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Emerging microalgae technology: A review

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Abstract

Cultivating microalgae has the potential to produce biofuels and bioproducts from solar energy with low land use and without competing with food crops. However, despite these key advantages, barriers remain to widespread, economical production from microalgae including: the relatively low solar energy conversion efficiency of photosynthesis, unknown optimal cultivation conditions, and the high energy and economic costs of cultivation and processing microalgal biomass. Thus, recent technological developments seek to address these barriers. To optimize cultivation conditions, devices taking advantage of advanced fluid and light handling techniques are being developed. These approaches drastically increase experimental throughput to find the ideal cultivation parameters. To apply optimal conditions, a range of cultivation approaches are being developed to deliver light and nutrients to microalgae to achieve high productivities. Finally, to extract maximal value out of microalgal biomass, downstream processing technology, such as hydrothermal liquefaction, is replacing costly conventional processes to produce fuels and high-value products from microalgae. Taken together, these technologies can allow microalgae to become competitive in the sustainable energy landscape – particularly to produce complex, high-value molecules.
Introduction

Addressing the future energy and climate concerns will require a diverse set of solutions, including low-carbon energy production, energy storage and carbon capture. Liquid fuels are integral for transportation owing to their high energy density and compatibility with existing transportation infrastructure\textsuperscript{1,2}. Photosynthesis can drive biological and synthetic processes to create complex molecules including biofuels that can substitute for long-chain fuel hydrocarbons which make up the primary energy source of greatest global demand\textsuperscript{1,2}. Moreover, microalgae are capable of producing high-value products for example, pigments, proteins and nutraceuticals\textsuperscript{3,4}.

Microalgae have advantages as biofuel feedstocks in terms of reduced land-use and do not compete directly with food as with crop-based fuels\textsuperscript{5,6}. However, despite these advantages biofuel production from algae still faces serious challenges: (1) Poor efficiency of photosynthetic solar energy capture\textsuperscript{1}; (2) sub-optimal culture conditions\textsuperscript{1}; and (3) High energy and economic costs of cultivation and downstream processing\textsuperscript{7–10}. New technology will be required to address these challenges. First, lab-based multi-parameter optimization is required to determine the ideal conditions for microalgae cultivation. For this to occur, technology and techniques to explore the extensive range of possible cultivation conditions is required. Secondly, once ideal cultivation conditions are determined photobioreactor technology must be advanced to ensure that those cultivation conditions can be applied uniformly and at reasonable capital and operating cost. Finally, once microalgae are grown efficiently, processing techniques are required to maximize extracted value while minimizing energy input. A number of quality reviews detail the current practices in microalgal cultivation, as well as niche developments\textsuperscript{11–14}. However, in recent years, key advances have been made in the development of novel and promising technologies pertaining to all aspects of biofuel and bioproduct production from microalgae. In this review, we detail these
emerging technologies relevant to microalgae production and processing, and discuss the gaps going forward. In this review, we overview emerging technologies in the realm of microalgal biotechnology. First, tools to screen for optimal photobioreactor conditions and techniques to implement this knowledge are presented. Next, we will discuss the light and carbon utilization considerations to be made while designing a photobioreactor, which leads into a discussion on porous substrate bioreactors - an emerging design. Lastly, downstream processing techniques will be discussed while highlighting emerging technologies for concentrating, extracting and upgrading microalgal biomass.

1 - Informing microalgal production

1.1 The complexity of microalgal photosynthesis

Microalgal productivity is dependent on a variety of parameters, namely temperature, the light characteristics, the aqueous environment, the gaseous environment, and the interactions between these variables. High throughput screening devices, particularly microfluidic devices, present an opportunity to rapidly explore the parameter space associated with microalgal productivity. Microfluidic devices for multiplexed studies of cell biology have already been developed and reviewed extensively\textsuperscript{15–17}. However, such devices are aimed at studying the role of the chemical environment, typically drugs. In contrast, platforms capable of studying photosynthetic organisms should be capable of multiplexing notably different variables, light, temperatures and dissolved gas concentrations. Thus, new screening platforms have been developed over recent years\textsuperscript{18–20}. Moreover, implementing this data can involve either direct operating conditions, or predictive models to be used with full scale modeling of the reactor\textsuperscript{21–25}. 
Aspects of the light environment to be studied include the intensity, spectral distribution and time variance. Briefly, increasing light intensity increases growth until a saturating intensity, where other nutrients are the limiting factor and additional light does not increase growth. A further increase in irradiance can become harmful to microalgae due to excess reactive oxygen species (ROS) production. The light spectrum plays a role, depending on a photon’s wavelength it can either drive photosynthetic growth, if absorbed by pigments associated with the photosynthetic apparatus, or induce signal transduction. Lastly, the time variance of light plays an important role, where under high frequency fluctuations, photosynthesis occurs at the same rate it would under the equivalent time-averaged irradiance, due to a mismatch in reaction rates. In short, multiple aspects of the light and chemical environment require comprehensive study and there is a growing need for the associated experimental tools.

1.2 Multiplexed screening of microalgae

Devices capable of multiplexing the light environment have been recently developed. These devices multiplex light environments using a diverse set of methods including individual light sources, fluid handling to act as neutral density filters, liquid crystal displays, and recently, photonic cavities (Fig. 1). The methods typically involve adapting an optical device to a microfluidic cell culture chamber. The short optical path of the cell culture chamber allows for a relatively constant light intensity through the depth, improving the accuracy of results. In contrast, flask-scale (10-1000 mL) studies of microalgal growth are obscured due to gradients of light intensity stemming from long optical paths, particularly at higher cell densities. However, none of these techniques provide the ability to simultaneously control the spectrum, irradiance, and time variance of the light environment. Light emitting diode (LED) arrays and liquid crystal displays (LCD) provide good control over irradiance and time variance with the
ability to adjust the relative intensity of three sections of the spectrum (corresponding to the red, green and blue sections of the visible spectrum). Fluid handling techniques of dyes (Fig. 1e and f)\textsuperscript{48,49} can in principle be used to control the spectrum, provided the dye or combination of dyes are available, however these methods are likely complex and expensive to fabricate. Moreover, they are also subject to issues of stability related to maintaining well balanced fluid flow of dyes when making gradients and have a slow response time. For example, Kim et al.\textsuperscript{48} designed a device which could switch from light to dark in two minutes at best, which is much too slow to mimic mixing effects. A more robust set of light intensities using dyes was shown by Lou et al.\textsuperscript{49} where channels of various heights are used to create multiple irradiance intensities, the benefit of robustness in this approach comes at the price of flexibility.

The use of plasmonics\textsuperscript{46} provides an academically interesting technique to control local irradiance intensity, but does not have any practical advantages over the use of dyes, LEDs or LCDs. Moreover, coping with the difficulty of characterizing the highly localized light environment in a plasmonic device is likely to be intimidating and not of interest to biologists. One possible exception is those that are interested in illuminating only a portion of a cell. However, this is more easily accomplished adapting current microscopy techniques (e.g. Fluorescence Recovery after photobleaching)\textsuperscript{29,50}. Phototoaxis experiments have been performed by integrating projectors
(specifically spatial light modulators) into the optical path of a microscope, devices of a similar design may be useful for high throughput heavily controlled screening, by integrating multiple light sources, similar to a laser scanning confocal microscope.

Photosynthetic organisms use CO$_2$ for growth, and thus can serve as a means of carbon capture. However, the CO$_2$ response of microalgae is complex, due to its role as a source of growth, acidification and the presence of the carbon concentrating mechanics. Therefore multiplexing the CO$_2$ concentration would be of value, particularly in conjunction with the light environment. A key area where multiplexing is lacking is in gas concentrations. Multiplexing the gas environment is distinct from the chemical environment, since the chemical environment can easily be adjusted by pipetting, including the use of automated pipetting systems. In addition, diffusion-based gradient generators have primarily developed for aqueous chemicals, and some gas diffusion

