We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

5,000
Open access books available

126,000
International authors and editors

145M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Recent Advances in Algal Biomass Production

Meghna Rajvanshi and Richard Sayre

Abstract

The promise of algae to address the renewable energy and green-product production demands of the globe has yet to be realized. Over the past ten years, however, there has been a substantial investment and interest in realizing the potential of algae to meet these needs. Tremendous progress has been achieved. Ten years ago, the price of gasoline produced from algal biomass was 20-fold greater than it is today. Technoeconomic models indicate that algal biocrude produced in an optimized cultivation, harvesting, and biomass conversion facility can achieve economic parity with petroleum while reducing carbon-energy indices substantially relative to petroleum-based fuels. There is also an emerging recognition that algal carbon capture and sequestration as lipids may offer a viable alternative to direct atmospheric CO_2 capture and sequestration. We review recent advances in basic and applied algal biomass production from the perspectives of algal biology, cultivation, harvesting, energy conversion, and sustainability. The prognosis is encouraging but will require substantial integration and field testing of a variety of technology platforms to down select the most economical and sustainable systems to address the needs of the circular economy and atmospheric carbon mitigation.

Keywords: algae, biofuel, biomass, carbon sequestration, carbon index

1. Introduction

Over the last ten years (since 2010) there has been accelerated investment in research for the development of commercially viable algal biomass and coproduct production systems [1–5]. The challenge for algae biomass production systems has been that unlike crop biomass production systems having thousands of years of development history, algae until very recently were not the target of integrated research and development (R&D) strategies focused on efficient production of food, fuel, and coproducts [6]. Recent estimates indicate that there are globally more than 150,000 species of single cell and multicellular algae having polyphyletic origins, complex and diverse metabolic machinery, occupying vast environmental niches with immense ranges of biotic and abiotic stress tolerance, and having growth or biomass production rates that range over two magnitudes in yield compared to traditional agricultural production [7]. The challenge for the industry has been to identify the best algal production systems that are suitable for commercially viable industrial applications. Beginning with algal biology much effort has focused on identifying the best performing algal strains. The criteria for down-selecting the best performing strains have included, identifying algae with the greatest biomass production rates, optimizing algal growth media, CO_2 exchange and culture

conditions, identifying algal strains that are the most resistant to pathogens and herbivory (minimizing pond crashes), and developing strains having enhanced performance characteristics through application of genetic engineering, breeding and genome editing tools [6]. Research and development for improved biomass production has also focused on developing enhanced cultivation, harvesting and biomass conversion technologies with the objective to achieve the lowest carbon emissions, recycle inorganic nutrients as efficiently as possible, minimize energy inputs at each stage in production, and integrate the algal biomass production systems into the existing energy infrastructure as seamlessly as possible.

In 2010, the US Department of Energy launched the largest government-funded integrated algal biomass, biofuels and bioproducts program carried out to date. The National Alliance for Advanced Biofuels and Bioproducts (NAABB) achieved notable advances in reducing the cost of producing biomass and making biofuels from microalgae. In three years NAABB developed and modeled a pathway to move the price point for producing a gallon gasoline equivalent (GGE) of fuel from microalgae from $150 to $8 a GGE [1–3]. More recently, the price point for a GGE produced from algal biomass has been reduced to < $5. Based on Reliance's demonstration scale studies, the technoeconomic modeling (TEM) for a 10 k barrels/ day (bpd) scale production of crude oil from microalgae was estimated to be at 100$/ barrel without any subsidy. The major factors contributing to the substantial cost reductions in producing fuel from algal biomass included, the discovery and development of more robust, high biomass producing algal strains for year-round consistent performance, identification of the best geographies to produce algal biomass, advance pond designs and improved culture mixing for effective light utilization, effective crop control methods that prevent pond crash and biomass loss, innovative harvesting techniques and effective water and nutrient recycling to maximize resource utilization. Also, advancements in biomass to biocrude conversion technologies including continuous flow hydrothermal liquefaction (HTL), the demonstration that algal biocrude coming from HTL could be used as a direct feedstock in existing oil refineries to produce fuels with performance characteristics similar to petroleum-based fuels, and the production of high value coproducts to offset the cost of producing fuels.

Stepping back, however, there remain many critical considerations that must be addressed if microalgal biomass is to be a commercial success in competition with other biomass sources in the world where the carbon energy index (g CO_2 emitted/ kJ energy produced) and the environmental impacts of any biomass production system must also be considered along with economics [6]. Beginning with first principles it is critical to identify what the thermodynamically most efficient biological mechanisms are for producing algal biomass that also have the highest carbon capture efficiency. Recent thermodynamic models suggest that the greatest energy efficiency for carbon capture and biomass production is achieved in algae that utilize light most efficiently and accumulate chemical energy in the form of carbohydrate polymers, e.g., starch rather than those that store oils [8, 9]. Additionally, algae with rapid division rates and/ or the ability to grow substantially in volume are likely to be greater biomass producers [10]. While most algal biofuel programs have focused on producing biomass from high lipid accumulating strains due to ease of conversion of lipids into biocrude it is becoming apparent that algae accumulating starch as a metabolic storage end product have the highest biomass production rates and thermodynamic efficiency [8–10]. While lipids have greater energy density and are more readily converted into fuels, starches have a greater chemical energy density per carbon per photon captured during photosynthesis [8]. One of the microalgal strains achieving the highest known biomass yields in cultivation is *Pseudoneochloris* which stores starch as an energy reserve, achieves high cell numbers at stationary phase of growth, and can increase its cellular volume as it grows by greater than 100-fold [8].

Cultivation systems are also a major cost factor in producing algal biomass. It is generally recognized that to produce low value algal biomass open pond production systems have the lowest capital and operating expenses and require less maintenance (to prevent fouling) than closed bioreactor cultivation systems. However, open pond systems require greater amounts of water to operate due to evaporation, have higher energy costs associated with concentrating more dilute cultures, and are more susceptible to contamination although biological contaminants in closed bioreactors may be more difficult to eradicate.

Regardless of the constraints and challenges mentioned above and the necessity to input higher capital investment in cultivation and downstream processing, production of microalgae biomass still stands out advantageous on many fronts in comparison to agriculture crops for food and fuel. Microalgae have high photosynthetic efficiency and short division time, making them highly suitable candidates for generating more biomass in less time. Growth rates of several microalgae have been reported to be 5–10 times higher than agriculture crops [9]. Moreover, microalgae can grow on low economic and ecological value lands and can utilize marine, brackish or fresh water for cultivation, depending on the species being used. CO_2 from industrial exhaust can be used for cultivation and nutrients from waste streams can be utilized for growth. Excess nutrients lost during harvesting process can be recycled back in the cultivation system, ensuring minimal wastage and maximum utilization [10, 11]. In contrast, agriculture depends on limited natural resources, like arable land and fresh water, with fresh water consumption being highest globally in agriculture. Over 80% of all water consumed globally is used for agricultural production. Agriculture also needs extensive application of fertilizers and pesticides to improve biomass productivity and prevent crop losses. However, nitrogen utilization is inefficient in crop plants, resulting in ~50% of nitrogen loss through leaching, soil erosion and gaseous evaporation [12]. Considering these facts, use of agricultural crops to meet growing biomass demands for food and energy will lead to land use change, environment pollution, loss of forest cover and biodiversity. Thus, from environment standpoint algal cultivation is much favored over traditional agriculture for feedstock production [12, 13].

Many microalgal species are good source of proteins, carbohydrates, lipids and other high value bioactive molecules, such as enzymes, pigments and vitamins. By altering the cultivation conditions or through metabolic engineering approaches, composition of algae can be manipulated to accumulate the specific biomolecule(s) of interest. Considering the higher growth rate and ability to accumulate high lipid content (≥30%), it is reported that microalgae can yield 58,700 L of oil/ ha as opposed to 172 L/ha for corn, 446 L/ha for soybean, 1892 L/ha for Jatropha and 5950 L/ha for oil palm [14]. Thus, the projected ability to produce oil from algae is ~10 times more compared to highest oil producing crop plant. Likewise, algae biomass can be a potential feedstock for bioethanol production because of its ability to accumulate starch even higher than 50% (w/w) of biomass under optimal conditions. Absence of lignin in algal cell wall makes its processing easier compared to lignocellulosic agricultural waste and woody biomass, where lignin removal is an additional step before processing for bioethanol production [13]. Moreover, lack of structural parts like leaves and roots in algae makes algal biomass more homogenous and might be less energy intensive to process compared to crop plants [13]. In an estimate, net energy from sugarcane ethanol and bagasse was 143 GJ/ha/year as opposed to 928 GJ/ha/year from microalgae, indicating microalgae to be significantly more efficient feedstock [15]. Protein is another commercially important component of algae biomass. Algae protein is comparable to other high-quality plant and animal protein sources, however, protein yield from algae happen to be between 4 and 15 tons/ha/year, which is significantly higher than 0.6–1.2 tons/ha/year,

reported for soybean [16]. Clearly, microalgae supersede traditional agriculture on multiple aspects, however, biomass harvesting is an area which is well established in case of crop plants but highly energy intensive in case of algae due to its small size and low biomass density [10].

Regarding algal biomass harvesting systems the general objective has been to develop algae harvesting and concentrating systems that have parasitic energy consumption values of less than 10% of the total algal biomass energy content [6]. To reduce the costs of fuel production, recent efforts have focused on the direct conversion of harvested algal biomass into separate fuel and coproduct fractions in a continuous flow system while efficiently recycling water and nutrients. One of the more promising technology developments in this sector has been the development of two-stage HTL which allows for the separate recovery of coproducts and biocrude feedstock while recycling water and nutrients back to the pond thus avoiding the energy intensive step of drying the algal biomass before biomass to fuel conversion. The appropriate selection of what high value coproduct(s) to produce from algal biomass is critical for economic viability when coproduct production is coupled with fuel production. From this perspective the coproduct should have sufficient value based on biomass yields to be economically sustainable without saturating markets to the point of driving coproduct prices so low as to be economically untenable. As modeled by the US-DOE PACE algal biofuels consortium a fully integrated algal cultivation, harvesting, co-product and fuel production system with integrated water and nutrient recycling has the potential to recover over 60% of the energy content of the algae as biocrude while producing valuable coproducts that have a large global market demand (**Figure 1**).

Optimizing algal biomass production and carbon sequestration also has the potential to address the existential threat of global climate change associated with greenhouse gas emissions. Currently, biological carbon capture and sequestration (BCCS) is one of the more feasible means to remediate the earth's atmosphere. As a BCCS system, algae are particularly attractive not only for their high areal

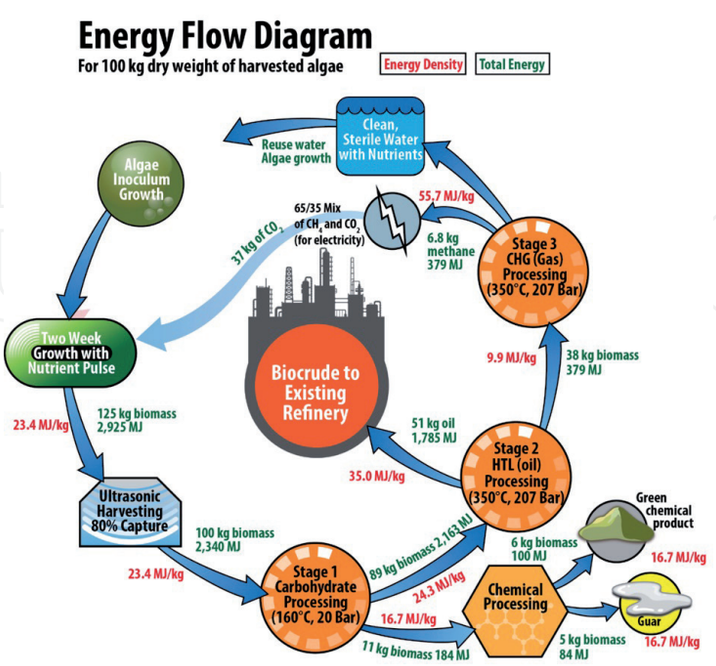

Figure 1.
PACE consortium working model for the integrated co-production of biofuels and co-products (green chemicals, polysaccharides (guar), and methane) from algae. Inorganic nutrients and wastewater are recycled. Algae are preloaded with nutrients (nutrient pulse) and grown in minimal media to reduce weedy species competition and continuously harvested at mid-log phase growth. HTL, hydrothermal liquefaction; CHG, catalytic gasification.

rates of carbon capture but also for their potential storage of carbon as lipids while recycling inorganic nutrients and water [17]. While not generally considered as a carbon sequestration material, lipids have several advantages over solid CO_2 as a carbon sequestration material [17]. Triacylglycerol ($C_{55}H_{98}O_6$) is 77% carbon by mass and has a density of 0.91 g/cm^3. In contrast, CO_2 is 27% carbon by mass and as a solid has a density of 1.96 g/ cm^3. Thus, lipids have a volumetric carbon density that is 32% greater than solid CO_2. Furthermore, being a liquid and not readily convertible to a gas, the ability of lipids to escape from deep geological sequestration is substantially less than CO_2 reducing potential long-term risk to aerobic organisms [17]. Overall, algae have great potential to address simultaneously fuel, food, green chemical, and environmental challenges.

In the following sections we will review recent advances in the sustainable production of algal biomass and coproducts for fuels and economic competitiveness with petroleum and non-algal coproduct production systems. Substantial achievements have been realized from an industry that has a truly short history compared to other biomass production systems.

2. Algal strains

Substantial efforts have focused on the identification of algal strains having maximum biomass yields under cultivation. Ideal biomass production strains must not only have fast growth rates but also must be robust and tolerate well abiotic (temperature, salinity, light) and biotic (pathogen, herbivore and weedy algae) stress conditions to minimize pond crashes and downtime in algal cultivation. There have been several large-scale algal surveys of wild algal species to identify those strains that perform well in cultivation [18]. In addition, screening systems for identifying strains with elevated performance characteristics in high light environments among others have led to some success in the identification of high performing algal strains [19]. Given that there as many as 150,000 species of algae have been identified and that limited resources have been available to screen algae for high biomass production, there remains a significant number of algae that remain to be assessed for biomass productivity in select environments [7]. In addition, substantial potential to improve algal productivity may also be achieved in traditional and molecular assisted breeding practices. Algae breeding efforts, except for laboratory strains such as *Chlamydomonas*, have been limited, however. This is because the means to induce gametogenesis to identify sexual mating types in most algae is not well understood. If the increased yield achieved through plant breeding are to serve as a prognosticator of the potential to enhance algal productivity it can be anticipated that algal breeding programs may enhance yields in the field by as much as ten-fold.

