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Review article

Assessing the potential of polyculture to accelerate algal biofuel production

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ABSTRACT

To date, the algal biofuel industry has focused on the cultivation of monocultures of highly productive algal strains, but scaling up production remains challenging. Algal monocultures are difficult to maintain because they are easily contaminated by wild algal strains, grazers, and pathogens. In contrast, theory suggests that polycultures (multispecies assemblages) can promote both ecosystem stability and productivity. A greater understanding of species interactions and how communities change with time needs to be developed. Ultimately a predictive model of community interactions is needed to harness the capacity of biodiversity to enhance productivity of algal polycultures at industrial scales. Here we review the agricultural and ecological literature to explore opportunities for increased annual biomass production through the use of algal polycultures. We discuss case studies where algal polycultures have been successfully maintained for industries other than the biofuel industry, as well as the few studies that have compared biomass production of algal polycultures to that of monocultures. Assemblages that include species with complementary traits are of particular promise. These assemblages have the potential to increase crop productivity and stability presumably by utilizing natural resources (e.g. light, nutrients, and water) more efficiently via tighter niche packing. Therefore, algal polycultures show promise for enhancing biomass productivity, enabling sustainable production and reducing overall production costs.

1. Introduction

Algae hold much promise as a viable feedstock for biofuels but there is still much discussion over how to make production more cost effective [1–7]. High biomass and lipid yields are achievable from algae cultured under controlled laboratory conditions at the bench scale or in incubators (e.g., photobioreactors). However, cultivation in outdoor pond facilities may be the only cost-effective way to produce algal biomass at the scales needed for the biofuel industry [3–9]. To date, the algal biofuel industry has focused on selecting for (or genetically modifying) algal strains that have rapid growth rates or high lipid contents in order to maximize lipid yields [3,4]. While this strategy can be effective at smaller scales and in the short term, shifting production from the laboratory to outdoor ponds raises a number of ecological challenges [10].

Monocultures are exceedingly difficult to maintain in outdoor settings, because they are easily contaminated through aerial colonization by wild algal strains, grazers, and pathogens [11–13]. In terrestrial systems, the agriculture industry has had to develop multiple layers of defense against such invaders (e.g. herbicides, fungicides, pesticides, genetically engineered organisms, etc.) to optimize yields of monoculture crops. Because aquatic systems are readily invaded by a host of microbes and invertebrates, algal crops are more susceptible to losses due to invading species. Specific interactions of concern include competition, predation (i.e., grazing pressure), and pathogenic interactions. Competition for nutrients and allelopathy (chemical warfare) with non-target algal strains can significantly reduce production rates and/or alter the quality of the biomass produced [14,15]. Predation by grazers or invasion by algal pathogens can decrease the stability of production by destroying mass cultures in a matter of days [16]. It has been estimated that 10 to 30% of annual production in open ponds is lost due to pond crashes induced via consumption of the algal crop by a contaminating grazer or invasion by a crop specific pathogen [11,17]. Additionally, these interactions can influence the temporal consistency of algal biomass production and/or cellular lipid content, thereby affecting the stability of the algal culture. Clearly, crop protection and management will be a critical need for maintaining predictable and stable crop production for the algal biofuel industry as production begins scaling up.

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In contrast to the monocultures grown for the biofuel industry, algae in natural ecosystems exist in diverse multispecies assemblages [12], where species abundance can fluctuate as environmental conditions change. Such changes are driven by spatial and temporal heterogeneity in resources such as light and nutrient availability that can change rapidly over time and with depth in the water column, creating multiple niches for species with different optimum light or nutrient conditions. In addition, the landscape of biotic interactions can also vary as grazers or pathogens can often be very selective for an algal strain or group of algae. When these stressors affect an algal population, other algal species within the assemblage can quickly multiply to fill the newly available niche. Although typically no single species in these complex natural system assemblages dominates for any extended period of time, overall algal production can stay relatively stable as long as nutrient concentrations are sufficient and algal species diversity is maintained.

There is much evidence from the agricultural and ecological literature that species diversity can promote both ecosystem stability and productivity [18–20]. These principles have recently been applied to biofuel production of grassland plants and algae with promising results—higher diversity can lead to higher biomass and lipid production than monocultures [21,22]. In addition to providing crop protection from losses due to grazing and infection, there is evidence that algae polycultures can provide a more stable crop [23]. Further, polycultures that effectively combine species with complimentary traits such as for nutrient and light utilization have greater potential for overyielding (i.e., higher productivity or increased biomass quality in the polyculture relative to the most productive monoculture strain [24]).

These studies provide encouraging indications that use of algal polycultures may be an effective approach to enhancing algal biomass production. Applying these principles to long-term, larger-scale production efforts will require a greater systematic understanding of species interactions and how algal communities change with time. Indeed, complex microbial communities are a reality of algal biofuel production at industrial scales. However, diversity is no guarantee of high productivity or stability as only a subset of species combinations display these desirable properties, and only under particular environmental contexts. Identifying consortia of species that consistently yield biomass with useful biochemical composition, and the conditions under which they outperform single species, is therefore a research priority.

In this review, opportunities that algal polycultures offer the biofuels industry for increasing algal biomass yields are highlighted. Specifically, the basic ecological principles that support the theoretical basis for increasing species diversity to increase crop stability and productivity are highlighted and examples are provided of studies that have shown promise for application to the industry. Recent advances in harvesting, extraction, and conversion technologies that make use of algal polycultures, and in turn make their cultivation a more viable solution than previously considered, are also described. Finally, the challenges and potential for improved biomass productivity and stability through implementation of a polyculture strategy are considered.

2. Ecological principles and polycultures

The relationship between ecological diversity and stability has been a subject of debate within the scientific community [25–28]. Theory predicts a positive correlation between diversity and stability in ecological communities [29], but there has been much discussion about how these factors should be defined and quantified. Definitions for ecological stability in the literature can broadly be broken into three categories: (1) the ability to stay unchanged (constancy), (2) the ability to return to the reference state after perturbance (resilience), and (3) persistence through time, and approaches to quantifying stability vary accordingly [30]. For example, biomass productivity may be maintained as the environment fluctuates if species that decline in abundance are replaced by others with different tolerances. Simply increasing the number of species does not necessarily increase the stability of a system if species are functionally equivalent or respond to the environment in a common fashion. That is, species must respond differently to environmental fluctuations or interact in ways that cause their populations to vary asynchronously in order for diversity to enhance ecosystem stability. The species that are present and what role they play in the ecosystem (i.e., functional diversity) is at least as important in determining both ecosystem stability and productivity [22,31].

Likewise, the relationship between diversity and productivity can be positive, either because diverse communities will randomly include highly productive species, or because in the face of fluctuating environmental conditions, diverse assemblages provide greater assurance that at least one species will grow well [32,33]. Within the planktonic (i.e., free floating) algae in natural aquatic ecosystems, the relationship between diversity and productivity is generally positive [12], even though maximum productivity can be seen during low diversity blooms that escape predation [34] which may not be sustainable over longer time periods. The algal biofuel industry will be faced with the trade-off between maximum productivity and crop stability for the long-term, sustained production of algal biomass [10].

