well as photosynthetic antenna [90]. Moreover, NblA1/A2-dependent protein turnover contributes to the maintenance of many amino acids (AAs) in NblA1/A2-dependently, while Lys pool markedly increased under sulfur starvation in cyanobacteria [94].

The internal C/N ratio is sensed by the PII protein, GlnB, in particular under N-limitation [95]. The global nitrogen regulator NtcA plays important roles for nitrogen regulation, where it senses αKG levels and regulates the genes involved in nitrogen assimilation. NtcA directly regulates the expression of *nrrA* gene which encodes a nitrogen-regulated response regulator of the OmpR family. NrrA is involved in induction of sugar catabolic genes as well under nitrogen starvation [96]. NrrA also regulates glycogen catabolism in *Anabaena* sp. by directly regulating expression of *glgP* gene encoding glycogen phosphylase and *sigE* gene encoding a group 2 σ factor of RNA polymerase [97]. Nrr controls cyanophycin accumulation and glycogen catabolism in cyanobacteria [98], where glycogen is accumulated, whereas the expression of sugar catabolic genes is widely upregulated under nitrogen starvation [99].

Microalgae produce certain biomass compounds under nutrient limitation [100]. In cyanobacteria such as *Synechocystis* sp., polyhydoxy butyric acid (PHB), one of polyhydroxy alcanoate (PHA), is accumulated under nitrogen or phosphate starvation, where PHB is formed from AcCoA via β-keto-thiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC), where PHA synthase is activated by acetyl phosphate (AcP) [101]. Since acetoacetyl-CoA reductase requires NADPH, the pathway modification that causes excess NADPH yields higher PHB production [102].

Cyanobacteria have nine sigma factors such as SigA-I, where RNA polymerase sigma factor SigE plays important roles under nitrogen starvation [103]. SigE activates the expression of the genes associated with degradation of glycogen and catabolic genes of glycolysis and PP pathway [104]. Moreover, SigE activates PHB synthetic pathway gene expression [105], and thus the overexpression of *sigE* allows higher PHB production under nitrogen limitation [106]. Moreover, SigE also activates the expression of hydrogenase gene, and thus the overexpression of *sigE* also allows higher hydrogen production under anaerobic condition [107].

Cyanobacteria have two types of sunscreen pigments such as scytonemin and mycosporine-like amino acids, where these secondary metabolites play roles against environmental stresses such as UV radiation and desiccation [108].

Systems biology approach and modeling of the metabolism

Although algae and cyanobacteria have been paid recent attention for the potential to the sustainable biosynthesis, unknown and uncharacterized gene and protein functions hamper the progress toward the future era of algae industrial biotechnology. The systems biology approach plays a crucial role for function prediction based on the database with proper metabolic modeling [109].

Some attempts have been made for the modeling of photosynthetic organisms [110,111], while mechanistic model of photosynthesis in microalgae has also been developed [112,113]. The sequential statistical analysis based on experimental design coupled with least squares multiple regression has been made to analyze the dependence of respiratory and photosynthetic responses upon concomitant modulation of light intensity as well as acetate, CO_2, nitrate, and ammonia concentrations in the culture of *C. reinhardtii* [114].

MFA may be considered to gain insight into the metabolism, where the optimal light intensity can be identified for the biomass yield of *C. reinhardtii* by considering the cell maintenance and biomass formation [115]. A mixed integer linear programming method was used to find the optimal flux distributions of *C. reinhardtii* cultivated under photoautotrophic conditions in photobioreactors functioning in physical light limitation based on the constraint-based model, which includes thermodynamic and energetic constraints on the functioning metabolism, highlighting the existence of a light-driven respiration depending on the incident photon flux density [116].

Flux balance analysis (FBA) based on the network consisting of 484 metabolic reactions and 458 intracellular metabolites for *C. reinhardtii* indicates that aerobic heterotrophic growth on acetate has a low yield on carbon, while mixotrophically and autotrophically grown cells are significantly more carbon efficient [117]. A genome-scale extension for *C. reinhardtii* has been made with the network consisting of 1,080 genes, associated with 2,190 reactions and 1,068 metabolites (named *i*RC1080), that enables quantitative growth prediction for a given light source, resolving wavelength and photon flux. This offers insight into algae metabolism and potential for genetic engineering and efficient light source design [118]. Another comprehensive literature-based genome-scale model with the network of 866 ORFs, 1,862 metabolites, 2,249 gene-enzyme-reaction-association entries, and 1,725 reactions has been developed (named AlgaGEM), where it predicted observable metabolic effects under autotrophic, heterotrophic, and mixotrophic conditions, and predicts increased hydrogen production when cyclic electron flow is disrupted, and the physiological pathway for H_2 production, which identified new targets for further improvement of H_2 yield [119].