2.1 Modulating cultivation conditions to impact oil and carbohydrate yields

Given the fast rates of cell division and the absence of dedicated higher-order cellular structures including tissue and organs it is not unexpected that microalgae have an enhanced capability to metabolically remodel cellular functions under different growth conditions. Algae frequently live boom and busts cycles in the nutrient deserts of lakes and the open oceans. Thus, it is imperative that algae have flexible metabolic systems to survive in unpredictable and ever-changing environments and be unencumbered by programmed cell fates associated with the differentiation and organization of cells into higher order tissues and organs.

One of the manifestations of this metabolic flexibility is the ability to shift the biochemistry of the major cellular energy storage products from low energy

density carbohydrates to high energy density hydrocarbons including triacylglycerol (TAG) and/ or polyterpenoids [20]. The metabolic shift from carbohydrate to hydrocarbon accumulation is typically induced by nutrient depravation. Upon shifting from a nitrogen-, sulfur- and/ or micronutrient-rich condition to a nutrient poor condition many algae will facultatively shift the metabolism of energy storage product accumulation from carbohydrates (starch) to hydrocarbons [21–25]. Hydrocarbons have more than 60% the energy density per fixed carbon of carbohydrates. Importantly, the facultative shift to hydrocarbon production allows algae to continue to generate and utilize reducing energy generated by the photosynthetic apparatus. Significantly, the accumulation of triacylglcerols may not only involve de novo synthesis but the remodeling of existing chloroplast membrane lipids into more fully reduced TAGs [26–29]. Given the desirability of hydrocarbons as a feedstock for biocrude production the ability to shift metabolism from carbohydrate to hydrocarbon production has been exploited to produce hydrocarbon rich biofuel feedstocks. The challenges with this strategy (nutrient deprivation) for facultative hydrocarbon production is that it can also lead to reduced rates of cell division and overall biomass accumulation. In a comprehensive empirical analysis of the impact of nitrogen deprivation on cell division rates, TAG accumulation, lipid remodeling, biomass accumulation and total caloric or biochemical energy accumulation in the green alga, *Chlorella sorokiniana*, it was demonstrated that upon shifting algae to a nitrogen-free growth medium there was a substantial increase in TAG accumulation and a redistribution of total cellular fatty acid profile to more energy dense saturated fatty acids [30]. Under the two-week nitrogen deprivation period employed in this study there was no statistically significant reduction in the rates of cell division or biomass (dry weight) accumulation. However, during the nutrient deprivation period the total chemical energy accumulated in biomass increased by greater than 60% associated with a 20-fold increase in TAG content. It is perhaps surprising that the two-week nitrogen deprivation period did not impair cell division and biomass accumulation suggesting that the alga had the capability to sequester nutrients and/ or catabolize and remodel existing nitrogen rich (proteins) molecules [30]. Not all algal species, however, exhibit similar responses to nutrient deprivation. For many algal species growth rates and biomass accumulation are substantially impaired during nutrient deprived growth conditions [31–33].

An additional practical application of nutrient deprivation for oil production is that growth in nutrient depleted media may reduce competition from weedy algal species [34]. This observation has led to the application of nutrient pulse technology to simultaneously induce oil accumulation during nutrient stress and inhibit the growth of weedy algal species. Under ideal growth conditions the limiting nutrients are withheld until there is an impairment in growth. At this transition point a pulse of the limiting nutrient is added to the growth media to support continued high growth rates [34]. Overall, the ability to induce oil production if managed well can lead to sustained high growth rates while enhancing the energy density of the biomass and the increased accumulation of biofuel feedstocks such as TAGs.

3. Genetic enhancement

Given the aforementioned challenges to breed wild algal strains for improved yield performance traits and the fact that substantial progress has been made in algal genomics and the development of robust genetic transformation systems substantial research efforts have focused on engineering microalgae with improved biomass performance traits. Most algal genetic engineering efforts have focused on the manipulation of metabolic pathways for increased biomass and coproduct

production. The production and accumulation of biomass can be broadly divided into four phases known as source (push), sink (pull), storage (accumulate) and turnover (metabolism). Providing an over-riding template on this simplistic model of biomass accumulation is the genetic and developmental control of cell size and cell division or replication rates. Source strength is effectively the primary photosynthetic processes associated with light conversion into chemical energy and the fixation of carbon dioxide into storage products. Sink strength refers to the impact of downstream metabolic processes on biomass accumulation including metabolic feedback control of carbon flux from photosynthesis to production of carbon storage products. The carbon storage products must also be compartmentalized in the cell to support night-time respiration and biomass accumulation. In algae, starch is first primary carbon storage product and is stored in plastids. Algae may also accumulate high energy density hydrocarbons including triacylglycerols or oils. Oil is stored in specialized droplets packaged by outer membranes having surface displayed amphipathic proteins or oil droplet proteins. The extent of accumulation of these storage compartments can be regulated at the level of gene expression and thus is the subject of genetic manipulation impacting overall product yields. However, algal cell division rates and control of cell volume are among the more important determinants of algal biomass production. While many single celled algae have fixed cell volumes that determine the timing of cytokinesis some single celled algae are capable of over 100-fold increases in cell volume as they grow while having variable rates of cell division [35, 36].

In the following paragraphs we focus on progress that has been made at the molecular level to engineer or breed algae with enhanced source and sink strength, increased storage product accumulation, and accelerated cell division rates leading to enhanced yields. As is evident from the success achieved to date two- to five-fold increases in the rate of biomass production and yields are feasible.

3.1 Alterations in source strength

The efficiency of solar energy conversion into chemical energy stored in biomass by plants and algae ranges from 3 to 5% of available solar energy. Theoretically, efficiencies as high as 11% for conversion of solar energy into the chemical energy in biomass can be achieved utilizing just the photosynthetically active radiation (400–700 nm) in the solar spectrum. Maximum efficiencies of energy conversion as high as 30% can be achieved using just red light (~650–700 nm) which is most efficiently harvested by the photosynthetic pigments [8, 37, 38]. Thus, it is conceivable that 2- to 4- fold increases in biomass yields are feasible through improvements in photosynthetic efficiency. It has long been recognized that the greatest potential for increasing photosynthetic efficiency is through enhanced light use efficiency by the photosynthetic apparatus (**Figure 2**) [39–41]. During photosynthesis, light saturates in all plants and algae at approximately one quarter of full sunlight intensity [38, 41]. Thus 75% of the energy captured by the photosynthetic pigments does no productive work leading to biomass production. Since the excess energy captured by the photosynthetic pigments does not drive electron transfer and carbon fixation processes it must dissipate through non-productive energy emission and/ or energy conversion pathways (heat, fluorescence, production of reactive oxygen species (ROS)) some of which (ROS) can lead to substantial damage to the photosynthetic apparatus further reducing biomass yields [42].

One approach to deal with the challenge of excess light absorption by the photosynthetic apparatus has been to reduce the optical cross section of the light-harvesting antenna complex to better couple the rate of light capture with rate-limiting electron transfer processes, i.e., plastohydroquinone oxidation by the

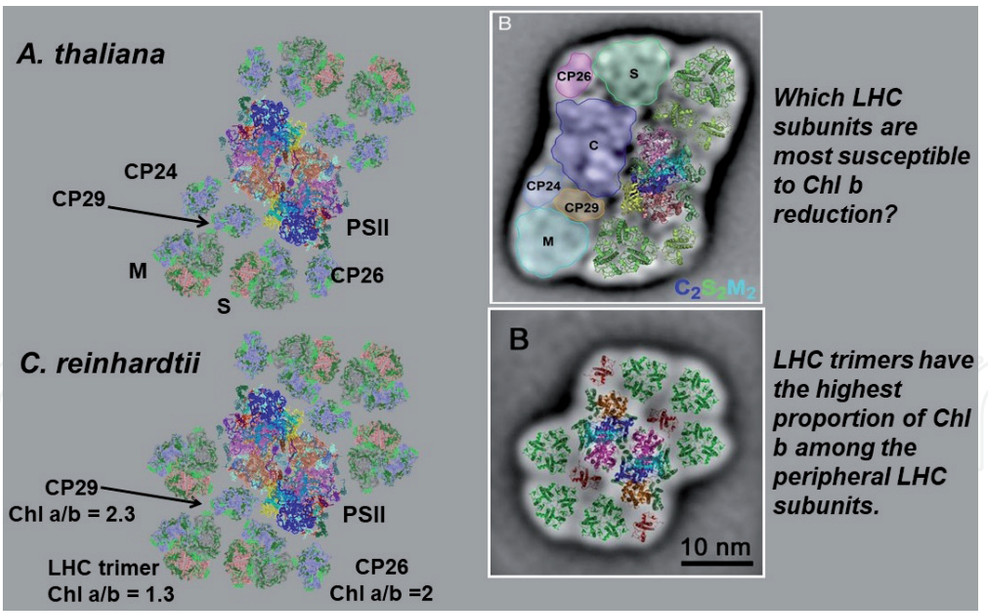

Figure 2.
Organization of the peripheral light harvesting antenna complexes adjacent to the dimeric photosystem II reaction center in plants and green algae. Chlamydomonas transgenics having chlorophyll a/b ratios of 5 have lost the equivalent of one peripheral light-harvesting complex II trimer (LHC trimer). Figure modified from Dr. Jun Minagawa.

cytochrome b6f complex and the development of an electron transport limiting trans-thylakoidal pH gradient [43, 44]. Various strategies have been developed to reduce the size of the light harvesting complex ranging from reducing the expression of the light harvesting complex proteins to targeted reductions in specific light harvesting pigment content often resulting in pleiotropic effects that indirectly affect photosynthetic efficiencies both negatively and positively [41, 45, 46]. Through the analysis of algae having a range in reduction in the light harvesting antenna size it has been empirically determined that the loss of approximately one third of the light harvesting apparatus (LHC2) results in maximum increases in photosynthetic efficiency of 20–30% and increases in biomass yield (40% greater) in both plants and green algae grown under outdoor cultivation conditions (**Figure 2**) [41, 45, 46]. A range in reductions of light harvesting antenna size were achieved by differential expression of the chlorophyllide a oxygenase gene (CAO) which produces chlorophyll b (Chl *b*). Chl *b* is present only in the light harvesting antenna complex proteins and not the photosynthetic reaction center. Since Chl *b* stabilizes the Chl *a/b* binding proteins, its reduction results in a corresponding loss in light harvesting antenna pigment-protein complexes. Significantly, a Chl *a/ b* ratio of 5 has been demonstrated both in plants and green algae to be optimal for achieving the greatest photosynthetic efficiency for plants and algae having altered light harvesting antenna sizes when grown at full sunlight intensity. Lesser or greater reductions in pigment (Chl *b*) content result in less than optimal photosynthetic performance due to indirect effects of Chl *b* reductions on the abundance of select light harvesting pigment-protein complexes, alterations in membrane architecture, reductions in energy transfer processes between the two photosystems, and increased susceptibility to photoinhibition [47, 48].

In nature, however, light intensities vary substantially over the course of the day, with depth in the canopy architecture or algal pond, and seasonally [48]. Theoretically, a light-harvesting apparatus that could be continuously adjusted in size to respond positively to differing light regimes would facilitate greater light use efficiency in dynamic light environments [47]. Recently, Negi et al. (2020) described a strategy for the continuous (daily) adjustment of the light-harvesting

antenna size in response to light intensity shifts in the green alga *Chlamydomonas reinhardtii* [47]. This dynamic antenna size regulation system is based on light regulated post transcriptional control of CAO activity. Protochlorophyllide a oxygenase (CAO) catalyzes the synthesis of Chl *b* which is found only in the peripheral, nuclear-encoded light-harvesting pigment-protein complexes. Light intensity-dependent regulation of the light-harvesting complex size was achieved using as a host a CAO minus mutant which had been engineered to express a gene fusion product between the 5′ light regulated element (LRE) and the CAO gene [46]. A light regulated translational repressor, NAB1, binds to the LRE element and at high light represses translation of the modified CAO transcript reducing Chl *b* synthesis and decreasing the light harvesting antenna size. In low light such as occurs in dense cultures CAO translational repression by the NAB1 protein is reduced resulting in increased Chl *b* levels and increased light harvesting antenna size. Significantly, when the LRE-CAO transgenics were grown as monocultures under conditions mimicking those of a commercial production pond the transgenics had biomass yields that were more than two-fold higher than their wild-type parental strains. These are the greatest increases in biomass yield observed to date for algae engineered for improved photosynthetic efficiency.

Significantly, additional enhancements in photosynthetic rate are feasible in algae with optimized light harvesting antenna sizes. When the LRE-CAO transgenics were exposed to elevated bicarbonate concentrations there was an additional 20% increase in photosynthetic rates indicating that improvements in downstream carbon fixation processes could further enhance photosynthetic efficiency and biomass yield [46]. Obviously, elevated chloroplast CO_2 concentrations could potentially suppress RubisCO oxygenase activity and photorespiration [49].

In addition to targeting single gene traits to enhance biomass productivity, engineering strategies based on altering the expression of master growth regulatory genes in algae has proven fruitful for increasing biomass yields. In *Chlamydomonas reinhardtii*, the blue light photoreceptor phototropin (Phot) plays a vital role in progression of the sexual life cycle [50, 51], the control of the eye spot size and light sensitivity and in the control of blue-light mediated changes in the expression of genes involved in the synthesis of chlorophylls, carotenoids, chlorophyll binding proteins [52]. Thus, it was anticipated that Phot expression could potentially play a role in regulating photosynthesis and biomass productivity. Negi et al., tested this hypothesis as well as identified downstream genes in the Phot regulatory pathway that were known to be master regulators of carbohydrate metabolism in plants including analogues of the *Arabidopsis* KIN10 and KIN11 genes [53].