Research has shown that polycultures can vary in productivity with respect to their respective monocultures [24], but that higher productivities (i.e., overyielding) are possible with polycultures [10,23,24,31,35]. Underyielding (i.e., when a polyculture yields less than the average of its component monocultures) can result, for example, when a polyculture is dominated by a fast-growing, low-yielding species [14,24] or due to allelopathy, where a secondary metabolite produced by one species is toxic to another [36]. In order to maximize the potential for overyielding, polycultures can be assembled based on their specific traits (e.g., light, nitrogen, micronutrients, etc.) to maximize biofuel industry resource use [37,38]. Functional richness, which is a measure of specific prominent characteristics (e.g., biochemical, physiological, and ecological), may be more influential on productivity in phytoplankton communities than species richness [31], and increased algal species richness has been shown to decrease grazer survival as inedible or defended taxa may interfere with feeding on preferred prey [10]. The uncertainties associated with polyculture yields highlight the need to be selective in polyculture assembly.

In laboratory experiments, rationally designed polycultures have shown the potential to outperform monocultures through overyielding [10,33,35,37,39,40]. This is accomplished by selecting species with desirable and complimentary traits that allow for more efficient utilization of light and nutrients, provide benefits toward crop protection, and/or resilience toward fluctuating environmental conditions. While some rationally designed polycultures have been shown to exceed productivity of monocultures in laboratory experiments, systems that utilize natural polycultures have demonstrated the benefits of diverse polycultures to algae production and culture stability on larger outdoor scales. For instance, a study of productivity of a production pond at Sapphire Energy over a complete annual cycle found that periods of high eukaryotic diversity and low prokaryotic diversity were associated with greater mean and lower variance in algal biomass yield [41]. This result indicates that algal diversity enhances productivity and stability, and that interactions between bacteria and prokaryotic algae drive diversity and productivity in industrial systems.

Another example of the functional role of diversity in aquatic ecosystems is illustrated by the Algae Turf Scrubber®, or ATS™, system [42]. The ATS is an engineered system using shallow, pulsed water flow in a planar surface channel configuration to grow an attached mat of indigenous benthic and planktonic algae in the form of "algal turf" characterized by a broad and dynamic species profile [43]. Since its initial development, ATS has been exploited at both pilot and multi-acre commercial scales to remove excess nutrient loading from fresh, estuarine, and marine surface water bodies [44–48] and from agriculture and municipal waste streams [49–53]. Pilot-scale ATS annualized biomass productivities approaching $20\ \mathrm{g\ m^{-2}\ d^{-1}}$ (AFDW) have been reported, but little work has been done thus far to optimize ATS biomass productivity and quality [43]. A rotating system analog to ATS is the rotating algal biofilm reactor (RABR) [54]. The indigenous polyculture in ATS systems has demonstrated stability and resilience over prolonged periods of operation, but biomass productivity and quality of harvested material (i.e., content profile of carbohydrates, proteins, lipids, other organics, and ash) has varied widely with conditions and details of cultivation and harvesting operations in water treatment applications. Improvements in these system properties may be achievable with a more intentional design of the cultivated algal community. Here we outline areas of opportunity for algal polyculture design.

2.1. Light

Photosynthesis is an inefficient process, in part because only a small portion of solar light (blue at ~ 430 nm or red at ~ 660 nm) is absorbed by chlorophyll *a*, the principal photosynthetic pigment in algae, and because much of the available light is quenched or dissipated before it can be used for photosynthesis. Commercial algal production rates only attain ~ 1–2% solar to product energy conversion efficiency, which corresponds to ~ 5–25% of the theoretical maximum productivity of photosynthesis. Stephens et al. [57] estimated that a 2% photosynthetic conversion efficiency supports practical annual yield of 20,000 L algal oil per hectare with current outdoor mass cultivation technology and available strains. They envisioned that much higher oil productivity (e.g. 60 to $100{,}000\ \mathrm{L\ ha^{-1}\ year^{-1}}$) could be attainable, assuming a maximum photosynthetic conversion efficiency of 10% [57]. Increasing light use efficiency has therefore been recognized as an area for significant improvement in biomass production. Several strategies to increase light use efficiency have been proposed, including genetically modifying algae photosynthetic apparatus to reduce scatter and dissipation of photons or pulsing light/dark cycles in algal photobioreactors.

Algal polycultures have the potential to utilize light resources more efficiently than monocultures if they include species of a variety of taxonomic groups [58,59]. Different algal taxa have evolved a suite of accessory pigments which are capable of absorbing light across a range of wavelengths [55] (Table 1). For example, while chlorophyll *a* is ubiquitous among algae, other pigments have a more limited distribution and function as accessory photosynthetic pigments.

Light spectrum can therefore be an important axis of niche differentiation which may reduce competition among algal species that absorb different spectra of light. Indeed, bench scale experiments have shown that the inclusion of algae from different functional groups (i.e. green algae, golden algae, cyanobacteria, and diatoms) increased not only light use efficiency, but also lipid yield [31,61]. Algal polyculture design can benefit from these studies, especially when considering situations where light may be limiting (e.g. off season, off peak times during the day, with depth in dense algal culture etc.).

2.2. Water and nutrient resources

Scaling up algal biomass production will put increasing demands on our nation's already strained water and nutrient supplies [62,63]. While algae need relatively low concentrations of nutrients and water, large scale production will compete with the agriculture industry for both irrigation and fertilizer resources, especially in areas where freshwater resources have reached the point of full allocation. The utilization of wastewater resources that are rich in nitrogen and phosphorus can supplement a portion, or completely replace, traditional fertilizer and water requirements may help significantly reduce algal production costs [64–67].

It is generally accepted that optimal algal biomass production requires nutrients at a molar ratio of C_{106}:N_{16}:P_1 (the Redfield Ratio) [68], suggesting that of these three major nutrients, nitrogen and phosphorus should become limiting before carbon. However, in commercial scale algal cultivation in nutrient-rich environments, dissolved carbon in water is usually the limiting factor for growth as carbon assimilation by algae is higher than the rate CO_2 diffusion from air into water [62,69–71]. Further, several studies have shown that deviations from Redfield stoichiometry can be due to various factors including habitat and nutrient use efficiency (the number of moles of carbon fixed into algal biomass per mole of cellular N or P) [72]. This plasticity in stoichiometry of algal biomass suggests that demands of N or P for algal biofuel ponds should be calculated based on pilot-scale or commercial-scale operating under varying environmental condition.