![Fig. 1](image-url)  
**Fig. 1** Summary of high throughput devices for microalgal screening: devices for creating multiplexed optical environments. (a) LED based approach$^{47}$. (b-c) LCD based approach$^{19,20}$. (d) use of photonic cavities$^{46}$. (e-f) Dye based approaches$^{48,49}$. Images a, b, c, e and f have been reproduce from Ref. 47, 19, 20, 48 and 49 with permission from The Royal Society of Chemistry. Image d, Reprinted with permission from, Integrated Microalgae Analysis Photobioreactor for Rapid Strain Selection, Soon Gweon Hong, Minsun Song, Sungjun Kim, Doyeon Bang, Taewook Kang, Inhee Choi, and Luke P. Lee ACS Nano 2016 10 (6), 5635-5642, DOI: 10.1021/acsnano.6b00803. Copyright 2017 American Chemical Society
devices have also been realized\textsuperscript{54}. However, these devices have been limited to under 10 levels. A large-scale gas multiplexing apparatus has yet to be demonstrated and would be greatly beneficial. Optimizing the production of high-value bioproducts is key to strengthening the business case for microalgae cultivation\textsuperscript{55}. In addition, biomanufacturing can be seen as a means to lower the capital cost of chemical synthesis, which can have a disruptive market effect\textsuperscript{56}. Typically, the accumulation of desired bioproducts depends on environmental conditions. Specifically, stressful conditions such as excess light, high salinity, and nutrient stress often lead to the hyperaccumulation of valuable bioproducts. Maximizing productivity is a balance between biomass generation, bioproduct accumulation, and cell viability\textsuperscript{57,58}. These complicating parameters mean that techniques to maximize experimental throughput are essential to mapping out the entire parameter space and identifying optimum productivity conditions. The previously mentioned screening platforms can be used to provide a diverse set of environmental parameters, however the readout is typically limited to cell density, or at best a fluorescent signal associated with lipid production. Unfortunately, the low sample volumes of microfluidic systems present an obstacle since most analytical chemical devices require volumes on the order of milliliters, whereas a microfluidic photobioreactor chamber rarely exceeds 100 nL. This presents an opportunity for improving the integration of on-chip measurement techniques such as Raman, and IR spectroscopy. Moreover, the integrated sample prep possible with centrifugal microfluidics or similar micro total analysis systems\textsuperscript{59}.

1.3 Scaling up knowledge

Transferring the light and chemical information from multiplexed studies to the design of a photobioreactor is more complex than matching the parameters of the reactor to the lab data, due to the heterogeneity of conditions within the reactor - specifically gradients in light intensity and
chemical concentration. Our recent work highlights the possible disconnect between small scale information and reactor scale data, by demonstrating that poorly absorbed green light is more effective than well absorbed red light at intensities when running a high density photobioreactor\textsuperscript{60}. The improved performance occurs due to a more uniform light distribution through the reactor. Being more representative of the light environment of a given microalgal cell, data from short optical that reactors (reactors where cells receive a similar irradiance intensity) should be used in conjunction with details regarding the large-scale gradients in full-scale photobioreactors.

Due to the combination of light gradients and mixing, microalgae in a large scale photobioreactor, receive a time varied irradiance\textsuperscript{34,42,43,61,62}. As mentioned earlier, the growth of microalgae is dependent on the time history of the light it receives. (Fig. 2a). Detailed design of a photobioreactor

![Fig. 2](image)

\textbf{Fig. 2} (a) Schematic illustrating a modelled photosynthetic response to a light profile experienced in a microalgal culture. (b) Three state model of photosynthesis, including the photosystem as being either resting, excited or inhibited. The decay from excited to resting dictates the growth rate. In this case the excitation from resting to excited state corresponds to the entire photosynthetic electron transport chain\textsuperscript{42,65-67} Reprinted from Journal of Theoretical Biology, 304, F. García-Camacho, A. Sánchez-Mirón, E. Molina-Grima, F. Camacho-Rubio, J.C. Merchuck, A mechanistic model of photosynthesis in microalgae including photoacclimation dynamics, 1, Copyright 2012, with permission from Elsevier. (c) A similar three state model, however the excitation from the ground state to the excited state relates to exciting photosystem II and the recovery step relates to water splitting. (d) A model similar to the model in (c), but taking into account that multiple steps are required to excite photosystem II. Images (c) and (d) have been reproduced from Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second Edition, Chapter 12: The essential role of timescales. Copyright Wiley 2013.
would require a model that is not too heavy computationally, and could be used in conjunction with a CFD (computational fluid dynamics) code to track macroalgal trajectories. To address this, multiple simple two or three state models have been developed (Fig. 2b and c). Generally, these models simplify the major photosynthetic reactions into a fast set of reactions and a slow set of reactions. Due to the mismatch in rates, there is a buildup of intermediate products to allow growth in the dark, and such dynamic light effects can be captured through suitable models.

The bulk of the models simplify photosynthesis by considering the light harvesting apparatus and the electron transport chain as a single Photosynthetic Unit (PSU), (Fig. 2b). Light drives the excitation of the photosynthetic unit, where the excited state corresponds to the cell having an increased concentration of ATP and NADPH. The ATP and NADPH are then used during the de-excitation process which represents the Calvin cycle. Moreover, these models have also captured the effect of photoinhibition. This simplified model has been used to capture growth in flashing light, where at a sufficiently high frequency (>5 Hz) the growth rate can reach the growth rate possible under the equivalent time-average irradiance. A photosynthetic unit can also become inhibited should it receive an excessively high irradiance for a sustained period. In contrast to the PSU style model, models from Zarmi and colleagues (Fig. 2c and d) relate to the mismatch between reactions along the photosynthetic electron transport chain. Though progress has been made in developing useable models, integrating all the relevant timescales associated with photosynthetic growth and induction timescales is required.

1.4 Genetic engineering and strain selection
Genetic engineering and strain selection can benefit from the fluid handling and automation associated with microfluidic techniques (Fig. 3). Digital microfluidics has shown promise in the reduction of labor associated with genetic engineering as well as savings on costly reagents. Strain selection methods have been improved using droplet microfluidics extensively. In addition, recent devices have emerged that leverage correlations between buoyancy and lipid content as well as phototaxis and photosynthetic growth. Tailoring strain selection and genetic engineering tools to be effective for microalgal screening would accelerate microalgal implementation.

Fig. 3  (a) Strain selection based on improved phototactic response, (b) Gene manipulation using droplet microfluidics, Reprinted with permission from Shih et al. Copyright 2015 American Chemical Society, and (c) Selection of high lipid producing yeast using buoyancy. Reprinted from Metabolic Engineering, 29, Leqian Liu, Anny Pan, Caitlin Spofford, Nijia Zhou, Hal S. Alper, An evolutionary metabolic engineering approach for enhancing lipogenesis in Yarrowia lipolytica, 36-45, Copyright 2015, with permission from Elsevier

1.5 Environmental Applications

In the context of this review, understanding the interactive effects of multiple variables has been useful for identifying conditions with high microalgal productivity, however this endeavor is also of great interest and importance in broader ecological contexts. The need for multiparameter control when cultivating microalgae mirrors the need to understand influential parameters in the
environmental context. However, the motivation to decouple the interactive effects of environmental parameters in ecological settings, in contrast to microalgal studies, stems from the need to identify detrimental combinations that cause harmful effects to global ecosystems as opposed to those which maximize productivity.

The natural environment is impacted simultaneously by both anthropogenic climate change and ecotoxic pollution. The onset of warming\textsuperscript{76}, CO\textsubscript{2} driven acidification\textsuperscript{77} and increased ultraviolet B radiation (UVB)\textsuperscript{78}, in conjunction with chemical pollutants\textsuperscript{79,80} act as stressors to global ecosystems. As with microalgae growth parameters, the effect of multiple anthropogenic stressors cannot always be inferred from studying constituent parameters independently due to complicated interactive effects\textsuperscript{81–83}. Furthermore, the increased complexity of macroorganisms and the interactions between them in ecosystems makes it even more difficult to decouple the effects of system variables. As a result, an effective assessment of multiple stressors requires studying all relevant stressors in parallel, with many different combinations\textsuperscript{83,84} (Fig. 4).
Methods, devices and techniques have been developed to achieve precise control over relevant stressor parameters in ecological studies, however the experimental throughput challenge, also present with microalgae cultivation optimization, has not been addressed. Commonly studied aquatic stressors include: CO$_2$-driven acidification, increased UVB radiation and warming. Acidification is typically controlled by injecting air/CO$_2$ mixtures into the headspace of a testing chamber (Fig. 5b), bubbling into aqueous solution (Fig. 5b, d), adding CO$_2$ saturated media (Fig. 5a, e-f) or adjusting pH directly (Fig. 5g); Ultraviolet B radiation by either UVB lamps or a combination of natural sunlight and filters; and warming through actively heating the system (e.g. Immersion water heaters) or passively warming, via enhancement of natural warming. Devices and systems have been developed to incorporate control over combinations of these parameters for multiplexed studies, however, the majority are large and costly, limiting the combinations and replicates that can be feasibly achieved. In general, these devices achieve control over relevant parameters generating environments capable of simple multi-stressor analysis (varying 1-2 stressors) but fall short in their...
inability to feasibly study sufficient combinations - with replicates - to decouple the effects of complex stressor interactions (varying 4+ stressors). That is, full factorial experiments are ideal in terms of predictive power and mechanistic insight. Tradition systems are practically limited to under 100 experimental units (Fig. 5) – not enough to perform multi-parameter full factorial experiments of relevant stressors.

