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Harvesting Microalgae for Food and Energy Products

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Microalgae are promising biological factories for diverse natural products. Microalgae tout high productivity, and their biomass has value in industrial products ranging from biofuels, feedstocks, food additives, cosmetics, pharmaceuticals, and as alternatives to synthetic or animal-derived products. However, harvesting microalgae to extract bioproducts is challenging given their small size and suspension in liquid growth media. In response, technologic developments have relied upon mechanical, chemical, thermal, and biological means to dewater microalgal suspensions and further extract bioproducts. In this review, the effectiveness and considerations were evaluated for the implementation of microalgae harvesting techniques. Nonbiological methods—filtration, chemical, electrical, and magnetic nanoparticle flocculation, centrifugation, hydrothermal liquefaction, and solvent-based extraction, as well as biological coculture-based methods are included. Recent advances in coculture algae-flocculation technologies that involve bacteria and fungi are summarized. These produce a variety of natural bioproducts, which show promise in fuel and food additive applications. Furthermore, this review addresses the developments of genetic tools and resources to optimize the productivity and harvesting of microalgae or to provide new bioproducts via heterologous expression. Finally, a glimpse of future biotechnologies that will converge to produce, harvest, and process microalgae using sustainable and cost-effective methods is offered.

1. Introduction

1.1. What Are Microalgae?

Algae are primarily photosynthetic aquatic organisms that possess little to no tissue differentiation. These diverse organisms vary in their size and physiology, and they belong to many different phylogenetic groups that are distinct from plants.[1] Microalgae are the subset of algae that are unicellular and range in size from several to a few hundred micrometers. Most microalgae diversity resides in freshwater or marine systems. Microalgae can grow in extreme environments such as deserts and polar regions,[2] and often show greater efficiency in synthesizing bioproducts compared to land plants.[3] Moreover, algae produce oxygen and sequester the greenhouse gas carbon dioxide at globally relevant scales,[4] and account for half of the oceans' net primary production.[5] They grow fast and can produce high-value biomass.[6] While microalgae are phylogenetically diverse, most biotechnology interest applies to the green algae (Chlorophyceae), diatoms (Bacillariophyceae), blue-green algae (Cyanobacteria), and Eustigmatophyceae (including *Nannochloropsis*), which are well-characterized species for valuable bioproducts.

1.2. Microalgae as Sustainable Biofactories

Microalgae have the potential to produce large amounts of valuable products sustainably, since they do not require arable land and can be produced using seawater, wastewater, or brackish water. Reduction in environmental impacts of fuels and food products will be important for the mitigation of climate change.[7] Microalgae are also being used in powerplants as a means to capture carbon dioxide and sequester it into biomass, which may provide opportunities for large-scale production of carbon-neutral energy and products.[8]

Vaccines and other pharmaceutical proteins are among the most high-value products that microalgae are used to produce, as well as effectively store and orally administer those products.[9] Other high-value products derived from microalgae include cosmetics,[10] food supplements and additives,[11] cooking oils,[12] and animal feed.[13,14] These have been developed as potentially more sustainable alternatives to synthetic or animal-derived products. Microalgae also provide feedstocks for biodiesel[15] and ethanol,[16] contributing to renewable and sustainable energy resource developments that may displace fossil fuels and food-derived fuels.

Genetic and synthetic biology approaches can accelerate the development of microalgae strains capable of producing novel specialty products[17–19] or producing conventional products with improved lipid content,[20] growth rate, and production efficiency.[21,22] Unlike field-grown transgenic crops, multiple

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layers of biocontainment can be employed to prevent the escape of genetically modified (GM) algae (and that of their transgenes).[23] Additional safety measures such as genetic addiction systems, kill switches, and bioreactor construction allow for the use of productive and specialized GM algae strains while protecting the environment.

1.3. Microalgal Markets

A 2018 estimate of the microalgae market valued it at US $1.7 billion, with a 5% compound annual growth from 2019 to 2027.[24] In response to the increasing demand for algae products, many efforts have been devoted to improving the harvesting methods or developing novel approaches for higher efficiency with a lower price tag in recent years (**Figure 1**). This is because harvesting incurs a substantial cost on microalgae producers, accounting for 3–15% of the total cost,[25] dependent on the harvesting method used. Microalgae have great potential as sources of diverse products, derived from atmospheric carbon dioxide, but require further innovations to challenge fossil fuels and terrestrial food crop feedstocks on price and quantity.

1.4. Microalgal Harvesting Technologies

Research into microalgae biology and technology is expansive. This review aims to summarize developments in techniques of harvesting microalgae and their products through conventional and newer biological flocculation methods, as well as the recent advancements in genetic tools for enhancing microalgae production systems.

2. Harvesting Microalgae

2.1. Conventional Harvesting Methods

Microalgae are cultivated in various types of indoor and outdoor bioreactors and open ponds (**Figure 2**A), which can produce a large amount of algal suspension that requires efficient and economical methods to harvest the biomass. These methods may be employed in combinations or alone, to concentrate algal suspensions or biofilms then manufacture final products. For most of the methods, harvesting (producing slurries of 2%–7% algal biomass) then dewatering (to concentrate to 15%–25% algal biomass) is conducted in series, prior to final extraction or drying of biomass.[26] Here we summarize the common techniques that have been used for harvesting algae (**Table 1**).

2.1.1. Centrifugation

Microalgae may be harvested by exploiting slight differences in density between algal cells and their culture media. As shown in Figure 2B, centrifugation enhances simple gravitational

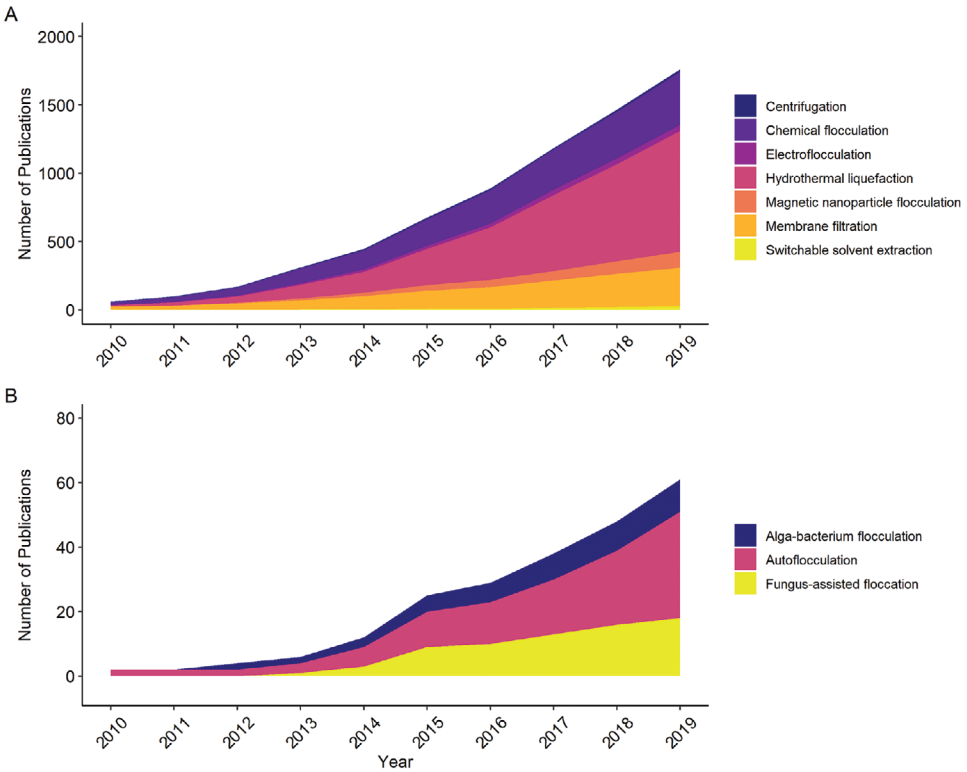

Figure 1. Cumulative publications related to microalgae harvesting technologies. A) Conventional and B) biological methods have both been employed for this task. Publication counts were determined by searching Web of Science for the respective search terms and "microalgae," then recording the number of returned hits. Number of publications for a given year is a cumulative number of all publications in Web of Science up to and including that year. Searches were performed on May 29, 2020.

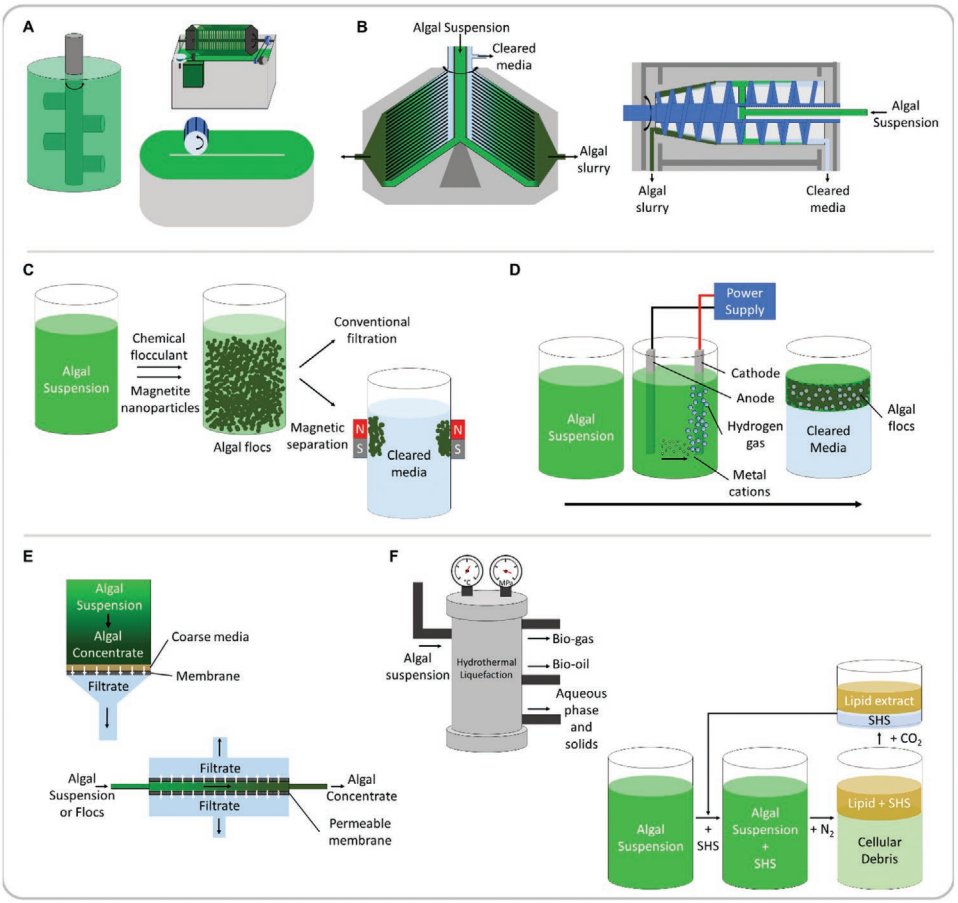

Figure 2. Microalgal production and harvesting techniques. A) Microalgae grown in closed bioreactors, rotating biofilm reactors, or open raceway ponds. B) Centrifugation methods including disc stack and decanter technologies. C) Chemical and magnetic nanoparticle flocculation. D) Electrocoagulation–floatation. E) Filtration dewatering by dead-end and tangential-flow filtration. F) Dewatering-independent technologies including hydrothermal liquefaction and switchable-hydrophobicity solvents (SHS).

sedimentation by applying centrifugal acceleration thousands of times greater than gravitational acceleration.[27] Centrifugation tends to be energy-intensive, but tradeoffs for flow rate and harvest efficiency can be used to reduce energy usage.[28] The high energy requirements of centrifugation favor higher-value algae products. Multiple types of centrifuges may be used based on capital costs, energy usage, maintenance, and biomass concentration, with disc stack and decanter centrifuges showing the most promise.[27]

While centrifugation produces satisfactory harvesting efficiency, low water content, and short process time, membrane filtration may allow for superior control of water content and lower cost from energy usage.[29] Due to the low water concentration achieved, centrifugation is a viable finishing step for algal biomass concentrated through other means.[25]

2.1.2. Chemical Flocculation

Induction of flocculation can be performed with a variety of chemical methods (Figure 2C), based on synthetic polymers such as polyacrylamides,[30] salts (especially alum, ferric chloride, various sulfates, and sodium hydroxide),[31–33] or biologically derived materials (e.g., eggshells, chitosan, and plant-derived compounds).[31,34] These approaches depend on a number of mechanisms to induce flocculation, including charge neutralization, electrostatic patch generation, bridging, and sweeping.[35]

Polymer flocculants, both synthetic and biologically derived, sometimes have high sensitivity to salt concentration[30] or pH,[36] which may be problematic for marine algae or algae grown in high pH media. Additionally, salt flocculants might contribute to high concentrations of metals to the extracted biomass.[31] Residual metals are less of a concern for lipid products, because the metals do not persist through processing.[32]

Despite some drawbacks, salt flocculants can produce high harvesting efficiencies, in excess of 99%, at low cost with reusable growth media.[32,37] Salt flocculants are effective across many species, both marine and freshwater.[31] Yet, biologically derived materials might be favorable for improved sustainability, lower toxicity, and the ability to keep cells intact, however, they have variable harvesting efficiencies.[31,34,38] While biologically derived flocculants may show a higher price per weight, occasionally the lower doses required make these preferable over salt flocculants for some species of microalgae.[31] Despite multiple benefits of chemical flocculation methods, the expense

Table 1. Performance of conventional harvesting methods.