Based on a comparison of the photosynthetic attributes of two independent Phot mutants to their independent parental strains Negi et al., [50] demonstrated that the Chl a/b ratios were significantly greater in Phot mutants (2.9) than in wild type (2.0) grown at low light indicative of a smaller light harvesting antenna size in Phot mutants. When grown at high light intensities there was a further reduction in Chl a/b ratio (3.4) in Phot mutants indicating an ability to reduce the size of the light harvesting antenna grown resulting in increased light use efficiency [50]. The net result was that for Phot mutants photosynthetic rates were light-saturated at intensities 3-fold greater than for wild-type cells resulting in substantially accelerated cell division rates and biomass accumulation. RNAseq experiments indicated that these increases in productivity in Phot mutants were associated alterations in the patterns of expression for genes encoding enzymes involved photosynthesis, carbon metabolism, and those controlling cell division rates. Phot mutants had a 2- to 5-fold increase in the expression levels of multiple rate-limiting enzymes including; the Rieske Fe-S protein, ribulose-1,5-bisphosphate carboxylase/oxygenase, sedoheptulose 1,7 bisphosphatase glyceraldehyde-3- phosphate dehydrogenase, carbonic

anhydrase, ADP glucose pyrophosphorylase, starch synthase, and genes involved in respiration and fatty acid biosynthesis. Additionally, genes involved in cell cycle control including; NIMA (never in mitosis), NEK2, NEK6 (NIMA related kinases), RCC1 (regulator of chromosome condensation, cyclin and cyclin-dependent kinases (CDK): Cyclin-dependent kinases, and MAT3 a homolog of retinoblastoma protein (MAT3/RB) were upregulated 2–15-fold in Phot mutants relative to their parental wild-type strains. The net result of this global alteration in gene expression was a two-fold increase in biomass productivity in Phot mutants relative to wild type [50].

Additional improvements in photosynthetic efficiencies have also been achieved by reducing apparent rate limitations in the Calvin–Benson–Bassham cycle (CBBC). Previous studies have demonstrated that the CBBC enzymes, fructose 1,6-bisphosphate aldolase (aldolase), sedoheptulose1,7-bisphosphatase (SBPase), and transketolase (TK), have the highest metabolic flux control coefficient values (maximum 0.55, 0.75, and 1.0, respectively) of any CBBC enzymes and thus have been targets for metabolic engineering to enhance carbon flux and accumulation in engineered plants and algae [54, 55]. Overexpression of the cyanobacterial dual functional fructose 1,6–/sedoheptulose 1,7-bisphosphatase (FBP/SBPase) and/ or plant SBPase was shown to significantly increase photosynthetic rates and growth in transgenic plants or algae [55, 56]. Similar to plants, mutagenesis studies in algae have demonstrated that hexokinase globally regulates genes involved in photosynthesis and hydrocarbon production and similar to Phot mutants can be manipulated to control biomass accumulation [57]. Thus, substantial gains in biomass productivity are feasible through targeted manipulations in both the light reactions and dark (CBBC) reactions of photosynthesis.

3.2 Alterations in carbon sink strength

Given the primary role of starch metabolism as carbon reserve and an intermediate in the production of hydrocarbons it is not unanticipated that alterations in starch metabolism may impact hydrocarbon and biomass yields [58]. For example, *Chlamydomonas* sta6 [ADP-glucose pyrophosphorylase] and sta7–10 [isoamylase] mutants having reduced capacity to synthesize starch had substantial increases in lipid accumulation during nitrogen deprivation relative to the wild-type controls but suppressed total biomass accumulation [58]. In addition, suppression of starch metabolism has been shown to impair upstream CBBC activity resulting in the dissipation of excess photosynthetically produced electrons through non-productive reduction of oxygen [54]. These results point to the central role of starch metabolism and accumulation in overall cellular homeostasis and biomass accumulation in algae and its impact on the thermodynamic efficiency of light energy conversion into chemical energy (biomass) [8, 58]. It has been estimated that carbohydrate metabolism can accounts for as great as 20% reductions in thermodynamic efficiency of photosynthesis [39]. These efficiencies can be further reduced by partitioning carbon into hydrocarbon storage products instead of starch. This is due to the central role of pyruvate (3C) metabolism in hydrocarbon (lipids, terpenes, and waxes) production. The production of acetyl CoA (2C) via the decarboxylation of pyruvate for hydrocarbon production results in the loss of 1/3 of the previously fixed carbon. In contrast, starch production from photosynthetically derived sugars has no associated decarboxylation steps. Hydrocarbons, however, have nearly twice the energy density of carbohydrates due to their more reduced state. Modeling studies indicate that the production of carbohydrates using solar photons is potentially 10–20% more efficient for solar energy conversion than hydrocarbon production [8]. Furthermore, the kinetics of lipid production are substantially slower than starch synthesis. Thus, algae that primarily store starch may accumulate biomass

faster than algae that store hydrocarbons as energy reserves. The ecological downside of starch storage, however, is that starch has high volumetric density (1.56 g/cm^3) while lipids have a density of 0.91 g/cm^3 or less than that of water. Thus, algae that store starch must invest energy in motility devices and associated energy expenditures to avoid sinking to depths where light availability may be limiting for photosynthetic growth. It might then be predicted that algae that store starch, e.g., *Chlamydomonas*, predominantly inhabit soil environments that provide physical support whereas lipid accumulating algae, e.g., *Nanochloropsis*, tend to occupy aquatic environments where they are less dense or near the density of water and can remain at levels in the water column where light is not limiting for photosynthesis. To date, the relative energetic costs needed to support motility in starch accumulators versus lipid accumulators remains to be assessed.

3.3 Product storage and metabolism

Following the metabolic engineering paradigm for increasing product yield, i.e., push, pull, sequester and block storage product turnover, less attention has been directed towards the metabolic engineering of storage and product turnover in microalgae. As stated previously, energy reserves in algae fall into two classes, carbohydrates, and lipids. The genetic manipulation of starch accumulation in algae has received much attention. The chloroplast is the site of starch synthesis and storage in plants and algae. In contrast to plant cells, however, microalgae typically have only a single chloroplast per cell since chloroplast division must be synchronized with cell division to ensure that each progeny has a chloroplast [59]. Thus, there is no differentiation of plastids in single-celled microalgae into specialized starch storing amyloplasts as occurs in plants. As a result, increasing starch storage sites is not a viable strategy for increasing starch accumulation. Starch accumulation in a plastid can be genetically manipulated, however. Structurally, starch is composed of two types of glucose polymers, amylose and amylopectin, that differ in their degree of branching. The glucose density of starch granules and their size is controlled by the levels of starch branching and debranching enzyme activities. Genetic manipulations of enzymes controlling starch branching has been shown to substantially impact biomass production [58].

Enhanced lipid storage in microalgae has been achieved by over-expression of enzymes implicated in fatty acid and TAG biosynthesis [60–63], or by repression of lipid catabolism [62, 63]. Additionally, genetic manipulations to decrease starch accumulation also leads to substantial increases in storage lipid accumulation per cell. A *C. reinhardtii* mutant blocked in starch accumulation nearly doubled the amount of lipids accumulated under nitrogen deprivation relative to the control strain, indicating that TAG can act as an alternate sink for excess carbon and photosynthetic reducing equivalents [62]. High energy dense hydrocarbons are primarily stored as TAGs in microalgae and contained in membrane bound lipid droplets. Lipid droplet size and numbers are regulated in part by the production of lipid droplet proteins which are present in the membranes surrounding lipid droplets. Reductions in the expression of major lipid droplet proteins using RNA silencing techniques has been shown to significantly decrease the size of lipid droplets [63]. However, genetic manipulations to increase TAG accumulation by enhancing lipid droplet protein production to our knowledge has not been reported to date. Overall, genetic manipulation of genes controlling select aspects of source, sink, storage, metabolism, and cell growth rates have all proven to enhance biomass yields. Integration of multiple aspects of carbon metabolism, storage and growth leading to enhanced biomass yields have been achieved by alterations in mastery regulatory genes. But much remains to be characterized to achieve maximum thermodynamic efficiency for conversion of photons to the chemical energy of biomass.

4. Cultivation

Cultivation is a vital starting point in algae biomass production and hence choice of production site, strain and cultivation system are very crucial in attaining high biomass productivity. In addition, seasonal influence, crop losses, harvesting processes and nutrient and water recycling are some of the primary governing factors influencing biomass yield and production economics (**Figure 3**). The following section will cover the recent advances in some of the key areas mentioned above.

4.1 Criteria for siting production facilities

First and critical aspect in establishing successful algae cultivation facility is selection of suitable cultivation site. Site selection is quite a complex task and involves considerable attention on terrain, land costs, sunlight availability, seasonal temperatures, proximity to CO_2 and water sources, well-connected transport system, power supply etc. Economical, non-arable flat land with constructible soil is needed for raceway pond installation. Availability of adequate acreage is also an important criterion, as algal cultivation facility should be of scale where production of algae meets economics [64]. Another very important aspect in algal cultivation is availability of enough sunlight. Therefore, it is important to select a geographic location, which is less prone to seasonal variations, receives less rainfall and is climatically suitable to the strain being cultivated. For example, low altitude regions having warm climate and average solar radiation availability for 250 h/month are considered as good sites climatically [65]. CO_2 is regarded as free of cost, but its transportation can add substantial cost to the algae production if the CO_2 generation facility is far from the cultivation site [66]. Water availability is another important criterion. Proximity to sea in case of marine microalgae cultivation and assessment of water scarcity footprint in the region in case of fresh water algae cultivation is essential while selecting a site [67]. Various site selection models that consider parameters, such as soil properties, water availability, growth rate, infrastructure proximity etc. have been reported for identification of a suitable site for algae cultivation [68, 69]. These models can serve as useful tools for algae production site selection.

4.2 Seasonal challenges for biomass yield

Microalgal outdoor cultivation is subjected to diurnal and seasonal variations in temperature, solar irradiance, photoperiod and humidity, which in turn affect physiological responses and biomass yield. For instance, light is essential for photosynthesis, but excess light leads to photoinhibition, oxidative stress, damage of proteins

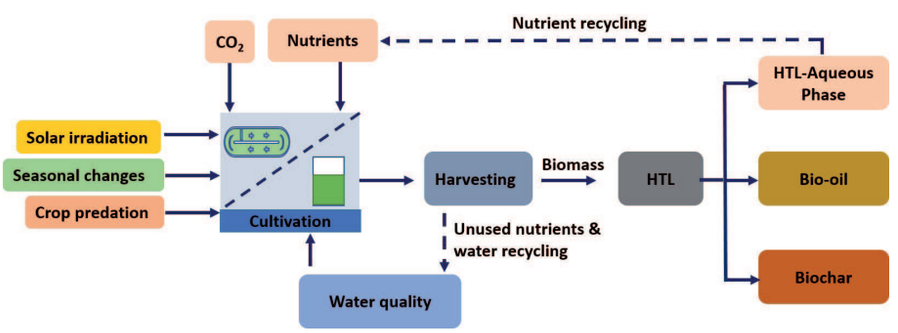

Figure 3.
Factors affecting algae biomass production.

involved in electron transfer and in turn affects CO_2 fixation in photosynthesis and biomass yield. Similarly, low light also reduces photosynthetic efficiency and thus biomass yield [70]. O_2 buildup in culture, which increases from morning till noon also can inhibit growth if O_2 concentration is more than 20 mg/L [71]. Temperature is another important factor, which is affected by light intensity, photoperiod and season. Optimal growth temperature for majority of algal strains lies between 20 and 25°C. However, temperatures above 35°C increases photorespiration, affects nutrient availability, increases the concentration of NH_3 in the medium, decreases CO_2 solubility and increases evaporation losses leading to salinity variations [71, 72]. The impact of these environmental variations is significant on biomass productivity but there are very few reports on quantification of effects of seasonal variations on algae biomass productivities in large scale production systems. For instance, growth performance of *Scenedesmus obtusiusculus* was studied in airlift extended loop photobioreactor operated in outdoor conditions. Yearlong study revealed that biomass productivity was maximum during spring (0.29 g/L/d) where irradiance (2035 μmol/m^2/s) and temperature (11–47°C) were highest, followed by autumn (0.22 g/L/d), summer (0.21 g/L/d) and winter (0.19 g/L/d). However, biomass productivity was much higher (0.97 g/L/d)under optimum laboratory conditions [73]. In another study, *Scenedesmus* sp. cultivated in outdoor pilot scale raceway ponds for waste water treatment resulted in biomass productivities, which ranged from 4 ± 0 g/m^2/d in December when average temperature was 13°C and irradiance was 300 ± 157 w/m^2 and 17 ± 1 g/m^2/d in July when average temperature was 23°C and irradiance was 468 ± 292 w/m^2 [74]. In another study, five microalgal sp. namely, *Chlorella vulgaris*, *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Euglena gracilis* and *Nannochloropsis oculata* were evaluated in open bioreactors with 30 L capacity in green house conditions. Experiments were conducted from March to April, June to July and Oct to Nov to evaluate growth response of these microalgae with seasonal variations. *C. vulgaris, B. braunii* resulted in highest growth during month of March and April when average temperature was 28.5°C and irradiance was 15.9 MJ/m^2/d. Whereas, *C. reinhardtii* and *N. oculata* grew best in the month of June when average temperature was 36.1°C and irradiance was 6.6 MJ/m^2/d. while, *E. gracilis* grew comparably during March and June months. In general growth response was low for all five microalgae tested during the month of Oct and Nov, when both temperature and irradiance were low [75].

It is clear from these studies that seasonal variations play significant role in microalgal biomass production and it is important to note that the effect of the environmental changes is strain specific. As complete control of abiotic factors is not possible at large scale outdoor cultivation complete, careful strains selection and adoption of right cultivation practices ensuring effective light and nutrient utilization can help in tackling the seasonal variability to some extent.

4.3 Recycling nutrients

Optimal microalgal growth relies on continuous and adequate supply of nutrients (nitrogen, phosphorous, carbon, potassium, trace elements and water) and sunlight. Nutrient input can be in the form of fertilizer and waste-water streams. Nutrient supply in the form of fertilizers can incur significant cost to the cultivation and is also a competition to fertilizer for agriculture [65]. Therefore, it is important to minimize nutrient losses during cultivation. One way is through stoichiometrically balanced nutrient management to minimize nutrient losses during cultivation [76] and other ways are by recycling of spent medium (water recycle) and nutrient recycling post biomass conversion process.