The microalgae technology reviewed here are compatible with ecologically relevant microalgal species and marine microbes and in contrast to the existing ecological techniques, are capable of generating sufficient combinations and replicates necessary for the multi-stressor challenge. Microalgae alone are responsible for about half of global primary productivity, however despite their importance to the carbon cycle, questions remain about how microalgae will respond to future environmental conditions. Particularly, growth responses to increasing CO₂ and temperature in a
diverse set of local environments has broad implications for global carbon fixation and ecosystem health. Beyond microorganisms, we see potential for some of the technology reviewed here to be scaled for larger, more complex organisms and systems (Fig. 6). That being said, the strengths of these technologies, namely small volumes and precise control of parameters, limit the scope that can be studied to simple mechanistic biological effects. This is because in ecology and ecotoxicology studies, a tradeoff between control and realism exists, and depending on the complexity of the system and response being studied, a larger more realistic system with less control might be necessary. For instance, a comprehensive study of ecosystem responses to eutrophication would not be possible with a small-scale highly controlled approach and would instead require larger volumes with multiple species of organisms to replicate complex ecosystem interactions. However, studying the effects of acidification, warming, increased UVB radiation and pollutant exposure on the larval development and growth of a single organism or small assemblage of organisms, is possible and well suited to this approach. Overall, we see diverse opportunities here for both emerging technologies in microalgae production, and in broader environmental application, in the development of more advanced microcosms uniquely tailored to multi-stressor ecological experiments.
2 – Optimizing the Photobioreactor

2.1 Major photobioreactor production factors

Photobioreactor optimization, driven by the promise of cost-effective biofuel production, is an iterative process of proving laboratory-scale production potential and evaluating pilot plant cost performance of proven designs. Several recent techno-economic and lifetime assessment studies using pilot plant data from conventional reactor designs (Fig. 7) together suggest a microalgae oil price point of 0.3 – 8 $US/L\textsuperscript{55,106–109} two orders of magnitude lower than the price predicted two decades ago\textsuperscript{110}, and not far from the current conventional diesel market price of ~ 0.7 $US/L\textsuperscript{111}. The validation of assessment models toward reducing price point uncertainties is complicated by...
the rarity of consistent, comparable pilot plant data\textsuperscript{107} and models that sometimes inconsistently define system boundaries and make questionable assumptions\textsuperscript{112}. However, assessment studies have consistently identified a set of production cost and productivity factors that inform photobioreactor optimization. The major production cost factors include labor, infrastructure, operational energy, product extraction, carbon supply and - depending on species demand - micronutrient supply (namely, nitrogen and phosphorous)\textsuperscript{106–108,112–116}. The relative size of these costs depends on the strategies employed as well as operation-specific parameters. The most important productivity factors are light and carbon\textsuperscript{1,55,112,115–121}. The remainder of this section summarizes reactor design approaches for efficient light and carbon utilization while mitigating
major production costs. This section concludes with an overview of unconventional reactor designs with novel means to improve productivity.

2.2 Light distribution

Efficient light utilization by cultures requires the intensity extremes in the reactor to span the optimal photosynthetic range for the species. Conventional reactors that optimally use sunlight at ~ 2000 µmol m⁻² s⁻¹ of 400 – 700 nm photosynthetically active radiation (PAR) must dilute captured intensities about ten times before transmission through a culture thickness that defines the optimal low-intensity. The spatial aspect ratios of flat panel reactors are ideal for sunlight dilution by capture-to-ground areas, and limit intensities by culture-volume-to-capture areas in ‘short light path’ designs (~ cm). Flat panel reactors achieve the greatest biomass
productivities and photosynthetic efficiencies of conventional designs\textsuperscript{55,68,120,123,124,127–129} though the necessary 1 – 10 cm gaps between adjacent panels that enables significant capture of diffuse light (~10 - 40% of solar radiation on clear days\textsuperscript{130}) also limits facility areal density\textsuperscript{55,120,123,126}. Closely stacked flat panels enable complete facility area usage, with compartment walls that can serve to effectively distribute light\textsuperscript{131,132}, though additional infrastructure is required for external light collection and concentration.

Light distribution by planar waveguides or ‘light guides’ between flat panel compartments enable similar intensity control as conventional flat panel reactors, with spatial dilution ratios of input-to-emission surface areas and low-intensities defined by inter-waveguide spacing\textsuperscript{129,133} (Fig. 8a). Optimal uniform emission from waveguides occurs by breaking total internal reflection, which occurs at sub-critical angles by means of tapering angle or a lengthwise distribution of internal or surface scatterers\textsuperscript{134–140}. Uniform emission at large supercritical angles is also possible by means of frustrated total internal reflection, induced by biomass contact with near-surface evanescent fields on unmodified waveguide surfaces\textsuperscript{141–143}. Planar-stack waveguide reactors employing micro-patterned waveguide surfaces\textsuperscript{133} and internal light-scattering particles\textsuperscript{134} have exceeded equivalent flat panel volumetric biomass productivities by 3 - 8× and densities by 3 - 4× even without control of the low-intensity limit, owing to the greater and more uniform illumination areas. Additionally, illumination intensity and distribution can be enhanced by modifying waveguide optical properties, as demonstrated with added broadband\textsuperscript{134,144} or wavelength-selective\textsuperscript{145} reflectors on waveguides, and waveguide integration with spectrum-converting luminescent particles\textsuperscript{146–151} or sensitizer-emitter pairs\textsuperscript{152}. Conversion of unproductive solar wavelengths to green is particularly beneficial for high-density production in suspension cultures due to greater light dilution achievable at low cell-absorption wavelengths, as demonstrated by
Ooms et al. (2016)\textsuperscript{60} (Fig. 8b). In contrast, solar wavelengths outside the PAR range may be captured by photovoltaics, as demonstrated in a biomass and electricity co-generation reactor\textsuperscript{153}. Effective utilization of the solar spectrum with the above approaches may also be combined with co-cultivation of microalgae with complementary absorbance spectra\textsuperscript{154,155}. Recent novel light distribution methods with less control over intensities relative to the above approaches include light-scattering open-pore glass sponges (Fig. 8c)\textsuperscript{156,157} and externally powered wireless LEDs in suspension\textsuperscript{158}. The reactor volume occupied by such internal emitter should be minimalized to ensure a high-density facility and mitigate infrastructure costs\textsuperscript{143,159}.

Despite lab-scale demonstrations of the numerous illumination-based production advantages offered by stacked-waveguide reactors, pilot plant investigations have not been realized, partly by difficulties in achieving a cost-effective external solar concentrator and light delivery system. Systems that use multiple and disparate optical elements demonstrate prohibitively large losses $> 50\%$ (typically using primary Fresnel lenses and secondary lenses or optical fibers)\textsuperscript{138,160–164}. Chained losses from reflection, absorption, aberration and unwanted breaking of total internal reflection are minimized by directly coupling concentration and dilution elements that are optically similar, for example, in consideration of acceptance angle and f/# focal ratio\textsuperscript{125,162,165}. In addition, to enable cost-effective production of low-value biomass, high costs of active solar tracking should be avoided with solar concentration factors optimized to large acceptance angles\textsuperscript{129,162,163,166}. Dye et al. (2011) developed a minimal optical system with $\sim70\%$ optical efficiency using 1-D Fresnel lenses that concentrate light directly into stacked reactor waveguides (Fig. 8a), though with active tracking\textsuperscript{165}. Planar solar concentrators, recently developed for photovoltaic applications, contain many spherical micro-optical lens-mirror pairs that have achieved overall $> 80\%$ optical efficiencies at concentration factors $\leq 100\times$ but with narrow acceptance angels ($< 1^\circ$) (Fig. 8d).\textsuperscript{167–}
The implementation of cylindrical lenses and/or active refractive index materials may improve acceptance angles to ±50° East-West and ±10° North-South\textsuperscript{170,171}. The high physical and optical compatibility of planar solar concentrators with light-diluting planar waveguides may then enable cost-effective solar stacked-waveguide reactors for biomass production\textsuperscript{172}. Further considerations for light-management strategies in photobioreactors and associated efficiencies, may be found in the recent review by Ooms, \textit{et al.} (2016)\textsuperscript{1}.

\textbf{2.3 Carbon distribution}

Efficient carbon utilization occurs in reactors that closely match the supply rate of dissolved carbon with culture demand\textsuperscript{173}. In reactors that maintain optimal pH, the carbon supply rate is mainly a function of the CO\textsubscript{2}\textsubscript{(g)}, aeration rate, concentration, and overall carbon mass transfer coefficient of the reactor (K\textsubscript{L}), which is dominated by that of the liquid film diffusion barrier at the gas-liquid interface (k\textsubscript{L}a)\textsuperscript{174–176}. In conventional reactors, aeration is accomplished using macrobubbles (1-2 mm diameter) with low k\textsubscript{L}a that necessitate elevated CO\textsubscript{2} concentrations and long bubble residence.