Methods	Technology	Efficiency	Microalgae	Advantages	Limitations	Refs.
Centrifugation	Disc stack, decanter centrifuges	98.8%	*Aurantiochytrium* sp.	Highly concentrated final product, no chemical addition	Loss of floating biomass or ruptured cells, expensive	[29]
Chemical flocculation	Synthetic polymers	80–100%	*Chlorella vulgaris, Nannochloropsis oculata*	High efficiency, low dosage	Sensitive to pH and salt concentration, potential toxicity	[30,36]
	Salts	>90% (Ferric chloride); 79–99% (Alum);86–93% (Various metal sulfates)	Many species; *Nannochloropsis salina*; *Chlorella vulgaris*	High efficiency, inexpensive, safe for algal growth	High metal concentration in biomass, cell lysis	[31–33]
	Natural products	99.86% (*S. potatorum* seed powder); 0–97% (Chitosan)	*C. vulgaris*; Many species	Improved sustainability, reduced cell damage, lower toxicity	Potentially more expensive, not effective for some species (especially marine)	[31,37]
Nanoparticle flocculation	Conjugated or naked magnetite nanoparticles	95.86% (naked); 98.45% (PEI); 90.9% (plant polyphenols); >95% (CPAM); 95% (Arg)	*Scenedesmus* sp.; *C. pyrenoidosa*; *C. vulgaris*; *Botryococcus braunii* and *C.* ellipsoidea; *Chlorella* sp.	Low dosage, low energy demand, reusability of flocculant	Expensive flocculant material	[39,41,42,45,48]
Electrocoagulation— floatation	Electric current used to induce floc formation	99%; 92%; 96.75%	*C. vulgaris*; *Nannochloropsis* sp.; *C. pyrenoidosa*	No chemicals added, fast, enhanced reusability of growth media	Replacement of electrodes, not effective in freshwater, high energy demand, large amount of metals in biomass	[50,53,54]
Filtration	Microfiltration	70–100%	*C. vulgaris*; Mixed culture	Greater initial flux, low final water content	Fouling from pore clogging, loss of damaged cell contents	[67,69]
	Ultrafiltration	100%	*C. vulgaris*	Avoids fouling, more efficient overall	Lower initial flux	[69]
Dewatering-independent methods	Hydrothermal liquefaction	38–66% bio-oil yield	*Chlorella* sp.; *Nannochloropsis* sp.; *Nannochloropsis* sp. and *Pavlova* sp.; *Nannochloropsis gaditana*	Avoids vaporizing water, converts biomolecules to bio-oil	Poorly characterized chemistry, need for solid management and capable equipment.	[71–73,155]
	Switchable solvent extraction	5–22% lipid yield	*B. braunii,; C. vulgaris*	Avoid water removal, less solvent than hexane extraction, higher yield than chloroform-methanol extraction	Toxic solvents, sample pretreatment	[74–76]

of flocculating chemicals and contamination of biomass are limiting factors to their widespread adoption.[25]

2.1.3. Nanoparticle Flocculation

Magnetite (Fe_3O_4) nanoparticles (MNPs) have been recently tested as a reusable means to capture microalgae from bulk algal suspensions (Figure 2C). MNPs have generally been applied for capturing *Botryococcus braunii*,[39] *Microcystis aeruginosa*,[40] *Chlorella*,[39,41,42] *Nannochloropsis*,[43,44] *Scenedesmus*,[41,42,45] and *Chlamydomonas* spp.[46] These particles, about 12 nm in size, attach to microalgae and allow for harvesting the algae–nanoparticle aggregate with magnets.[45]

MNPs can be functionalized to improve properties including stability and harvesting efficiency,[45] typically by improving MNPs' adherence to negatively charged algal cells via electrostatic attraction with a positively charged coating material.[47] Coatings including cationic polyacrylamide (CPAM),[39,46] polyethyleneimine (PEI),[40,41] and plant polyphenols[42] were able to produce harvesting efficiencies of >95% for *B. braunii* (1.8 g algal dry weight L^{-1}), *Chlorella ellipsoidea* (0.7 g L^{-1}), and *Chlamydomonas* sp. (1.2–1.5 × 10^7 cells mL^{-1}), 98.45% ± 0.35% for *Chlorella pyrenoidosa* (0.5 g L^{-1}) and 97.5% for *M. aeruginosa* (1.5 × 10^6 cells mL^{-1}), and 90.9% of *Chorella vulgaris* (3.06 g L^{-1}), respectively. Amino acids can additionally be used to functionalize MNPs, with arginine-functionalized MNPs showing 95% harvesting efficiency.[48] However, another study produced a higher efficiency (a theoretical 95.68% from parameter optimization) using nonfunctionalized nanoparticles, compared to those functionalized with cetyltrimethylammonium bromide (CTAB), PEI, or amine.[45]

One proposed benefit of MNPs is their reusability. The nanoparticles can be cleaned by increasing the pH and rinsing[45,49] and/or ultrasonication[42,45] followed by recoating[42] if needed. In the examined studies, harvesting efficiencies of reused particles remained high, from 90.9% to 80.2% after 10 cycles[42] and 90% to 84.1% after five cycles.[45] MNPs can be cost-effective, at an estimated US $347 ton^{-1} of harvested algae, even without reuse and at a 1:1 ratio of biomass to MNP mass.[48]

2.1.4. Electrocoagulation

Microalgae can be induced to form flocs with the application of electric current (Figure 2D), via the charge neutralization of algal cells that results in sweeping flocculation as uncharged cells stick together.[50] The charge neutralization results from the in situ production of metal flocculating salts such as iron and aluminum hydroxides, a process known as electrocoagulation (EC) or electroflocculation.[51] EC primarily favors algae growing in highly conductive media, such as saltwater[52] and wastewater,[51] as these require reduced electricity inputs. In contrast, freshwater algae such as *Chorella vulgaris* require the addition of salts such as 1.5 g L^{-1} NaCl, which increases the cost.[50] Additionally, electrolysis of water produces hydrogen microbubbles on the cathode, which contribute to harvesting by flotation of algae, contributing in one study to at most 36.6% to the total harvest.[50] The use of this flotation process is considered as electrocoagulation–floatation.

Multiple factors influence the efficiencies of EC, including electrode material, pH, temperature, current density, and algal cell density.[51] Harvesting using EC has can produce efficiencies of 99%,[50] 96.75%,[53] and 92%.[54] While harvesting may approach 100% efficiency and energy use may be under 1 kWh kg^{-1} of algae, the method is much less energy efficient than bioflocculation combined with filtration,[55] and may have somewhat lower flocculation efficiency than ferric chloride (96.75% compared to 98.84%).[53] However, at optimum conditions, the cleared media from electrocoagulation could support greater algal growth, with a media composed of 20% fresh and 80% recycled media producing 0.68 g L^{-1} biomass for $FeCl_3$ flocculated compared to 0.76 g L^{-1} for EC harvested and 0.98 g L^{-1} for new media.[53] Also, the conditions favoring the highest harvesting efficiency may consume 10 times more electricity per kilogram than the most energy-efficient conditions,[52] so harvesting efficiency may not be the primary target for optimization. Replacing the sacrificial anode and fouling of the cathode are other challenges with EC.[56] As with salt flocculation, high metal concentration in the final products can be problematic.[54]

2.1.5. Filtration

As shown in Figure 2E, the use of permeable membranes to capture microalgae includes macrofiltration, microfiltration (MF), and ultrafiltration (UF). These approaches can be optimized with different pore sizes, each of which can be used in cross-flow (tangential-flow) or dead-end filtration systems with applied pressure or vacuum.[57] Membranes may experience fouling (clogging of pores via use), which is dependent on pore size, favoring membranes with finer pores in most cases.[58] While smaller pore sizes limit flux (rate of filtration) with pure water, the reduced fouling may justify the use of UF membranes.[59–61] Fouling may depend on system flow, whereby alternating the direction of flow into the filter[62] or using cross-flow systems with higher bulk flow velocity[63] improve filtration performance by reducing fouling. Sand or other coarse materials have been used to avoid fouling of the membrane, with improvements in cost effectiveness.[64] Membrane composition is important, as it can lead to substantial differences in flux for a given pore size with pure water,[59] and can induce fouling by the adsorption of polysaccharides or other macromolecules on hydrophobic membranes.[60,61] Negatively charged surface coatings can similarly improve resistance to fouling.[65] Negatively charged membrane surfaces can alternately be created by applying current to conductive ceramic filters, with comparable benefits to fouling resistance.[66]

Filtration methods often show a 100% retention rate of cells (cells in retentate per cells in initial suspension), however, this may exclude any cell debris not captured by the membrane.[58] Recovery rate (cells available to downstream processes per cells in initial suspension) may be a more useful measure.[58] Although less frequently measured, it has been measured previously at 70–89%,[67] set at 80%,[68] or used synonymously with retention rate.[69]

2.1.6. Dewatering-Independent Methods

The previous methods in Sections 2.1.1–5 produce concentrated algal slurries having most of the water volume removed. The resulting slurry must then still be extracted to access lipids or other desired compounds residing in algal cells. Other methods exist (Figure 2F), including hydrothermal liquefaction (HTL) and solvent-based methods, which do not require an initial harvesting step, but rather are completed in situ in the algal suspension at growing density. Alternatively, algae may be grown out of suspension in algal-biofilm reactors, which can harvest biomass by scraping off adhered cells.