4.3.1 Water recycling

During growth not, all the nutrients are used completely, and these unused nutrients will be lost if the water is not recycled post harvesting. Water recycling is important not just for nutrient recycling but also from an economics perspective. Water reuse reduces the need to acquire new water for cultivation, thus reducing the water foot print for cultivation and lowering energy usage in pumping water from source to site [77]. There is a finite possibility that water recycling can affect subsequent growth performance of the algae if the recycled water quality does not meet required standards. Primary factors influencing recycled water quality can be increased salinity of the water, use of chemical based harvesting system, accumulation of extracellular metabolites (protein, carbohydrate, fatty acids, nitrogen rich small organic molecules, cell wall debris and other particulate matter) which may be directly inhibit algal growth or increase the dissolved organic carbon (DOC) leading to increased bacterial load and gradual accumulation of toxic metabolites [77–79]. However, multiple studies, both at small and large scale have successfully demonstrated recycling of water without negatively affecting algal growth. Recycled water obtained after electro-flocculant, bio-flocculant, nanno-chitosan, filtration, and centrifugation based harvesting methods had shown no negative effect on the growth of tested algal species [80–84]. Flocculation-based methods have been predicted to be better for water recycling than other methods because they do not lyse the cells and help in reducing dissolved organic matter during harvesting [78]. Farooq et al. (2015) compared chemical flocculation ($FeCl_3$ or alum) of *Chlorella vulgaris* against centrifugation and showed that recycled media obtained after centrifugation or flocculation with $FeCl_3$ had positive effect on growth and lipid productivity. However, recycled medium obtained through treatment with alum even in low dose (<5 ppm) inhibited the growth of *C. vulgaris* due to the toxic effect of residual Al in the recycled water [85]. Similar results were obtained in case of *Scenedesmus* sp., where growth was affected in recycled medium, when alum (1 mM) was used to harvest the cells [84]. Likewise, in another study strain dependent growth inhibition was observed due to accumulated DOC in recycled water. Growth of *Navicula* sp. and *Chlorella* sp. were comparable to fresh medium, while growth of *Staurosira* sp. was completely inhibited in reused medium [79]. It is important to note that stage at which the culture is harvested also affects DOC concentration. Water recycled from exponentially growing cells was found to be more supportive of growth than cells in late log phase or stationary phase, conditions that lead to the maximum accumulation of growth inhibitory substances secreted by algae. As DOC accumulation is more during late log and stationary phases due to the release of secondary metabolites into extracellular space, it is better to avoid recycling water from cultures harvested from these phases [78]. Pretreatment of water before recycling can be considered to improve water quality for long term cultivation with recycled medium. Filtration, high speed centrifugation and sterilization methods have been studied for pretreatment, but their commercial scale application is questionable [77]. In one study, activated carbon was used to process recycled medium to remove humic and fulvic acid like growth inhibitors. This step moderately improved growth of *Nannochloropsis oceanica* in recycled water [86]. Recently, advanced oxidation process has been evaluated for pretreatment of recycled water. It was observed that UV/peroxydisulfate and UV/H_2O_2 processes are quite effective in addressing organic matter load in the water. Oxidation method could degrade and converts inhibitory substances into nutrient source for algal growth. This method helps in utilization of DOC in recycled water rather than its removal [87].

Thus, recycling of spent medium is commercially viable and practically feasible option, which not only helps in saving loss of unused nutrients but also reduces the overall nutrient input.

4.3.2 Nutrient recycling from HTL aqueous phase

Hydrothermal liquification (HTL) is a potential technology to convert wet algal biomass into bio-oil with biochar and aqueous phase (AP) as byproducts. AP is substantial portion because high moisture containing (~10–20% algal slurry) biomass is used as feedstock in HTL [88]. AP is nutritionally rich, containing organic carbon as short chain organic acids, like acetic and propionic acid, nitrogen as NH_4^+, nitrate and other nitrogen containing compounds, phosphorous as orthrophosphates and other macro and micro nutrients [89]. This makes AP a potential nutrients source for microalgae when recycled back into cultivation, which are otherwise lost. It is also reported that even harmful algal blooms are also good feedstock for HTL and AP produced is promising nutrient source for microalgae cultivation [90]. AP also has growth inhibitory compounds like phenols, amides, pyrazines, indole, metal ions like Ni etc., which either must be removed or diluted to the extent that they are no more growth inhibitory [89, 91]. Composition of AP is quite variable and depends on algal feedstock used for HTL, processing parameters, biomass loading and use of AP separation method from bio-oil. For instance, high protein content in feedstock leads to higher organic carbon and nitrogen content in AP [92]. Likewise, increasing resident time in HTL process also has shown to result in increased total nitrogen in the AP. Since, the concentration of nutrients and toxic compounds is often high in AP, substantial dilution of AP is needed to bring concentration of nutrients in the usable range and dilute growth inhibitory toxic elements. There are multiple studies reported where AP is used as sole nutrient source for algal cultivation or a supplement with systematic heavy dilutions made either with water or combination of water and standard nutrient medium. Outcome of these studies is quite variable and was dependent on AP composition and strain being used for cultivation. When AP was used as sole nutrient source, growth of the tested algae was relatively compromised. For instance, AP obtained from *Spirulina* HTL was used as sole nutrient source for cultivating *Chlorella minutissima,* where AP consisted ~16,200 mg/L N and 795 mg/L P along with other nutrients. Biomass productivity obtained was 0.035 g/L/d at 0.2% AP (500X dilution), which was significantly less than BG11 control, having 0.07 g/L/d productivity [91]. Likewise, APs obtained from HTL of *Chlorella vulgaris, Scenedesmus dimorphous* or *Spirulina platensis* as feedstocks were also evaluated as sole nutrient source at various dilutions to grow these stains. Growth of *Chlorella* and *Scenedesmus* was less in comparison to standard medium even at 400X dilution, however, *Spirulina* showed comparable growth in AP and standard medium [93]. Alba et al. (2013), presented comparative account of AP diluted with water versus standard medium for cultivation of *Desmodesmus* sp. A substantial reduction in growth was observed when AP was diluted with water, however, when mixture of water and AP was enriched with standard medium, growth comparative to standard medium was observed. This study clearly indicates that it is not just N and P content that is important for growth but balancing AP in such a way that other macro and micro nutrients are also not limiting is essential for successful use of AP for cultivation [94]. Similar results were obtained in other studies, where AP diluent was enriched with desired nutrients [95–100]. Interestingly, Lopez Barreiro et al. (2015) observed that growth in AP diluted with standard medium was strain dependent. *Nannochloropsis gaditana* and *Chlorella vulgaris* could grow well in AP diluted with standard medium, however, *Phaeodactylum*

tricornutum and *Scenedesmus almeriensis* showed poor performance [98]. Apart from deficiency of essential nutrients in AP, other factors which have been reported for inhibited growth are presence of phenolic compounds [91], high Ni concentration [93], NH_3 toxicity [92, 101], limitation of carbon availability and generation of toxic metabolites [102]. HTL technology is evolving to address these issues. In direct HTL at temperature 300°C or above, protein converts into pyrazines, pyrroles and amines, whereas, polysaccharides convert into cyclic ketones and phenols [103]. These non-fuel components lower bio-oil quality, in the process polysaccharides are lost and toxic metabolites are generated and accumulated in AP. To improve quality of bio-oil and prevent loss of polysaccharides, sequential HTL (SEQHTL) is developed, where AP is recovered in first stage of HTL operated at lower temperature (~160°C) [104]. Polysaccharides constitute major portion in the AP from SEQHTL in contrary to AP from direct HTL, where N and P dominate. In nutrient reuse experiments using AP from SEQHTL, it was shown that *Chlorella sorokiniana and Chlorella vulgaris* could utilize 77% and 64% of hydrolyzed polysaccharides, respectively, however, *Galdieria sulphuraria* could not use the polysaccharides from AP, suggesting again that the utilization of nutrients from AP of HTL is strain dependent [88]. Apart from altering HTL conditions and dilution of AP, other ways to reduce toxicity of AP is through removal of toxic substance by absorbents like activated charcoal, zeolite and ion exchange resins. In recent study it was shown that AP treated with ion-exchange resin, Dowex 50WX8 supported the growth of *Chlorella vulgaris* at 100X dilution similar to control medium and better than activated charcoal treated AP [105].

Thus, outcome of multiple studies suggests that for successful utilization of HTL-AP for algal cultivation, selection of right strain is crucial, which can grow mixotrophically and can utilize N as NH_4^+. Appropriate dilution of AP or treatment with absorbents to reduce toxic metabolites load and supplementation with limiting nutrients are also essential for overcoming growth inhibition in AP.

4.4 Pond crashes and mitigation

Large scale algae cultivation ponds and photobioreactors are usually prone to contamination by unwanted foreign organisms due to nonsterile cultivation conditions. Moreover, suboptimal cultivation conditions (light, temperature, nutrients), poor culture mixing, old and sick cells, allow predators and contaminants overtake and crash the culture [106]. Common contaminants in algae cultivation include, grazers (ciliates, rotifers, flagellates, crustaceans, amoeba), pathogens (bacteria and virus) and parasites (fungi, vampyrellids). Multiple studies have reported culture crash due to these organisms. For instance, chytrid contamination in *Haematococcus pluvialis* [107], *Poterioochromonas* sp. (flagellate) [108] and *Euplotes* sp. (ciliate) [109] contamination in *Chlorella,* pleomorphic bacterial (FD111) contamination in *Nannochloropsis* [110], *Colpoda steinii* (ciliate) contamination in *Synechocystis* sp. [111], *Amoeboaphelidium protococcarum* (amoeba) contamination in *Scenedesmus* sp. [112] etc. are some of the studies where contamination resulted in collapse of the culture at mass scales. Since culture crash results in substantial biomass loss, a scalable, environmentally friendly and economical crop control measures are crucial.

Various chemical and physical methods are available for crop protection; however, selection of a method at large scale depends on its activity against predators, non-toxicity towards algae of interest, scalability and cost effectiveness. In case of chemical methods, availability, stability of the chemical and its environmental toxicity should also be considered [109]. Various chemicals belonging to antimicrobials, fungicides, herbicides, oxidants, pesticides, natural compounds, antiparasitic,

antifeeding categories have been evaluated to control predators in algae cultivation. Majority of chemicals tested at lab scale are not suitable for large scale operation because of environmental toxicity or they are very expensive for use in algae cultivation. However, copper has been successfully used to selectively control rotifer- *Brachionus calyciflorus* at 1.5 ppm concentration in open pond cultivation of *Chlorella kessleri* [113]. Similarly, sodium hypochlorite (NaOCl) at a dosage of 0.45 to 0.6 mg Cl/L with dosing frequency of every two hours also inhibited predation by *B. calyciflorus* while no growth inhibition was observed in *C. kessleri* [114]. Use of NaOCl might be practically more feasible in open ponds as chlorine dissipates rapidly, leaving no long-lasting residual effects. Moreover, it is effective at lower dosage in comparison to commonly used insecticides Fenitrothion (6.7 mg/L) and Chlorpyrifos (12 mg/L) for controlling *Brachionus calyciflorus* [115, 116]. Recently, Karuppasamy et al. (2018), have screened around 100 chemicals and out of these 21 were effective against *Euplotes* sp. and *Oxyrrhis* sp., and did not have noticeable detrimental effect on *Chlorella vulgaris.* Further, considering cost, availability, stability and effectiveness, benzalkonium chloride (a quaternary amine) at a concentration of 2 mg/L was evaluated and recommended for preventing pond crash [109]. Apart from chemical control, temporary alteration of cultivation conditions has also been reported to be effective in pond crash mitigation. For instance, limitation of P in the medium does not affect algae severely but affects growth of zooplanktons. Slowest zooplankton growth was observed under high light/P ratio [117]. Flynn et al. (2017) also reported through predictive modeling that low level of P stress can be strategically applied to create suboptimal conditions to zooplankton growth without causing detrimental conditions for algal growth [118]. Another potential strategy to control certain type of predators is use of high level of CO_2 in the culture medium. Ma et al. (2017) demonstrated that CO_2 purging temporarily lowered *C. sorokiniana* GT-1 culture pH to 6–6.5 and helped in controlling *Poterioochromonas malhamensis* by lowering its intracellular pH and resulting in cell death. This strategy can be implemented for controlling *P. malhamensis* and other protozoans in large scale cultivation [119]. In addition, CO_2 asphyxiation was found to be effective in causing acute mortality of all zooplankton species in $t < 10$ min [120]. *Poterioochromonas* sp. contamination could also be controlled through cultivation at high pH (>pH 11) as reported in *Synechocystis* sp. PCC 6803 cultures [121].

Apart from chemical methods, there are multiple physical methods, which have been developed for grazer control in algae cultivation. Hydrodynamic cavitation (HC), ultrasonication, foam flotation, pulse electric field, filtration and electromagnetic stratagem are some of the technologies used for crop protection. HC is considered as simple and economical method to kill zooplanktons in waste-water treatment. Kim et al. (2017) have extended this technology in controlling rotifers in algal cultivation. This method could successfully control 99% rotifers in four passes with little effect on *Nannochloropsis* [122]. Likewise, flagellate *Poterioochromonas* sp., a deleterious contaminant in *Chlorella* mass cultivation was disrupted using ultrasonication. This method was tested at 60 L scale and has potential to be used at mass scale. Ultrasonication was also shown to be effective in controlling fungi, amoeba, and ciliates [108]. Electrocution is another technology, which was successfully tested outdoors in 1 and 20 m^2 ponds. Here, 5–10 mA current was applied through graphite rods for 6 h or more to control ciliates and dinoflagellates, however, algae growth was not affected [123]. Pulse electrophoresis is another technology which has been used to effectively control rotifers in tubular PBR. Technology however, can be used for freshwater algal cultures [124]. Umar et al. (2018) evaluated foam flotation, a physiochemical method to remove ciliates *Tetrahymena pyriformis* from *C. vulgaris* culture grown in PBR. Addition of SDS at 40 mg/L concentration lysed ciliates without affecting algal cells [125].

It is clear from the above description that there are multiple methods available to control the crop loss. However, not all methods are equally effective in controlling all types of predators. Therefore, careful selection of a chemical or physical method based on algae and its intended use is needed to prevent the pond crashes or to control the predators without affecting the algal growth.

4.5 Harvesting efficiencies and energy targets

Harvesting and dewatering of microalgae is a very challenging process due to their small cell size (<20 μm), low biomass concentration (0.2–1 g/L in ponds and 2–9 g/L in PBRs) [126], density comparable to water (1.08–1.13 g/mL) and negative charge on algal cells, keeping cells in suspension due to repulsive forces [127]. Common harvesting technologies of microalgae include flocculation, centrifugation, sedimentation, filtration and flotation. These methods can be used individually or in combination to improve the effectiveness and economics of harvesting. For example, flocculation can be combined with sedimentation or dissolved air flotation (DAF), DAF can be combined with filtration or centrifugation. First stage of algae harvesting is generally called primary harvesting process, which concentrates cells up to 2–7% and the second stage is called secondary harvesting or dewatering. It uses primary harvested biomass as feed and further concentrates it up to 15–25% [128]. Fasaei et al. (2018) have discussed 28 combinations of primary and secondary harvesting and recommended filtration followed by centrifugation or flocculation followed by membrane filtration and a finishing step with spiral plate technology or centrifugation as economically attractive solutions. Further, when initial biomass concentration and separation techniques are considered, the estimated operational costs and energy consumption for various harvesting methods were estimated to be in the range of 0.1–2 €/kg and 0.1–5 kWh/kg, respectively. Based on these estimates, harvesting cost was projected to be between 3 and 15% of the production cost, which is significantly lower than the earlier estimate of 20–30%, reported in other studies [129, 130].