\textbf{Fig. 8} Internal light management strategies in photobioreactors. (a) Fresnel lenses concentrate and directly couple sunlight into waveguides\textsuperscript{165}. (b) Photographs of columns of cyanobacteria suspensions visualizing the penetration depth of different wavelengths\textsuperscript{60}. (c) Photobioreactor using glass sponge (structure inset) to distribute light\textsuperscript{156}. (d) Ray-tracing in a planar solar concentrator \textsuperscript{168}
times to meet culture demand\cite{117,177,178}. On-demand injection of elevated CO\(_2\) in (closed) reactors then results in greater carbon utilization efficiencies (50-96\%\cite{117,179-181}) than with continuous aeration (< 10\%\cite{177,182}) due to the elevated concentration lost to atmosphere. By increasing continuous aeration flow rate, or \(k_{\text{L}a}\) with higher-pressure bubbles, productivity is improved at greater operational energy cost and much reduced carbon utilization efficiency\cite{117,121,177,178,183}.

However, to reduce carbon supply cost when an inexpensive, high-concentration source (i.e. flue gas) is not available, purchased CO\(_2\)(g) supply rate must be lowered, which necessitates continuous aeration to meet culture demand. Production and cost considerations using carbon supplied from flue gas are reviewed in references\cite{181,184-187}. With CO\(_2\) supply concentrations less than the 1 – 5\% typically used in laboratory and pilot plant reactors\cite{188,189}, macrobubbling aeration rates that optimize carbon utilization efficiency and productivity cannot meet culture demand\cite{117,177,178}.

The recent development of microbubbling technologies (10-50 \(\mu\)m diameter) enable greater mass transfer coefficients and longer residence times, which provide dissolved CO\(_2\) saturation without the need for elevated gas-phase concentration, or, continuous aeration with superior carbon utilization efficiency\cite{185,190-193}. Notably, bubble retention is maximized in tall reactors compatible with the thin format for efficient light utilization in flat panel and stacked waveguide reactors\cite{55,120}.

Microbubble generation methods include fluidic oscillation applied to microporous diffusers (Fig. 9a)\cite{190,194} and break-through pressures applied to microporous membranes\cite{195-197}. A few potential disadvantages of microbubbling have been identified: (i) bursting in the culture provides shear forces which can kill cells\cite{185}; (ii) light scattering from bubble interfaces can reduce light penetration\cite{185,198}; and (iii) generation may require large energy use compared to macrobubble aeration. Further quantification is required for a fair economic comparison of these technologies\cite{199}.

Aeration is also required to strip oxygen waste from the culture to concentrations recommended
below 1 mM, or at most four times the atmospheric equilibrium concentration in water\textsuperscript{200}. Especially susceptible to toxic oxygen buildup, enclosed tube reactors require aeration nodes every ~ 80 m of tube length primarily to manage oxygen holdup rather than replenish dissolved CO\textsubscript{2} \textsuperscript{201–203}.

An alternative to bubbling aeration, the direct supply of dissolved CO\textsubscript{2} to a culture combined with oxygen stripping may be provided by microporous membrane contactors, which have been widely used for microalgae-based wastewater treatment\textsuperscript{183,201,204,205}. Membrane contactors do not necessarily require operational energy for elevated pressure or flow\textsuperscript{205,206}, but mass transfer coefficients are generally lower than for microbubbling (and greater than for macrobubbling)\textsuperscript{175,195,198,205,207–210}.

A major disadvantage of current membrane contactor materials is opaqueness that blocks internal light transmission, which reduces productivity and limits reactor scalability, as exhibited in the series of studies that showed reduced productivity in stacked-waveguide reactors (mentioned above) when opaque hollow fiber membranes were added to improve carbon availability (Fig. 9b)\textsuperscript{211–213}. The use of transparent nanoporous materials provide a compromise between internal light transmission and mass transfer barriers to CO\textsubscript{2} supply and can be doubly used as internal waveguides in scalable reactor designs (Fig. 9c)\textsuperscript{206,214–216}.
The novel light and carbon management technologies cultivation reviewed here have proven laboratory-scale production advantages, but their potential to enable cost-effective production upon scale-up in pilot plant reactors has not been evaluated. Furthermore, novel reactor designs that undercut conventional pilot plant production costs by $> 50\%$ may be required for economic biofuel production, according to some assessment studies $^{107,113,114,217,218}$. Being more complex than conventional approaches, these novel technologies may be economically feasible only for high-value product generation using suspension cultures. Irrespective of light and carbon management technologies, however, the management of large, commercial-production suspension culture volumes significantly contributes to production costs by each of the major factors outlined above – namely, infrastructure (by liquid volume), product extraction (by biomass harvesting and de-watering), operational energy and carbon supply (by flow and aeration pumping), and labor (by

**2.4 Biofilm photobioreactors**

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**Fig. 9** Carbon supply technologies in photobioreactors. (a) Microbubbles 20–100 µm diameter generated by fluidic oscillation (cm scale)$^{190}$. (b) Schematic of reactor with hollow fiber membrane contactors for carbon supply interspacing planar waveguides for internal light supply $^{212}$. (c) Conceptual illustration of transparent, gas-permeable waveguide implemented in ref. $^{216}$. 

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facility size and complexity related to each factor)\textsuperscript{11,55,112,116,219–223}. Microalgal biofilms feature 2-3 orders of magnitude reduced liquid volumes\textsuperscript{220,224} and investigations of their potential to reduce water-related production costs of low-value biomass began in \textasciitilde 2013\textsuperscript{222,225,226}.

Previously, microalgal biofilm reactors were developed mainly for municipal wastewater treatment, and later for coupled biomass and solely-biomass production\textsuperscript{227–231}. In these reactors, biofilms self-establish by natural recruitment and colonization, for which initial attachment is sensitive to and species-dependent on substrate properties (e.g., hydrophobicity, surface roughness and energy components) and materials, including waveguide substrates that enable illumination from both sides\textsuperscript{140,232–234}. Lipid content and production in biofilm reactors coupled with wastewater treatment can achieve comparable lipid content and biomass productivity as suspension cultures\textsuperscript{235–239}. Biofilms are submerged in media, optimally in a thin \textasciitilde mm layer using vertical drip irrigation, or intermittent contact with atmosphere and media on moving platforms (Fig. 10a). The thin liquid layer enhances gas transfer\textsuperscript{222,225,235,237,238,240} but the still-present gas-liquid boundary resistance (k\textsubscript{L}a) necessitated media aeration by bubbling for cultivation in some lab-scale studies\textsuperscript{237,241}. However, a year-long study of rotating biofilm reactors without active aeration or elevated CO\textsubscript{2} supply demonstrated greater productivities compared to conventional reactors based on suspension culture ground areas\textsuperscript{242}.

Porous substrate photobioreactors (PSBRs), developed in the last few years for biomass generation, isolate biofilms from direct media contact between porous substrates and gaseous surface exposure (Fig. 10b-e), which minimizes both k\textsubscript{L}a and the chance of spreading contaminants by liquid flow\textsuperscript{223,225,226,243,244}. By forcing cell attachment onto substrates - typically printing paper - stable biofilm cultivation of \textgreater 80\% of at least 90 strains have been demonstrated\textsuperscript{223,226} using this “attached cultivation” (previously “Twin-layer”) system. These species include those relevant to biofuel
production, and at least one species sensitive to hydrodynamic shear forces\textsuperscript{161} that are absent in PSBRs\textsuperscript{245}. Other investigated substrates include solid and fibrous natural and synthetic polymers\textsuperscript{189,228,238}. Dissolved micronutrients are supplied to biofilms by perfusion though substrates from gravitational media flow from a pumped reservoir. Reactors are typically vertical-stack, as for flat panels, with light capture and cultivation occurring on both sides of the vertical flow reservoir. Micronutrient diffusion is mainly driven by concentration gradients from cell consumption\textsuperscript{189,246,247}, since the evaporation-driven component alone was shown insufficient for cultivation\textsuperscript{223,248}. Owing to the greater surface area, evaporative water loss from PSBRs compared to conventional (open pond) reactors are greater by ground area, but less per unit of biomass produced\textsuperscript{249–251}. PSBRs have consistently demonstrated superior productivity and light utilization efficiencies compared to conventional reactor cultivation. Most PSBR studies evaluate isolated biomass production, but some consider high-value product generation – for example, astaxanthin\textsuperscript{3,4} and to a lesser extent animal\textsuperscript{189} and aquaculture feedstock\textsuperscript{225} – and coupled operations with wastewater remediation\textsuperscript{235,252}; see Hoh \textit{et al.} (2016) for a review of these coupled systems\textsuperscript{228}. 
Table 1 shows the cultivation conditions in different PSBRs that resulted in biomass productivities (linear growth regime) and maximum standing-crop densities achieved over the reported growth durations, based on cultivation surface area. Typically, PSBR productivities of 5 - 16 g\(_{DW}\) m\(^{-2}\) d\(^{-1}\) are sustained for about a dozen days, but as high as 31 g\(_{DW}\) m\(^{-2}\) d\(^{-1}\) was achieved for three days using *Halochlorella rubescens* \(^{189,220,246,253,254}\). With 2 - 20 cm gaps between single PSBR layers that dilute solar intensities by \(\sim 10\times\) (comparable to flat-panel facility layouts) \(^{220,222,246,253}\), facility footprint productivities are typically 50 - 80 g\(_{DW}\) m\(^{-2}\) d\(^{-1}\), as demonstrated using *Scenedesmus obliquus* and *Halochlorella rubescens*. These footprint productivities are several times that of the same species in conventional photobioreactors\(^{189,222,253}\). The highest facility footprint