Hydrothermal liquefaction uses high pressure (5–40 MPa) and temperature (200-600 °C) to convert wet biomass into a hydrocarbon mixture sometimes called "bio-oil" or "biocrude."[70] The process is energy efficient because it avoids dewatering and a phase change to steam, while converting additional biomolecules to hydrocarbon products.[70] Yields of bio-oil have been measured at 66% of algal dry weight for *Nannochloropsis* sp., yet in this experiment, the algae were preconcentrated to standardize conditions across strains.[71] Catalysts such as Na_2CO_3 or CaO may be used to improve yields, but only for some strains and under particular reaction conditions.[72,73]

Another method uses compounds classified as switchable hydrophilicity (or polarity) solvents. These are solvents, dependent on the ionic state, which may solubilize in aqueous solution as hydrophilic or separate with extracted lipids as hydrophobic. This polarity change may be accomplished by bubbling CO_2 to make the solvent hydrophilic, or N_2 to convert to a hydrophobic and lipophilic state.[74] Experiments with *B. braunii* have yielded 16% lipid per dry weight from freeze-dried and 8% from aqueous algae[74] and 22% from freeze-dried algae,[75] which is better than comparable hexane extraction producing 7.8% and 5.6% for freeze-dried and aqueous samples, respectively. For *C. vulgaris*, extraction with the solvent C_6DIPA-Im from 80% water content microalgae sludge produced 12.38% ± 0.18% lipid, compared with 10.48% ± 0.31% for chloroform–methanol extraction.[76] While switchable solvents can avoid the use of methanol and chloroform in extraction from aqueous algae suspensions, they may still require drastic pH change (up to 13) and produce small yields of lipids.[74] However, newer techniques avoid heating or drastic pH changes, and can be reusable with high efficiency (83.6 ± 3.6% of initial efficiency over five cycles).[76]

To reduce or eliminate the needs for algal removal from liquid media, algal biofilm or "algal turf" reactors have been developed.[77,78] These reactors function by rotating a substrate material, through liquid growth media then air. Some designs have employed steel mesh or polycarbonate disks,[79] cotton fabric,[77,78] or cotton rope[78] as substrates. After a period of growth, algal cells are scrapped from the substrate, producing concentrated algal sludge (143–168 g kg^{-1} dry weight biomass for the disk system).[79] Such systems may expand growth area without increasing pond size, have greater productivity than suspended cultures, and show improved regrowth times.[77,78] This production methods shows effects on the final algal product, however, with potential for much lower lipid content but increased protein content compared to a flat panel bioreactor.[77] Additionally, algal biofilm reactors can be effective at removing total dissolved solids from wastewater.[80]

2.2. Bioflocculation Methods

Unlike conventional methods, bioflocculation seeks to use biological agents to collect microalgae, which avoids potentially toxic flocculants or compromised products (**Figure 3**). Bioflocculation methods are also considered more cost-efficient, sustainable, and environmentally friendly than their conventional counterparts.[81] As with many of the conventional methods, bioflocculation is followed by further steps in the production process. Current progress in bioflocculation techniques is summarized in **Table 2**.

2.2.1. Autoflocculation

Autoflocculation occurs when algal cells bind to each other to form aggregates in the culture, which can be triggered by introducing flocculating algae into a culture of nonflocculating algae, by changing culture conditions such as pH, and by cultural aging (Figure 3A). Algal strains that can autoflocculate are described in Table 2.

Alga–Alga Flocculation: The addition of flocculating algae can trigger the flocculation and concentrating of the nonflocculating algae of interest through cell–cell interactions. The method is simple and effective, and no chemical flocculants are required. Similar cultivation conditions can be used for both the flocculating and target algae, and the medium can be recycled for cultivation.[81,82] The method is of particular interest because the flocculating algae can also provide valuable biomass such as lipids and proteins,[83] which increases the industrial value of the final product.

Although autoflocculation does not occur in all species, many self-flocculating microalgae have been identified including *Ankistrodesmus falcatus*,[81] *Chlorella vulgaris* JSC-7,[84] and *Scenedesmus obliquus* AS-6-1,[85] and these algae can be used for harvesting other microalgae of interest (Table 2). For example, *Phormidium* sp., a filamentous cyanobacterium, could capture the small green alga *Chlorella* sp. (1.3–7.6 µm) with its dense and tangled filaments, and form large granules that ranged from 600 to 2000 µm in size, which promoted sedimentation of both microalgae with a recovery efficiency higher than 99%.[86] Studies on the autoflocculation of *C. vulgaris* JSC-7 and *S. obliquus* AS-6-1 have suggested that the self-flocculation of these two strains was due to their cell wall-associated polysaccharides.[84,85] The crude extract of cell wall-bound substance of *C. vulgaris* JSC-7 was able to isolate over 80% of nonflocculating alga *Chlorella vulgaris* CNW11 at a low dosage of 0.5 mg L^{-1}.[84] Similarly, the crude and purified extracellular substances of *Scenedesmus obliquus* AS-6-1 could harvest freely suspended *S. obliquus* FSP-3 with over 80% flocculating efficiency.[85] Another study on the oleaginous *Ettlia texensis*, an autoflocculating green alga, has shown that extracellular polymeric substances and particular glycoproteins are responsible for the self-flocculation, and they also facilitated efficient bioflocculation of other microalgal species including *Chlorella vulgaris* SAG211-11b.[82]

Alga–alga flocculation occurs when the flocculating cells or algal flocculants patch or capture adjacent nonflocculating algal cells or by the development of bridges among the cells through neutralization of charges in the culture. The method does not require the addition of chemicals, nor the cost in procuring them. Also, since no extra chemicals are used, the algal medium can be readily reused for a new batch, without expensive filtration or chemical treatments, and the algal biomass can be directly processed for the final products. However, one major limitation of this method is that the process may take considerable time and the efficiency is relatively low, depending on the species. However, using crude extracts or exudates instead of intact cells as flocculants can improve the harvesting efficiency. For example, the green alga *Coelastrum* cf. *pseudomicroporum* cultivated with urban wastewater was enriched in carotenoid (up to 0.47 mg L^{-1}). Further, its exudates could be used to flocculate the green alga *Scenedesmus ellipsoideus* with over 95% harvesting efficiency.[87] Using water-soluble extracts of the marine alga *Skeletonema marinoi*, *Nannochloropsis oculata*, a marine alga enriched in omega-3 eicosapentaenoic acid (EPA), was harvested with 95% efficiency after 6 h settling.[88]

pH-Induced Autoflocculation: In response to changes in growth conditions, especially high acidic or alkaline pH, many algal species can form large cell–cell flocs and settle by gravity (Table 2). Algal autoflocculation may occur along with algal photosynthesis, which consumes CO_2, a weak acid when dissolved, increasing pH levels and promoting autoflocculation.[89] However, the process is slow and regulation of pH using acids (i.e., HNO_3) and bases (i.e., NaOH) can rapidly change the cultural pH and induce high-efficiency autoflocculation in minutes.[90,91] The low and high pH levels are known to reduce the intensities of the negative charge on the surface of algal cells, thereby promoting self-aggregation.[90,92] Both conditions may inhibit the growth of algae and the pH level required to induce autoflocculation is species-dependent because the flocculation is mainly based on the cell wall composition. Using HNO_3, pH decreases for the freshwater microalgae *Chlorococcum ellipsoideum*, *Chlorococcum nivale*, and *Scenedesmus* sp. achieved flocculation efficiencies over 90%, with the optimum efficiency observed at pH 4.0–4.5 and staying stable from pH 1.5 to 4.0.[90] These self-flocculating algae were observed to form large flocs, where the target microalgae such as *Chlorella zofingiensis* and *C. vulgaris*, two small microalgae not flocculating responsive to pH decrease, were attracted and trapped, and settled together in the flocs by gravity.[93]

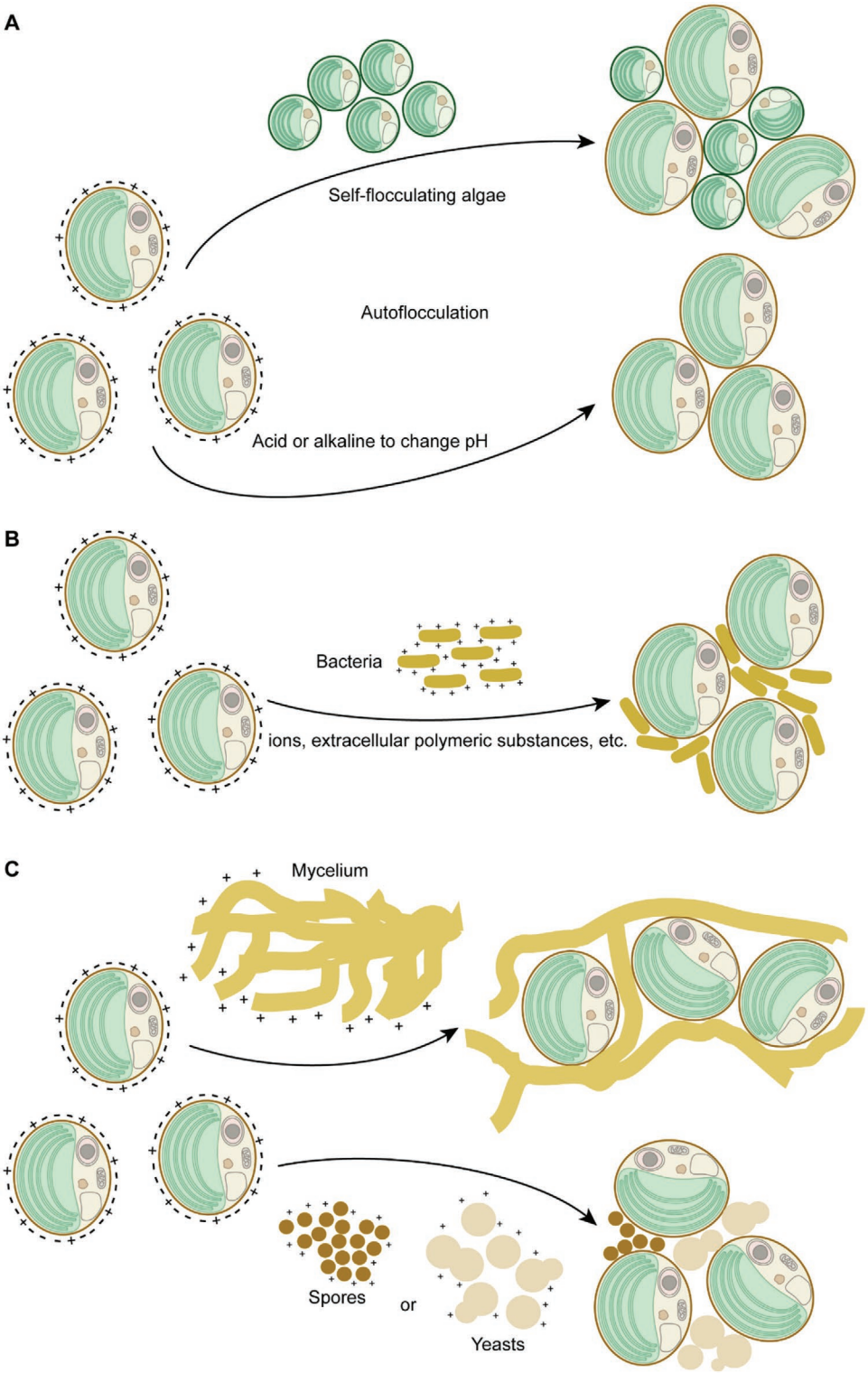

Figure 3. Harvesting microalgae with biological materials. A) Algal autoflocculation. B) Bacterium-based flocculation. C) Fungal pelletization and flocculation.

Similar to the acidic conditions, alkaline pH levels can trigger autoflocculation by surface charge neutralization and cell aggregation. In the cultures of the marine microalgae *Nannochloropsis* sp. and *Phaeodactylum tricornutum*, adjusting pH to 9.0–9.3 led to effective flocculation with the highest efficiencies above 90%.[92] Although high pH levels may affect cell

Table 2. Autoflocculation of microalgae.