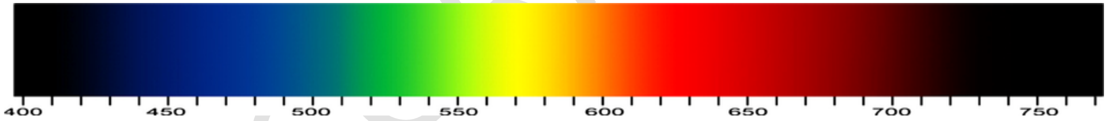
Algal diversity enhances resource use efficiency. More than 3000 phytoplankton samples analyzed from Scandinavian lakes and the Baltic Sea showed that phytoplankton diversity and nutrient use efficiency were positively correlated and both community composition and nutrient use efficiency were stabilized by diversity [73]. Species combinations with more divergent traits may use resources in complementary ways, and produce more biomass than even their most productive species [24]. Cardinale [74] argued that species rich communities take greater advantage of the niche opportunities in an environment, and this allows diverse systems to capture a greater percent of available nutrients [74]. Fertilization with nutrients often reduces algal diversity by eliminating the potential for niche partitioning, leading to a system where only one resource (usually light) is limiting to growth [74,75]. Spatial and/or temporal heterogeneity created by changing nutrient stoichiometry (N:P ratio) in different patches of algal culturing units influences the relative fitness of species across patches, allowing for spatial niche differentiation to generate complementary resource use [76].

A number of investigations have demonstrated the utility of wastewaters for algal cultivation in both mono [77–79] and polycultures [44–54,77], with the latter typically demonstrating higher yields and/or more stable productivity. Similar to nutrient stoichiometry and light availability, the full utilization of the complex niche space in wastewater streams will be facilitated by incorporating polycultures as wastewaters commonly contain a complex combination of factors that can negatively influence cultivation of individual algal strains. Although, potentially viable, intentional polyculture constructs designed as a function of wastewater characteristics have yet to be demonstrated in the literature. However, strategies that cultivate naturally selected polycultures, as described for the ATS systems, are currently being employed for wastewater

Table 1

Distribution of major algal pigments in algal classes (modified from Hoek et al. [60]).

Major pigment	Maximum absorption wavelength (nm)[a]	Cyanophyta	Prochlorophyta	Glaucophyta	Rhodophyta	Heterokontophyta/ Eustigmatophyceae	Heterokontophyta/ Bacillariophyceae	Heterokontophyta/Phaeophyceae	Haptophyta	Cryptophyta	Dinophyta
Chlorophylls											
Chlorophyll a	663, 430	+	+	+	+	+	+	+	+	+	+
Chlorophyll b	645, 435		+								
Chlorophyll c	634, 583						+	+	+	+	+
Phycobilins											
Phycocyanin	615	+		+	+					+	
Allophycocyanin	650	+		+	+						
Phycoerythrin	565	+			+					+	
Carotenes											
α-carotenes	447, 475				+					+	
β-carotenes	453, 480	+	+	+	+	+	+	+	+	+	+
Xanthophyll											
Zeaxanthene	454, 481	+	+	+	+						
Lutein	450										
Violaxanthene	442					+		+			
Fucoxanthene	446						+	+	+		+
Diatoxanthene	453						+		+		+
Diadinoxanthene	448, 478						+		+		+
Neoxanthene	442										



+ = presence of pigments.

[a] Absorption of pigments dissolved in acetone.

treatment [44–54] and have the potential to be scaled to achieve large-scale biofuel production.

2.3. Temperature variation

The effect of algal diversity on the stability of community biomass production in a variable environment depends on whether populations fluctuate synchronously or out of phase with one another [80]. The degree of synchrony in population dynamics depends on niche differentiation in terms of whether species respond similarly or differently to environmental conditions. For instance, if all species grow better at higher temperatures, then all populations will increase in the summer and decline in the winter [81]. Alternatively, if species compete for resources and differ in their response to the physical or biological environment, then decline in one species should be compensated directly by rise in another, maintaining community biomass. Ecological engineering should aim at identifying combinations of species that maximize asynchrony in population dynamics to ensure stable production and maintain resilience in the face of climate and contamination variations.

Although many variables affect the productivity of outdoor cultures, temperature and light intensity may be the most important [82]. Algal raceways, such as those used for algae biofuel production, are particularly vulnerable to temperature variations in the spring and fall [83] as well as other climatic controls such as precipitation, solar radiation, humidity, and wind that also exhibit seasonal patterns [84]. Seasonal temperature variation results in suboptimal growth temperatures, which negatively impacts biomass productivity. Fig. 1 depicts an example of typical variation in productivity over a year using a biomass growth model, where seasonal temperature variation is a key variable [85]. Productivity during spring (March through May) and fall (September through November) are estimated to be approximately 47% and 34% lower, respectively, than during peak summer months (June through August) [85].

Engineering and biological solutions for minimizing the impact of seasonal and diurnal temperature variation have been pursued, including novel raceways designs (e.g., ARID raceway) [83,86] and algal crop rotation with cold-tolerant strains [87,88]. Effective strategies for optimizing the annual yield of ponds will certainly include both engineering and biological solutions, as the temperature-dependent growth rates of algal species vary considerably among strains [89]. Generally, algae growth rates increase with rising temperatures until they reach an optimal growing temperature around 20 to 25 °C [89], at which point, further increases in temperature are accompanied by decreases in growth rate. After 30 °C, growth rates decrease dramatically for most strains currently investigated for biofuel production. Butterwick et al. found large inter-species differences in the ability of algae to grow at low temperatures (i.e., less than 10 °C) [89]. Their results indicated that some strains were better suited for cool seasonal growth and that adopting a crop-rotation approach to enhance productivity seemed reasonable. In addition, Thomas et al. [90] showed that the temperatures for optimum growth ranged from 30 °C to less than 5 °C for algae isolated from across a broad latitudinal gradient [90]. Although crop rotations with cold tolerant algae have the potential to normalize productivity over an annual period by extending the seasonal window for cultivation, crop rotation may be best suited for winter when temperatures are consistently suboptimal and a productive cold-tolerant strain could thrive. In the absence of crop rotation, a diverse polyculture composed of strains with differing temperature optima would be able to respond quickly to fluctuating daily temperatures during the spring and fall. Whereas a monoculture, limited to the adaptability of a single strain, would be less productive.

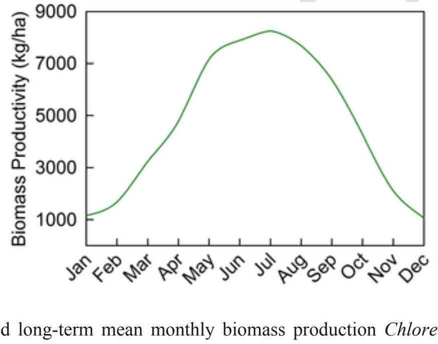


Fig. 1. Modeled long-term mean monthly biomass production *Chlorella* sp. 1412 in Sarasota FL [85].