FBA approach has also been employed for cyanobacteria with the emphasis on the alleged glyoxylate shunt and the role of photorespiration in cellular growth and analyzed the diurnal light/dark cycles of the metabolism [120]. Genome-scale metabolic model of *Synechococcus elongates* PCC7942 (named *i*Syf715) has also been developed with the network of 851 reactions and 838 metabolites, and the applicability has been demonstrated for autotrophic growth conditions [121].

Metabolic modification for various biofuel and biochemical production

Algae have the potential for the genetic modification of their lipid pathways by upregulation of fatty acid biosynthesis or by downregulation of β-oxidation. By knocking out or modifying enzymes responsible for the synthesis of polyunsaturated lipids in the cell, it may be possible to dramatically increase the production of mono-unsaturated lipids [122]. Under optimal growth condition, the wild-type *Chlamydomonas* strains accumulate very low amount of oil (<1 μg per 10^6 cells) [45]. When cells are subjected to nitrogen starvation, oil content can be increased more than tenfold (up to 10 μg per 10^6 cells) [43-45]. Intracellular TAG amounts also fluctuate during the diurnal cycle because TAGs produced during the day provide a carbon and energy source for the night [123]. This is the major

factor to yield loss in open pond microalgae cultivation. Researchers have not been able to achieve efficient homologous recombination in the nuclear genome of the commonly transformed laboratory algal strain *C. reinhardtii*, but this is not the case for the marine alga and biofuel candidate *Nannochloropsis* [124].

As a result of genetic engineering, some obligate photoautotrophs, formerly unable to partake in a sweet diet, have been given a taste of heterotrophy through the introduction of hexose transporters [125]. In the starchless mutant of *Chlamydomonas*, the flux through starch production redirects to lipid accumulation under nitrogen-starved condition. The strain should be designed to switch off completely for starch accumulation under nitrogen-starved condition. Blocking oil turnover processes might help increase the level of oil accumulated, as was observed in *Arabidopsis* leaves where the oil content was increased tenfold by knocking out a lipase gene [126].

Most metabolic engineering investigations have been made using such typical model organisms as *Synechocystis* sp. and *Synechococcus elongatus* sp. as well as *Aanabaena* sp., whereas much more complex genetic engineering is required for algae [16].

Bioethanol can be produced by introducing pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) genes from *Z. mobilis* into the shuttle vector and then transform *Synechosystis* sp. [127,128] (Table 1).

As shown in Figure 4, 2-ketoisovalerate can be converted to isobutyraldehyde by introducing ketoacid decarboxylase gene *kivd* from *Lactococcus lactis* into the genome of *S. elongatus* PCC7942 [61]. The flux to 2-ketoisovalerate can be improved by introducing *alsS* gene from *Bacillus subtilis* and the *ilvC* and *ilvD* genes from *E. coli* into the chromosome of *S. elongatus*. Carbon fixation can be improved by overexpression of RubisCO genes to increase the productivity of isobutyraldehyde. Iso-butanol can then be produced by introducing alcohol dehydrogenase YqhD from *Escherichia coli* [61].

2-Methyl-1-butanol (2 MB) can be produced in *S. elongatus* by expressing heterologous enzymes for citramalate pathway [129] to direct pyruvate toward isoleucine sysnthesis pathway, where 2-keto-3-methyvalerate is converted to 2 MB by Kivd and YqhD [130] (Table 1).