Methods	Microalgae	Flocculant	Optimization	Efficiency	Refs.
Alga–alga autoflocculation	*Chlorella* sp.	*Phormidium* sp.	Algal granule formation	≈99%	[86]
	Chlorella vulgaris	*Ankistrodesmus falcatus*; *Chlorella vulgaris* JSC-7; *Chlorococcum nivale*; *Chlorococcum ellipsoideum*; *Ettlia texensis*; *Scenedesmus obliquus* AS-6-1; *Scenedesmus* sp.	CO_2 enriched airflow; gently mixing after the addition of flocculant algae	40–85%	[81,82,84,85,93,156]
	Chlorella zofingiensis	*Chlorococcum nivale*; *Chlorococcum ellipsoideum*; *Scenedesmus* sp.	CO_2 enriched airflow	60–80%	[93]
	Nannochloropsis oculata	*Skeletonema marinoi*	Crude extract as flocculant	95%	[88]
	Neochloris oleoabundans	*Tetraselmis suecica*	CO_2 enriched airflow	≈70%	[81,156]
	Scenedesmus ellipsoideus	*Coelastrum* cf. *pseudomicroporum*	Algal exudates as flocculant	>95%	[87]
	Scenedesmus obliquus FSP-3	*Scenedesmus obliquus* AS-6-1	Addition of 0.6 mg L^{-1} cell wall-associated polysaccharide	88%	[85]
pH-induced autoflocculation	*Chlorococcum ellipsoideum*	pH 4.0–4.5	CO_2 enriched airflow	>90%	[90]
	Chlorococcum nivale	pH 4.0–4.5	CO_2 enriched airflow	>90%	[90]
	Scenedesmus sp.	pH 4.0–4.5	CO_2 enriched airflow	>90%	[90]
	Nannochloropsis sp.	pH 9.0–9.3	CO_2 enriched airflow; addition of Mg^{2+}	≈90%	[92]
	Phaeodactylum tricornutum	pH 9.0–9.3	CO_2 enriched airflow; addition of Mg^{2+}	≈90%	[92]
	Chaetoceros calcitrans	pH 10.2	Addition of polyelectrolyte flocculant	>90%	[94]
	Nannochloropsis oculata	pH 10.4	CO_2 enriched airflow	90%	[95]
	Chlorella vulgaris	pH 10.5–11	CO_2 enriched airflow; addition of Ca^{2+} and Mg^{2+}; slaked lime for low cost	>90%	[92,98]
	Chaetoceros muelleri #862	pH 11.5	Concentrated algal culture (0.42 g L^{-1})	100%	[101]
	Scenedesmus quadricauda #507	pH 11.6	Concentrated algal culture (0.54 g L^{-1}); addition of synthetic ocean water	≈95%	[101]
	Chlorococcum sp. R-AP13	pH 12	Re-use medium after flocculation	94%	[91]
	Ettlia sp. YC001	pH 12.5	CO_2 enriched airflow	>90%	[96]

growth, increasing pH in the culture was observed to effectively enhance flocculating efficiency. For example, at pH 10.2, the diatom *Chaetoceros calcitrans* self-flocculated with an efficiency of over 90%, whereas no obvious autoflocculation was observed at pH 8.0.[94] Autoflocculation of the marine alga *Nannochloropsis oculata* started at pH 9.5 and reached the highest efficiency of 90% at pH 10.4.[95] Increasing pH to over 12.0 was reported to dramatically improve the flocculation efficiencies to over 90% in the freshwater microalgae *Chlorococcum* sp. R-AP13[91] and *Ettlia* sp. YC001.[96] As shown in Table 2, pH adjustment promotes autoflocculation in various microalgae, however, it did not work for some microalgal species such as the marine alga *Isochrysis* sp., which had a flocculating efficiency less than 30% after pH adjustment.[97] Resistance to flocculation by pH may result from cell surface properties of *Isochrysis* sp., but has not been investigated. Thus, to increase the harvesting efficiency of algal autoflocculation, pH alteration was often applied with other flocculating stimulators including the addition of metal ions such as calcium and magnesium[95,98–100] and supplement of natural and synthetic flocculants such as synthetic ocean water[101] and Magnafloc LT 25 and LT 27.[94]

Autoflocculation by Other Stimulates: In addition to pH adjustment, other stimulates have been reported to facilitate algal autoflocculation: changes in concentration of dissolved oxygen;[102,103] alteration in the cultural nutrients such as ammonium[104] and nitrate;[100] and, culture aging.[85,91] In general, autoflocculation-based techniques provide efficient, economical, and environment-friendly strategies to harvest microalgae, without a heavy dose of chemical flocculants. The flocs are relatively easy to process for final products and the medium could be effectively recycled for future cultivation.

2.2.2. Bioflocculation with Microbial and Other Natural Flocculants

Bioflocculation has been considered as a sustainable and environmentally friendly technique for algae harvesting. Instead of chemical flocculants, bioflocculation methods isolate microalgae with natural materials such as bacteria (**Table 3**) and fungi (**Table 4**). The bioflocculants can be cocultured with the target microalgae or cultivated separately before adding to the algal culture, dependent on the interaction between the algae and flocculants. The mechanism of bioflocculation is diverse and the applications of bioflocculation are discussed here.

Alga–Bacterium Flocculation: Although there are some concerns about bacterium contamination in the biomass that

Table 3. Bacterium-based flocculation.

Methods	Microalgae	Flocculant	Efficiency	Refs.
Alga–bacterium flocculation	*Chlorella vulgaris*	*Bacillus* sp.; *Flavobacterium* sp.; *Paenibacillus polymyxa* AM49; *Terrimonas* sp.; *Sphingobacterium* sp.	80–94%	[107,108,115]
	Chlorella pyrenoidosa	*Citrobacter freundii*	≈90%	[109]
	Chlorella zofingiensis	*Escherichia coli*	≈70%	[110]
	Nannochloropsis oceanica	*Solibacillus silvestris*	90%	[111]
	Scenedesmus dimorphus	*Escherichia coli*	≈80%	[110]
	Scenedesmus sp.	*Paenibacillus polymyxa* AM49	95%	[116]
	Synechocystis UTEX 2470	*Klebsiella pneumoniae* NY1	>90%	[114]

might affect the final products for food or feed purposes, bacterial flocculation is an efficient, economical, and environment-friendly approach to harvest microalgae, especially for bioenergy products.[35] In fact, many bacterial species coexist with algae in industrial-scale cultures of open ponds and even in closed bioreactors. The interaction between bacteria and algae is mostly species-specific, and it can be mutually beneficial to each other and increase the productivity of certain biomass such as lipids.[105,106] Although the mechanism of bacterial flocculation is not yet clear, it is believed that charged functional groups in bacteria aggregate algal cells by neutralizing the charge and electrostatic patch (Figure 3B). In the green alga *C. vulgaris*, a 94% flocculating activity was observed in the culture containing various bacteria compared to 2% self-flocculation in the axenic culture. Three alga-associated bacteria, *Flavobacterium* sp., *Terrimonas* sp., and *Sphingobacterium* sp., were identified to promote the flocculation by forming large flocs with the *C. vulgaris* cells.[107] Two strains of the soil bacterium *Bacillus* sp. (y3 and y6) were able to flocculate *C. vulgaris* cells and promote the algal growth, likely by regulating ROS and antioxidant system response of the algal cells.[108]

Other studies have also reported successful practices of harvesting oleaginous microalgae using bacteria. For example, the freshwater green alga *C. pyrenoidosa* was flocculated by *Citrobacter freundii* with over 90% efficiency.[109] Oil-producing green algae *Scenedesmus dimorphus* and *Chlorella zofingiensis*, also known for being a promising producer of the antioxidant astaxanthin, were harvested by coflocculating with *Escherichia coli*, and the efficiency can be increased with UV irradiation and polyethylenimine-coating on the bacterial cells.[110] Culture broth of *Solibacillus silvestris*, a bacterial strain isolated from activated sludge, showed 90% flocculating

Table 4. Harvesting microalgae by alga–fungus interaction and natural flocculants.

Methods	Microalgae	Flocculant	Efficiency	Refs.
Alga–fungus interaction	*Chlamydomonas reinhardtii*	*Saccharomyces bayanus*	95%	[132]
	Chlorella protothecoides (*Auxenochlorella protothecoides*)	*Aspergillus fumigatus*	≈90%	[123]
	Chlorella pyrenoidosa	*Aspergillus fumigatus*; *Mucor circinelloides*	>90%	[109,122]
	Chlorella sorokiniana	*Isaria fumosorosea*	97%	[157]
	Chlorella sp.	*Penicillium* sp.; *Pleurotus ostreatus*	65–98%	[120,134]
	Chlorella vulgaris	*Altermaria alternata*; *Aspergillus fumigatus*; *Aspergillus niger*; *Aspergillus nomius*; *Aspergillus oryzae*; *Aspergillus* sp.; *Cunninghamella echinulata*; *Mucor circinelloides* UMN-B34; *Mucor hiemalis*; *Nigrospora oryzae*; *Saccharomyces pastorianus*	>90%	[121,124–127,133,158,159]
	Chroococcus sp.	*Aspergillus lentulus*	>90%	[130]
	Nannochloropsis oceanica	*Mortierella elongata*	≈70%	[128]
	Nannochloropsis sp.	*Aspergillus nomius*	94%	[125]
	Picochlorum sp. HM1	*Saccharomyces bayanus*	75%	[132]
	Pseudokirchneriella subcapitata	*Aspergillus fumigatus*	>90%	[159]
	Scenedesmus quadricauda	*Aspergillus fumigatus*	>90%	[159]
	Synechocystis PCC 6803	*Aspergillus fumigatus*	>90%	[129]
	Tetraselmis suecica	*Aspergillus fumigatus*	≈90%	[123]
Natural flocculants	*Chlorella* sp.	*Moringa oleifera* (seed powder)	>95%	[160,161]
	Chlorella vulgaris	*Moringa oleifera* (seed powder); *Strychnos potatorum* (seed powder)	≈85%	[37,162]
	Chlorella vulgaris	Seafood wastewater	0.49 g L^{-1}	[163]

efficiency on the marine alga *Nannochloropsis oceanica*. Subsequent chemical analysis revealed an efficient bioflocculant produced by *S. silvestris*, which is a proteoglycan composed of 75% carbohydrate and 25% protein. This reusable proteoglycan flocculant does not affect the algal growth and it can work without the addition of metal ions.[111] Other bacterium-derived materials have been used for harvesting microalgae such as poly-gamma-glutamic acids (γPGA). With no damage to the cell integrity, γPGA from *Bacillus subtilis* has been shown to efficiently (>90%) flocculate various oleaginous microalgae including *Botryococcus braunii*, *C. vulgaris*, *Nannochloropsis oculata*, and *Phaeodactylum tricornutum*.[112] Similar γPGA produced by the soil bacterium *Bacillus licheniformis* was used to isolate the thermo-tolerant freshwater alga *Desmodesmus* sp. F51 with a flocculating efficiency of up to 99%.[113]

In addition to bioenergy products, bacterial flocculation has been reported for wastewater treatment. By screening and isolation of bacterial strains from wastewater sediments, a bacterial strain *Klebsiella pneumoniae* NY1 was identified with high flocculating activity, which produced a high level (14.9 g L^{-1}) of MNXY1, a bioflocculant containing 26% protein and 66% carbohydrate. MNXY1 was used to flocculate the cyanobacterium *Synechocystis* sp. UTEX 2470 (>50% in 10 min and 95% in 1 h) at a laboratory scale and removed 72% suspended solids from the wastewater at a dose of 44 mg L^{-1}.[114] Many efforts have been made to increase the efficiency of bacterial flocculation. For example, the addition of 6.8×10^{-3} M calcium could increase the flocculating efficiency of *C. vulgaris* cells from 72% to 83% when using *Paenibacillus polymyxa* AM49 as the flocculant.[115] A subsequent study optimized the method using 1% bioflocculant from the culture broth of *P. polymyxa* AM49 and 8.5×10^{-3} M $CaCl_2$ and 0.2×10^{-3} M $FeCl_3$ as coagulants and achieved 95% flocculating efficiency in high density (2.35 g L^{-1}) culture of the green alga *Scenedesmus* sp. KCTC AG20831.[116] The recycled medium showed an 8% decrease in the biomass yield, which could be recovered by the addition of 20% fresh medium supplements. Purification of flocculants from bacteria avoids the potential biohazard concern of bacterial contamination in the final products but increases the production cost for preparing the flocculants. Future bioprocess and genetic engineering approaches could promote the commercial application of bacterial flocculation by increasing the productivity of flocculating mass, improving affinities towards the target microalgae, and engineering the bacterial flocculants for value-added bioproducts.