Flocculation is most common primary harvesting technique, where cell aggregation is achieved through charge neutralization by cationic flocculants, polymers and metal salts like ferric chloride, alum, aluminum sulfate and ferric sulfate [128]. The flocks formed in association with chemicals are either allowed to settle under gravity in a settling tank or floated by attaching micro-bubbles to their surface using a DAF. Energy consumption range for this process as reviewed by Mo et al. (2015) is 0.1–14.8 kWh/m^3 [131]. Chemical flocculation has resulted in variable outcome as harvesting efficiency of flocculation is dependent on the flocculent dosage, pH of the culture medium, surface charge and salinity. Under optimal conditions, greater than 90% harvesting efficiency was achieved in many studies, for instance, flocculation of *Chlorella sorokiniana, Chaetoceros muelleri, Chlorella vulgaris* and *Scenedesmus costatum* with chitosan [132–134]. Likewise, *Chlorococcum* sp. and *Dunaliella tertiolecta* were harvested with more than 90% harvesting efficiency using $Al_2(SO_4)_3$ or $Fe_2(SO_4)_3$ as flocculants [135]. However, chemical flocculation in large scale algae production may not be economically viable because of high cost of chemical and high dosage requirement. Also, accumulation of residual flocculant in the harvested water and with microalgae might affect the downstream process and may pose environmental concerns [136].

Filtration is another promising harvesting method, which can give 100% biomass recovery and clean biomass, as the process is devoid of chemical input. However, low flux, frequent membrane fouling and high cost of filtration process are key bottlenecks in the large-scale operations. To improve filtration performance and reduce membrane fouling, filtration process has been clubbed with accessory

technologies, like aeration [137], vibration [138], use of electro membrane [139] and rotating disk [140]. Bilad et al. (2012) used submerged microfiltration equipped with vibrator for harvesting *Chlorella vulgaris* and *Phaeodactylum tricornutum* and reported energy consumption of 0.27 kWh/m^3 (0.64 kWh/kg) and 0.25 kWh/m^3 (0.98 kWh/kg), respectively [138]. Corresponding energy for electrocoagulation flocculation process is reported to be 1.3–9.5 kWh/m^3 for the same species, which was substantially high [141]. Recently, pilot scale ultra-filtration membrane trial clubbed with air assisted backwashing technology has been successfully used to harvest *Scenedesmus acuminatus*. The culture was concentrated from 0.5 g/L to 136 g/L with 93% biomass recovery. The energy consumption reported was 0.59 kWh/kg dry biomass [142]. Though filtration is less energy intensive [130], further improvements in filtration technology is required and can also be achieved by using membranes with advanced hydrophilic material and introducing negative surface charges [126].

Centrifugation is another physical method of harvesting, but the harvesting efficiency is less than filtration and highly depends on the gravitational forced applied. Centrifugation is also energy intensive, difficult to scaleup, requires high maintenance and considered expensive for low value products like oil. Using centrifugation as sole harvesting method is not recommended as energy consumption and cost of harvesting is significantly higher compared to a process, where centrifugation is used as secondary harvesting method. In a study where Evodos spiral plate centrifuge was solely used to harvest 10,000 L of *Chlorella* culture, energy consumption was 55 kWh/m^3, as opposed to 5.5 kWh/m^3, when centrifugation was used as secondary harvesting step [128]. Other common centrifuge types are disc stack and decanter. Disc stack is the most common industrial centrifuge with reported energy consumption ~1 kWh/m^3. However, energy consumption was further reduced to 50% by design changes, like modifying flow paths of rotor, reduction of aerodynamic losses by air removal outside rotor and use of direct drive instead of belt or gear drive [143]. In case of decanter centrifuge, energy consumption ranged between 1.3–8 kWh/m^3. In another study by National Renewable Energy Laboratory (NREL), energy consumption in concentrating microalgae from 13–20% using centrifuge was estimated to be 1.3 kWh/m^3, with a dewatering efficiency of 97% [144].

In conclusion, it is clear from above description that significant developments are made in harvesting technology but none of the techniques seems to be economical and efficient enough. Combination of two to three technologies have been proposed to give economically viable solution but still significant optimization and innovation is necessary in current technologies and there is substantial scope for development of new, cheaper and more efficient harvesting technologies.

5. Commercial scale up

High cost of biomass production and subsequent extraction processes have limited the progress of upscaling of microalgae for commercial fuel and other value-added products. The technoeconomic analyses reported thus far have a wide variation in the cost estimates, primarily due to non-existence of standardized cost assumptions across different geographic locations. For example, in a study conducted in the US, production of microalgal biomass is estimated at $4.92/kg with current technology status [145]. In another study conducted in Europe, production cost was estimated to be €4.95, 4.16, and 5.96/kg of biomass from open ponds, horizontal tubular and flat panel photobioreactors, respectively [146]. Even the biomass production cost drops down to $0.5/ kg, still scaling-up of microalgae

for standalone production of biofuel is economically infeasible due to swift competition with fossil fuel [145]. Hence, cost reduction and integration of additional revenue generation steps could help in successful scale-up.

While microalgae are primarily sought-after for biodiesel production through utilization of lipids, valorization of other components through a biorefinery approach, as proposed in many studies might enhance the chances of commercialization. Microalgae are traditionally utilized for food and feed, cosmetics, nutraceutical and pharmaceutical applications because of the presence of high content of protein, carbohydrate, pigments, antioxidants, ω-3 fatty acids and other industrially important chemicals. Extraction of these compounds as co or byproducts can improve the overall process economics [147, 148]. In microalgal biorefinery, valorization of different components of microalgae biomass is achieved through a series of unit operations for extraction, purification and biomass conversion [149]. Based on the type of the primary product being extracted, biorefineries can be classified as energy driven or material driven biorefinery. In energy driven biorefinery, oil for biofuel is extracted first, and the de-oiled biomass is used for extraction of value-added products or in a bioconversion processes like fermentation, anaerobic digestion, pyrolysis, hydrothermal liquification (HTL) etc. The best possible sequence of extraction of compounds for valorization of biomass can be evaluated through cost effectiveness assessment (CEA), which is the ratio of total outcomes from a biorefinery to the total cost of producing products [150]. Also, for successful biorefinery scheme, the net energy ratio (NER) assessment is important. It is the ratio of energy output over energy input and should be greater than unity. Higher the values of CEA and NER are, higher would be the feasibility of that biorefinery scheme [148, 150].

Several microalgae biorefineries have been proposed and tested in the literature but their implementation at large scale is still far from reality. **Table 1** summarizes some of the recent biorefinery approaches reported in the literature and **Figure 4** represents various possible biorefinery approaches. Razon and Tan (2011) evaluated a biorefinery for production of biodiesel and biogas from *Haematococcus pluvialis* and *Nannochloropsis* [151]. The NER was less than one for both the cases indicating negative energy balance, even when best performance estimates were taken for unit operations. However, economics of the system can be improved if cultivation is integrated with waste-water plant, thus eliminating the need for chemical fertilizers. Also, wet extraction should be followed thus saving on drying cost [151]. Similarly, Andersson et al. (2014) evaluated the biodiesel and biogas production through integration of cultivation with waste-water treatment plant, flue gas as carbon source and excess heat from industrial cluster. Production of biodiesel and biogas in biorefinery scheme resulted in net positive outcome compared to biogas alone [152]. In another biorefinery approach, Ansari et al. (2015) evaluated lipid extracted algae (LEA) for its use as protein or reducing sugar source and observed comparable yields of these products from whole algae and LEA as source material. Also, oven drying over sun drying and microwave assisted lipid extraction resulted in highest lipid yield compared to other methods tested [153]. In another study first protein was recovered from *Botryococcus braunii* under alkaline conditions, followed by lipid extraction for biodiesel and finally spent biomass was used for bio-oil production through pyrolysis. This biorefinery process resulted in 10% protein recovery, 2% lipid recovery and 33% bio-oil recovery. Bio-oil being obtained in this scheme was at neutral pH and hence non-corrosive for combustion engines but bio-oil recovery from spent biomass was less than that of whole algae. Due to poor recovery of lipids, their extraction step can be omitted and scheme can be simplified [154]. Recently, in *Chlorella vulgaris* biorefinery, where protein extraction was integrated with pyrolysis, extraction under alkaline condition (12 pH) at

Microalgae	Biorefinery products	Remarks	Reference
Nannochloropsis sp.	Biodiesel; Carotenoids; Bio hydrogen	CO_2 super critical fluid extraction plus ethanol (20 wt.%) could extract 45% (dry weight basis) of lipids and recover 70% of the pigments Dark fermentation of left-over biomass by *E. aerogenes* yielded maximum 60.6 mL H_2/g Dry biomass of alga.	[159]
Dunaliella tertiolecta	Biodiesel; Bioethanol	Enzymatic saccharification of de-oiled biomass followed by fermentation resulted in the yield of 0.14 g ethanol/g residual biomass equivalent of 82% of the theoretical fermentation yield.	[160]
Botryococcus braunii	Protein; lipid; bio-oil	Protein extraction followed by bio-oil recommended. Neutral pH was found in bio-oil from microalgal biomass.	[154]
Scenedesmus obliquus	Lipid; Protein/ Reducing sugars	Microwave assisted extraction from oven dried samples provided highest lipid yield. Protein and reducing sugar yield comparable in lipid extracted algae vs. whole algae. Sun drying resulted in poor outcome.	[153]
Nannochloropsis sp.	Lipid; fuel gases; Nitrogen as NH_4^+	75% recovery of energy in SCWG process and 100% recovery of N from lipid extracted hydrochar.	[156]
Spirogyra sp.	Carotenoids; Biohydrogen	Electrocoagulation and solar drying reduced the energy requirements by 90% for harvesting and dewatering. 0.12 g/100 g dry biomass of total pigments with 56% free astaxanthin, 16% beta-carotene & 5% of lutein and canthaxanthin. Fermentation of residual biomass produced hydrogen yield of 47 mL/g d.w. Carotenoid extraction with acetone is expensive and hydrogen yields have to improve by increasing sugar content in the biomass through altered cultivation practices.	[161]
Scenedesmus acutus	Bioethanol; biodiesel	Whole algal slurry after acid pretreatment is directly used for ethanol fermentation. No losses of fermentable sugars in the solids, which are otherwise separated from the sugar rich supernatant. $0.95/ GGE cost reduction in biofuel production.	[157]
Phaeodactylum tricornutum	Pigments; Fatty acids	Green processes: pressurized liquid extraction (PLE) and microwave-assisted solvent extraction (MAE) were evaluated for extraction of bioactive compounds. Optimum extraction conditions were 50°C, 100% EtOH, 20 min for PLE, while optimum conditions for MAE were 30°C, 100% EtOH and 2 min. Higher recovery of fucoxanthin enriched with EPA were obtained with PLE method.	[162]

Microalgae	Biorefinery products	Remarks	Reference
Chlorella vulgaris	Protein; bio-oil	Hydrolysis with sonication under alkaline conditions yielded high protein recoveries. Scheme is economically feasible if extracted protein is used for food application. Profit is 1.51 $/Kg of microalgae biomass	[155]
Chlorella vulgaris	Lutein; Protein	Pulse electric field treatment enhanced the lutein (2.2 ± 0.1-fold) and chlorophyll yields (5.2 ± 3.4-fold) compared to non-treated cells single-stage ethanol extraction process. Protein extraction cost estimated to be US$4.16/kg of protein with 50% extraction yield and 57% purity. Further improvement in yield and purity is needed to make this biorefinery economically viable.	[163]

All the studies mentioned are conducted at lab scale.

Table 1.
Experimental demonstration of microalgal biorefinery approaches.

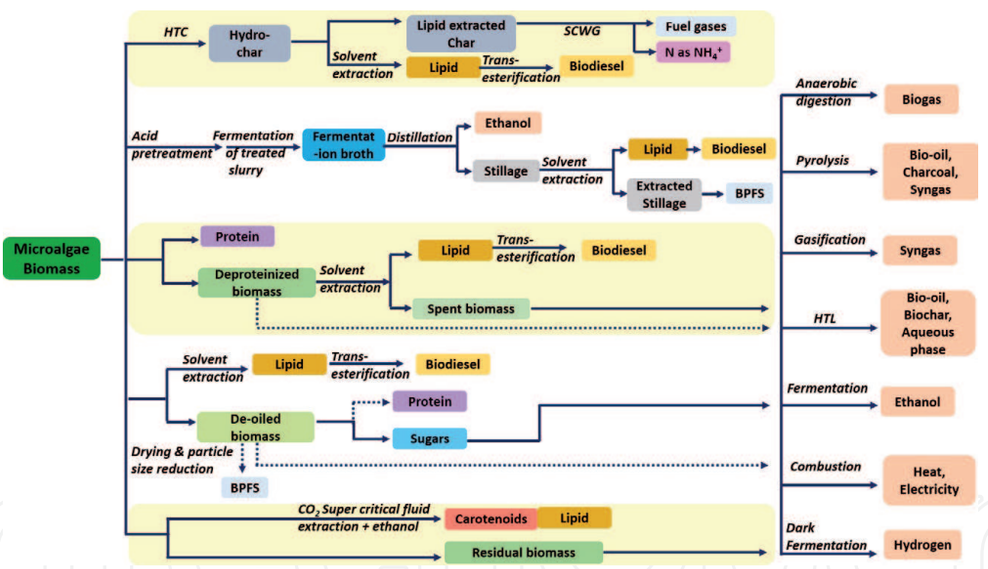

Figure 4.
Possible microalgal biorefinery approaches (dotted line: Alternate route, HTL: Hydrothermal liquification, HTC: Hydrothermal carbonization, SCWG: Super critical water gasification, BPFS: Bioplastic feed stock, stillage: Fermentation broth after removal of ethanol, extracted stillage: Broth after extraction of ethanol and lipids, spent biomass: Deproteinized and de-oiled biomass).