Litchman et al. described three ways in which natural phytoplankton communities respond to changing environmental conditions, each of which have important implications to how algae could be effectively cultured for biofuel production [91]. First, when faced with changing conditions, such as a cooler temperature, a species can adapt to the new conditions as long as the conditions are within a tolerable range through either phenotypic plasticity or genetic changes. Algae with a broad thermal tolerance will be better able to adapt to seasonal fluctuations. For the monoculture cultivation model to be successful for algal biofuel production, a commercial strain must be capable of growth at both high temperatures in the summer and the lower, fluctuating temperatures experienced in the spring and fall. A second mode of response to changing conditions, observed by Litchman et al. in natural communities, occurs when a dominant species fails to adapt to new conditions and instead is replaced by a species better suited for the current conditions [91]. This change in species abundance is termed species sorting. As diversity within a culture increases, the probability that at least one member of the community will be better suited for the current environmental conditions and the biotic interaction landscape increases. A final mechanism of species adaptation to changing conditions is through natural selection, where the new conditions select for mutations, horizontal gene transfers, or recombination events that confer some competitive advantage. Ras et al. considered this third mechanism for adaptation to be unlikely in the case of algae biofuels due to the rapid temperature fluctuations that shallow algal ponds are likely to undergo during the spring and fall. The fluctuating conditions would not prevail long enough to select for generational adaptation [92]. A polyculture approach to algae biofuels could provide sufficient diversity to minimize decreases in growth rate and productivity due to fluctuating temperature.

A recent study investigated the response of monocultures and polycultures to daily fluctuating temperatures [93]. The experiment was carried out by acclimating 15 monocultures to three temperatures: 12, 18, and 24 °C for a 2-week period. Mixtures with varying species richness (i.e., 2, 3, 6, 9, and 12 strains) were assembled from the same 15 strains and were incubated at the same constant temperatures. After 14 days, Phase II of the experiment began to determine the effect of fluctuating temperatures on culture productivity. During this phase, the culture temperatures were each increased by 4 °C for a period of 7 h. The temperature oscillations were repeated daily for 7 days. Species richness had a positive effect on growth rate at each temperature level (i.e., 12, 18, and 24 °C). Overyielding was observed for both the constant and fluctuating temperature environments, with the strongest effect being observed at 18 °C. Overyielding was attributed to complementary effects, where increased diversity resulted in a more efficient utilization of resources through resource partitioning among species with different temperature-growth profiles. The authors did not find evidence to support the selection effect or the emergence of a dominant strain. This study emphasizes the importance of biodiversity in determining a phytoplankton community's response to varying temperatures and indicates that polycultures could greatly improve annual algal biomass productivity over a monoculture.

2.4. Synergistic and/or probiotic relationships

Algae and bacteria have long been known to grow in close association with one another. This is particularly evident in marine systems where nutrients are scarce and limit growth. For example, iron availability often limits phytoplankton primary production due to iron's poor solubility at near neutral pH [94]. Mutualistic and commensalistic interactions among bacteria and algae have been shown to promote algal growth by increasing Fe availability [95,96]. As a result of bacteria-algae associations, algal growth can often be sustained at low Fe concentrations that would not support growth in the absence of associated bacteria [95]. Bacteria overcome iron limitation by secreting siderophores, small iron-chelating organic molecules, to increase the solubility of iron [97]. Siderophore secreting bacteria in close association with microalgae under photoautotrophic conditions have been found to enhance iron uptake in algae by 20 fold [96]. Associations between bacteria and algae can improve nutrient utilization and promote algal productivity, suggesting that polyculture production may benefit from community constructs that incorporate more than just photosynthetic community members.

Some algae depend on prokaryotes for vitamins essential for growth. Croft et al. found that 50% of the microalgae surveyed are vitamin B_{12} auxotrophs and require an external source of cobalamin for growth [98,99]. Cobalamin, produced only by prokaryotes, is essential to the production of the amino acid methionine in about half of microalgal species. Those that do not require vitamin B_{12} (about half), utilize a different enzyme that performs the same function in the absence of vitamin B_{12} [98]. Microalgae may obtain the necessary cofactor from bacteria either directly or indirectly by scavenging from the environment [98–101]. In exchange for the essential cofactor, microalgae support the bacteria with photosynthate [98,99,101], this exchange is an example of how bacteria and algae mutually benefit from their association.

Phytohormones are a class of small molecules that regulate plant growth and development and include the plant hormones indole-3-acetic acid (auxin), cytokinin, gibberellin, ethylene, and abscisic acidin; this is in addition to brassinosteroids, jasmonic acid, and salicylic acid. The effect of the addition of exogenous phytohormones to *Chlorella* cultures has been a topic of active research for several decades. Addition of a wide variety of phytohormones to *Chlorella* cultures impacted growth rate and the concentration of cellular constituents (such as chlorophyll carotenoids, proteins, and nucleic acids) (for review see [102]). The phytohormones that displayed positive effects on *Chlorella* growth include indole-3-acetic acid (IAA). Bacterial species, such as *Azospirillum brasilense* and *Azospirillum lipoferum*, that produce IAA enhance the growth of microalgae in co-cultures and when co-immobilized in alginate beads with *Chlorella* [103]. Mutant strains of *Azospirillum* sp. that were defective for the production of IAA did not enhance the productivity of the algae grown in co-culture but the wild-type, growth-enhancing, phenotype could be rescued through introduction of exogenous IAA into the mixed cultures [104].

Other algal bacterial interactions are less well characterized. A number of bacteria have been co-isolated with microalgae from natural sources or from xenic laboratory cultures. These bacteria tend to group phylogenetically with the Alphaproteobacteria, Gammaproteobacteria and the Cytophaga–Flavobacterium–Bacteroides cluster [105–107]. Park et al. [108] demonstrated that the addition of *Brevundimonas* sp., which is isolated from xenic *Chlorella ellipsoidea* cultures, to *axenic C. ellipsoidea* cultures results in improved algal growth [108]. The bacteria grew in tight association with the algae surface in the so-called phycosphere; however, the molecular basis of the growth-promoting effect has yet to be determined. Modest growth enhancement was also demonstrated for *Chlorella sorokiniana* in co-culture with a strain of *Microbacterium trichotecenolyticum*, which was originally isolated from a xenic laboratory culture [109].

In nitrogen-limited areas of the open ocean, N-fixing cyanobacteria can have symbiotic relationships with other algae (e.g. diatoms, green algae) [110–112]. The benefit for either partner was characterized in a prymnesiophyte–cyanobacterial symbioses [113]. The prymnesiophyte receives fixed N in exchange for transferring fixed carbon to the cyanobacteria, *Candidatus* sp. Currently there is no report available that employs an algae-cyanobacterial symbiosis for mass culture of algae. This strategy may provide a free source of N to cultivate algae and prevent down-stream pollution of water bodies by minimizing fertilizer demands.

2.5. Biomass quality

Biofuels can be produced from a great variety of algal feedstocks and depending on the origin and quality of the feedstock, the properties of the produced fuel can vary. For example, different strains of algae and cyanobacteria can vary widely in their relative lipid, carbohydrate, and protein content. Further, each algal strain has the ability to regulate their metabolism in response to environmental conditions. Nutrient limitation has been found to lead to lipid accumulation in a number of microalgal species [114,115]. In particular, N-starvation suppresses protein synthesis in algae, so that the carbohydrate formed by photosynthesis is preferentially channeled to making lipids [116]. Nitrogen deficiency also results in the biosynthesis of lipid enriched in saturated and mono-unsaturated fatty acids [117]. Temperature also has a major effect on the fatty acid composition of algae with low temperatures typically inducing the formation of unsaturated fatty acid in algae [116].