1-Butanol can be produced from AcCoA by anaerobic dark condition by engineered *S. elongatus* by introducing acetyl transferase (AtoB) from *E. coli*, 3-hydroxybutyryl-CoA dehydrogenase (Hbd) from *Clostridium acetobutylicum*, trans-2-enoyl-CoA reductase (Ter) from *Treponema denticola*, crotonase (Crt) from *C. acetobutylicum*, and bifunctional aldehyde/alcohol dehydrogenase (AdhE2) from *C. acetobutylicum* [131]. 1-Butanol can be also produced by aerobic culture of *S. elongates* PCC7942, where condensation of AcCoA is made by consuming ATP with CO_2 evolution [132], where ATP-dependent malonyl-CoA synthesis enzyme NphT7 was introduced, and NADH-dependent enzymes were replaced by NADPH-dependent enzymes in this strain. Butanol tolerance of *Synechocystis* can be improved by 150% by evolution by gradually increasing butanol concentration from 0.2% to 0.5% (*v*/*v*) [133] (Table 1).

Lactic acid has been used in the food and pharmaceutical industries and for biodegradable polymers [134], and this can be produced in *Synechosystis* after heterologous expression of LDH [135,136]. Since Pyk is inhibited under light condition in *Synechosystis*, heterologous expression of Pyk can enhance the pyruvate production and in turn enhance the lactate production, where Pyk is allosterically activated by fructose 1,6-bisphosphate (FBP) in the case of Pyk-F originated form *E. coli*, while the original Pyk does not show such characteristics [136]. Moreover, Ppc may be knocked down to direct the carbon flow from PEP towards lactate production via PYR, but the cell growth is depressed, since Ppc is also an important pathway for CO_2 fixation [136]. In most bacteria, LDH requires cofactor NADH, whereas NADPH is abundant in *Synechosystis* as mentioned before, and thus NADPH-dependent LDH may increase the lactate production, where this may be partly attained by introducing the LDH from *B. subtilis*, of which LDH co-utilizes NADH and NADPH [136,137] (Table 1).

Isoprene is a volatile compound and utilized in the synthesis of rubber etc., where isoprene is easily evaporated from the culture broth, and thus the toxicity to the cell can be relaxed by evaporation, where it can be trapped in the gas phase. Isoprene can be synthesized by *Synechocystis* sp. PCC6803 by introducing *lspS* gene from vine *Pueraria montana* and utilizing the naturally occurring methyl-erythritol-4-phosphate (MEP) pathway (Figure 4) [138] (Table 1).

Ethylene is another volatile compound, where this can be also produced by *Synechocystis* sp. PCC6803 by introducing ethylene forming pathway gene *efe* from *Pseudomonas syringae pv phaseolicola* (Figure 4) [139] (Table 1).

Fatty acid and fatty alcohol production can be made by *Synechocystis* sp. PCC6803 by overexpression of endogenous fatty acyl-ACP synthase gene *slr1609* (Figure 4) [140]. Only fatty acids can be produced and excreted outside of the cell by modification of *Synechocystis* sp. PCC6803 [141,142]. Fatty alcohols such as hexadecanol and octadecanol can be produced by introducing fatty acyl-CoA reductase genes from jojoba, which catalyze a fatty-acyl-ACP (Figure 4) [143] (Table 1).

Eucaryotic algae have also been considered for fatty acid production, where they can accumulate lipids up to about 70% of dry biomass [41,144]. The limitations of using algae are the complexity of the eukaryotic system and less available genetic tools, although some attempts have been done [145]. Some efforts are being made to