50°C for 90 min followed by sonication resulted in 80% protein recovery. Bio-oil obtained from protein extracted biomass was better in quality and comparable in quantity with whole algal biomass extraction. Based on technoeconomic analysis, it was proposed that the extracted protein if used for food application then the profit can increase by 1.51 USD/ kg of microalgae biomass [155]. Lu and Savage (2015) processed *Nannochloropsis* slurry through hydrothermal carbonization (HTC) and resultant hydrochar was further used for lipid extraction. Lipid extracted residual char was converted into fuel gases through a process called super critical water gasification and almost complete recovery of N as NH_4^+ was achieved, which can be used for nutrient recycling. This scheme is attractive as multiple products can

be extracted efficiently and use of HTC process in the beginning eliminates the energy intensive step of drying for lipid extraction [156]. Dong et al. (2016) tested another biorefinery approach, where *Scenedesmus acutus* slurry was subjected to fermentation after acid pretreatment at 155°C for 15 min. Ethanol was recovered through distillation of fermentation broth and lipids were recovered from stillage by solvent extraction. Advantage of this approach was that monomeric sugar was fully utilized in the fermentation process as sugar-rich liquor was not separated from solid residues post pretreatment. Also, using whole cell algal slurry (post pretreatment) for fermentation resulted in microalgal biofuel cost reduction by $0.95/GGE [157]. In another study, production of bioplastic feed stock (BPFS) and biofuel were integrated in algal biorefinery. Open raceway pond (ORP) cultivation followed by utilization of dried biomass as BPFS was found to be the most economical with minimum selling price (MSP) estimated to be $970/ ton. Other scenarios, were, lipid extraction or fractionation prior to use of biomass as BPFS. These biorefineries with an estimate of MSP of $1370 and $1460/ ton of lipid extracted or fractionated biomass, respectively, were proposed to be competitive if cultivation cost is reduced [158].

Though biorefinery concept gives greater product and economic flexibility, the technologies needed for processing of residual streams of microalgae biomass are still in nascent stages of development and hence many biorefinery models are faced with technoeconomic hurdles. Cultivation, harvesting and drying are highly cost and energy intensive steps and needs substantial innovations and advancements to improve economics. Economics of downstream processing steps, which include cell disruption, extraction, purification and biomass conversion are not thoroughly assessed and reported, moreover, technology for multiproduct extraction is neither fully mature nor evaluated at large scale [164]. The economic analyses reported on biorefineries thus far are mostly based on small scale studies and limited knowledge on end-to-end biorefinery trials at large scale, affects the reliability of economic analysis [152]. Other significant challenges in successful implementation of microalgae biorefineries are; consistent availability of algal biomass, variation in microalgal composition based on cultivation conditions and strain specificity. Therefore, adequate control of cultivation parameters and selection of appropriate strain is important. When biorefinery products are intended for food industry, then the production process from cultivation to final product should adhere to regulations set by regulatory agencies in respective geographic locations [149]. Product stability is another key challenge and must be ensured throughout the storage period.

In conclusion, though microalgae are an excellent feedstock for implementation of biorefinery approaches, a concerted effort is still needed to make the production process economically viable and environmentally sustainable.

6. Conclusions

6.1 Will algal biomass production ever be economically viable?

Though microalgae technologies have evolved tremendously in the past decade and have shown greater promise as renewable feedstocks for food, fuel and other high value products, their commercial scale production is still in its infancy. Companies like Sapphire Energy, Aurora Biofuels, Solazyme, and Algenol started with the aim of producing biofuel from algae at a large scale but could not sustain their operations due to economic infeasibility. Some companies have stopped the operations, while others changed their focus to produce algae for food or other non-fuel products. Considering the technoeconomic analysis of fuel production from

microalgae, production of algae for food, nutraceutical, cosmetics etc. has higher chances of success, as these products provide lot of opportunities to innovate and higher value of these compounds in comparison to fuel can fetch higher returns on investments. However, it must be noted that the market for these products is either substantially small or still in early stages of evolution. Moreover, availability of several cheap alternatives and lack of awareness among people about algae products are also critical stumbling blocks in market acceptability of algal products.

To bring microalgae production into mainstream both cost and market awareness must be improved. Integrated biorefinery approaches, discussed in detail in previous section, can be a viable option in this direction if technological and financial challenges are overcome. For that, focused research in both fundamental and applied areas to bridge the gap between lab to field translatability is imperative. Understanding biology for high biomass production and tweaking production strains through mutation, genetic and metabolic engineering approaches to increase the efficiency of accumulating desirable products and building the capability to withstand biotic and abiotic stresses would be a step towards success of commercial scale algal biomass production. In parallel, optimization of unit operations in cultivation, harvesting and downstream processing by improving their efficiency, lowering cost and finally integrating biological and engineering systems to ultimately develop economically viable end-to-end process is crucial for success. Lastly, government support in terms of well-defined policy, setting clear renewable energy targets, funding and subsidies on environmentally sustainable technologies would be a strong push in making algal biomass production at commercial scale a reality.

6.2 Next generation systems

It is clear from the discussion above that substantial improvements are needed in multiple processes of algal biomass production. Next generation systems should focus on improving pond design and better hydrodynamics, which can enhance fluid mixing and minimize dead zones resulting in improved biomass productivity, reduction in contaminant growth and pond crashes. Pond design should also support improved light and dark cycle leading to better light utilization, thus enhancing biomass productivity. Cost reduction through innovative low-cost pond lining is another important focus area for next generation systems. Development of efficient and inexpensive CO_2 delivery systems, where CO_2 wastage can also be minimized is an area of active research and such novel delivery methods should be part of next generation systems. Harvesting incurs significant cost to the algal biomass production, hence, combining two or more harvesting strategies and identifying coagulation, flocculation and dewatering chemical recipes that also can work effectively under saline conditions for microscopic algae will add in improving economics of biomass production. Strain modification and developing robust strains should also be the focus area of next generation systems. One example is propiconazole resistant *Chlorella* strain developed through mutagenesis, also harbors trait of high temperature tolerance. These two traits make the strain apt for cultivation in outdoor conditions [165].

6.3 Biological carbon capture and sequestration

There is growing recognition that the greatest existential threat facing the planet is anthropomorphic climate change. There is growing evidence that reductions in carbon emissions may not be sufficient to push global temperatures beyond a tipping point that would lead to an inhabitable planet for much of life as we know it today. Perhaps the greatest irony is that the geological sequestration of microalgal

biocrudes may be one of the most efficient and sustainable means to sequester atmospheric carbon [35, 166, 167]. Instead of extracting non-renewable petroleum (ancient algal biomass) from the earth it may become necessary to sequester atmospheric carbon by returning algal biocrude to the earth perhaps through the same pumps and wells that were used to extract petroleum. Carbon capture by algae is sustainable given efficient recycling of water and nutrients. The major concern is public inertia to mitigate carbon and economics. The costs associated with algal biocrude or carbon sequestration may be attractive. The economics of algal biocrude sequestration can be offset in part by the co-production of high volume/ low value animal feeds (proteins and carbohydrates) and the production of high value commodities minimizing the need for governmental financial support of atmospheric carbon mitigation technologies. To date, an algal BCCS system linked with food and valuable coproduct production has not been modeled for carbon capture efficiency and costs. The challenge for the next generation of algal scientists and economists is to consider whether algal BCCS is a workable solution to mitigate atmospheric carbon and address the looming specter of climate change.

Acknowledgements

Richard Sayre acknowledges financial support from the New Mexico Consortium to write this article. Meghna Rajvanshi acknowledges Dr. Santanu Dasgupta and Dr. Ajit Sapre from Reliance Industries Ltd., India for providing the opportunity and support in the preparation of this chapter.

Author details

Meghna Rajvanshi[1] and Richard Sayre[2*]

1 Reliance Industries Limited, Mumbai, India

2 New Mexico Consortium, Los Alamos, USA

*Address all correspondence to: rsayre@newmexicoconsortium.org

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Unkefer CJ, Sayre RT, Magnuson JK, Anderson DB, Baxter I, Blaby IK, et al. Review of the algal biology program within the National Alliance for advanced biofuels and bioproducts. Algal Research. 2017;**22**:187-215

[2] Lammers PJ, Huesemann M, Boeing W, Anderson DB, Arnold RG, Bai X, et al. Review of the cultivation program within the National Alliance for advanced biofuels and bioproducts. Algal Research. 2017;**22**:166-186

[3] Marrone BL, Lacey RE, Anderson DB, Bonner J, Coons J, Dale T, et al. Review of the harvesting and extraction program within the National Alliance for advanced biofuels and bioproducts. Algal Research. 2018;**33**:470-485

[4] Benedetti M, Vecchi V, Barera S, Dall'Osto L. Biomass from microalgae: The potential of domestication towards sustainable biofactories. Microbial Cell Factories. 2018;**17**(1):173

[5] Fabris M, Abbriano RM, Pernice M, Sutherland DL, Commault AS, Hall CC, et al. Emerging Technologies in Algal Biotechnology: Toward the establishment of a sustainable, algae-based bioeconomy. Frontiers in Plant Science. 2020;**11**:279

[6] Barry A, Wolfe A, English C, Ruddick C, Lambert D. 2016 National Algal Biofuels Technology Review; USDOE Office of Energy Efficiency and Renewable Energy (EERE), Bioenergy Technologies Office (EE-3B), 2016 DOE/EE-1409; Other: 7421 United States 10.2172/1259407 Other: 7421 EE-LIBRARY English.

[7] AlgaeBase is a global algal database of taxonomic, nomenclatural and distributional information. https://www.algaebase.org/.

[8] Subramanian S, Barry AN, Pieris S, Sayre RT. Comparative energetics and kinetics of autotrophic lipid and starch metabolism in chlorophytic microalgae: Implications for biomass and biofuel production. Biotechnology for Biofuels. 2013;**6**(1):150

[9] Zullaikah S, Utomo AT, Yasmin M, Ong LK, Ju YH. 9. Ecofuel conversion technology of inedible lipid feedstocks to renewable fuel. In: Azad K, editor. Advances in Eco-Fuels for a Sustainable Environment: Woodhead Publishing. 2019. pp. 237-276

[10] Pienkos PT, Darzins A. The promise and challenges of microalgal-derived biofuels. Biofuels, Bioproducts and Biorefining. 2009;**3**(4):431-440

[11] Rösch C, Roßmann M, Weickert S. Microalgae for integrated food and fuel production. GCB Bioenergy. 2019;**11**(1):326-334

[12] Campbell BM, Beare DJ, Bennett EM, Hall-Spencer JM, Ingram JSI, Jaramillo F, et al. Agriculture production as a major driver of the earth system exceeding planetary boundaries. Ecology and Society. 2017;**22**:4

[13] Van Iersel S. Algae-Based Biofuels. A Review of Challenges and Opportunities for Developing Countries. Rome (Italy): FAO; Global Bioenergy Partnership GBEP, c/o Environment, Climate Change and Bioenergy Division, Food and Agriculture Organization of the United Nations FAO, Viale delle Terme di Caracalla, 00153 Rome (Italy); Ecofys, Utrecht (Netherlands); 2009. Medium: X; Size: 60 pages p

[14] Chisti Y. Biodiesel from microalgae. Biotechnology advances. 2007;**25**(3):294-306

[15] Chisti Y. Response to Reijnders: Do biofuels from microalgae beat biofuels

from terrestrial plants? Trends in Biotechnology. 2008;**26**(7):351-352

[16] MMv K, Bikker P, IMvd M, CMCvd P-S, Vereijken JM. Cultivation, Processing and Nutritional Aspects for Pigs and Poultry of European Protein Sources as Alternatives for Imported Soybean Products. Lelystad: Wageningen UR Livestock Research; 2013. pp. 1570-8616

[17] Sayre R. Microalgae: The potential for carbon capture. Bioscience. 2010;**60**(9):722-727

[18] Neofotis P, Huang A, Sury K, Chang W, Joseph F, Gabr A, et al. Characterization and classification of highly productive microalgae strains discovered for biofuel and bioproduct generation. Algal Research. 2016;**15**:164-178

[19] Weissman JC, Likhogrud M, Thomas DC, Fang W, Karns DAJ, Chung JW, et al. High-light selection produces a fast-growing *Picochlorum celeri*. Algal Research. 2018;**36**:17-28

[20] Breuer G, Lamers PP, Martens DE, Draaisma RB, Wijffels RH. The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains. Bioresource Technology. 2012;**124**:217-226

[21] Cakmak T, Angun P, Demiray YE, Ozkan AD, Elibol Z, Tekinay T. Differential effects of nitrogen and sulfur deprivation on growth and biodiesel feedstock production of *Chlamydomonas reinhardtii*. Biotechnology and Bioengineering. 2012;**109**(8):1947-1957

[22] Gong Y, Guo X, Wan X, Liang Z, Jiang M. Triacylglycerol accumulation and change in fatty acid content of four marine oleaginous microalgae under nutrient limitation and at different culture ages. Journal of Basic Microbiology. 2013;**53**(1):29-36

[23] Lv J-M, Cheng L-H, Xu X-H, Zhang L, Chen H-L. Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. Bioresource Technology. 2010;**101**(17):6797-6804

[24] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering. 2009;**102**(1):100-112

[25] Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, et al. Oil accumulation in the model green alga *Chlamydomonas reinhardtii*: Characterization, variability between common laboratory strains and relationship with starch reserves. BMC Biotechnology. 2011;**11**(1):7

[26] Benning C. Mechanisms of lipid transport involved in organelle biogenesis in plant cells. Annual Review of Cell and Developmental Biology. 2009;**25**(1):71-91

[27] Fan J, Andre C, Xu C. A chloroplast pathway for the de novo biosynthesis of triacylglycerol in *Chlamydomonas reinhardtii*. FEBS Letters. 2011;**585**(12): 1985-1991 Epub 2011/05/18

[28] Martin GJO, Hill DRA, Olmstead ILD, Bergamin A, Shears MJ, Dias DA, et al. Lipid profile remodeling in response to nitrogen deprivation in the microalgae *Chlorella* sp. (Trebouxiophyceae) and Nannochloropsis sp. (Eustigmatophyceae). PLoS ONE. 2014;**9**(8):e103389-e

[29] Lourenço SO, Barbarino E, Marquez UML, Aidar E. Distribution of intracellular nitrogen in marine microalgae: Basis for the calculation of specific nitrogen-to-protein conversion factors. Journal of Phycology. 1998;**34**(5):798-811

[30] Negi S, Barry AN, Friedland N, Sudasinghe N, Subramanian S, Pieris S, et al. Impact of nitrogen limitation on biomass, photosynthesis, and lipid accumulation in *Chlorella sorokiniana*. Journal of Applied Phycology. 2016;**28**(2):803-812

[31] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. Journal of Bioscience and Bioengineering. 2006;**101**(2):87-96

[32] Huerlimann R, de Nys R, Heimann K. Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnology and Bioengineering. 2010;**107**(2):245-257

[33] Sharma KK, Schuhmann H, Schenk PM. High lipid induction in microalgae for biodiesel production. Energies. 2012;**5**(5):1532-1553

[34] Shi T-Q, Wang L-R, Zhang Z-X, Sun X-M, Huang H. Stresses as first-line tools for enhancing lipid and carotenoid production in microalgae. Frontiers in bioengineering and biotechnology. 2020;**8**:610

[35] Aketo T, Hashizume R, Yabu Y, Hoshikawa Y, Nojima D, Maeda Y, et al. Characterization of a novel marine unicellular alga, *Pseudoneochloris* sp. strain NKY372003 as a high carbohydrate producer. Journal of Bioscience and Bioengineering. 2020;**129**(6):687-692

[36] Matsuo T, Iida T, Ohmura A, Gururaj M, Kato D, Mutoh R, et al. The role of ROC75 as a daytime component of the circadian oscillator in *Chlamydomonas reinhardtii*. PLoS Genet. 2020;**16**(6):e1008814-e

[37] Okada K, Fujiwara S, Tsuzuki M. Energy conservation in photosynthetic microorganisms. The Journal of General and Applied Microbiology. 2020;**66**(2):59-65

[38] Blankenship RE, Tiede DM, Barber J, Brudvig GW, Fleming G, Ghirardi M, et al. Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. Science. 2011;**332**(6031):805-809

[39] Zhu X-G, Song Q, Ort DR. Elements of a dynamic systems model of canopy photosynthesis. Current Opinion in Plant Biology. 2012;**15**(3):237-244

[40] Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, et al. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proceedings of the National Academy of Sciences. 2015;**112**(28):8529-8536

[41] Perrine Z, Negi S, Sayre RT. Optimization of photosynthetic light energy utilization by microalgae. Algal Research. 2012;**1**(2):134-142

[42] Demmig-Adams B, Garab G, Iii W, Govindjee. Non-photochemical quenching and energy dissipation in plants, algae and cyanobacteria 2014.