Biodiesel is produced by transesterification, where triglyceride lipids are transformed into fatty acid methyl esters. The properties of biodiesel are mainly determined by fatty acid unsaturation and chain length [118]. Biodiesel with a high degree of unsaturated fatty acid exhibits better lubricity and cold-temperature flow properties [119], but oxidative stability, heat of combustion and ignition quality (measured by the cetane number) decreases with unsaturation. While no single fatty acid is ideal for all fuel properties, a good compromise can be achieved by growing multiple species with different fatty acid properties.

The specific species and/or strain structure of polycultures may also affect the quality of the algal community biomass. For example, an experiment testing all possible combinations of six different algal species, where strains were selected based on known carbohydrate production potentials, had six polyculture treatments that resulted in overyielding in the estimated total annual carbohydrate production by 1.5 to 30% [120]. Importantly, in this work not all polyculture constructs with equal diversity levels resulted in overyielding, as also noted elsewhere [10,24]. These findings suggest that species richness alone is not predictive for determining the potential overyielding of polycultures in terms of total biomass or biomass quality. Rather, the structure of the algae community (i.e., specific species or strains present, richness, and distribution of each species) influences net productivity and quality metrics. Suggesting that polyculture strain selection should be influenced by quality characteristics and combined with strategies that result in greater stability and resilience to contaminant invasion to tailor the quality of the polyculture biomass yield.

2.6. Crop protection

One of the primary challenges for large scale production of algal biomass is the negative biotic interactions that limit algal population densities, productivity rates, and biomass yields [16,121]. Grazing by rotifers, infection by fungal and bacterial pathogens [122,123], and competition from invasive algal species can affect overall crop yield through the reduced quantity and/or quality of biomass [124]. Therefore, successful large-scale production of algal biofuels will likely require a holistic and broad-spectrum approach that parallels traditional agricultural techniques, whereby invasive weeds and insects are controlled through mechanical, chemical, and biological means. Increasing the species and strain diversity of an algal crop has the potential to reduce the effect of biotic contaminants on algal productivity and potentially minimize the necessity of mechanical and chemical controls on contaminants [10,125].

While the overall impact of predation and pathogens to large-scale algal biomass production has not been reported on an annual basis, epidemics, grazers, and wild competitors have all been implicated in crashes of cultivated algae. Importantly, mass cultures can be destroyed within days of invasion [16,121], therefore controlling contaminants is a critical need for algae biofuels to reach their potential [16]. Mitigating crop losses can have a substantial impact on overall algal yields and on costs from reseeding ponds that have crashed due to grazing and/or infection.

Polycultures that contain strains with a wide range of cell diameters and different shapes (which affect feeding by predators) may dampen the impact of infection or predation, thereby imparting greater stability. Similarly, polycultures may contribute to increased annual productivity by providing stability during periods when predators or pathogens are most active [126]. In the case of the benthic polyculture assemblages cultivated in engineered systems such as ATS™, the algal community's diversity, productivity, and stability depend on the complex interdependencies of the colonization processes. These processes involve filamentous species capable of forming attachments to the ATS™ system floway substrate in cohabitation with both attached, solitary, and unattached planktonic species that reside within the algal mat matrix [43]. In such systems, modification of the selected physical aspects of system design and operation (such as floway substrate material, texture, and the hydrodynamics of the pulsed, turbulent water flow) will also have an impact on the colony's species diversity profile and collective dynamic biological performance in terms of culture stability and productivity [44,127].

Sustained production of the algal monoculture has been successful for only a few strains of extremophiles such as *Spirulina* and *Dunaliella*, where conditions (such as high salinity and pH) provide protection from predation; however these operating conditions are not always practical or consistent with high productivity. Pond crashes are a major threat to algal biofuels; therefore, crop protection strategies should be considered a high priority research area. Park et al. [66] report that within a period of 3 months, their attempts to grow algal monocultures in outdoor ponds failed due to contamination [66]. At an industrial scale, Sapphire Energy's test facility has also demonstrated the vulnerability of algal strains to invasion by contaminating microorganisms and has used real-time polymerase chain reaction to track invasion by a contaminant [10]. Pond crashes are a significant threat to algal biofuel production and can occur rapidly, with referenced rates of predatory consumption of algae on the order of 200 algal cells min^{-1} rotifer^{-1} [128] and with the high potential of complete decimation of a culture [129]. Accordingly, investments have been made to develop technologies to assess shifts in the microbiome in an attempt to predict/prevent pond crashes [128].

Population diversity has been shown to dampen the impacts of both predation and parasitism [130]. The persistent negative effects of rotifers have been observed for monocultures, whereas polycultures were more resilient following biological perturbation, specifically rotifer addition [23]. Consortia may be more resistant and/or resilient to invasion by other species for a variety of reasons, including diversity of algal size, presence of silica frustules (diatoms), specificity of predator-prey dynamics [23,131,132], and production of compounds that inhibit predators and pathogens [133]. Interference by unpalatable or defended taxa may reduce grazer consumption and indirectly benefit undefended taxa through associational defense. In addition, the dilution effect suggests that a diverse host community may slow the spread of invasions [134,135]. Dense monocultures may be uniquely vulnerable to the spread of specialized pathogens. However, disease agents that encounter a range of potential hosts varying in competency may spread more slowly, resulting in weaker epidemics and smaller impacts on host populations.

A handful of recent polycultures studies, focused on algal biofuel production, report new developments relating to crop protection. Corcoran and Boeing [23] assessed combinations of two groups of phytoplankton with differing growth rates and susceptibility to grazing [23]. They found that productivity was driven primarily by species combination, while species richness was more important in terms of stability. Shurin et al. [10] reports the potential of polycultures to increase algal biofuel based on nutrient load, ecological trade-offs, and predator susceptibility [10]. They randomly assembled and tested communities consisting of 1, 2, 5, or 10 species of phytoplankton (representatives from *Chlorophyta*, *Cyanophyta*, *Bacillariophyta*, and *Heterokontophyta*), generating 31 unique diversity and composition treatments. These treatments were challenged by the addition of a grazer. On average, polycultures were found to be about 29% more productive than the mean component species in a monoculture, but they were about 19% less than the most productive species alone. However, one exception comprised of two cyanobacteria and three chlorophytes produced more biomass than any other combination and two times more than any monoculture. Both community richness and species composition were found to influence biomass productivity. In addition, polycultures, in general, utilized more diverse nutrients and better resisted grazers. However, the most productive polycultures were not necessarily the most resistant to predation, demonstrating that it may not be possible to optimize for all desired population characteristics simultaneously. Furthermore, there are examples in the literature where, depending on the species present, polycultures were less productive when compared to monocultures [10,14,136]. This again emphasizes that strain composition is a critical parameter and that not all polycultures are expected to out-perform monocultures.