Table 1 Biofuel and biochemical production by cyanobacteria

Product	Species	Titer or productivity	Overexpressed or knockout gene (s)	Cultivation	Reference
Ethanol	*Synechococcus*	230 mg/L in 28 days	*pdc, adh*	Shake flask	[210]
	Synechocystis	552 mg/L in 6 days	*pdc, adh*	Photobioreactor	[127]
	Synechocystis	608 mg/L in 18 days	*pdc, adh*	Photobioreactor	[128]
Isobutyraldehyde	*Synechococcus*	1,100 mg/L in 8 days	*alsS, ilvC,D, kivd,rbcls*	Bottle with NaHCO3	[61]
Isobutanol	*Synechococcus*	18 mg/L	*kivd, yqhD*	Shake flask with NaHCO3	[61]
	synechococcus	450 mg/L in 6 days	*alsS, ilvC, D, kivd, yqhD*	Shake flask with NaHCO3	[61]
2 Methyl-1-butanol	*Synechococcus*	2 mg/L	*kivd, yqhD, cims*	Shake flask with NaHCO3	[61,130]
1-Butanol	*Synechococcus*	14.5 mg/L in 7 days	*hbd, crt, adhE2, ter, atoB*	Bottle under anoxic cond.	[131]
	Synechococcus	30 mg/L in 18 days	*ter, nphT7, bldh, yqhD, phaJ, B*	Shake flask	[132]
Fatty alcohol	*Synechocystis*	0.2 mg/L in 18 days	*far*	Photobaioreactor with 5% CO_2	[143]
	Synechocystis	0.02 mg/L/OD	*far,aas*	Shake flask	[140]
	Synechocystis	2.87 mg/gDCW	Δ*sll0208,* Δ*sll0209*	Flask	[211]
Fatty acids	*Synechocystis*	197 mg/L in 17 days	*tesA, accBCDA, fatB1, B2, tesA137*	1% CO_2 bubbling	[142]
Alka (e) nes	*Synechocystis*	0.162 mg/L/OD	*accBCDA*	Shake flask	[143]
	Synechocystis	2.3 mg/l/OD	*sll0208, sll0209*	Shake flask	[212]
Hydrogen	*Synechococcus*	2.8 μmol/h/mgChlorophyll-a	*hydEF, hydG, hydA*	Anaerobic condition	[153]
	Synechococcus	54 mmol/1,017 cells in 4 days	Δ*ldh*	Anoxic condition	[213]
L-Lactate	*Synechocystis*	0.0178 mmol/gDCW/h	*ldh, sth*	Shaking incubator	[214]
	Synechocystis	0.2512 mmol/gDCW/h	*pyk, ldh*	Shaking incubator	[136]
D-Lactate	*Synechocystis*	2.17 g/L in 24 days	*gldA, sth*	Photoautotropic with acetate	[215]
1,2-propanediol	*Synechococcus*	Approximately 150 mg/L	*mgsA, gldA, yqhD*	Shake flask	[216]
Isoprene	*Synechocystis*	50 μg/gDCW/d	*lspS*	Sealed culture	[138]
Ethylene	*Synechocystis*	26 μmol/gDCW/h	*efe* (RS1010)	Rotary shaker	[217]
	Synechocystis	111.6 μmol/gDCW/h	*efe* (slr068)	Rotary shaker	[217]
	Synechococcus	84.8 μmol/gDCW/h	*efe* (pUC303)	Flask	[218]
	Synechococcus	80.5 μmol/gDCW/h	*efe* (psbAI)	Flask	[219]
Acetone	*Synechocystis*	36.0 mg/L in 4 days	*ctfAB*, *adc*, Δ*phaCE*, Δ*pta*	Flask	[148]
PHAs	Synechocystis	1.4 mg/100 mgDCW	*sigE*	Bubbled with 1% CO_2 in the air	[106]
	Synechocystis	533.4 mg/L in 21 days	Δ*slr1829*, Δ*slr1830*	Flask	[220]

identify stress conditions and key enzymes for fatty acid synthesis in the green algae *Haematococcus pluvialis* [146]. Effects of light conditions on fatty acid production were also investigated for *Nannochloropsis* [147].

Acetone can be used as the precursor for isopropanol. This can be produced in *Synechocystis* PCC6803 by introducing CoA transferase (CtfAB) and acetoacetate decarboxylase (Adc) from *C. acetobutylicum* for converting AcCoA to acetone (Figure 4) [148], where the PHB-forming pathway genes *phaCE* and the acetate-forming pathway gene *pta* may be disrupted. Alpha-olefin production can be also made by *Synechococcus* sp. PCC7008 [149] (Table 1).

Methane can be produced by co-culture of *C. reinhardtii* and methanogenic bacteria, where glyconate is produced from the former, while methane is produced from the latter by assimilating glyconate [150]. *Rhodobacter* are non-sulfur photosynthetic bacteria that produce hydrogen (H_2) from acetate etc. Hydrogen production may be enhanced by introducing aldehyde dehydrogenase (ALDH) gene from *Rhodospirillum rubrum* into *Rhodobacter sphaeroides* [151]. Hydrogen production can be enhanced by introducing exogenous hydrogenase into nitrogen fixing [152] and non-nitrogen fixing cyanobacteria [153]. Hydrogen production can be improved by introducing hydrogenase from *Clostridium thermocellum* into *Rhodopseudomonas*