Alga–Fungus Pelletization and Flocculation: Eukaryotic microalgae and cyanobacteria are known to interact with fungi and yeasts. In fact, these organisms can form natural symbiotic consortia known as lichens, which have unique morphology and metabolisms.[117,118] Many fungal species, especially filamentous ones, have self-pelletizing abilities. Taking advantage of the algal–fungal interaction, the hyphal tissue (mycelium) of filamentous fungi can capture or trap microalgae and form large pellets for harvesting the small algal cells (**Figure 4**). In addition, some fungal strains produce large amounts of spores that can flocculate microalgae,[119,120] somewhat similar to the cell coagulation in the coculture of yeast and algae. In most cases, fungal mass is prepared separately in a fermenter or bioreactor, and the resultant materials are applied to harvest algae by copelletization or flocculation. The coculture of algae and fungi can also form large flocs, where fungal cells could have positive or negative impacts on the productivity of the algae, dependent on species combinations and culture conditions. In general, fungal pelletizing and flocculation methods can be economical for harvesting microalgae and producing valuable biomass as feedstocks for various food and energy products (Table 3).

Copelletization of fungi and algae has been reported in many combinations of species. For example, many efforts have been made to harvest *Chlorella*, a genus of small (typically 2–10 μm) unicellular eukaryotic green algae with high photosynthetic efficiency and valuable biomass, using filamentous fungi as adsorbates: fast-growing *Mucor circinelloides* was used to harvest oleaginous *C. vulgaris*[121] and sewage-cultured *C. pyrenoidosa*[109] with over 97% efficiency; oleaginous (10% dry weight) *Aspergillus fumigatus* was reported to collect over 99% of *C. pyrenoidosa* cells within 3 h and 95% of wastewater-grown cells within 3.5 h,[122] compared to over 90% of heterotrophic *C. protothecoides* (also known as *Auxenochlorella protothecoides*) cells in 24 h.[123] Other *Aspergillus* fungi have been applied for harvesting *C. vulgaris* including citric acid-enriched *Aspergillus niger* (over 90% efficiency with symbiotic activities between the two strains),[124] aflatoxin-producing *Aspergillus nomius* (over 94%),[125] food-fermenting *Aspergillus oryzae* (almost 100%),[126] and two *Aspergillus* spp. UMN F01 and UMN F02 can copellet with almost 100% of algal cells and efficiently remove nutrients in wastewater treatment.[127]

In addition to *Chlorella*, fungus–alga copelletization has been used for harvesting other microalgae species. Mycelia of *A. fumigatus*, *A. nomius*, and *Mortierella elongata*, a widespread soil fungus, were applied for isolating the unicellular EPA-producing marine microalgae *Tetraselmis suecica*,[123] *Nannochloropsis* sp.[125] and *N. oceanica*,[128] respectively. The filamentous *A. fumigatus* could utilize secreted free fatty acids from the unicellular cyanobacterium *Synechocystis* PCC 6803 and form pellets with over 90% of the algal cells.[129] Another unicellular cyanobacterium *Chroococcus* sp. isolated from wastewater was harvested with the pellets of *Aspergillus lentulus* FJ172995.[130] Although the mechanism of the interaction between fungal mycelium and microalgae is not entirely clear, the results of electron microscopy have suggested that intact hyphae were required for the attachment of algal cells.[122] Fungal mycelium is usually positively charged because of the cell wall materials such as polysaccharides, which could attract the negatively surface charged microalgae and form large flocs in the culture.[129] In addition to the ionic attraction, a more recent study has shown structural attachment of algal cells to hyphae, where cells of the marine alga *N. oceanica* lost their outer coat during coculture with the soil fungus *M. elongata*, most likely due to the activities of digesting enzymes released by the fungus. The exposed fibrous extensions underneath the smooth coat may contribute to anchor the algal cells to the hyphae and form alga–fungus pellets.[131]

In addition to mycelium, many fungal species can produce large amounts of spores as flocculants for harvesting microalgae. For example, spores of *A. lentulus* and *Penicillium* sp. were used to flocculate *Chroococcus* sp.[130] and *Chlorella* sp.[120] cells, respectively. Along with the spore germination and hyphal growth, the coagulation of spores and algae could form larger

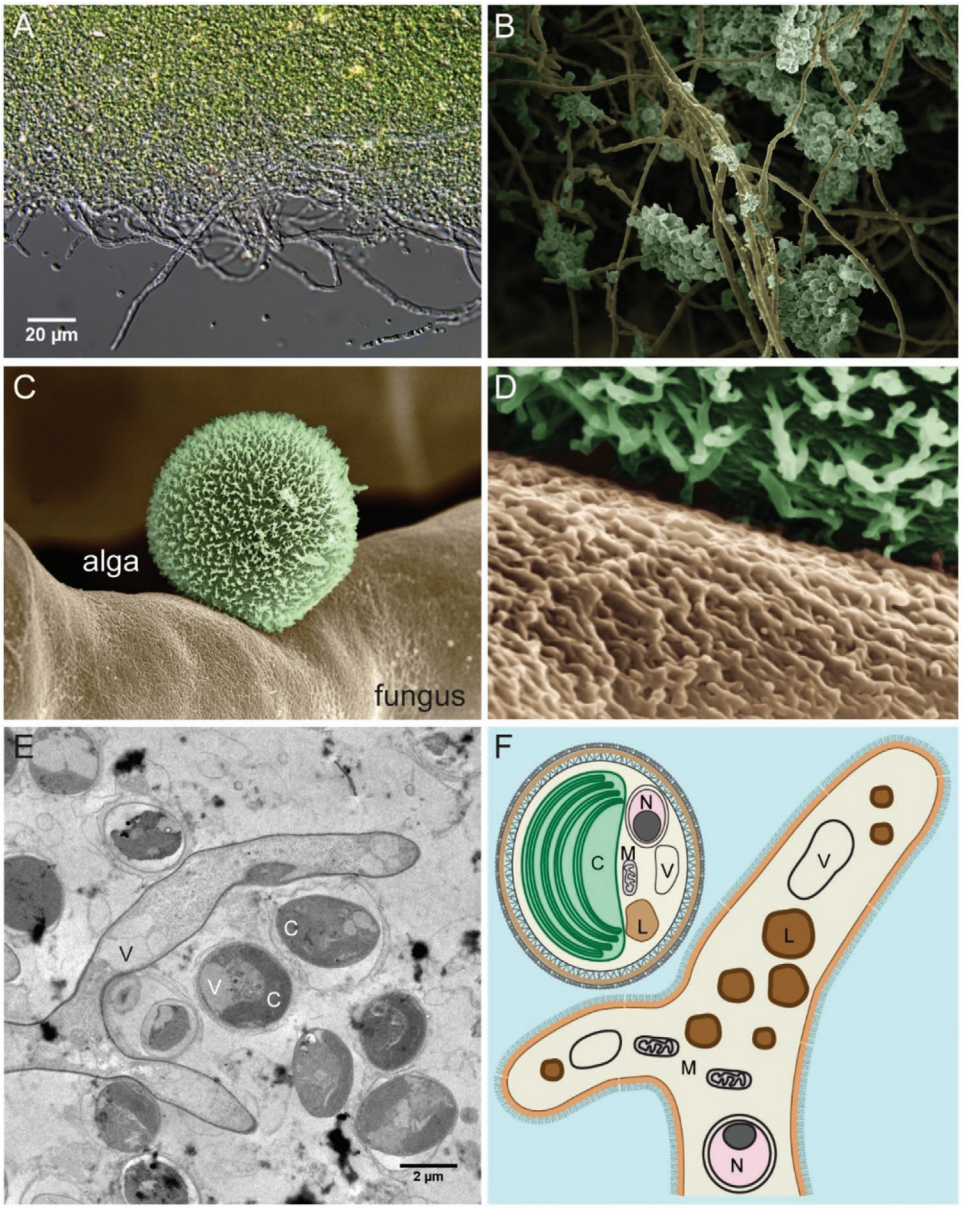

Figure 4. Interaction between filamentous fungi and microalgae. A) Differential interference contrast micrograph of copelletization of microalgae (*Nannochloropsis oceanica*) and mycelium (*Mortierella elongata*). B–D) Scanning electron microscopy images showing alga-fungus interaction. E) Transmission electron microscopy on alga–fungus aggregates. F) Cross-section cartoon of the algal and fungal cells shown in (E). C, chloroplast; N, nucleus; M, mitochondrion; V, vacuole; L, lipid droplet. Images are original to the authors.

flocs that facilitate the sedimentation and more efficient harvesting. By comparing pellet- and spore-assisted methods using fungal materials of *Penicillium* sp. in the algal culture of *Chlorella* sp., it was reported that fungal pelletization required only half of the glucose input and less settling time (2.5 vs 28 h) than the spore flocculation with slightly lower recovery efficiency (98.2% vs 99.3%), which was considered as a more promising approach to harvest microalgae. Similar to the coagulation of spores and algae, unicellular yeast strains have been used to harvest microalgae. Self-flocculating yeast *Saccharomyces bayanus* cells were able to flocculate 80% of the freshwater alga *Chlamydomonas reinhardtii* and 60% of the marine alga *Picochlorum* sp., which could be increased to 90% and 75% using 0.1 g L^{-1} extracellular proteins instead of 1:1 mixing whole cells with algae.[132] Chemically modified lysates of *Saccharomyces cerevisiae*, a brewery by-product with positive surface charge, was used to flocculate *C. vulgaris* cell and achieved over 90% efficiency at a dosage of 0.4 mg g^{-1}.[133]

Fungus-assisted pelletization and flocculation methods have a low-input cost and have performed well in harvesting microalgae. However, some efficient fungal species reported in the literature may be pathogenic to plants and animals, which raises concerns for producing the food and feed products, or reuse of aqueous media. Employing edible and nonpathogen

species of fungi such as *Pleurotus* spp. (mycelium of oyster mushroom) and *Mortierella* spp. (usually nonpathogenic, much fewer spores than *Aspergillus* and *Mucor* fungi) may solve this problem.[128,134] Moreover, similar to the alga–alga flocculation methods, the fungal materials can provide valuable biomass to the final products such as ω-3 and ω-6 polyunsaturated fatty acids, antioxidants, and other nutraceuticals.

In order to develop sustainable, economical, and environment friendly methods for harvesting microalgae, a diverse range of natural and biological flocculants are being investigated for flocculating microalgae such as seed powders of *Moringa oleifera* and *Strychnos potatorum* (Table 4). Along with the progress of novel genetic and processing engineering approaches, bioflocculation techniques have a promising future in the application for harvesting microalgae.

3. Genetic Tools and Prospects

3.1. Application of Genetic Tools in Microalgae

Like many other organisms, microalgae have been the subject of advancements in genomics and molecular biology in the last decade. Genomes have been sequenced for many important strains, such as *Nannochloropsis* spp.,[135–137] *Chlorella variabilis*,[138] and *Chlamydomonas* spp. providing genome-enabled resources and biotechnology development.[139] While gene annotation is in early stages for these species, the development of mutant libraries and efficient transformation techniques is progressing.[140] The use of CRISPR/Cas9 in *N. oceanica* facilitates the engineering of strains absent of any selection marker,[141] which may enhance biosafety if such strains are grown in outdoor cultivation. *Nannochloropsis* shows additional promise because of the development of additional genetic engineering techniques.[142,143]

Genetic tools have been used to enhance desirable traits in microalgae. Repression of phosphoenolpyruvate carboxylase in *C. reinhardtii* with CRISPRi was shown to increase lipid production by 94.2%.[144] Also in *C. reinhardtii*, a knockout mutant of phospholipase A_2 generated with CRISPR/Cas9 increased lipid productivity by 64.25%.[145] Similar genetic engineering experiments have been successful in *N. oceanica*, with a 32% increase in growth rate, a 46% increase in biomass accumulation, and a 41% increase in lipid accumulation associated with overexpression of a nuclear-encoded candidate RuBisCo activase gene.[146] Deletion of a homolog of fungal $Zn(II)_2Cys_6$-encoding genes in *Nannochloropsis gaditana* increased partitioning of carbon into lipids and yielded twice as much lipid over the wild type.[147] Genetic engineering can additionally be used to generate high-value compounds, such as terpenoids in *C. reinhardtii*,[19] or isobutyraldehyde biofuel in *Synechococcus elongatus*.[17]

3.2. Potential Applications of Synthetic Biology to Microalgae Harvesting

Harvesting cells for high-value bioproducts has led to genetic engineering for ease of collecting. These changes occur via induction of a genetic circuit (such as an environmentally sensitive promoter driving a gene of interest), and may cause behaviors such as cell flocculation or lysis. Synthetic biology is a field that concerns the use of interchangeable DNA "parts" for the creation of novel biological systems and functions.[148]

As many of the most widely grown microalgae are eukaryotic, studies in yeast (*Saccharomyces*) may be more informative for transferring useful genetic circuits. *Saccharomyces* spp. can produce flocculation proteins, encoded by *FLO* genes, which are glycosylphosphatidylinositol-linked glycoproteins capable of between cell anchoring and result in flocculation when induced.[149] Work has already been underway to use proteins from flocculating yeasts to flocculate microalgae, either by the addition of the yeast or its proteins,[132] or by algal expression of flocculation promoting proteins.[150]

Other potential methods of using genetic engineering techniques to enhance harvesting have been less explored. Some microalgae are flagellated, such as *Chlamydomonas* spp., and can respond to stimuli such as light in a phototaxic response.[151] Phototaxis may be used to condense cells to a smaller volume while siphoning off media, without mechanical or chemical concentration techniques.