3. Conversion

Until recently, the primary conversion pathway for algal biofuels involved the production and extraction of a high lipid content algal monoculture followed by lipid upgrading [3–7], although challenges remain with lipid extraction from wet biomass [140] required to avoid the energy and environmental costs associated with drying the biomass. The combination of monoculture cultivation approaches and focus on lipid production as the dominant pathway to fuels also face challenges with respect to the maintenance of robust, long-term culture health and productivity [16,17,66,121–124,128,129] and sustainable scale-up from the standpoint of resource demand and utilization [62,63,141]. Polyculture approaches can potentially help address these challenges. However, polyculture algal consortia also face technical challenges in terms of downstream processing (e.g., harvesting, dewatering, and extraction and/or conversion to fuels). When compared to monocultures, polycultures tend to have lower content of neutral lipids and varied content and ratios of major organic biochemical constituents (i.e., proteins, carbohydrates, lipids, and other compounds). Harvested polyculture biomass can also have larger inorganic ash content (a combination of biogenic and exogenous ash material) that depends on the algae species profile and the conditions under which it is grown and harvested. This includes water quality (e.g. use of clean vs. wastewater with silt, sand, and other particulate content), water chemical composition (e.g., fresh vs. saline with salt content), cultivation and harvesting approaches used and cultivation location (which can lead to greater ash content from environmental contamination). For example, the presence of macroalgae (typically high in ash) or diatoms (containing silica frustules) in harvested polyculture material can add biogenic ash, while additional exogenous ash can be introduced through the environmental contamination in open cultivation systems and from salts in saline water systems, as noted earlier. To be clear, monocultures otherwise grown in relatively clean fresh or saline water can also have elevated ash content approaching or greater than 20%, on a dry weight basis, depending on species (e.g. diatoms vs. green), water conditions (fresh vs. salt), and cultivation system and location factors (e.g. open systems in arid regions subject to contamination from atmospheric dust, blowing sand, etc.) [142].

Recent technologies for the processing and conversion of whole algal biomass (e.g., hydrothermal liquefaction – HTL and the combination of biochemical conversion with HTL) allow for relatively efficient conversion of all organic constituents of the biomass (lipids, carbohydrates, protein, and other) into fuel intermediates regardless of the neutral lipid content of the algae. HTL mimics natural geologic processes that converted ancient plant material to petroleum. HTL uses heated, compressed water to dissolve organic compounds, and its elevated ion production can accelerate the acid-catalyzed, hydrolytic decomposition of algal biomacromolecules [143,144]. Output from HTL typically consists four separable fractions [144–159]: (1) an oily biocrude that can be upgraded using refining techniques to produce hydrocarbon fuels, (2) an aqueous co-product (ACP) phase, (3) a solid residue that may be suitable for use in soil amendments, absorbents, catalysts, and asphalt [145–150], and (4) a possible gaseous phase, depending on feedstock and processing conditions [152]. The ACP can be further processed to produce additional energy products and process heat [150,152,153], hydrogen for co-processing [152,153,160], and the capture and recycling of water, nutrients (N, P, etc.) and CO_2 [143,150,152,153]. The HTL process is well suited to the conversion of wet algal biomass that has been partially dewatered to about 10–20% solids content, with the feedstock itself providing the water needed for processing. This avoids the severe energy input costs and environmental penalties associated with having to dry the biomass, and also provides a conversion process potentially capable of effectively dealing with variations in the organic content (lipids, carbohydrates, proteins, etc.) of polyculture biomass. This has made it possible to re-consider the use of polycultures as a viable option for biofuel production, regardless of the neutral lipid content of the biomass [145,161,162]. Elevated non-organic ash content in the wet algal biomass feedstock will be an issue for HTL, in that its presence adds material loading to the processing stream that does not contribute to fuel production, requires additional system processing capacity (and associated capital and operating costs) to accommodate, and reduces the effective fuel product yield per mass unit of material processed [140,163–168]. Ash content can also contribute to corrosion or fouling of HTL systems and components, depending on the type and amount of ash, the processing conditions, and the type of materials used in the equipment [153–159]. Varying degrees of ash content impacts on HTL processing have been only sparely reported in the literature [153–159], however no systematic investigation has been done thus far to better quantify the impacts and establish practical limits for cost-effective HTL processing [169]. Ash impact mitigation approaches include taking steps to possibly reduce the introduction of exogenous ash content into the harvested material in the first place, and applying pretreatment to the wet harvested material to reduce the existing ash content prior to HTL processing [157,163–166,168].

Whole processing of the entire biomass via HTL is a promising technology because of the relatively high yields of biocrude possible from the non-ash organic content of the biomass (i.e., up to 66 wt% in some cases) [151]. Roberts et al. [145], Chen et al. [147], and Pate et al. [164,168] used polyculture algae biomass produced from wastewater and contaminated surface water as HTL feedstock and demonstrated that the bio-crude oil yields and energy recovery were comparable to those converted from monoculture microalgae [145,147,164,168]. In typical one-step direct hydrothermal liquefaction process some portion of valuable polysaccharides, reducing sugar, and protein are decomposed into bio-gas and biochar through hydrolysis, dehydration, and polymerization. The Chen group from Washington State University suggested to use sequential hydrothermal liquefaction process in which sugar and protein were separated first at a lower temperature, and the remaining biomass was then converted to bio-oil at a higher temperature [170]. More recently, Sandia National Laboratories (SNL) researchers have shown promising work on tandem biochemical and HTL processing of whole polyculture algal biomass, including algal turf, to allow for high-efficiency conversion of the carbohydrate and protein fractions to ethanol and isobutanol, extraction of neutral lipids, and HTL processing of remaining organic residue [163,171].

3.1. Downstream processing of polyculture algal biomass: a case study

SNL has recently demonstrated that acid pretreatment of both raceway pond monoculture algae biomass and polyculture algal turf biomass is sufficient for solubilization and hydrolysis of about 90% of the carbohydrate fraction to monomeric sugars. The dominant sugars that were recovered from this process include mannitol and glucose. The protein content of the biomass was approximately equal to the total quantity of carbohydrates. Together, these biochemical fractions account for approximately 75% of the mass balance of the total AFDW biomass. SNL also demonstrated that the combination of dilute acid hydrolysis and protease digestion was sufficient to solubilize and hydrolyze greater than 80% of the protein fraction to amino acids suitable for bioconversion [164,165,172–174]. Production of a variety of chemicals from algal proteins and carbohydrates, including isopropanol, isobutanol, butanediol, C4 di-acids, amyl alcohol, tert-butyl alcohol, phenyl alcohol, and the sesquiterpene, caryophyllene could enhance the economic sustainability of algal biofuel production. Bench scale batch HTL processing of algal turf at SNL showed conversion yields greater than 40% [163,166] of the organic fraction of the feedstock into biocrude. The tandem combination of biochemical pre-processing of polyculture algal turf biomass to first convert proteins and carbohydrates to alcohols, followed by HTL processing of the organic residue, has also demonstrated reduction of the N content in the biocrude to less than 1%. This is in comparison to the ~ 5% N content in the biocrude produced from the whole algae HTL conversion [163,166]. This processing technology, combined with pretreatment to reduce ash content in the wet polyculture material prior to processing, has the potential to increase the flexibility of processing polyculture feedstocks with varying organic composition into fuels. Fig. 2 shows the SNL process flow diagram and process yields obtained, based on bench scale test results using polyculture algal turf biomass material having the composition (non-ash components) of 8% lipids, 39% protein, 34% carbohydrate, and 19% other organics. Also shown at the top of Fig. 2 are projections of annual fuel production potential using the two processing pathways, in terms of gasoline gallons equivalent (GGE) fuel per acre, for assumed levels of annual average daily algal turf productivity (AFDW) of 15 g m^{-2} per day and 25 g m^{-2} per day.