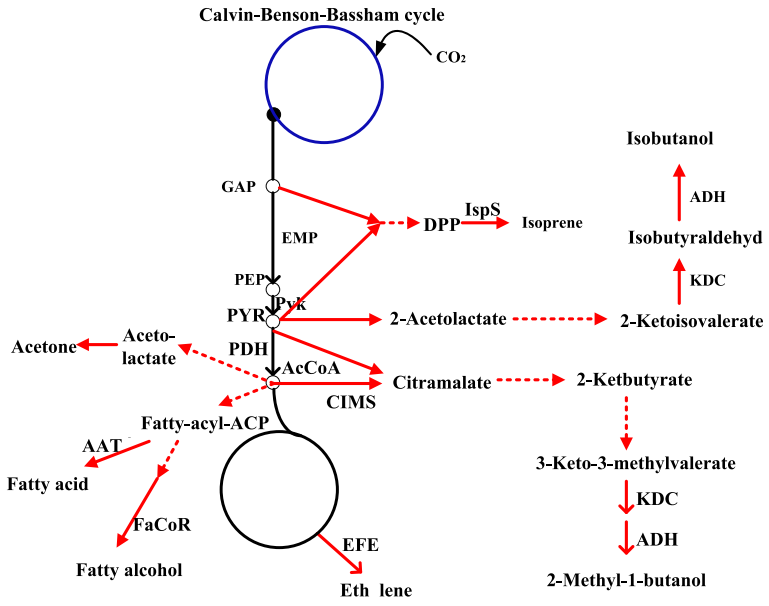

Figure 4 A variety of metabolic pathways for the production of biofuels and biochemicals.

palustris CGA009 and cultivated at 38°C by considering the outdoor bioreactors [152]. Hydrogen can be produced by introducing a hydrogenase HydA from *C. acetobutylicum* into *S. elongates* [153], and its production can be significantly improved in *Arthosporia* by continuously removing it from the culture broth [154].

Another important aspect of utilizing photosynthetic organisms is their ability of producing pharmaceuticals due to the reduction of ketones [155-157]. Microalgae are also attractive for their production of antioxidants, where *Fischerella ambigua* and *Chlorella vulgaris* show higher antioxidant activities [158]. Phenolic compounds have also antioxidant properties, where their production can be enhanced in *Spirulina platensis* by manipulating light intensities [159].

Phycocyanin is also attractive, where its production by *Arthospira* (*Spirulina*) *platensis* was investigated in marine environment [160]. The pigment sesquiterpene β-caryophyllene can be produced by *Synechocystis* sp. PCC6803 with the aid of a β-caryophyllene synthase gene from *Artemisia annua* [161], where this compound is used in the fragrance and cosmetic industry, and in natural remedies for its anti-inflammatory and antimicrobial properties. Lycopene is also important food additives and pigment, and can be produced by a purple non-sulfur bacterium, *R. rubrum*, by deletion of downstream phytoene desaturase gene *crtC* and *crtD* [162].

Cyanobacteria can excrete fructose, lactate, and glucose by introducing transport genes from *E .coli* [134]. Many cyanobacteria naturally produce sucrose as an osmotic response to their saline habitat together with manipulation of transport and secretion genes [163].

The biodegradable plastic such as PHB can be produced by *Rhodovulum sulfidophilum* P5 using inexpensive nitrogen and carbon sources [164]. PHB can be also produced in the filamentous cyanobacterium *Nostoc muscorum* under phosphate limitation by recombinant *Synechocystis* sp. PCC6803 [165] (Table 1). Glycogen can be produced in halophilic bacterium *A. palentensis* by manipulating growth condition [166]. Even ammonia can be produced by nitrogen-fixing cyanobacteria *Anabaena* sp. ATCC 33047 [167].

Cultivation and harvesting methods

Raceway pond

Microalgae can use sunlight more efficiently than other crop plants to produce oil [168]. The oil production capacity is almost one or two times higher than any other crop [169]. The open pond system is better for large-scale production. The main disadvantage of open pond systems is that by being open to the atmosphere, they lose water by evaporation at a rate similar to land crops and are also susceptible to contamination [122,170]. Some protozoa may contaminate the system and hamper the growth of microalgae.