[43] Kiirats O, Cruz J, Edwards G, Kramer D. Feedback limitation of photosynthesis at high CO_2 acts by modulating the activity of the chloroplast ATP synthase. Functional Plant Biology. 2009;**36**(10-1):893-901

[44] Baker NR, Harbinson J, Kramer DM. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant, Cell & Environment. 2007;**30**(9):1107-1125

[45] Friedland N, Negi S, Vinogradova-Shah T, Wu G, Ma L, Flynn S, et al. Fine-tuning the photosynthetic light harvesting apparatus for improved photosynthetic efficiency and biomass yield. Scientific Reports. 2019;**9**(1):13028

[46] Negi S, Perrine Z, Friedland N, Kumar A, Tokutsu R, Minagawa J, et al. Light regulation of light-harvesting antenna size substantially enhances photosynthetic efficiency and biomass yield in green algae†. The plant journal. 2020;**103**(2):584-603

[47] Wu G, Ma L, Sayre RT, Lee C-H. Identification of the optimal light harvesting antenna size for high-light stress mitigation in plants. Frontiers in Plant Science. 2020;11(505).

[48] Mirkovic T, Ostroumov EE, Anna JM, van Grondelle R, Govindjee, Scholes GD. Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. Chemical Reviews. 2017;**117**(2):249-293

[49] Wang Y, Duanmu D, Spalding MH. Carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*: Inorganic carbon transport and CO2 recapture. Photosynthesis Research. 2011;**109**(1-3):115-122

[50] Negi S, Sayre RT, Starkenburg SR. Improved Productivity and Bioproduct Formation in Phototropin Knock/out Mutants in Microalgae. USA Patent WO 2016/197/136 A2. 2016.

[51] Huang K, Beck CF. Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(10):6269-6274 Epub 2003/04/25

[52] Im CS, Eberhard S, Huang K, Beck CF, Grossman AR. Phototropin involvement in the expression of genes encoding chlorophyll and carotenoid biosynthesis enzymes and LHC apoproteins in *Chlamydomonas reinhardtii*. The Plant Journal. 2006;**48**(1):1-16

[53] Baena-González E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. Nature. 2007;**448**(7156):938-942

[54] Saroussi S, Karns DAJ, Thomas DC, Bloszies C, Fiehn O, Posewitz MC, et al. Alternative outlets for sustaining photosynthetic electron transport during dark-to-light transitions. Proceedings of the National Academy of Sciences. 2019;**116**(23):11518-11527

[55] Banerjee S, Banerjee S, Ghosh AK, Das D. Maneuvering the genetic and metabolic pathway for improving biofuel production in algae: Present status and future prospective. Renewable and Sustainable Energy Reviews. 2020;**133**:110155

[56] Yang B, Liu J, Ma X, Guo B, Liu B, Wu T, et al. Genetic engineering of the Calvin cycle toward enhanced photosynthetic CO_2 fixation in microalgae. Biotechnology for Biofuels. 2017;**10**(1):229

[57] Roth MS, Westcott DJ, Iwai M, Niyogi KK. Hexokinase is necessary for glucose-mediated photosynthesis repression and lipid accumulation in a green alga. Commun Biol. 2019;**2**(347):019-0577

[58] Work VH, Radakovits R, Jinkerson RE, Meuser JE, Elliott LG, Vinyard DJ, et al. Increased lipid accumulation in the *Chlamydomonas reinhardtii* sta7-10 starchless isoamylase mutant and increased carbohydrate synthesis in complemented strains. Eukaryotic Cell. 2010;**9**(8):1251-1261 Epub 2010/06/18

[59] de Vries J, Gould SB. The monoplastidic bottleneck in algae and plant evolution. Journal of Cell Science. 2018;**131**(2):203414

[60] Leyland B, Boussiba S, Khozin-Goldberg I. A review of diatom lipid droplets. Biology. 2020;**9**:38

[61] Wang X, Wei W, Li N-J, Yuan W, Ding Y, Yang W-D, et al. Heterogeneous expression of human PNPLA3 triggers algal lipid accumulation and lipid droplet enlargement. Algal Research. 2018;**31**:276-281

[62] Ischebeck T, Krawczyk HE, Mullen RT, Dyer JM, Chapman KD. Lipid droplets in plants and algae: Distribution, formation, turnover and function. Seminars in Cell & Developmental Biology. 2020

[63] Moellering ER, Benning C. RNA interference silencing of a major lipid droplet protein affects lipid droplet size in *Chlamydomonas reinhardtii*. Eukaryotic Cell. 2010;**9**(1):97-106

[64] US-DOE. Algae Cultivation for Carbon Capture and Utilization Workshop Summary Report. Orlando, Florida: 2017.

[65] Slade R, Bauen A. Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. Biomass and Bioenergy. 2013;**53**:29-38

[66] Bhujade R, Chidambaram M, Kumar A, Sapre A. Algae to economically viable low-carbon-footprint oil. Annual Review of Chemical and Biomolecular Engineering. 2017;**8**:335-357

[67] Xu H, Lee U, Coleman AM, Wigmosta MS, Wang M. Assessment of algal biofuel resource potential in the United States with consideration of regional water stress. Algal Research. 2019;**37**:30-39

[68] Sun N, Skaggs RL, Wigmosta MS, Coleman AM, Huesemann MH, Edmundson SJ. Growth modeling to evaluate alternative cultivation strategies to enhance national microalgal biomass production. Algal Research. 2020;**49**:101939

[69] Venteris ER, McBride RC, Coleman AM, Skaggs RL, Wigmosta MS. Siting algae cultivation facilities for biofuel production in the United States: Trade-offs between growth rate, site constructability, water availability, and infrastructure. Environmental Science & Technology. 2014;**48**(6):3559-3566

[70] Janssen M. Chapter 4 - microalgal photosynthesis and growth in mass culture. In: Legrand J, editor. Advances in Chemical Engineering. Academic Press; 2016. pp. 185-256

[71] Posadas E, Alcántara C, García-Encina PA, Gouveia L, Guieysse B, Norvill Z, et al. 3 - microalgae cultivation in wastewater. In: Gonzalez-Fernandez C, editor. Muñoz R, editors. Microalgae-Based Biofuels and Bioproducts: Woodhead Publishing; 2017. pp. 67-91

[72] Emerson K, Russo RC, Lund RE, Thurston RV. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. Journal of the Fisheries Research Board of Canada. 1975;**32**(12):2379-2383

[73] Morales M, Sánchez L, Revah S. The impact of environmental factors on carbon dioxide fixation by microalgae. FEMS Microbiology Letters. 2017;**365**(3)

[74] Posadas E, MdM M, Gomez C, Acién FG, Muñoz R. Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chemical Engineering Journal. 2015;**265**:239-248

[75] Metsoviti MN, Papapolymerou G, Karapanagiotidis IT, Katsoulas N. Comparison of growth rate and nutrient content of five microalgae species cultivated in greenhouses. Plants (Basel). 2019;**8**(8):279

[76] Rajvanshi M, Gautam K, Manjre S, Krishna Kumar GR, Kumar C, Govindachary S, et al. Stoichiometrically balanced nutrient management using a newly designed nutrient medium for large scale cultivation of *Cyanobacterium aponinum*. Journal of Applied Phycology. 2019;**31**(5):2779-2789

[77] Farooq W, Suh WI, Park MS, Yang J-W. Water use and its recycling in microalgae cultivation for biofuel application. Bioresource Technology. 2015;**184**:73-81

[78] Loftus SE, Johnson ZI. Cross-study analysis of factors affecting algae cultivation in recycled medium for biofuel production. Algal Research. 2017;**24**:154-166

[79] Loftus SE, Johnson ZI. Reused cultivation water accumulates dissolved organic carbon and uniquely influences different marine microalgae. Frontiers in bioengineering and biotechnology. 2019;**7**(101)

[80] Fon Sing S, Isdepsky A, Borowitzka MA, Lewis DM. Pilot-scale continuous recycling of growth medium for the mass culture of a halotolerant *Tetraselmis* sp. in raceway ponds under increasing salinity: A novel protocol for commercial microalgal biomass production. Bioresource Technology. 2014;**161**:47-54

[81] Erkelens M, Ball AS, Lewis DM. The influences of the recycle process on the bacterial community in a pilot scale microalgae raceway pond. Bioresource Technology. 2014;**157**:364-367

[82] Hadj-Romdhane F, Jaouen P, Pruvost J, Grizeau D, Van Vooren G, Bourseau P. Development and validation of a minimal growth medium for recycling *Chlorella vulgaris* culture. Bioresource Technology. 2012;**123**:366-374

[83] Farid MS, Shariati A, Badakhshan A, Anvaripour B. Using nano-chitosan for harvesting microalga *Nannochloropsis* sp. Bioresource Technology. 2013;**131**:555-559

[84] Kim D-G, La H-J, Ahn C-Y, Park Y-H, Oh H-M. Harvest of *Scenedesmus* sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Bioresource Technology. 2011;**102**(3):3163-3168

[85] Farooq W, Moon M, Ryu B-G, Suh WI, Shrivastav A, Park MS, et al. Effect of harvesting methods on the reusability of water for cultivation of *Chlorella vulgaris*, its lipid productivity and biodiesel quality. Algal Research. 2015;**8**:1-7

[86] Zhang X, Lu Z, Wang Y, Wensel P, Sommerfeld M, Hu Q. Recycling *Nannochloropsis oceanica* culture media and growth inhibitors characterization. Algal Research. 2016;**20**:282-290

[87] Wang W, Sha J, Lu Z, Shao S, Sun P, Hu Q, et al. Implementation of UV-based advanced oxidation processes in algal medium recycling. Science of The Total Environment. 2018;**634**:243-250

[88] Chen L, Zhu T, Fernandez JSM, Chen S, Li D. Recycling nutrients from a sequential hydrothermal liquefaction process for microalgae culture. Algal Research. 2017;**27**:311-317

[89] Leng L, Li J, Wen Z, Zhou W. Use of microalgae to recycle nutrients in aqueous phase derived from hydrothermal liquefaction process. Bioresource Technology. 2018;**256**:529-542

[90] Kumar V, Kumar S, Chauhan PK, Verma M, Bahuguna V, Joshi HC, et al. Low-temperature catalyst based hydrothermal liquefaction of harmful macroalgal blooms, and aqueous phase nutrient recycling by microalgae. Scientific Reports. 2019;**9**(1):11384

[91] Jena U, Vaidyanathan N, Chinnasamy S, Das KC. Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. Bioresource Technology. 2011;**102**(3):3380-3387

[92] Li Z, Haifeng L, Zhang Y, Shanshan M, Li B, Zhidan L, et al. Microalgae production for bioenergy from post hydrothermal liquefaction wastewater: Effects of strain, nutrients concentration and inoculum size on microalgae culture. International Journal of Agricultural and Biological Engineering. 2017;**10**:194-204

[93] Biller P, Ross AB, Skill SC, Lea-Langton A, Balasundaram B, Hall C, et al. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Research. 2012;**1**(1):70-76

[94] Garcia Alba L, Torri C, Fabbri D, Kersten SRA, Brilman DWF. Microalgae growth on the aqueous phase from hydrothermal liquefaction of the same microalgae. Chemical Engineering Journal. 2013;**228**:214-223

[95] Bagnoud-Velásquez M, Schmid-Staiger U, Peng G, Vogel F, Ludwig C. First developments towards closing the nutrient cycle in a biofuel production process. Algal Research. 2015;**8**:76-82

[96] Hognon C, Delrue F, Texier J, Grateau M, Thiery S, Miller H, et al. Comparison of pyrolysis and hydrothermal liquefaction of *Chlamydomonas reinhardtii*. Growth studies on the recovered hydrothermal aqueous phase. Biomass and Bioenergy. 2015;**73**:23-31

[97] Yao C, Wu P, Pan Y, Lu H, Chi L, Meng Y, et al. Evaluation of the integrated hydrothermal carbonization-algal cultivation process for enhanced nitrogen utilization in *Arthrospira platensis* production. Bioresource Technology. 2016;**216**:381-390

[98] López Barreiro D, Bauer M, Hornung U, Posten C, Kruse A, Prins W. Cultivation of microalgae with recovered nutrients after hydrothermal liquefaction. Algal Research. 2015;**9**:99-106

[99] Godwin CM, Hietala DC, Lashaway AR, Narwani A, Savage PE, Cardinale BJ. Algal polycultures enhance coproduct recycling from hydrothermal liquefaction. Bioresource Technology. 2017;**224**:630-638

[100] Edmundson S, Huesemann M, Kruk R, Lemmon T, Billing J, Schmidt A, et al. Phosphorus and nitrogen recycle following algal bio-crude production via continuous hydrothermal liquefaction. Algal Research. 2017;**26**

[101] Talbot C, Garcia-Moscoso J, Drake H, Stuart BJ, Kumar S. Cultivation of microalgae using flash hydrolysis nutrient recycle. Algal Research. 2016;**18**:191-197

[102] Selvaratnam T, Reddy H, Muppaneni T, Holguin FO, Nirmalakhandan N, Lammers PJ, et al. Optimizing energy yields from nutrient recycling using sequential hydrothermal liquefaction with *Galdieria sulphuraria*. Algal Research. 2015;**12**:74-79

[103] Yang W, Li X, Li Z, Tong C, Feng L. Understanding low-lipid algae hydrothermal liquefaction characteristics and pathways through hydrothermal liquefaction of algal major components: Crude polysaccharides, crude proteins and their binary mixtures. Bioresource Technology. 2015;**196**:99-108

[104] Martinez-Fernandez JS, Chen S. Sequential hydrothermal liquefaction characterization and nutrient recovery assessment. Algal Research. 2017;**25**:274-284

[105] Chen PH, Venegas Jimenez JL, Rowland SM, Quinn JC, Laurens LML.