4. Scale

The economics at commercial-scale production currently favor algal biomass cultivation in outdoor raceways [176] due to the significantly higher projected costs for biofuel production using closed photobioreactors (PBRs) [5–7], although PBRs are expected to play a role in the production of inoculum in support of maintaining outdoor cultivation at commercial scales [177]. A major challenge for raceway cultivation is the identification of microalgae polycultures that have the potential to exhibit high seasonal or annual biomass productivities in outdoor ponds. Even if rationally designed polycultures grow well in the laboratory under a given set of incubation conditions (e.g., room temperature and relatively low light intensities), there is no guarantee, even in the absence of predators and invasive species, that the same polycultures will achieve high productivities in outdoor pond cultures that are subjected to a wide range of daily and seasonal water temperature and light fluctuations. Furthermore, a rationally designed polyculture that is cultivated in open outdoor ponds faces the risk of being taken over by local species. This risk may be minimized by assembling the polyculture only with very fast growing and stable strains that are difficult to outcompete by invasive species. Davis et al. [178] demonstrated the stable biomass growth in a mixed outdoor pond culture of *Nannochloropsis salina* and *Phaeodactylum tricornutum* [178]. Similarly, a binary designed culture consisting of fast growing *Chlorella* and *Scenedesmus* strains remained free of invasive species in an outdoor pond culture experiment (PNNL unpublished data).

Although large-scale microalgae polycultures have been successfully operated in the context of wastewater treatment [49,179–181], these polycultures were not scaled-up from the laboratory; instead they assembled naturally from the available pool of indigenous microorganisms (e.g., cyanobacteria, microalgae, and heterotrophs). Similarly, open polyculture algal turf production using ATS systems for removing excess nutrients in both wastewater and other open surface waters in the environment, which have been contaminated by non-point sources, utilize a naturally assembled mix of indigenous benthic and planktonic microorganisms. The ATS approach is currently being investigated by SNL and other collaborators as poten-

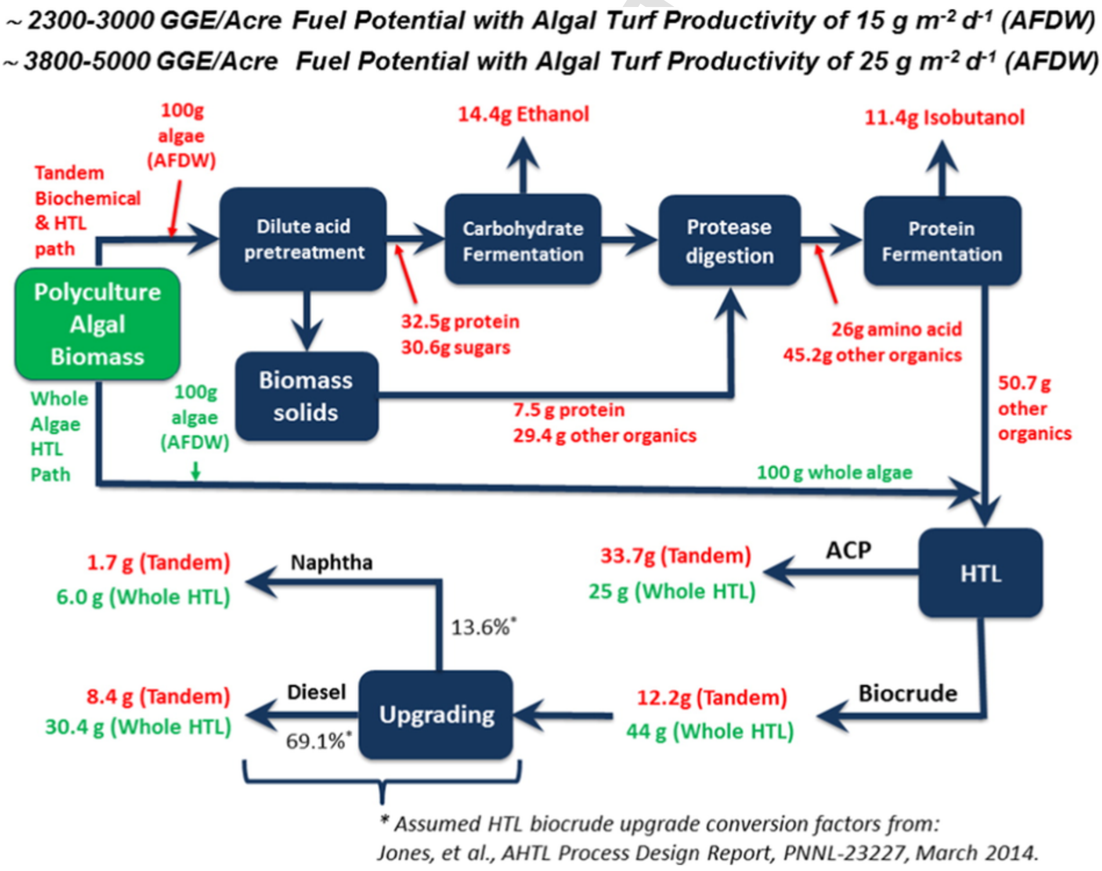


Fig. 2. Examples of preliminary product yield and mass balance flows based on SNL evaluation of alternative biochemical and HTL processing pathways for converting polyculture algal biomass to fuel intermediates and blendstock fuels [166].

tially being more cost effective than open raceway ponds for the scale-up of algal biomass production for fuels [163,164,166,167]. Among the conditions under which this may be possible is where single-pass operation of ATS systems at large scales can achieve sufficiently high annual average AFDW biomass productivities based on the nutrients and inorganic carbon available in the source water. This would avoid the cost and logistics of needing to add nutrients or supplemental CO_2, which would be an operational and cost advantage relative to the recirculating raceway pond approach. However, this requires further investigation and comparative performance assessment before definitive conclusions can be made. High-throughput approaches for evaluating the performance of polycultures representing different combinations of algal strains are needed to advance ecological engineering to a reliable industrial practice.