An effective culture system may consist of the following criteria: (1) effective illumination area, (2) optimal gas-liquid transfer, (3) easy to operate, (4) low contamination level, (5) low capital and production cost, and (6) minimal land area requirement [171]. The system can be made of paddle wheel to avoid microalgae biomass sedimentation, and CO_2 may be sparged at the bottom of the raceway as carbon source [172].

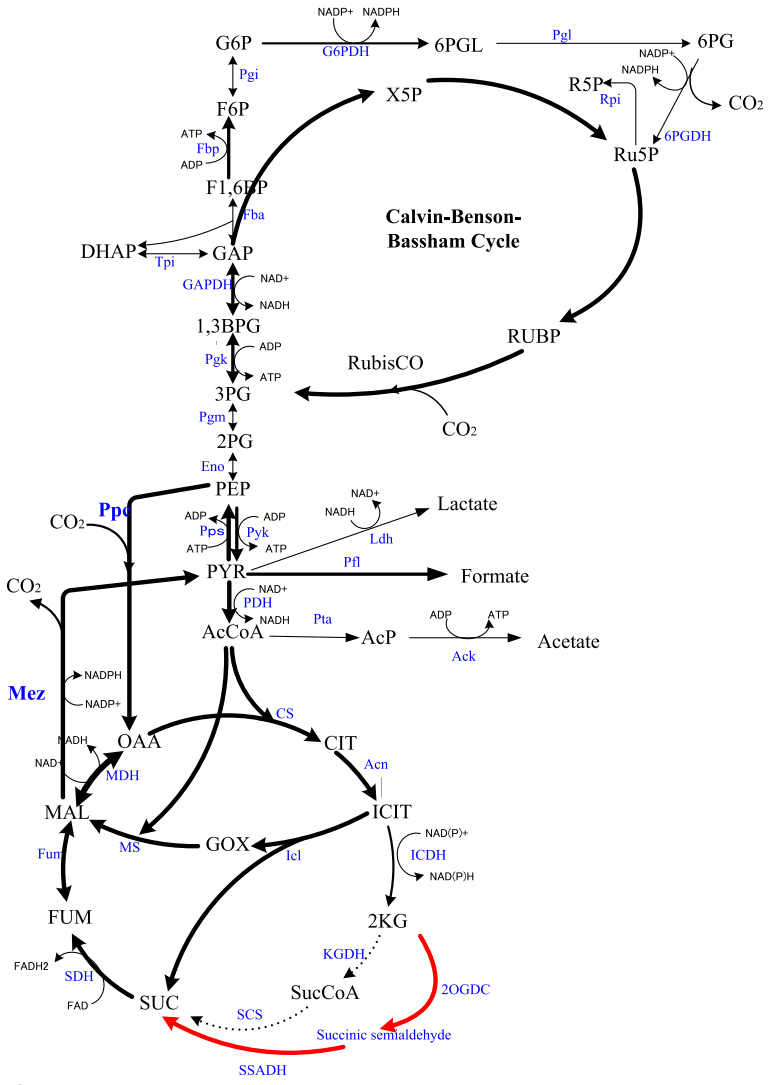

Figure 5 TCA cycle in cyanobateria.

Photobioreactor

Closed photobioreactor may be considered to overcome the limitations encountered in raceway pond [170]. There are several designs of closed photobioreactor such as air-lift tubular, flat plate, and vertical-column reactors and culture parameters such as nutrient levels, temperature, amount of inlet CO_2, etc. [173]. The reactor permits selective culture strain, in which optimum growth condition can be maintained to give high biomass and lipid productivity. The tubuler photobioreactor may be one of the most typical cultivation apparatus, where a vertical tubuler photobioreactor can increase the residence time of sparged gas, giving higher CO_2 utilization efficiency [174]. The higher intensity of light cannot reach to most of the cell in the large-scale photobioreactor. As a consequence, the metabolism also changes from light to dark condition. This phenomenon is undesirable for large-scale production.

Harvesting of algal biomass

The microalgae need to be separated from water to recover their biomass for downstream processing. There are several methods for microalgae harvest: (1) bulk harvesting- to separate microalgae from suspension such as natural gravity sedimentation, flocculation, and floatation, and (2) thickening to concentrate the microalgae slurry after bulk harvesting such as centrifugation and filtration [169].