Microalgal production of flocculating compounds, such as polymers, tannins, or carbohydrates could result in flocculation once cells are induced at the ideal cell density. Yet another method may use manipulation of cell buoyancy by expression of proteins responsible for gas vesicle formation in bacteria or archaea, which are fully encodable and used in medical imaging.[152] Some cyanobacteria possess such gas vesicles and are capable of forming dense surface blooms.[153] Skimming buoyant cells may allow less resource-intensive harvesting, but gas vesicles have not been expressed successfully in eukaryotic hosts.

Microalgae provide promise as engineerable and containable cell factories. Because of fast generation time and reproduction, genetic engineering and synthetic biology techniques show promise for enhancing the productivity, value, and harvestability of microalgae. While sourcing natural strains has shown results in some traits,[154] genetic tools can improve upon natural variability to tailor strains for specific applications. The combination of genetic improvement of microalgae and the development of better harvesting technologies can be integrated to increase yields and decrease costs for microalgae products.

4. Conclusions

As the use of fossil fuels generates CO_2, contributing to global climate change and related environmental impacts, photosynthetic organisms have the potential to contribute to carbon-neutral and sustainable technologies and products. Microalgae are a promising source of these products, including biofuels, food additives, cosmetics, pharmaceuticals, and animal feed, which can be produced with limited freshwater or arable land in many cases. Despite the promise of microalgae feedstocks, harvesting bioproducts from microalgae poses challenges because of the small size and recalcitrance of their cells. Technologies have been developed to deal with the harvesting challenge, ranging from conventional centrifugation, filtration, and flocculation to newer solvent and thermal reactors and finally coculture-based methods.

The diverse spectrum of microalgae products necessitates various technologies as producers balance cost, yield, purity, available materials, and environmental considerations, as well as the numerous types of microalgae required to synthesize these products. Several harvesting and extraction methods have a role to play, as combination and specialization contribute to effective and efficient harvesting. Additionally, genetic approaches could further enhance the productivity of microalgae, allow for new products, and may enhance harvesting by a variety of mechanisms. Microalgae harvesting continues to pose multiple challenges, but improvements in techniques and associated genetic engineering tools offer promise to make microalgae products as viable, sustainable fuels, foods, and high-value natural products.

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Conflict of Interest

The authors declare no conflict of interest.

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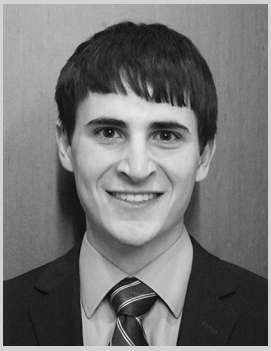
[1] G. I. McFadden, *J. Phycol.* **2001**, *37*, 951.
[2] P. Varshney, P. Mikulic, A. Vonshak, J. Beardall, P. P. Wangikar, *Bioresour. Technol.* **2015**, *184*, 363.
[3] A. Taubert, T. Jakob, C. Wilhelm, *Plant Biotechnol. J.* **2019**, *17*, 1538.
[4] J. A. Raven, *Eur. J. Phycol.* **2017**, *52*, 506.
[5] C. B. Field, M. J. Behrenfeld, J. T. Randerson, P. Falkowski, *Science* **1998**, *281*, 237.
[6] K. W. Chew, J. Y. Yap, P. L. Show, N. H. Suan, J. C. Juan, T. C. Ling, D. J. Lee, J. S. Chang, *Bioresour. Technol.* **2017**, *229*, 53.
[7] O. Edenhofer, R. Pichs-Madruga, Y. Sokona, S. Kadner, J. C. Minx, S. Brunner, S. Agrawala, G. Baiocchi, I. A. Bashmakov, G. Blanco, J. Broome, T. Bruckner, M. Bustamante, L. Clarke, M. C. Grand, F. Creutzig, X. Cruz-Núñez, S. Dhakal, N. K. Dubash, P. Eickemeier, E. Farahani, M. Fischedick, M. Fleurbaey, R. Gerlagh, L. Gómez-Echeverri, S. Gupta, J. Harnisch, K. Jiang, F. Jotzo, S. Kartha, S. Klasen, C. Kolstad, V. Krey, H. Kunreuther, O. Lucon, O. Masera, Y. Mulugetta, R. B. Norgaard, A. Patt, N. H. Ravindranath, K. Riahi, J. Roy, A. Sagar, R. Schaeffer, S. Schlömer, K. C. Seto, K. Seyboth, R. Sims, P. Smith, E. Somanathan, R. Stavins, C. von Stechow, T. Sterner, T. Sugiyama, S. Suh, D. Ürge-Vorsatz, K. Urama, A. Venables, D. G. Victor, E. Weber, D. Zhou, J. Zou, T. Zwickel, Technical Summary. in *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (Eds: O. Edenhofer, R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. vonStechow, T. Zwickel, J. C. Minx), Cambridge University Press, Cambridge, UK **2014**.
[8] C. Stewart, M. A. Hessami, *Energy Convers. Manage.* **2005**, *46*, 403.
[9] Y. M. Dyo, S. Purton, *Microbioly* **2018**, *164*, 113.
[10] F. Apone, A. Barbulova, M. G. Colucci, *Front. Plant Sci.* **2019**, *10*, 756.
[11] I. Barkia, N. Saari, S. R. Manning, *Mar. Drugs* **2019**, *17*, 304.
[12] R. B. Draaisma, R. H. Wijffels, P. M. Slegers, L. B. Brentner, A. Roy, M. J. Barbosa, *Curr. Opin. Biotechnol.* **2013**, *24*, 169.
[13] K. M. Allen, H. M. Habte-Tsion, K. R. Thompson, K. Filer, J. H. Tidwell, V. Kumar, *Sci. Rep.* **2019**, *9*, 6178.
[14] C. Kalbe, A. Priepke, G. Nürnberg, D. Dannenberger, *J. Anim. Physiol. Anim. Nutr.* **2019**, *103*, 574.
[15] X. Wu, R. Ruan, Z. Du, Y. Liu, X. Wu, R. Ruan, Z. Du, Y. Liu, *Energies* **2012**, *5*, 2667.
[16] J. Kopka, S. Schmidt, F. Dethloff, N. Pade, S. Berendt, M. Schottkowski, N. Martin, U. Dühring, E. Kuchmina, H. Enke, D. Kramer, A. Wilde, M. Hagemann, A. Friedrich, *Biotechnol. Biofuels* **2017**, *10*, 56.
[17] S. Atsumi, W. Higashide, J. C. Liao, *Nat. Biotechnol.* **2009**, *27*, 1177.
[18] M. Sun, K. Qian, N. Su, H. Chang, J. Liu, G. Chen, *Biotechnol. Lett.* **2003**, *25*, 1087.
[19] K. J. Lauersen, T. Baier, J. Wichmann, R. Wördenweber, J. H. Mussgnug, W. Hübner, T. Huser, O. Kruse, *Metab. Eng.* **2016**, *38*, 331.
[20] J. Xue, Y. F. Niu, T. Huang, W. D. Yang, J. S. Liu, H. Y. Li, *Metab. Eng.* **2015**, *27*, 1.
[21] H. G. Koh, N. K. Kang, S. Jeon, S. E. Shin, B. R. Jeong, Y. K. Chang, *Biotechnol. Biofuels* **2019**, *12*, 122.
[22] T. Ogawa, M. Tamoi, A. Kimura, A. Mine, H. Sakuyama, E. Yoshida, T. Maruta, K. Suzuki, T. Ishikawa, S. Shigeoka, *Biotechnol. Biofuels* **2015**, *8*, 80.
[23] W. J. Henley, R. W. Litaker, L. Novoveská, C. S. Duke, H. D. Quemada, R. T. Sayre, *Algal Res.* **2013**, *2*, 66.
[24] Transparency Market Research, *Microalgae-Based Products Market - Global Industry Analysis, Size, Share, Growth, Trends, and Forecast, 2019–2027*, https://www.transparencymarketresearch.com/microalgae-based-products-market.html (accessed: May 2020).
[25] F. Fasaei, J. H. Bitter, P. M. Slegers, A. J. B. van Boxtel, *Algal Res.* **2018**, *31*, 347.
[26] G. Shelef, A. Sukenik, M. Green, *Microalgae Harvesting and Processing: A Literature Review*, Solar Energy Research Institute, Golden, CO **1984**.
[27] S. L. Pahl, A. K. Lee, T. Kalaitzidis, P. J. Ashman, S. Sathe, D. M. Lewis, in *Algae for Biofuels and Energy* (Eds: M. A. Borowitzka, N. R. Moheimani), Springer, Dordrecht, the Netherlands **2013**, pp. 165–185.
[28] A. J. Dassey, C. S. Theegala, *Bioresour. Technol.* **2013**, *128*, 241.
[29] K. Kim, H. Shin, M. Moon, B. G. Ryu, J. I. Han, J. W. Yang, Y. K. Chang, *Bioresour. Technol.* **2015**, *198*, 828.
[30] D. Bilanovic, G. Shelef, A. Sukenik, *Biomass* **1988**, *17*, 65.
[31] S. Lama, K. Muylaert, T. B. Karki, I. Foubert, R. K. Henderson, D. Vandamme, *Bioresour. Technol.* **2016**, *220*, 464.
[32] V. M. Rwehumbiza, R. Harrison, L. Thomsen, *Chem. Eng. J.* **2012**, *200-202*, 168.
[33] L. Zhu, T. Hu, S. Li, Y. K. Nugroho, B. Li, J. Cao, P. L. Show, E. Hiltunen, *Biomass Bioenergy* **2020**, *132*, 105433.
[34] M. Shurair, F. Almomani, R. Bhosale, M. Khraisheh, H. Qiblawey, *Bioresour. Technol.* **2019**, *281*, 250.
[35] D. Vandamme, I. Foubert, K. Muylaert, *Trends Biotechnol.* **2013**, *31*, 233.
[36] F. Roselet, D. Vandamme, M. Roselet, K. Muylaert, P. C. Abreu, *BioEnergy Res.* **2017**, *10*, 427.
[37] S. Abdul Razack, S. Duraiarasan, A. S. Santhalin Shellomith, K. Muralikrishnan, *Biotechnol. Rep.* **2015**, *7*, 150.