![Fig. 10 Biofilm-based photobioreactors. (a) Intermittently submerged rotating biofilm photobioreactor using cotton cord as a substrate \(^{239}\). (b) “Twin-layer” biofilm reactors cultivating different species \(^{225}\). (c) Photograph of a partly harvested biofilm of *Halochlorella rubescens* with \(\sim 160\) g\(_{DW}\) m\(^{-2}\) grown on printing paper in a porous substrate photobioreactor \(^{246}\). (d-e) Layout and microscale conceptual illustration of porous substrate photobioreactors \(^{225,257}\).](image-url)
productivities of conventional reactors is $\sim 40 \text{ gDW m}^{-2} \text{ d}^{-1}$

$^{11,255,256}$. PSBRs have also achieved extremely high standing-crop densities of $\sim 200 \text{ gDW m}^{-2}$ for *Halochlorella rubescens* and *Haematococcus pluvialis*, and 60 $\text{ gDW m}^{-2}$ for *Botryococcus braunii* $^{4,246,253}$. By footprint area, the standing crop density of *Botryococcus braunii* $^{247,253}$ can be as high as 560 $\text{ gDW m}^{-2}$.

**Table 1**: Growth conditions in different single-layer porous substrate photobioreactors (PSBRs) for biomass productivity (linear growth regime) and maximum standing crop densities achieved in growth durations that include lag phase. Productivities and densities are based on cultivation surface area. Biomass values are by substrate growth area and determined between 25°C and 32°C. Bracketed values are for an indoor stacked-reactor configuration.

<table>
<thead>
<tr>
<th>Species</th>
<th>Biomass productivity† and standing crop density††</th>
<th>Growth period (days)</th>
<th>Light cycle (hours per day)</th>
<th>PPFD (µmol m$^{-2}$ s$^{-1}$)</th>
<th>CO$_2$(g) % v/v</th>
<th>Inoculation density (g$_{\text{DW}}$ m$^{-2}$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Spirulina platensis</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$†</td>
<td>8 - 12</td>
<td>3</td>
<td>24</td>
<td>100 - 200</td>
<td>0.5</td>
<td>7 - 11</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$††</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haematococcus pluvialis</em> CCAC 0125</td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>12 - 16</td>
<td>10-16</td>
<td>14</td>
<td>400 - 500</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>200</td>
<td>16</td>
<td>14</td>
<td>400 - 1015</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Halochlorella rubescens</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>30</td>
<td>3</td>
<td>24</td>
<td>1023 - 1486</td>
<td>2 - 3</td>
<td>2 - 5</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>205</td>
<td>31</td>
<td>14</td>
<td>1011</td>
<td>$\sim$ 0.04</td>
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</tr>
<tr>
<td><em>Botryococcus braunii</em> SAG 807-1</td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>6.5</td>
<td>8</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>60</td>
<td>8</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Botryococcus braunii</em> FACHB 357</td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>6.5, 5.5</td>
<td>2, 10</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>62</td>
<td>10</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Pseudochlorella sp.</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>6 - 8</td>
<td>6</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td>3 - 5</td>
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<td>$\text{gDW m}^{-2}$</td>
<td>$\sim$ 40</td>
<td>6</td>
<td>24</td>
<td>100</td>
<td>1</td>
<td>3 - 5</td>
</tr>
<tr>
<td><em>Scenedesmus dimorphus</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>107</td>
<td>10</td>
<td>24</td>
<td>100</td>
<td>1.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Acutodesmus obliquus</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>$\sim$ 8</td>
<td>7</td>
<td>24</td>
<td>100</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>$\text{gDW m}^{-2} \text{ d}^{-1}$</td>
<td>110 (70.9)</td>
<td>3</td>
<td>24</td>
<td>135</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$\text{gDW m}^{-2}$</td>
<td>83.7 (450)</td>
<td>8</td>
<td>24</td>
<td>100 (135)</td>
<td>2</td>
<td>10.6 (200)</td>
</tr>
</tbody>
</table>

Light utilization efficiencies in PSBRs are superior to that of conventional reactors at moderate-high continuous light intensities 100 - 500 µmol m$^{-2}$ s$^{-1}$ with atmospheric CO$_2$$^{222,246,253,254}$ and at 25°C - 32°C.
greater intensities with elevated CO\textsubscript{2} due to the shift from light- to carbon-limited regimes\textsuperscript{224,246}. For example, at 700 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} and a dilution factor of 10\times, \textit{Scenedesmus} productivity and photosynthetic efficiency was \sim 7\times that reported of an open pond suspension culture under the same conditions\textsuperscript{222,257}. At moderate intensities \sim 200 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1}, the addition of minimally elevated CO\textsubscript{2(g)} concentration (e.g. 0.12 - 0.5\%) improves carbon utilization efficiencies and enhances productivities, for example, by \sim 25\times with \textit{Halochlorella rubescens} (Fig. 11a)\textsuperscript{189,224,246,247,254}. Of the carbon supply technologies reviewed here, PSBR may enable the closest approach to achieving the ultimate goal of using free, dilute atmospheric CO\textsubscript{2} to meet high-density culture demand\textsuperscript{176}.

The recent development of a comprehensive kinetic model of PSBRs\textsuperscript{259}, combined with light and chemical microprofiling studies\textsuperscript{224,239,257} has provided great insight into light and carbon utilization in PSBRs. The fixed culture permits cell photoacclimation to local light intensities by pigment content expression, resulting in deeper light penetration (more light available per-cell)\textsuperscript{224,227,257} and tolerance to high light intensities that would inhibit production in suspension cultures (Fig. 11 b-c)\textsuperscript{246}. Also, density-dependent forward scattering in high-density biofilm cultures greatly enhances light dilution\textsuperscript{223,248,257,259,260}. With a greater photosynthetically active culture fraction than achievable in suspension cultures, PSBRs achieve greater biomass productivity and light utilization efficiency than conventional reactors, especially under high-light conditions\textsuperscript{246,257}. In addition, the minimal liquid layer on biofilms reduces reflection losses compared to submerged biofilm and suspension cultures and optimizes \textit{k}_{\text{La}}\textsuperscript{222,223} . The latter enables high rates of CO\textsubscript{2} uptake and O\textsubscript{2} removal and results in the greatest net photosynthetic productivity at the biofilm surface, rather than deeper down - as is the case for submerged biofilms\textsuperscript{224}. The high-density production of dissolved O\textsubscript{2} occurs throughout the photoactive culture fraction, however, which leads to supersaturated concentrations in the bulk that drives respiration (negative production) in
cells below the light compensation point (Fig. 11d-e)\textsuperscript{224}. From this point, increased biofilm thickness and respiratory culture fraction is observed as a decline in productivity from the linear growth regime \textsuperscript{225,227,246,253,257}. 
Aside from growth parameters of light and carbon, PSBR production is optimized via the species-dependent inoculation density of substrates and a cultivation period that avoids respiration losses. The onset of respiration can occur before ten days of cultivation and has occurred at 31...
days at the latest in studies to-date\textsuperscript{222,224,225,232,239,246,253,257,259}. Biomass may be harvested by simple scraping, and contains 75-80\% water content, which is comparable to centrifuged biomass from suspension cultures that initially contain \textasciitilde99\% water\textsuperscript{128,223}. Compared to conventional reactors, harvesting biomass in PSBRs is highly energetically efficient and does not require flocculation\textsuperscript{7,250}. In summary, compared to conventional reactors, PSBRs exhibit superior light, carbon and water utilization efficiencies, and biomass productivities by facility area that do not necessarily suffer upon modular scale-up of the 2-D culture. PSBRs are then a promising technology for economically feasible production of low-value biomass.