Flocculation, the aggregation and sedimentation (or flocculation) of algal biomass, is also a very common primary harvesting method used to concentrate algae. Algal strains can also be engineered such that the addition of a polymer or a change in an environmental variable triggers flocculation [175,176].

Conventional flocculation method poses several disadvantages: (1) high dosage of multivalent slat is required

to achieve satisfactory result, (2) it produces large quantity of sludge that increases the difficulty to dehydrate the biomass, and (3) flocculation efficiency is highly dependent on pH level [169,177]. By introduction of a coagulant that is positively charged into the culture medium, the negative charge surrounding the microalgae cells will be neutralized. At the same time, flocculant can be added to promote agglomeration by creating bridges between the neutralized cells to become dense flocs and settle down due to natural gravity [178]. Another possible method to harvest microalgae is through immobilization, in which microalgae are embedded in an entrapment matrix and continuously grow within the matrix. Only alginate gel entrapment method is feasible to immobilize microalgae so far [179]. Some of the advantages of using alginate gel are the requirement of only mild condition during immobilization process with negligible toxicity and high transparency [179,180]. Immobilized microalgae beads can be applied in diverse research areas such as for high-value product synthesis, organic pollutant removal, heavy metal removal, and toxicity measurement (biosensor) [180-186]. A few issues need to be addressed in immobilization of microalgae before the process can be upgraded to commercialization stage such as (1) stability, (2) leakage of microalgae cells, and (3) mass transfer limitation.

Downstream processing

Oil extraction

Effective lipid extraction is required particularly for microalgae with low lipid content, since the loss of the lipid during extraction process brings a serious problem for the production cost of microalgae biofuels [187]. The energy consumed in lipid extraction from dried microalgae biomass is a relatively small portion to the overall energy [172,188]. Various cell disruption methods are microwave application, sonication, bead beating, autoclaving [189,190], grinding, osmotic shock, homogenization, freeze drying [189], and 10% (*w*/*v*) NaCl addition [190,191].

Solvent extraction method

The solvent must be inexpensive, nontoxic, volatile, nonpolar, and it must selectively extract the lipid of the cell [189]. The potential of using co-solvent mixtures of ionic liquids and polar covalent molecules has been shown for lipid extraction [192]. The Soxhlet extraction method uses hexane, while the Bligh and Dyer's method uses mixture of chloroform and methanol as solvents to extract lipids [193]. The other solvents include benzene and ether, but hexane has gained more popularity as a chemical for solvent extraction, and it is also relatively inexpensive [194]. Although n-hexane is widely used to extract oil from various seed crops, it is inefficient to extract microalgae lipid. This is because microalgae lipid contains high concentration of unsaturated fatty acid, while n-hexane is a nonpolar solvent. Thus the selectivity of lipid towards the solvent is reduced [187]. Methanol and n-hexane are not sustainable, since both solvents are conventionally derived from nonrenewable fossil fuels. On the other hand, ethanol is a greener solvent, since it has a low toxicity level and can be derived from renewable source such as sugar-based plant (e.g., sugar cane and sweet sorghum) and lignocellulosic material (e.g., weed and corn stover) [170]. The ethanol, however, gives low extraction efficiency. Ultrasonication and microwave can be also used for cell disruption. The cell wall-less mutant is better for oil extraction.

Super critical fluid extraction

Several supercritical fluids are CO_2, ethane, methanol, ethanol, benzene, toluene, and water [195,196]. The basic principle of this technology is by achieving a certain phase (supercritical) that is beyond the critical point of a fluid, in which meniscus separating the liquid and vapor phase disappears, leaving only a single homogeneous phase [196]. Supercritical CO_2 has received much interest typically in extraction of pharmaceutical and health-related products from microalgae [197-200]. In fact, supercritical CO_2 offers several advantages in comparison with chemical solvent extraction such as (1) nontoxic and provide nonoxidizing environment to avoid degradation of extracts, (2) low critical temperature (around 31°C) which prevents thermal degradation of products, (3) high diffusivity and low surface tension which allow penetration of pores smaller than those accessible by chemical solvents, and (4) easy separation of CO_2 at ambient temperature after extraction [195,197,200].