[38] C. Banerjee, S. Ghosh, G. Sen, S. Mishra, P. Shukla, R. Bandopadhyay, *Carbohydr. Polym.* **2013**, *92*, 675.
[39] S. K. Wang, F. Wang, Y. R. Hu, A. R. Stiles, C. Guo, C. Z. Liu, *ACS Appl. Mater. Interfaces* **2014**, *6*, 109.
[40] C. Wang, Y. Yang, J. Hou, P. Wang, L. Miao, X. Wang, L. Guo, *Algal Res.* **2020**, *45*, 101756.
[41] Y. Liu, W. Jin, X. Zhou, S. F. Han, R. Tu, X. Feng, P. D. Jensen, Q. Wang, *Bioresour. Technol.* **2019**, *290*, 121771.
[42] Y. Zhao, X. Wang, X. Jiang, Q. Fan, X. Li, L. Jiao, W. Liang, *Environ. Sci. Pollut. Res.* **2018**, *25*, 26246.
[43] Y. R. Hu, F. Wang, S. K. Wang, C. Z. Liu, C. Guo, *Bioresour. Technol.* **2013**, *138*, 387.
[44] P. Y. Toh, B. W. Ng, A. L. Ahmad, D. C. J. Chieh, J. Lim, *Nanoscale* **2014**, *6*, 12838.
[45] A. Abo Markeb, J. Llimós-Turet, I. Ferrer, P. Blánquez, A. Alonso, A. Sánchez, J. Moral-Vico, X. Font, *Water Res.* **2019**, *159*, 490.
[46] J. Ma, W. Xia, X. Fu, L. Ding, Y. Kong, H. Zhang, K. Fu, *J. Cleaner Prod.* **2020**, *248*, 119276.
[47] T. Wang, W. L. Yang, Y. Hong, Y. L. Hou, *Chem. Eng. J.* **2016**, *297*, 304.
[48] P. Liu, T. Wang, Z. Yang, Y. Hong, X. Xie, Y. Hou, *Sci. Total Environ.* **2020**, *704*, 135286.
[49] K. Lee, S. Y. Lee, R. Praveenkumar, B. Kim, J. Y. Seo, S. G. Jeon, J. G. Na, J. Y. Park, D. M. Kim, Y. K. Oh, *Bioresour. Technol.* **2014**, *167*, 284.
[50] N. Fayad, T. Yehya, F. Audonnet, C. Vial, *Algal Res.* **2017**, *25*, 1.
[51] S. Gao, J. Yang, J. Tian, F. Ma, G. Tu, M. Du, *J. Hazard. Mater.* **2010**, *177*, 336.
[52] N. Uduman, V. Bourniquel, M. K. Danquah, A. F. A. Hoadley, *Chem. Eng. J.* **2011**, *174*, 249.
[53] M. Mubarak, A. Shaija, T. V. Suchithra, *J. Environ. Chem. Eng.* **2020**, *8*, 103612.
[54] T. R. Shuman, G. Mason, D. Reeve, A. Schacht, A. Goodrich, K. Napan, J. Quinn, *Chem. Eng. J.* **2016**, *297*, 97.
[55] R. R. Soomro, T. Ndikubwimana, X. Zeng, Y. Lu, L. Lin, M. K. Danquah, *Front. Plant Sci.* **2016**, *7*, 113.
[56] M. Y. A. Mollah, P. Morkovsky, J. A. G. Gomes, M. Kesmez, J. Parga, D. L. Cocke, *J. Hazard. Mater.* **2004**, *114*, 199.
[57] G. Singh, S. K. Patidar, *J. Environ. Manage.* **2018**, *217*, 499.
[58] W. Mo, L. Soh, J. R. Werber, M. Elimelech, J. B. Zimmerman, *Algal Res.* **2015**, *11*, 1.
[59] N. Rossi, P. Jaouen, P. Legentilhomme, I. Petit, *Food Bioprod. Process.* **2004**, *82*, 244.
[60] N. Rossignol, L. Vandanjon, P. Jaouen, F. Quéméneur, *Aquacult. Eng.* **1999**, *20*, 191.
[61] E. Clavijo Rivera, L. Villafaña-López, S. Liu, R. Vinoth Kumar, M. Viau, P. Bourseau, C. Monteux, M. Frappart, E. Couallier, *Process Biochem.* **2020**, *89*, 199.
[62] L. Yang, L. Wang, S. Ren, B. Pan, J. Li, X. Zhang, Y. Chen, Q. Hu, *J. Biosci. Bioeng.* **2019**, *128*, 103.
[63] G. W. Meindersma, J. Augeraud, F. H. P. Vergossen, *J. Membr. Sci.* **1997**, *125*, 333.
[64] J. Fret, L. Roef, L. Diels, S. Tavernier, W. Vyverman, M. Michiels, *Algal Res.* **2016**, *13*, 116.
[65] R. Huang, Z. Liu, B. Yan, Y. Li, H. Li, D. Liu, P. Wang, F. Cui, W. Shi, *J. Membr. Sci.* **2020**, *595*, 117488.
[66] L. Hua, H. Cao, Q. Ma, X. Shi, X. Zhang, W. Zhang, *Environ. Sci. Technol.* **2020**, *54*, 2012.
[67] B. Petruševski, G. Bolier, A. N. Van Breemen, G. J. Alaerts, *Water Res.* **1995**, *29*, 1419.
[68] T. De Baerdemaeker, B. Lemmens, C. Dotremont, J. Fret, L. Roef, K. Goiris, L. Diels, *Bioresour. Technol.* **2013**, *129*, 582.
[69] H. Elcik, M. Cakmakci, *Environ. Technol.* **2017**, *38*, 1585.
[70] A. A. Peterson, F. Vogel, R. P. Lachance, M. Fröling, M. J. Antal, J. W. Tester, *Energy Environ. Sci.* **2008**, *1*, 32.
[71] R. Shakya, S. Adhikari, R. Mahadevan, S. R. Shanmugam, H. Nam, E. B. Hassan, T. A. Dempster, *Bioresour. Technol.* **2017**, *243*, 1112.
[72] R. Shakya, J. Whelen, S. Adhikari, R. Mahadevan, S. Neupane, *Algal Res.* **2015**, *12*, 80.
[73] A. Sánchez-Bayo, R. Rodríguez, V. Morales, N. Nasirian, L. F. Bautista, G. Vicente, *Processes* **2020**, *8*, 6.
[74] C. Samorì, C. Torri, G. Samorì, D. Fabbri, P. Galletti, F. Guerrini, R. Pistocchi, E. Tagliavini, *Bioresour. Technol.* **2010**, *101*, 3274.
[75] A. R. Boyd, P. Champagne, P. J. McGinn, K. M. MacDougall, J. E. Melanson, P. G. Jessop, *Bioresour. Technol.* **2012**, *118*, 628.
[76] W. Tang, K. Ho Row, *Bioresour. Technol.* **2020**, *296*, 122309.
[77] M. Gross, W. Henry, C. Michael, Z. Wen, *Bioresour. Technol.* **2013**, *150*, 195.
[78] L. B. Christenson, R. C. Sims, *Biotechnol. Bioeng.* **2012**, *109*, 1674.
[79] W. Blanken, M. Janssen, M. Cuaresma, Z. Libor, T. Bhaiji, R. H. Wijffels, *Biotechnol. Bioeng.* **2014**, *111*, 2436.
[80] J. Peng, K. Kumar, M. Gross, T. Kunetz, Z. Wen, *Water Environ. Res.* **2020**, *92*, 766.
[81] S. Salim, R. Bosma, M. H. Vermuë, R. H. Wijffels, *J. Appl. Phycol.* **2011**, *23*, 849.
[82] S. Salim, N. R. Kosterink, N. D. Tchetkoua Wacka, M. H. Vermuë, R. H. Wijffels, *J. Biotechnol.* **2014**, *174*, 34.
[83] M. J. Griffiths, S. T. L. Harrison, *J. Appl. Phycol.* **2009**, *21*, 493.
[84] M. A. Alam, C. Wan, S. L. Guo, X. Q. Zhao, Z. Y. Huang, Y. L. Yang, J. S. Chang, F. W. Bai, *J. Biosci. Bioeng.* **2014**, *118*, 29.
[85] S. L. Guo, X. Q. Zhao, C. Wan, Z. Y. Huang, Y. L. Yang, M. Asraful Alam, S. H. Ho, F. W. Bai, J. S. Chang, *Bioresour. Technol.* **2013**, *145*, 285.
[86] O. Tiron, C. Bumbac, E. Manea, M. Stefanescu, M. N. Lazar, *Sci. Rep.* **2017**, *7*, 4646.
[87] B. Úbeda, J. Á. Gálvez, M. Michel, A. Bartual, *Bioresour. Technol.* **2017**, *228*, 210.
[88] R. L. Taylor, J. D. Rand, G. S. Caldwell, *Mar. Biotechnol.* **2012**, *14*, 774.
[89] A. Sukenik, G. Shelef, *Biotechnol. Bioeng.* **1984**, *26*, 142.
[90] J. Liu, Y. Zhu, Y. Tao, Y. Zhang, A. Li, T. Li, M. Sang, C. Zhang, *Biotechnol. Biofuels* **2013**, *6*, 98.
[91] S. B. Ummalyma, A. K. Mathew, A. Pandey, R. K. Sukumaran, *Bioresour. Technol.* **2016**, *213*, 216.
[92] Z. Wu, Y. Zhu, W. Huang, C. Zhang, T. Li, Y. Zhang, A. Li, *Bioresour. Technol.* **2012**, *110*, 496.
[93] J. Liu, Y. Tao, J. Wu, Y. Zhu, B. Gao, Y. Tang, A. Li, C. Zhang, Y. Zhang, *Bioresour. Technol.* **2014**, *167*, 367.
[94] Z. Harith, F. Yusoff, M. Mohamed, M. Shariff, M. Din, A. Ariff, *Afr. J. Biotechnol.* **2009**, *8*, 5971, https://doi.org/10.5897/AJB09.569.
[95] N. A. T. Tran, J. R. Seymour, N. Siboni, C. R. Evenhuis, B. Tamburic, *Algal Res.* **2017**, *26*, 302.
[96] C. Yoo, H. J. La, S. C. Kim, H. M. Oh, *Biotechnol. Bioeng.* **2015**, *112*, 288.
[97] R. M. Knuckey, M. R. Brown, R. Robert, D. M. F. Frampton, *Aquacult. Eng.* **2006**, *35*, 300.
[98] D. Vandamme, I. Foubert, I. Fraeye, B. Meesschaert, K. Muylaert, *Bioresour. Technol.* **2012**, *105*, 114.
[99] Z. Wang, J. Hou, D. Bowden, J. M. Belovich, *J. Chem. Technol. Biotechnol.* **2014**, *89*, 714.
[100] T. D. P. Nguyen, M. Frappart, P. Jaouen, J. Pruvost, P. Bourseau, *Environ. Technol.* **2014**, *35*, 1378.
[101] S. Huo, Z. Wang, S. Zhu, F. Cui, B. Zou, W. You, Z. Yuan, R. Dong, *Energies* **2014**, *7*, 6186.
[102] B. Q. Liao, H. J. Lin, S. P. Langevin, W. J. Gao, G. G. Leppard, *Water Res.* **2011**, *45*, 509.
[103] B. M. Wilén, P. Balmér, *Water Res.* **1999**, *33*, 391.
[104] J. Lv, X. Wang, W. Liu, J. Feng, Q. Liu, F. Nan, X. Jiao, S. Xie, *Int. J. Environ. Res. Public Health* **2018**, *15*, 434.
[105] B. Kim, R. Praveenkumar, J. Lee, B. Nam, D. M. Kim, K. Lee, Y. C. Lee, Y. K. Oh, *Bioresour. Technol.* **2016**, *219*, 608.
[106] S. Magdouli, S. K. Brar, J. F. Blais, *Biomass Bioenergy* **2016**, *92*, 20.