Nutrient recycle from algae hydrothermal liquefaction aqueous phase through a novel selective remediation approach. Algal Research. 2020;**46**:101776

[106] Borowitzka MA, Vonshak A. Scaling up microalgal cultures to commercial scale. European Journal of Phycology. 2017;**52**(4):407-418

[107] Han D, Li Y, Hu Q. Biology and Commercial Aspects of *Haematococcus pluvialis*. In: Richmond A, Hu Q, editors. Handbook of Microalgal Culture. 2013. pp. 388-405

[108] Wang Y, Gong Y, Dai L, Sommerfeld M, Zhang C, Hu Q. Identification of harmful protozoa in outdoor cultivation of *Chlorella* and the use of ultrasonication to control contamination. Algal Research. 2018;**31**:298-310

[109] Karuppasamy S, Musale AS, Soni B, Bhadra B, Gujarathi N, Sundaram M, et al. Integrated grazer management mediated by chemicals for sustainable cultivation of algae in open ponds. Algal Research. 2018;**35**:439-448

[110] Lee PA, Martinez KJL, Letcher PM, Corcoran AA, Ryan RA. A novel predatory bacterium infecting the eukaryotic alga *Nannochloropsis*. Algal Research. 2018;**32**:314-320

[111] Troschl C, Fritz I, Sodnikar K, Drosg B. Contaminations in mass cultivation of cyanobacteria: Highly resilient *Colpoda steinii* leads to rapid crash of *Synechocystis* sp. cultures and is inhibited by partially anoxic conditions. Algal Research. 2017;**28**:229-234

[112] Letcher PM, Lopez S, Schmieder R, Lee PA, Behnke C, Powell MJ, et al. Characterization of *Amoeboaphelidium protococcarum*, an algal parasite new to the *Cryptomycota* isolated from an outdoor algal pond used for the production of biofuel. PLoS One. 2013;**8**(2):e56232

[113] Pradeep V, Van Ginkel SW, Park S, Igou T, Yi C, Fu H, et al. Use of copper to selectively inhibit *Brachionus calyciflorus* (predator) growth in *Chlorella kessleri* (prey) mass cultures for algae biodiesel production. International journal of molecular sciences. 2015;**16**(9):20674-20684

[114] Park S, Van Ginkel SW, Pradeep P, Igou T, Yi C, Snell T, et al. The selective use of hypochlorite to prevent pond crashes for algae-biofuel production. Water Environment Research. 2016;**88**(1):70-78

[115] Snell TW, Moffat BD, Janssen C, Persoone G. Acute toxicity tests using rotifers: IV. Effect of cyst age, temperature, and salinity on the sensitivity of *Brachionus calyciflorus*. Ecotox Environ Safe. 1991;**21**(3):308-317

[116] Ferrando MD, Sancho E, Andreu-Moliner E. Chronic toxicity of Fenitrothion to an algae (*Nannochloris oculata*), a rotifer (*Brachionus calyciflorus*), and the Cladoceran (*Daphnia magna*). Ecotox Environ Safe. 1996;**35**(2):112-120

[117] Hessen DO, Færøvig PJ, Andersen T. Light, nutrients, and P:C ratios in algae: Grazer performance related to food quality and quantity. Ecology. 2002;**83**(7):1886-1898

[118] Flynn KJ, Kenny P, Mitra A. Minimising losses to predation during microalgae cultivation. Journal of Applied Phycology. 2017;**29**:1829-1840

[119] Ma M, Yuan D, He Y, Park M, Gong Y, Hu Q. Effective control of *Poterioochromonas malhamensis* in pilot-scale culture of *Chlorella sorokiniana* GT-1 by maintaining CO_2-mediated low culture pH. Algal Research. 2017;**26**:436-444

[120] Montemezzani V, Duggan IC, Hogg ID, Craggs RJ. Screening of potential zooplankton control

technologies for wastewater treatment high rate algal ponds. Algal Research. 2017;**22**:1-13

[121] Touloupakis E, Cicchi B, Benavides AMS, Torzillo G. Effect of high pH on growth of *Synechocystis* sp. PCC 6803 cultures and their contamination by golden algae (*Poterioochromonas* sp.). Applied Microbiology and Biotechnology. 2016;**100**(3):1333-1341

[122] Kim D, Kim EK, Koh HG, Kim K, Han J-I, Chang YK. Selective removal of rotifers in microalgae cultivation using hydrodynamic cavitation. Algal Research. 2017;**28**:24-29

[123] Venkata Subhash G, Rangappa M, Raninga S, Prasad V, Dasgupta S. Raja Krishna Kumar G. electromagnetic stratagem to control predator population in algal open pond cultivation. Algal Research. 2019;**37**:133-137

[124] Rego D, Redondo LM, Geraldes V, Costa L, Navalho J, Pereira MT. Control of predators in industrial scale microalgae cultures with pulsed electric fields. Bioelectrochemistry. 2015;**103**:60-64

[125] Umar A, Caldwell GS, Lee JGM. Foam flotation can remove and eradicate ciliates contaminating algae culture systems. Algal Research. 2018;**29**:337-342

[126] Sinha A, Pandharbale A, Patel JR. Current issues with a sustainable approach in the industrial-scale operations of microalgae. In: Gayen K, Bhowmick TK, Maity SK, editors. Sustainable downstream processing of microalgae for industrial application. Florida, USA: CRC Press (Taylor and Francis Group); 2019. pp. 231-291

[127] Park JBK, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology. 2011;**102**(1):35-42

[128] Sharma KK, Garg S, Li Y, Malekizadeh A, Schenk PM. Critical analysis of current microalgae dewatering techniques. Biofuels. 2013;**4**(4):397-407

[129] Fasaei F, Bitter JH, Slegers PM, van Boxtel AJB. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Research. 2018;**31**:347-362

[130] Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: Process options and economics. Biotechnology advances. 2003;**20**(7):491-515

[131] Mo W, Soh L, Werber JR, Elimelech M, Zimmerman JB. Application of membrane dewatering for algal biofuel. Algal Research. 2015;**11**:1-12

[132] Xu Y, Purton S, Baganz F. Chitosan flocculation to aid the harvesting of the microalga *Chlorella sorokiniana*. Bioresource Technology. 2013;**129**:296-301

[133] Chang Y-R, Lee D-J. Coagulation–membrane filtration of *Chlorella vulgaris* at different growth phases. Drying Technology. 2012;**30**(11-12):1317-1322

[134] Chen G, Zhao L, Qi Y, Cui Y-L. Chitosan and Its derivatives applied in harvesting microalgae for biodiesel production: An outlook. Journal of Nanomaterials. 2014;**2014**:217537

[135] Eldridge RJ, Hill DRA, Gladman BR. A comparative study of the coagulation behaviour of marine microalgae. Journal of Applied Phycology. 2012;**24**(6):1667-1679

[136] Branyikova I, Prochazkova G, Potocar T, Jezkova Z, Branyik T. Harvesting of microalgae by flocculation. Fermentation. 2018;**4**(4):93

[137] Castaing JB, Massé A, Séchet V, Sabiri NE, Pontié M, Haure J, et al. Immersed hollow fibres microfiltration (MF) for removing undesirable micro-algae and protecting semi-closed aquaculture basins. Desalination. 2011;**276**(1):386-396

[138] Bilad MR, Vandamme D, Foubert I, Muylaert K, Vankelecom IFJ. Harvesting microalgal biomass using submerged microfiltration membranes. Bioresource Technology. 2012;**111**:343-352

[139] Kim D-Y, Hwang T, Oh Y-K, Han J-I. Harvesting *Chlorella* sp. KR-1 using cross-flow electro-filtration. Algal Research. 2014;**6**:170-174

[140] Hwang K-J, Wu S-E. Disk structure on the performance of a rotating-disk dynamic filter: A case study on microalgae microfiltration. Chemical Engineering Research and Design. 2015;**94**:44-51

[141] Vandamme D, Pontes S, Goiris K, Foubert I, Pinoy L, Muylaert K. Evaluation of electro-coagulation-flocculation for harvesting marine and freshwater microalgae. Biotechnology and Bioengineering. 2011;**108**

[142] Wang L, Pan B, Gao Y, Li C, Ye J, Yang L, et al. Efficient membrane microalgal harvesting: Pilot-scale performance and techno-economic analysis. Journal of Cleaner Production. 2019;**218**:83-95

[143] Szepessy S, Thorwid P. Low energy consumption of high-speed centrifuges. Chemical Engineering & Technology. 2018;**41**(12):2375-2384

[144] Davis R, Markham J, Kinchin C, Grundl N, Tan ECD, Humbird D. Process design and economics for the production of algal biomass: Algal biomass production in open pond systems and processing through dewatering for downstream conversion. Golden, CO (United States): National Renewable Energy Lab. (NREL); 2016. NREL/TP-5100-64772 United States 10.2172/1239893 NREL English

[145] Kumar D, Singh B. Algal biorefinery: An integrated approach for sustainable biodiesel production. Biomass and Bioenergy. 2019;**131**:105398

[146] Norsker N-H, Barbosa MJ, Vermuë MH, Wijffels RH. Microalgal production — A close look at the economics. Biotechnology advances. 2011;**29**(1):24-27

[147] Koyande AK, Show P-L, Guo R, Tang B, Ogino C, Chang J-S. Bio-processing of algal bio-refinery: A review on current advances and future perspectives. Bioengineered. 2019;**10**(1):574-592

[148] Zhu L. Biorefinery as a promising approach to promote microalgae industry: An innovative framework. Renewable and Sustainable Energy Reviews. 2015;**41**:1376-1384

[149] Chandra R, Iqbal HMN, Vishal G, Lee H-S, Nagra S. Algal biorefinery: A sustainable approach to valorize algal-based biomass towards multiple product recovery. Bioresource Technology. 2019;**278**:346-359

[150] Roux J-M, Lamotte H, Achard J-L. An overview of microalgae lipid extraction in a biorefinery framework. Energy Procedia. 2017;**112**: 680-688

[151] Razon LF, Tan RR. Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*. Applied Energy. 2011;**88**(10):3507-3514

[152] Andersson V, Broberg Viklund S, Hackl R, Karlsson M, Berntsson T. Algae-based biofuel production as part of an industrial cluster. Biomass and Bioenergy. 2014;**71**:113-124

[153] Ansari FA, Shriwastav A, Gupta SK, Rawat I, Guldhe A, Bux F. Lipid extracted algae as a source for protein and reduced sugar: A step closer to the biorefinery. Bioresource Technology. 2015;**179**:559-564

[154] Muñoz R, Navia R, Ciudad G, Tessini C, Jeison D, Mella R, et al. Preliminary biorefinery process proposal for protein and biofuels recovery from microalgae. Fuel. 2015;**150**:425-433

[155] Phusunti N, Cheirsilp B. Integrated protein extraction with bio-oil production for microalgal biorefinery. Algal Research. 2020;**48**:101918

[156] Lu Y, Savage PE. Supercritical water gasification of lipid-extracted hydrochar to recover energy and nutrients. The Journal of Supercritical Fluids. 2015;**99**:88-94

[157] Dong T, Knoshaug EP, Davis R, Laurens LML, Van Wychen S, Pienkos PT, et al. Combined algal processing: A novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Research. 2016;**19**:316-323

[158] Beckstrom BD, Wilson MH, Crocker M, Quinn JC. Bioplastic feedstock production from microalgae with fuel co-products: A techno-economic and life cycle impact assessment. Algal Research. 2020;**46**:101769

[159] Nobre BP, Villalobos F, Barragan BE, Oliveira A, Batista AP, Marques P, et al. A biorefinery from *Nannochloropsis* sp. microalga–extraction of oils and pigments. Production of biohydrogen from the leftover biomass. Bioresource Technology. 2013;**135**:128-136

[160] Lee OK, Kim AL, Seong DH, Lee CG, Jung YT, Lee JW, et al. Chemo-enzymatic saccharification and bioethanol fermentation of lipid-extracted residual biomass of the microalga, *Dunaliella tertiolecta*. Bioresour Technol. 2013;**132**:197-201

[161] Pacheco R, Ferreira AF, Pinto T, Nobre BP, Loureiro D, Moura P, et al. The production of pigments & hydrogen through a *Spirogyra* sp. biorefinery. Energy Conversion and Management. 2015;**89**:789-797

[162] Gilbert-López B, Barranco A, Herrero M, Cifuentes A, Ibáñez E. Development of new green processes for the recovery of bioactives from *Phaeodactylum tricornutum*. Food Research International. 2017;**99**:1056-1065

[163] Soto-Sierra L, Kulkarni S, Woodard SL, Nikolov ZL. Processing of permeabilized *Chlorella vulgaris* biomass into lutein and protein-rich products. Journal of Applied Phycology. 2020;**32**(3):1697-1707

[164] Dixon C, Wilken LR. Green microalgae biomolecule separations and recovery. Bioresources and Bioprocessing. 2018;**5**(1):14

[165] Kumar C, Soni N, Soni BR, Das G, Dasgupta S, Reliance Industries Limited, India, Assignee. Propiconazole Resistant Mutants of *Chlorella* Species. United States of America Patent US 10,694,752 B2. 2020.

[166] Krause-Jensen D, Lavery P, Serrano O, Marbà N, Masque P, Duarte CM. Sequestration of macroalgal carbon: The elephant in the blue carbon room. Biology Letters. 2018;**14**(6):20180236

[167] Packer M. Algal capture of carbon dioxide; biomass generation as a tool for greenhouse gas mitigation with reference to New Zealand energy strategy and policy. Energy Policy. 2009;**37**(9):3428-3437