Transesterification of oils

The most suitable catalyst for transesterification of oils with low free fatty acids (FFA) content is necessary. The presence of high free fatty acid content in microalgae lipid (more than 0.5% *w*/*w*) prevents the use of homogeneous base catalyst for transesterification reaction [201-203]. Alkaline metal alkoxides, even in small concentration of 0.5 mol %, are highly active catalysts [194]. Metal alkoxides (e.g., potassium methoxide) in methanol are better options than metal hydroxides (NaOH, KOH). In a short reaction time of about 30 min, they give high yields of about 98% [194]. They performed better in absence of water, which makes them inappropriate for industrial processes [204]. FFA will react with base catalyst to form soap leading to lower biodiesel yield and increase the difficulty to separate biodiesel from glycerol.

Acid catalyst (e.g., H_2SO_4) is not sensitive towards FFA level in oil, and thus esterificaiton (FFA is converted to alkyl ester) and transesterification can occur simultaneously. A chemically catalyzed transesterification process requires a high amount of energy, and separation of catalyst from the

product is cost effective. Glycerol, produced as a byproduct of alcoholysis, readily adheres to the surface of immobilized lipase and decreases its enzyme activity, where glycerol removal is a complex process, which may hinder the large-scale operations [189].

Free fatty acids contained in extracted oil have to be removed prior to reaction in order to maintain activity of the alkaline catalysts. In the superheated method, free fatty acid and triglycerides are converted to fatty acid methyl ester directly. Academic and commercial enterprises have realized the importance of such research on this topic, and a direct transesterification method for total microalgal lipid content has produced appreciable levels of biodiesel even when there are undetectable levels of neutral lipids [205,206]. The advantages of non-catalytic alcoholysis reaction for the production of biodiesel are as follows: (1) the purification process to remove catalyst after reaction is not required, (2) the by-product (glycerol) can be directly utilized in other industry, (3) not only the triglycerides but also the free fatty acid might be converted into fatty acid methyl ester, (4) neutralization process for removal of free fatty acid is not required prior to the reaction process, and (5) the yield of the total system will be improved, and the cost required for the process will be reduced.

Improvement of product recovery

In a large-scale operation, the presence of microorganisms, medium composition, and process condition may cause emulsion formation, which lowers the product recovery efficiency. A better understanding on the mechanism of emulsion formation is necessary for the performance improvement based on nanotechnology as well as electrochemical properties such as ion, charge, viscosity, interface stabilization [207].

Conclusions

The fluctuation in global prices of crude oil, increasing threats to the environment by exhaust emissions, global warming, and threats of supply instabilities have adversely impacted the developing countries, more so to the petroleum-importing countries. The rising of sea level caused by green-house gas (CO_2) also threatens the most populated areas of the world. It is important to find a safe alternative fuel to relieve the escalating energy crisis and to protect the environment. Photosynthetic microorganisms have emerged as one of the most promising sources for biodiesel production.

Although algae and cyanobacteria have been paid recent attention from the point of view of sustainable biosynthesis as well as biofuel and biochemical production, the cell growth rate is significantly lower as compared to the typical biofuel-producing microorganisms such as *E. coli* and yeast. It is, therefore, strongly desirable to design microbial cell factories by means of a synthetic biology approach with in-depth understanding of the metabolic regulation mechanism with the aid of a systems biology approach such as modeling.

Several attempts are being made for improving the efficiency of capturing light energy and CO_2 fixation as mentioned before. One of the reasons of the lower cell growth rate in cyanobacteria may be due to an incomplete TCA cycle lacking KGDH and succinyl CoA synthetase (SCS). Recent investigation on *Synecococcus* sp. PCC7002 indicates that the genes encoding αKG decarboxylase (or 2-oxoglutarate decarboxylase) and succinic semialdehyde dehydrogenase are present, where NADPH instead of NADH is produced without producing guanosine triphosphate (GTP) by substrate level phosphorylation (Figure 5) [208]. It is important to elucidate the nature of such TCA cycles in cyanobacteria and plants from the point of view of functional significance of the metabolic feature in a broader evolutionary context [209]. Further investigation is necessary to improve the cell growth rate with balanced energy generation and biosynthesis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

DS and KS investigated the references and drafted the manuscript. Both authors read and approved the final manuscript.

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