In response to the pressing need for a rational approach to scale up microalgae cultures and to accelerate the transition of laboratory strains to outdoor ponds, two different outdoor pond simulators have been developed and tested recently: the Phenometrics Environmental Photobioreactors (ePBR™) and the LED-lighted and temperature-controlled indoor raceway ponds at Pacific Northwest National Laboratory (PNNL). In addition to inherent imprecisions of these indoor pond simulators (as discussed in both the ePBRs and PNNL's) LED-lighted and temperature-controlled ponds simulate only the abiotic environment of the outdoor ponds (i.e., light, temperature, pH, salinity, and mixing) while protecting the cultures, to some degree, from predators and invasive species. Consequently, the biomass productivities measured in these simulated cultures, including polycultures, are likely to reflect the best-case scenario in terms of the expected outdoor pond performance.

Assuming that most industrial-scale cultures are grown under conditions where neither essential nutrients (i.e., N, P, trace-elements, and CO_2) nor mass-transfer processes (i.e., poor mixing) limit microalgae growth, incident sunlight intensity and pond water temperature that are determined by the climate at the given geographic location are the key environmental factors affecting biomass productivity. Given these climatic constraints, biomass productivity can be optimized by selecting strains that exhibit high growth rates under the prevailing seasonal light and water temperature conditions.

Matching a microalgae strain and its inherent physiological characteristics to the diurnal/seasonal variations in light and temperature conditions in outdoor ponds to optimize biomass productivity is a complex task. To aid in this effort a strain-specific biomass growth model has recently been developed and was used in conjunction with PNNL's biomass assessment tool to generate annual biomass productivity maps for outdoor ponds located at different geographic locations in the United States [84,185,186]. The model requires that each strain must be extensively characterized prior to generating maps. The model takes into account a strain's maximum specific growth rate as a function of temperature, light intensity, pH, and salinity. The model also considers the strain's biomass loss rate in the dark as a function of temperature, the average light intensity during the preceding light period, culture depth and biomass concentration [187].

In polycultures containing two or more strains, the characteristics of each contributing strain will affect the overall biomass productivity observed in outdoor ponds. For illustration, using a binary co-culture as the simplest case, any of the following differences in physiological characteristics between the two contributing strains is likely to affect not only the overall biomass productivity but also the relative abundance of each strain as a function of time: (a) *temperature optimum* – the strain that grows faster at a higher temperature will outcompete the strain with the lower temperature optimum during the afternoon hours when the pond water temperatures are the highest; (b) *saturating light intensity* – assuming both strains have the same maximum specific growth rate at a given temperature, at their respective saturating light intensity, the strain with the lower saturating light intensity will have an advantage over the competing strain under conditions of low light (i.e., in dense cultures and at dawn and dusk); (c) *pH optimum* – the pH set point and the variations in pH due to periodic CO_2 sparing and subsequent CO_2 uptake by the growing culture will determine the relative abundance of each strain based on their respective pH optima and response functions; (d) *salinity optimum* – the continuous increase in medium salinity as a result of water evaporation is likely to increase the relative abundance of the strain with the highest salt tolerance; and (e) *biomass loss due to dark respiration* – the strain that loses the least biomass during the night period will have a competitive advantage and contribute more to the overall biomass productivity of the pond culture. In the presence of continually changing environmental conditions, these differences in physiological characteristics among polyculture strains also explain "the paradox of the plankton" [188], i.e., the ability of highly diverse phototrophic organisms to coexist without ever achieving a stable equilibrium distribution or one specie outcompeting all others, as would be predicted by the competitive exclusion principle [189].

Predicting biomass productivity and relative strain abundance of outdoor pond polycultures subjected to diurnal and seasonal light and temperature fluctuations is extremely complex. Consequently, to facilitate rational assembly of engineered polycultures with potential to achieve higher biomass productivities than their respective monocultures, it will be necessary to develop a biomass growth model with experimentally determined physiological input parameters for all contributing strains. This model could be used, in conjunction with the biomass assessment tool, to identify the best geographic location and growth season for each designer polyculture and also select the pond's operational conditions (i.e., pond depth, dilution rate, pH set point, and salinity range) that are likely to result in optimal seasonal or annual biomass productivities.

5. Path forward

Understanding the interactions among algae, grazers, and microbes; their response to the environment; and their influence on biomass productivity, represent some of the most substantial challenges impeding commercialization of algal biofuels. Searching for general principles governing overyielding, symbiosis, consumer-resource interactions, nutrient recycling, and niche partitioning may simplify the identification of stable and productive communities and environments. Aquatic microbial communities employed for large-scale algal cultivation are likely to become unavoidably diverse through contamination with non-target organisms. Identifying the traits that govern the coexistence and dynamics of species that come together to form a community is the first step toward applying ecological principles toward generating stable and productive bioenergy technologies.

Communities of algae grown for biofuel applications (i.e., polycultures) should include species with distinct but complementary ecological roles to utilize resources more effectively than monocultures. This technique has shown promise to outperform monocultures [23,125,190], resulting in increased biomass production and carbon fixation [31], increased nutrient removal efficiency [10], increased resistance to grazing [10,23], increased production of carbon storage compounds [37], and enhanced resistance to pathogens [191]. Although, use of polycultures has the potential to limit the effect of grazers, pathogens, and other microbial competitors on algal production, the diversity of these grazers and competitors is broad, meaning use of polycultures alone is not expected to completely limit the ability of these organisms to influence algal production. Therefore, additional crop protection strategies that can be employed in conjunction with polycultures will likely be necessary to achieve predictable and relatively continuous yields of algal biomass from open cultivation systems.

Current techniques for grazer control in algal cultivation systems are limited but are beginning to expand [17,192,193]. State-of-the-art approaches include chemical interventions that either selectively kills grazers/pathogens or otherwise provides a selective advantage for the algal crop (i.e., pesticide application) [17,192,194]; periodic modulation of chemical conditions in the algal cultivation system filtration (e.g., pH reduction and NH_3 addition) [192,195]; early detection of grazers and other contaminants to time harvest cycles, thereby limiting grazer effects on productivity [128,196]; application of continuous strong selective conditions to limit the growth and viability of unwanted competitors (e.g., hypersaline conditions) [197]; and genetic modification of the algal cultivar to change protein expression, thereby altering palatability of the algal cell [198]. While each of these approaches can reduce the number and impact of grazers, they can also represent unique regulatory or economic hurdles and limit the diversity of the algal strains compatible with each control mechanism.

Progress toward large-scale algal biofuel production will likely require a combined approach that incorporates polycultures for enhanced stability and productivity in combination with extrinsic controls on grazing/predation pressure and contaminant introduction. Also required will be further development, refinement, and scale-up of post-harvest pretreatment, processing, and conversion of harvested polyculture algae biomass using chemical, biochemical and/or thermochemical processes for fuel production at commercial scale that can cost-effectively deal with relatively low neutral lipid content and relatively higher inorganic ash content. Improving cultivation and harvesting systems to reduce exogenous ash content in harvested polyculture algae material, especially associated with the ATS approach, is also needed and recommended.

Uncited references

[56,137–139,175,182–184]

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