2.5 Alternative immobilized growth formats

The productivity and cost advantages of high-density and fixed culture cultivation enabled by biofilms and exploited in PSBRs can be alternatively achieved using matrix-immobilized microalgae. Immobilization matrices are widely used in wastewater remediation applications for the isolation of the culture from the environment and the ease of biomass reclamation\textsuperscript{261–266}. Alginate gels are popular immobilization matrices due to their relatively low cost and gentle encapsulation\textsuperscript{264}. We recently demonstrated high-density biomass production in a periodically harvested air-exposed alginate-immobilized microalgal culture, with comparable light utilization efficiencies material costs as reported of PSBRs\textsuperscript{223,246,267} (Fig. 12a). The different physiological state of matrix-immobilized compared to free microalgae can benefit production, depending on the species, density, and immobilization matrix, and include greater pigment concentration and lipid variety\textsuperscript{265,268,269}, lower senescence\textsuperscript{270}, comparable biomass productivities and light use efficiencies\textsuperscript{262,264,271,272}. Mutual symbiosis between multiple immobilized species can also benefit production, as recently reviewed in Santos and Reis (2014)\textsuperscript{263}. Spatially organized co-cultivation
(Fig. 12b) and synthetic mutualism with alginate-immobilized microalgae were recently separately demonstrated in proof-of-concept studies\textsuperscript{273–275}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{alginate_immobilization.png}
\caption{Alginate-immobilized cultivation of (a) partially harvested, air-exposed cyanobacteria culture\textsuperscript{267} and (b) printed scaffold with human bone cells and microalgae (green) species\textsuperscript{275}.}
\end{figure}

\section*{3 - Optimizing downstream processing}

Unlike terrestrial plants, microalgae are harvested as wet biomass which requires different downstream processing from crop-based biomass. To complete the microalgae-to-biofuel conversion route, pretreatment of biomass such as harvesting, thickening and dehydration are typically applied to simplify conversion processes\textsuperscript{7,255,276–278}. However, given the high latent heat vaporization for water (2,265kJ/kg) the energy intensity and associated cost of drying is a fundamental challenge for microalgal biofuels. Given the fact that most microalgae have a total energy content on the order of 18,000 kJ/kg\textsuperscript{279,280}, drying a microalgae slurry at a concentration below \textasciitilde20 wt\% (which contains \textasciitilde3600 kJ/kg of energy) is of limited practicality for biofuel application. As such, the strategy to operate a microalgae operation is to either avoid massive drying or produce high-value products to offset the high processing costs. In this section, we will first discuss the dewatering and drying processes, then describe how dry biomass, wet biomass, microalgae in culture and direct secretion can be implemented. Microalgae in culture involve
directly using cells, wet biomass is typically concentrated from harvested cells and dry biomass is obtained by drying wet biomass. Each area will be briefly reviewed while highlighting emerging technologies.

3.1 - Harvesting and dewatering processes

Initial harvesting is typically accomplished by either screening or sedimentation, where the concentration is increased from 0.1% of total suspended solids (TSS) to a slurry of about 2-7% TSS\textsuperscript{7,11,278,281}. In terms of screening, microstrainers and vibrating screens are the most commonly used. Standalone sedimentation by gravity is highly energy efficient for large cells\textsuperscript{281,282} but is unsuitable for many types of microalgae. Sedimentation can be enhanced by forming larger aggregates of cells, typically by chemical flocculation and coagulation. Ultrasound-assisted harvesting forces cell aggregation at acoustic nodes.\textsuperscript{283} This technique is free of chemical additives, operated continuously and has the flexibility of adopting different types and sizes of cells by adjusting the operating parameters such as acoustic energy density, contrast factor, and ultrasound frequency. Lab-scale experiments have demonstrated a concentration factor of 11.6 can be achieved at a flow rate of 25 mL/min.\textsuperscript{283} Other initial harvesting techniques includes air flotation\textsuperscript{284,285}, and electric field assisted harvesting\textsuperscript{7,278}. As discussed, biofilm cultivation\textsuperscript{143,286} has unique harvesting advantages but further development is required to make them cost and energy effective for biofuel production.

After initial harvesting, thickening techniques can be applied to concentrate the algae suspension to above 15% of TSS\textsuperscript{7,278,281}. Two major thickening techniques are centrifugation and filtration. Thickening saves a significant amount of energy compared to directly drying the harvested biomass\textsuperscript{10,287,288} but requires extra capital investment. The energy cost for this process depends on the characteristics of the cells, system design, and desired output concentration. Moreover, as the
desired output concentration increases, the energy cost associated with incremental percentage of dry biomass climb steeply.\textsuperscript{279,288} Centrifugation is commonly used in the lab and provides rapid water removal but it is very energy intensive, thus not practical at industrial scale for bioenergy applications, but is widely used to produce high-value products such as pigments, polyunsaturated fatty acids (PUFAs), phycobiliproteins, enzymes and toxins.\textsuperscript{151,289–291} Filtration is conceptually simple but potentially difficult to operate due to the two major issues: 1) the pore size needs to be small to increase efficiency but not too small to cause clogging issues and reduce flow rates; 2) easy recovery of algal biomass from the filter is desired without using the backwash which will lead to re-dilution of the product.\textsuperscript{236,278,279}

Dehydration is used to achieve the final high level of biomass concentration so that conventional extraction methods and infrastructure used for terrestrial plants can be used effectively. Typical dehydration techniques include solar drying, spray drying, freeze drying and belt drying. Solar drying is relatively easy to implement but it is hindered by the lack of control (overheating, weather dependency), long drying time and possible biomass degradation.\textsuperscript{11,292–295} Other drying processes are far too costly and energy intensive to be applicable for bioenergy applications.

3.2 - Processing for dry microalgae

Once microalgae biomass is completely dried through dehydration techniques, conventional physical disruption followed by chemical extraction and conversion techniques can be directly applied. Effective physical disruption can offset the need for harsh processing conditions required in the process of chemical extraction.\textsuperscript{276,290,295} Cell homogenization and bead milling are commonly used in the industry, and emerging disruption techniques such as microwave, pulsed electric field and ultrasonic are still under development.\textsuperscript{282,295,296} Among these techniques, microwave-assisted extraction (MAE) has attracted lots of attention due to its advantage of easy
operation, high energy transfer efficiency, rapid heating and relatively low cost. MAE has been investigated to be highly effective for pigments\textsuperscript{297} and lipids\textsuperscript{298} extraction from microalgae. MAE of fucoxanthin from Cylindrotheca closterium for 5 min enabled maximal extraction equivalent to 60 min of conventional solvent extraction method.\textsuperscript{297} With the addition of ionic liquids, MAE has been applied to extract lipids from wet microalgal biomass where extraction rates were increased by an order of magnitude in most cases.\textsuperscript{299} However, the cost associated with these disruption techniques is still too high for biofuel production. The requirements for physical disruption depend on the characteristics of the microalgae and specific extraction techniques could be simplified, or even eliminated, by genetic engineering or extraction process enhancement.

The most commonly used lipid extraction technique for dry microalgal biomass is organic solvent extraction which is found to be highly effective and cost efficient\textsuperscript{282,296}. Supercritical CO\textsubscript{2} extraction is not suitable for biofuel production due to its high initial and maintenance cost, but its application has increased in the nutraceutical and biochemical industry in recent years\textsuperscript{300–303}. The major advantage of using supercritical CO\textsubscript{2} as solvent is to produce contamination-free products and the associated cost can be offset by the high marginal value bioproducts. Other extraction techniques such as accelerated solvent, two-phase solvents and switchable-solvent extractions have been proposed and demonstrated to improve the extraction efficiency of intracellular components from microalgae\textsuperscript{304–306}.

Lipids extracted from microalgae can be further converted to biodiesel through several pathways: chemical transesterification, enzymatic conversion and catalytic upgrading. Among these, chemical transesterification is relatively mature and has been used widely for biodiesel production with conversion efficiencies above 90\%.\textsuperscript{307–309} However, algal oils are typically highly complex in contrast with vegetable oils, containing fatty acids, phospholipids, carotenoids, chlorophyll and
other components in various composition\textsuperscript{279,310}. Therefore, in order to accelerate this reaction, obtain higher conversion rate and reach the full potential of commercialization, a full understanding of strain specific oil composition is required. In contrast, enzymatic conversion uses biological catalysts (lipases) instead of acids or bases to convert lipids into biodiesel with less processing energy, easier removal of glycerol and catalysts and less alkaline wastewater pollution. Although, enzymatic approaches have advantages over conventional methods, other challenges associated with the cost of lipase, operational life, tolerance of the environment for enzymes and harvesting strategies for products need to be addressed for this path to run at a commercial scale\textsuperscript{311–313}. Catalytic upgrading of algal lipids into renewable gasoline, jet fuel and diesel can also be achieved by hydrotreating processes which react oils with hydrogen at high temperature and pressure in the presence of catalysts\textsuperscript{314–316}. This conversion process is commonly used in the petroleum industry to upgrade crude oil to produce a wide multitude of performance specified fuels. Ideally, this process leverages existing techniques and infrastructure albeit with catalysts and process parameters tuned for algal feedstocks.