[107] J. Lee, D. H. Cho, R. Ramanan, B. H. Kim, H. M. Oh, H. S. Kim, *Bioresour. Technol.* **2013**, *131*, 195.
[108] Y. Li, Z. Zhang, Y. Duan, H. Wang, *Bioresour. Technol.* **2019**, *280*, 188.
[109] J. Jiang, W. Jin, R. Tu, S. Han, Y. Ji, X. Zhou, *Waste Biomass Valorization* **2020**, https://doi.org/10.1007/s12649-020-00979-6.
[110] M. Agbakpe, S. Ge, W. Zhang, X. Zhang, P. Kobylarz, *Bioresour. Technol.* **2014**, *166*, 266.
[111] C. Wan, X. Q. Zhao, S. L. Guo, M. Asraful Alam, F. W. Bai, *Bioresour. Technol.* **2013**, *135*, 207.
[112] H. Zheng, Z. Gao, J. Yin, X. Tang, X. Ji, H. Huang, *Bioresour. Technol.* **2012**, *112*, 212.
[113] T. Ndikubwimana, X. Zeng, Y. Liu, J. S. Chang, Y. Lu, *Algal Res.* **2014**, *6*, 186.
[114] M. Nie, X. Yin, J. Jia, Y. Wang, S. Liu, Q. Shen, P. Li, Z. Wang, *J. Appl. Microbiol.* **2011**, *111*, 547.
[115] H. M. Oh, S. J. Lee, M. H. Park, H. S. Kim, H. C. Kim, J. H. Yoon, G. S. Kwon, B. D. Yoon, *Biotechnol. Lett.* **2001**, *23*, 1229.
[116] D. G. Kim, H. J. La, C. Y. Ahn, Y. H. Park, H. M. Oh, *Bioresour. Technol.* **2011**, *102*, 3163.
[117] R. Honegger, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1991**, *42*, 553.
[118] T. Spribille, V. Tuovinen, P. Resl, D. Vanderpool, H. Wolinski, M. C. Aime, K. Schneider, E. Stabentheiner, M. Toome-Heller, G. Thor, H. Mayrhofer, H. Johannesson, J. P. McCutcheon, *Science* **2016**, *353*, 488.
[119] J. Zhang, B. Hu, *Bioresour. Technol.* **2012**, *114*, 529.
[120] J. Chen, L. Leng, C. Ye, Q. Lu, M. Addy, J. Wang, J. Liu, P. Chen, R. Ruan, W. Zhou, *Bioresour. Technol.* **2018**, *259*, 181.
[121] A. Rajendran, B. Hu, *Biotechnol. Biofuels* **2016**, *9*, 112.
[122] A. Bhattacharya, M. Mathur, P. Kumar, S. K. Prajapati, A. Malik, *Algal Res.* **2017**, *21*, 42.
[123] N. Muradov, M. Taha, A. F. Miranda, D. Wrede, K. Kadali, A. Gujar, T. Stevenson, A. S. Ball, A. Mouradov, *Biotechnol. Biofuels* **2015**, *8*, 24.
[124] S. Gultom, C. Zamalloa, B. Hu, *Energies* **2014**, *7*, 4417.
[125] M. M. R. Talukder, P. Das, J. C. Wu, *Biochem. Eng. J.* **2014**, *91*, 53.
[126] W. Zhou, M. Min, B. Hu, X. Ma, Y. Liu, Q. Wang, J. Shi, P. Chen, R. Ruan, *Sep. Purif. Technol.* **2013**, *107*, 158.
[127] W. Zhou, Y. Cheng, Y. Li, Y. Wan, Y. Liu, X. Lin, R. Ruan, *Appl. Biochem. Biotechnol.* **2012**, *167*, 214.
[128] Z.-Y. Du, J. Alvaro, B. Hyden, K. Zienkiewicz, N. Benning, A. Zienkiewicz, G. Bonito, C. Benning, *Biotechnol. Biofuels* **2018**, *11*, 174.
[129] A. F. Miranda, M. Taha, D. Wrede, P. Morrison, A. S. Ball, T. Stevenson, A. Mouradov, *Biotechnol. Biofuels* **2015**, *8*, 179.
[130] S. K. Prajapati, P. Kumar, A. Malik, P. Choudhary, *BioEnergy Res.* **2014**, *7*, 1430.
[131] Z.-Y. Du, K. Zienkiewicz, N. Vande Pol, N. E. Ostrom, C. Benning, G. M. Bonito, *eLife* **2019**, *8*, e47815.
[132] E. Díaz-Santos, M. Vila, M. de la Vega, R. León, J. Vigara, *Algal Res.* **2015**, *8*, 23.
[133] G. Prochazkova, P. Kastanek, T. Branyik, *Bioresour. Technol.* **2015**, *177*, 28.
[134] S. Luo, X. Wu, H. Jiang, M. Yu, Y. Liu, A. Min, W. Li, R. Ruan, *Bioresour. Technol.* **2019**, *282*, 325.
[135] A. Vieler, G. Wu, C. H. Tsai, B. Bullard, A. J. Cornish, C. Harvey, I. B. Reca, C. Thornburg, R. Achawanantakun, C. J. Buehl, M. S. Campbell, D. Cavalier, K. L. Childs, T. J. Clark, R. Deshpande, E. Erickson, A. Armenia Ferguson, W. Handee, Q. Kong, X. Li, B. Liu, S. Lundback, C. Peng, R. L. Roston, Sanjaya, J. P. S , A. TerBush, J. Warakanont, S. Zäuner, E. M. Farre, E. L. Hegg, N. Jiang, M. H. Kuo, Y. Lu, K. K. Niyogi, J. Ohlrogge, K. W. Osteryoung, Y. Shachar-Hill, B. B. Sears, Y. Sun, H. Takahashi, M. Yandell, S. H. Shiu, C. Benning, *PLoS Genet.* **2012**, *8*, e1003064.
[136] R. Radakovits, R. E. Jinkerson, S. I. Fuerstenberg, H. Tae, R. E. Settlage, J. L. Boore, M. C. Posewitz, *Nat. Commun.* **2012**, *3*, 686.
[137] D. Wang, K. Ning, J. Li, J. Hu, D. Han, H. Wang, X. Zeng, X. Jing, Q. Zhou, X. Su, X. Chang, A. Wang, W. Wang, J. Jia, L. Wei, Y. Xin, Y. Qiao, R. Huang, J. Chen, B. Han, K. Yoon, R. T. Hill, Y. Zohar, F. Chen, Q. Hu, J. Xu, *PLoS Genet.* **2014**, *10*, e1004094.
[138] G. Blanc, G. Duncan, I. Agarkova, M. Borodovsky, J. Gurnon, A. Kuo, E. Lindquist, S. Lucas, J. Pangilinan, J. Polle, A. Salamov, A. Terry, T. Yamada, D. D. Dunigan, I. V. Grigoriev, J. M. Claverie, J. L. van Etten, *Plant Cell* **2010**, *22*, 2943.
[139] I. K. Blaby, C. E. Blaby-Haas, N. Tourasse, E. F. Y. Hom, D. Lopez, M. Aksoy, A. Grossman, J. Umen, S. Dutcher, M. Porter, S. King, G. B. Witman, M. Stanke, E. H. Harris, D. Goodstein, J. Grimwood, J. Schmutz, O. Vallon, S. S. Merchant, S. Prochnik, *Trends Plant Sci.* **2014**, *19*, 672.
[140] X. Li, W. Patena, F. Fauser, R. E. Jinkerson, S. Saroussi, M. T. Meyer, N. Ivanova, J. M. Robertson, R. Yue, R. Zhang, J. Vilarrasa-Blasi, T. M. Wittkopp, S. Ramundo, S. R. Blum, A. Goh, M. Laudon, T. Srikumar, P. A. Lefebvre, A. R. Grossman, M. C. Jonikas, *Nat. Genet.* **2019**, *51*, 627.
[141] E. Poliner, T. Takeuchi, Z. Y. Du, C. Benning, E. M. Farré, *ACS Synth. Biol.* **2018**, *7*, 962.
[142] E. Poliner, E. Clark, C. Cummings, C. Benning, E. M. Farre, *Algal Res.* **2020**, *45*, 101664.
[143] J. Verruto, K. Francis, Y. Wang, M. C. Low, J. Greiner, S. Tacke, F. Kuzminov, W. Lambert, J. McCarren, I. Ajjawi, N. Bauman, R. Kalb, G. Hannum, E. R. Moellering, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E7015.
[144] P. H. Kao, I. S. Ng, *Bioresour. Technol.* **2017**, *245*, 1527.
[145] Y. S. Shin, J. Jeong, T. H. T. Nguyen, J. Y. H. Kim, E. S. Jin, S. J. Sim, *Bioresour. Technol.* **2019**, *271*, 368.
[146] L. Wei, Q. Wang, Y. Xin, Y. Lu, J. Xu, *Algal Res.* **2017**, *27*, 366.
[147] I. Ajjawi, J. Verruto, M. Aqui, L. B. Soriaga, J. Coppersmith, K. Kwok, L. Peach, E. Orchard, R. Kalb, W. Xu, T. J. Carlson, K. Francis, K. Konigsfeld, J. Bartalis, A. Schultz, W. Lambert, A. S. Schwartz, R. Brown, E. R. Moellering, *Nat. Biotechnol.* **2017**, *35*, 647.
[148] M. A. Scaife, A. G. Smith, *Biochem. Soc. Trans.* **2016**, *44*, 716.
[149] P. Govender, J. L. Domingo, M. C. Bester, I. S. Pretorius, F. F. Bauer, *Appl. Environ. Microbiol.* **2008**, *74*, 6041.
[150] E. Díaz-Santos, M. Vila, J. Vigara, R. León, *J. Appl. Phycol.* **2016**, *28*, 1611.
[151] M. E. H. Feinleib, G. M. Curry, *Physiol. Plant.* **1971**, *25*, 346.
[152] A. Lakshmanan, G. J. Lu, A. Farhadi, S. P. Nety, M. Kunth, A. Lee-Gosselin, D. Maresca, R. W. Bourdeau, M. Yin, J. Yan, C. Witte, D. Malounda, F. S. Foster, L. Schröder, M. G. Shapiro, *Nat. Protoc.* **2017**, *12*, 2050.
[153] A. Mlouka, K. Comte, A. M. Castets, C. Bouchier, N. Tandeau De Marsac, *J. Bacteriol.* **2004**, *186*, 2355.
[154] L. Rodolfi, G. C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, M. R. Tredici, *Biotechnol. Bioeng.* **2009**, *102*, 100.
[155] H. Li, M. Wang, X. Wang, Y. Zhang, H. Lu, N. Duan, B. Li, D. Zhang, T. Dong, Z. Liu, *Sci. Total Environ.* **2018**, *635*, 70.
[156] S. Salim, M. H. Vermuë, R. H. Wijffels, *Bioresour. Technol.* **2012**, *118*, 49.
[157] S. Mackay, E. Gomes, C. Holliger, R. Bauer, J. P. Schwitzguébel, *Bioresour. Technol.* **2015**, *185*, 353.
[158] S. Xie, S. Sun, S. Y. Dai, J. S. Yuan, *Algal Res.* **2013**, *2*, 28.
[159] D. Wrede, M. Taha, A. F. Miranda, K. Kadali, T. Stevenson, A. S. Ball, A. Mouradov, *PLoS One* **2014**, *9*, e113497.
[160] S. H. Abdul Hamid, F. Lananan, H. Khatoon, A. Jusoh, A. Endut, *Int. Biodeterior. Biodegrad.* **2016**, *113*, 310.
[161] S. H. Abdul Hamid, F. Lananan, W. N. S. Din, S. S. Lam, H. Khatoon, A. Endut, A. Jusoh, *Int. Biodeterior. Biodegrad.* **2014**, *95*, 270.
[162] C. M. L. L. Teixeira, F. V. Kirsten, P. C. N. Teixeira, *J. Appl. Phycol.* **2012**, *24*, 557.
[163] T. D. P. Nguyen, T. N. T. Tran, T. V. A. Le, T. X. Nguyen Phan, P. L. Show, S. R. Chia, *J. Biosci. Bioeng.* **2019**, *127*, 492.



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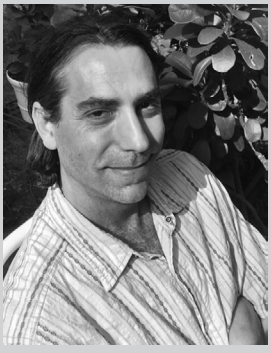
Abigail E. Bryson obtained her B.S. degree in genetics from Michigan State University in 2019 and is currently working toward her Ph.D. Her research interests include plant biotechnology for health and sustainability.



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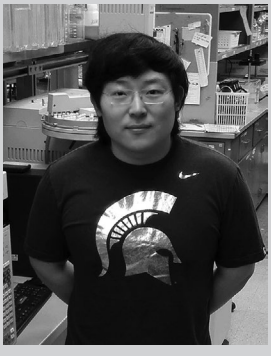

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