Recent research in algal downstream processing, has shifted from a lipid-centric approach to a more holistic approach. If one can obtain value from all feedstock components the commercialization of microalgae technology will be more economic viable. For example, residual carbohydrates and proteins from microalgae can be used to enrich the nutrition in animal feed or further processed to produce biofuels through other conversions such as fermentation, anaerobic digestion and hydrothermal liquefaction. Another approach to achieve commercially feasibility in microalgae industry is to produce high-value bioproducts.\textsuperscript{13,39,55,317,318} These bioactive components present a great interest in pharmaceutical, cosmetic and nutraceutical industries and can easily offset the high costs associated with biomass processing. The understanding of microalgae and
development of technology gained in this approach will accelerate the accomplishment of producing sustainable energy from microalgae.

### Table 2 Summary of extraction and conversion techniques for different concentrations of microalgae biomass.

<table>
<thead>
<tr>
<th>Feedstock Type</th>
<th>Conversion/Extraction Techniques</th>
<th>Main Products</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry powder</td>
<td>Mechanical disruption and chemical extraction</td>
<td>Antioxidants, pigments, PUFA, additives</td>
<td>151,319</td>
</tr>
<tr>
<td></td>
<td>Direct Combustion</td>
<td>Heat</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>Pyrolysis</td>
<td>Bio-oil</td>
<td>321</td>
</tr>
<tr>
<td>Microalgae slurry (&gt; 15% TSS)</td>
<td>Hydrothermal Liquefaction</td>
<td>Biocrude</td>
<td>322–325</td>
</tr>
<tr>
<td></td>
<td>Supercritical water gasification</td>
<td>Syngas</td>
<td>326–328</td>
</tr>
<tr>
<td>Microalgae in culture (&lt; 10% TSS)</td>
<td>Fermentation</td>
<td>Bioethanol</td>
<td>329–335</td>
</tr>
<tr>
<td></td>
<td>Anaerobic digestion</td>
<td>Methane</td>
<td>336–340</td>
</tr>
<tr>
<td></td>
<td>Direct secretion</td>
<td>Hydrogen, alcohols or alkanes</td>
<td>341–343</td>
</tr>
<tr>
<td></td>
<td>Microbial fuel cells</td>
<td>Electricity</td>
<td>344–352</td>
</tr>
</tbody>
</table>

Without extracting lipids, direct combustion and pyrolysis have been investigated to directly produce energy from whole algae. Direct combustion of dry microalgae biomass releases the largest amount of energy. However, multiple life cycle assessments\(^{10,353,354}\) indicate drying the microalgae into powders costs more energy than the biomass contained, plus the challenges associated with ash content and emission control make direct combustion of microalgae is impractical on a commercial scale\(^ {355–357}\). Moreover, the energy released from direct combustion is in the form of heat which is less desirable than liquid biofuels used for transportation. Pyrolysis is a thermal decomposition process in absence of oxygen at high temperature (300 °C – 1000 °C), converting organic components into a wide range of products with hydrocarbon rich liquid (bio-
oil) as the desired product.\textsuperscript{358–360} Recent research\textsuperscript{321} indicated that fast or flash pyrolysis of microalgae with reaction time of 2-3 s, reaction temperature around 500 °C with heating rate of 600 °C/s is capable of achieving 18 – 24 % liquid yields with higher heating value of 29 MJ/kg. Pyrolysis bio-oil from microalgae has lower oxygen content and viscosity and larger higher heating value than that from woody biomass but still requires extensive refining for use in conventional fuel engines. More importantly, similar to direct combustion, the major roadblock in pyrolysis is removing the moisture content in the biomass which cause the overall process to be energy negative.

3.3 Processing for wet microalgae biomass

Avoiding the energy and cost intensive thickening and drying processes dramatically alleviates the energy burden for microalgae biofuel applications. Two promising conversion processes
applicable to wet biomass at a concentration above 15% TSS are hydrothermal liquefaction (HTL) and supercritical water gasification (SCWG).

HTL is one of the most promising conversion pathway for biofuel production due to the ability to employ raw wet feedstock, a fast reaction rate, a high-energy return on investment, good characteristics of biocrude, a low production of char, and the potential for recycling of nutrients (Fig. 13a and b). Similar to natural formation of petroleum-based fossil fuels, HTL converts biomass into biocrude at high temperature (200-380 °C) and pressure (5-28 MPa) in the presence

![Image](image_url)

**Fig. 13** Summary of current downstream processing techniques for wet microalgae biomass (a-f) and cells in culture (g-j). (a-b) Hydrothermal liquefaction$^{322,323}$. (c) Supercritical water gasification$^{327}$. (d-e) Ionic liquid treatment for wet extraction$^{381,382}$. (f) Astaxanthin extraction for hydrothermal disrupted cells$^{380}$. (g) Fermentation of pretreated wet biomass$^{329}$. (h-i) Anaerobic digestion of algal biomass$^{337,338}$. (j) Microalgae-microbial fuel cells$^{402}$. 
of water, albeit on a timescale of minutes by chemically and physically cracking down large biomolecules into small fractions\textsuperscript{322,324,325,361,362}. Compared to other thermochemical conversion techniques such as pyrolysis, the higher heating value of HTL biocrude is about 35 MJ/kg, much higher than typical pyrolysis bio-oil with a value of 20-25 MJ/kg\textsuperscript{363}.

A fundamental challenge in HTL of biomass is optimizing the processing conditions (temperature, pressure, residence time and heating rate) to obtain optimal yield and efficiency\textsuperscript{361,364,365}. To this end, HTL\textsuperscript{324,366,367} with a fast heating rate was investigated and resulted in higher biocrude productivity. However, the heating rate in this experiment was limited at 230 °C/min which was not able to explore the peak productivity with respect to heating rate. By eliminating the limits from conventional batch reactors, microdevices\textsuperscript{322} that allow direct observation of HTL (Fig. 13a) have been developed to precisely control processing parameters and perform real-time monitoring. The results indicate the higher heating values of biocrude approached saturation within 1 min due to early mechanical disruption of cells that enabled solvent extraction. Therefore, the HHV alone does not present the quality of biocrude whereas chemical composition and physical characteristics should also be considered.

A sequential HTL process\textsuperscript{368} was also performed to maximize the biocrude productivity and minimize the bio-char formation. A low temperature (140 – 200 °C) HTL was used as the first step to disrupt the cell and release intracellular products, polysaccharides in this case. The second step utilize a higher temperature (220 – 300 °C) HTL that converts the remaining biomass to biocrude which resulted in overall higher biocrude productivity and less bio-char. Lower polysaccharide content resulting in decreased bio-char production also agrees with HTL of individual categories of biomass feedstocks which indicates polysaccharides generates more solid products than protein and lipids.\textsuperscript{364} Similar to HTL, Hydrothermal carbonization (HTC) uses reaction temperatures in
the lower range (180 – 250 °C) with slightly elevated pressure (2 – 10 MPa) which tends to produce more solid products instead of liquid products\textsuperscript{369–371}. A recent comparison of torrefaction and HTC of lignocellulosic biomass indicates that HTC as the wet torrefaction method is the more favorable process in terms of energy content, hydrophobicity, and inorganic components of the solid products\textsuperscript{372}.

Analogous to HTL, SCWG reacts at higher temperature (400 - 700 °C) and pressure (> 22 MPa) to decompose biomolecules to produce syngas containing hydrogen, carbon monoxide and methane with a small quantity of solid and liquid products\textsuperscript{326–328,373} (Fig. 13c). Gasification is commonly combined with Fischer-Tropsch Synthesis (FTS) to convert syngas into liquid fuels\textsuperscript{374–376}. The major advantage of this pathway is the flexibility to produce a wide variety of fuels and products with known properties. Recent research indicates the issues for developing this process are: precipitation of inorganic salts, an unclear reaction mechanism associated with reaction temperature, pressure, heating rate and wall effects, the requirement of effective catalyst and high-temperature-resistant materials, and high energy costs\textsuperscript{359,371,373}. Conventional gasification (in an environment of insufficient oxidizer) of microalgae in a temperature range of 800 – 1000 °C was also studied and due to the requirement of dry biomass, this pathway for biofuel production usually resulted in negative net energy\textsuperscript{11,357,377}.

Another interesting application of subcritical water is to lyse cells for extraction of high-value intracellular products\textsuperscript{378–380}. A particular challenge is breaking robust cell walls to allow extraction, particularly with wet biomass\textsuperscript{380}. Lab scale tests have shown that hydrothermal (Fig. 13f) and ionic liquids (ILs) (Fig. 13d and e) can achieve comparable efficiencies as mechanical processing to dry biomass. Hydrothermal disruption of the cell wall at 200 °C effectively enabled solvent extraction of astaxanthin to achieve more than 95% efficiency as opposed to control with a 7.5%
in extraction efficiency.\textsuperscript{380} ILs have been used as extracting agents to replace organic solvents or pre-treatment material to disrupt the cell wall to enhance extraction. An extraction efficiency of 82\% was achieved using 1-Ethyl-3-methylimidazolium ethylsulfate, but a germination process of \textit{H. pluvialis} cysts is required to weaken the cell wall.\textsuperscript{381} ILs are also applied in a pre-treatment process to achieve > 70\% extraction efficiency, but the experiment utilized dry cell powders as feedstock which normally has weaker cell walls and the reusability of ILs needs improvement for large scale production.\textsuperscript{382}

There are also other approaches to directly extract intracellular lipids from wet biomass\textsuperscript{277,383–385}, but due to either high cost or low extraction efficiency none of them have been commercialized yet. For biofuel productions, since the current wholesale price for diesel is down to about 70 cents per liter, bringing additional steps or expensive additives into the process is most likely a non-starter.

\section*{3.4 - Processing for microalgae in culture}

To minimize the energy used in removing water from biomass, biologic conversion processes involving fermentation, anaerobic digestion, and direct secretion have been studied. In these pathways, biofuels can be either produced directly from microalgae culture or after initial harvesting with minimal energy cost. Lastly this section includes microalgae-microbial fuel cell approaches whereby microalgae are employed for direct electricity production.

Fermentation is a well-established method used in alcohol production to convert carbohydrates into ethanol by yeast. There are two major approaches to produce ethanol from microalgae: 1) like yeast, some microalgae such as \textit{Chlorella} and \textit{Chlamydomonas} can produce alcohols through heterotrophic fermentation\textsuperscript{330,331,386,387}; 2) microalgae containing a significant amount of
carbohydrates can be used as a sugar source for yeast fermentation\textsuperscript{332,333,335}. In the first approach, sugars can either be generated internally from the synthesis of microalgae or fed to algae externally, however the process lacks the productivity of yeast fermentation. The second approach usually requires pretreatment to promote hydrolysis of the cell wall to: 1) access to intracellular components; and 2) release fermentable sugars. Although research\textsuperscript{334} indicated the sugar released from wet microalgal biomass was lower than that from dried biomass, drying the biomass prior to conversion does not provide an energy return on investment\textsuperscript{353,354,357}. Recent research\textsuperscript{329} (Fig. 13g) demonstrated that acid-catalyzed pretreatment prior to lipids extraction released more than 90\% of fermentable sugars for wet microalgae biomass. Although noticeable achievements have been made in this pathway, significant breakthroughs are required to produce biofuels economically at industry scale with this approach.

Anaerobic digestion used in wastewater treatment to produce methane has the potential to exploit the entire organic carbon content of microalgae without the requirement for drying (Fig. 13h and i). It can also significantly benefit from the direct use of existing infrastructure and experience in the field of wastewater treatment\textsuperscript{336–338,388,389}. Methane as the main product in the process not only can be used as fuel but can also be converted to bioplastics to increase the value\textsuperscript{339,340,390}. However, this pathway is hindered by the low practical methane yields mainly due to the resistance of microalgal cell walls. Rigid cell walls of microalgae not only reduce the amount of digestible substrate but also limit the access of microorganisms to intracellular components resulting in low reaction rate\textsuperscript{388,389,391,392}. Thermal, chemical and mechanical pretreatments of microalgae were used to improve methane yields but the energy cost involved in the extra steps is higher than the energy gain from increased methane production\textsuperscript{336,389,393}. By tuning the pH and retention time, providing heat treatment and addition of methanogen inhibitors, this process can be altered to
produce hydrogen\textsuperscript{387,394} instead of methane but it requires significant development to be commercially available. The more likely role for anaerobic digestion is in combination with other methods to fully harvest the remaining value of biomass and therefore maximize the biofuel production.

3.5 Direct secretion from living cells

Direct secretion of products such as hydrogen\textsuperscript{395,396}, alcohols\textsuperscript{342,397,398} and alkanes\textsuperscript{341,343,399} from microalgae culture can be achieved through genetic engineering. Hydrogen production from \textit{Scenedesmus obliquus} was first observed by Gaffron and Rubin\textsuperscript{400} and having an anaerobic environment was later found to be critical for this process.\textsuperscript{401} Sulfur deprivation was used to inhibit the activity of PSII and enhance the activity of hydrogenase enzymes for hydrogen production. Breakthroughs in genetic engineering processing parameter control on sulfur quantity and immobilization of cells are expected to increase hydrogen productivity by further inhibiting PSII activity and increasing the activity of hydrogenase enzymes. Secreted hydrogen can be easily collected, however the low conversion efficiency remains a major roadblock for this pathway. Direct secretion of alcohols and alkanes appears to be a promising alternative but most information in this area is proprietary. Secretion of triterpene from Botryococcus braunii was reported to reach a volumetric productivity of 22.5 mg/L/photo-h at a high cell concentration of 20 gDW/L.\textsuperscript{283} These pathways usually require cheap source of CO\textsubscript{2} or sugars as feedstock and development to boost the feedstock-to-product conversion rates.

Microalgae-microbial fuel cells biologically convert solar energy to electrical energy and while the technology is maturing, the development of this conversion strategy is early stage\textsuperscript{345,346,348,349,402}. Microbes at the anode generate electrons, protons and CO\textsubscript{2} by digestion of organics and the microalgae at the cathode takes CO\textsubscript{2}, light, proton and electrons to grow through
photosynthesis (Fig. 13j). Also known as biological photovoltaics, or BPVs, this approach has seen many recent advances. Microfluidic approaches were applied to perform a qualitative investigation of several key factors including cell density, electron mediator concentration and light intensity and indicated the major obstacle for power production from BPVs is the transport of reducing equivalents across the cytoplasmic membrane.\textsuperscript{344,350} Understanding of electron transport mechanism is also believed to be essential for selection of photosynthetic microbes to enhance electrical output.\textsuperscript{347,352} Furthermore, the overall performance of BPVs is strongly associated with surface morphology and corresponding material characteristics\textsuperscript{403}, manipulation of thylakoid terminal oxidases\textsuperscript{404,405} and types of feedstocks\textsuperscript{406}. One of the challenges with this approach is the need for electrodes which tend to block light paths in a way similar to the CO\textsubscript{2} delivery mechanisms discussed earlier. A proof-of-concept cell was developed whereby both light and electrons were delivered via a metallic/plasmonic surface\textsuperscript{351}, however, that approach is not well suited to large scale production for a number of reasons. In general, there are many challenges with microalgal-microbial fuel cell approaches including technical obstacles, high operation costs and low power output that need to be solved for this process to be economically feasible. Similar to some other approaches above, this strategy can be more readily adopted in a wastewater treatment context, where the primary objective is remediation and power production is a side benefit. We note however, that this research area is adapting quickly and may well produce surprises \textsuperscript{407}.

**Perspective**

The potential for biofuel and bioproducts from microalgae to replace fossil fuels and convert CO\textsubscript{2} into valuable products with low land use and without competing with food supply is, of course, very attractive. However, in practice, the limitations of production from microalgae currently
constrain widespread adoption. This is especially true given the competitive technological landscape in sustainable energy with impressive developments in photocatalysis and electrocatalysis (coupled with inexpensive solar photovoltaics) side-stepping many of the limitations of photosynthesis – particularly the low conversion efficiency\textsuperscript{408,409}.

Nevertheless, microalgae remain the only sustainable and scalable option for the production of complex molecules such as nutraceuticals and long-chain hydrocarbons from solar energy. As such, overcoming the limitations of producing fuels and products from microalgae remains important. To this end, technology to inform and optimize microalgal cultivation and processing is rapidly developing.

As these technologies develop, removing current barriers is essential for application beyond a narrow niche. Multiplexed experimental platforms to discover optimal culture conditions provide a great advantage over conventional techniques in terms of throughput, they currently suffer from key limitations. First, they lack versatility in terms of spectral control. Second, the complexity of these devices limit their usability and consequently their industrial adoption. Finally, there is a great opportunity for improving the readouts possible with multiplexed platforms. The development of cultivation technology must not only maximize productivity – but must also produce in a manner conducive to efficient harvesting and downstream processing. Further, most of the technologies discussed in this review currently remain at lab-scale. While critical information can be obtained at the laboratory scale, production and processing technology will need to be scaled to make a widespread impact. Simplification of effective but complex lab-scale approaches will be essential for growth, and widespread adoption.

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