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Wicker Rebecca J., Kumar Gopalakrishnan, Khan Eakalak, Bhatnagar Amit

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Rebecca J. Wicker [a]*, Gopalakrishnan Kumar [b], Eakalak Khan [c], Amit Bhatnagar [a]

[a] *Department of Separation Science, Lappeenranta-Lahti University of Technology, Sammonkatu 12, 50130 Mikkeli, Finland*

[b] *Faculty of Science and Technology, University of Stavanger, 4021 Stavanger, Norway*

[c] *Department of Civil and Environmental Engineering and Construction, University of Nevada, Las Vegas, 89154 USA*

*Corresponding author: Rebecca J. Wicker
Phone: +358 50 406 26 66
E-mail: rebecca.wicker@lut.fi

Abstract

The current COVID-19 pandemic is forcing radical change in the global energy economy. All energy sectors have experienced profound economic contraction in 2020, with the notable exception of renewable energy, which has grown by nearly 3%. These unprecedented circumstances offer a pristine opportunity to create new jobs, technologies, and infrastructure, aimed at engineering a climate-friendly and sustainable energy future. This review explores several pathways to renewable bioenergy by developing microalgal biorefinery systems which capitalize upon the unique abilities of microalgae to sequester carbon and produce biomass for bioenergy feedstock, without compromising food security or land use. This review further highlights the necessity of synergistically coupled upstream and downstream techniques to realize the economic viability of microalgal biorefinery systems, and details possible product pathways using either whole or fractionated biomass. The zero-waste circular biorefinery approach, when specifically tailored to local conditions, in terms of regional climate, economics, infrastructure, and available resources, is the answer to economically competitive microalgal bioenergy. The most promising emergent methods for microalgal biomass valorization to fungible bioenergy are reviewed herein.

Keywords: Biorefinery, microalgal biotechnology, biomass upgrading, biofuel production, renewable energy

1. Introduction

The world is facing an unprecedented challenge in combatting global energy crises, pollution, and climate change simultaneously. Despite international legislative efforts [1], carbon dioxide (CO_2) emissions from fuel combustion have risen significantly in recent years, reaching 32.8 billion tons in 2018. This increase in CO_2 emission is a direct result of the 2.3% growth in worldwide energy demand in 2018 (the highest in ten years), with fossil fuels meeting nearly 80% of this consumption increase. Oil continues to dominate the transport sector, totaling 93% of fuel consumption in 2017, despite an increase in biofuel production. The transport sector consumes the most energy, responsible for ~33% of global energy consumption [2]. Compounding the energy and emissions crises, population-driven urbanization and industrialization are driving the deposition of toxic materials into the environment, many of which are fiscally valuable, but cannot be efficiently recycled using technologies available. Multifunctional solutions to address these intricately linked issues must be developed within current infrastructures and frameworks to establish cohesion between scientific, economic, and legislative spheres.

The events of 2020 have caused a sharp and unprecedented global reduction in oil consumption due to worldwide COVID-19 lockdowns and travel restrictions [3,4]. In marked contrast with the instability of fossil energy markets, renewable energy has been the only energy sector to grow in 2020, with foreign direct investments in renewables surpassing those directed towards oil and gas for the first time [3–5]. Global use of renewables across all sectors in 2020 increased by approximately 1.5% compared to 2019. The most notoriously carbon-intensive fuel sources, oil and coal, are taking the greatest economic hits, with capital investments in these sectors falling by at least 18%. The inertia of these market fluxes is irreversible, but they present an unprecedented opportunity to direct this momentum towards paradigm change. Now is the ideal time reshape the global

energy economy by investing in cleaner, renewable energy technologies and a sustainable, circular bioeconomy.

The argument for fungible biofuel technology is not merely environmental, or even economic, nor is it solely a technological challenge. Fossil fuels have caused geopolitical tension for the past century, since they were first used to drive the machine of military conquest during the first World War [6]. Fossil resources are not equally distributed across the planet, allowing a relatively small number of countries to control the majority of oil reserves and therefore the global market; in the most unfortunate cases, providing a premise for conflict. According to the International Renewable Energy Agency, shifting towards renewable energy economies will empower developing nations to achieve energy independence, energy security, and the creation of wealth [7].

Energy technologies are evolving faster than energy demands. For example, the production efficiency of hydrogen gas is being rapidly improved, creating a fuel source that cannot be used in the internal combustion engine which powers much of the modern world. Thus, the global energy crisis requires fungible "drop-in" fuels, especially for transport vehicles larger than the average car. Although research is beginning to focus on using biofuels in airplanes, cargo ships and other large vessels are powered primarily by heavy fuel oil [3].

To be considered as a drop-in fuel (i.e. competitive in all aspects with fossils), a biofuel must adhere to the following criteria:

(1) The total lifecycle (production, processing, refining, and combustion) must be net emissions-neutral or -negative

(2) The biofuel lifecycle must also be energy-balanced; the energy required to produce fuel must be significantly lower than the energy it provides

(3) The cost of biofuel production must be considerably lower than the economic return of the fuel products, such that the biofuel products are economically competitive with their fossil counterparts.

This review is focused towards green practices; as such, only methods that stringently conform to these three criteria are reviewed herein. This review distinguishes itself from other technology comparisons in the field by recommending an overall process chain (Fig. 1), from harvesting to biofuel production, with priority on the most efficacious and environmentally sound technologies as established by this review. Each technology can potentially find a place within a zero-waste circular biorefinery scheme towards a climate-friendly global energy economy. This review provides insights and critical comparisons of emergent technologies, from cell harvest to biomass conversion, for valorization of microalgal and microbial biomass to fungible bioenergy, as well as recommendations for developing locally relevant biorefinery schemes.

2. Microalgal biofuels

Fungible, economically viable hydrocarbon fuels can be generated by bio-mediated recycling of anthropogenic waste into bioenergy products by microalgae (which, in this review, includes prokaryotic cyanobacteria as well as eukaryotic green algae, diatoms, and euglenoids). Microalgae boast higher photosynthetic efficiency, greater biomass production, and faster growth than their terrestrial plant counterparts [8], and, especially when augmented by heterotrophic bacteria in consortia, are highly adept at removing nutrients and other contaminants from wastewater and incorporating them into biomass [9–11]. Gautam et al. [11] demonstrated species-specific synergistic interactions between eukaryotic microalgae and prokaryotic cyanobacteria, which enhanced biomass production, lipid accumulation, and nitrogen fixation rates. Moreover, both eukaryotic and prokaryotic microalgae possess a diversity of carbon-concentrating mechanisms for

the acquisition of inorganic carbon, including dissolved CO_2 and carbonate species, during autotrophic photosynthesis [12]. Many species can also utilize organic carbon sources in low-light conditions; a process called heterotrophic metabolism, or mixotrophic when both types of carbon and light are available [13,14]. Heterotrophic metabolism may prove itself integral to economizing algal biofuel production pathways; Johnson and Wen [15] reported that the heterotrophic green alga *Schizochytrium limacinum* could grow effectively using crude glycerol recycled from conventional transesterification as its primary carbon source.

The concept of upgrading waste and recycling by-products back into production pathways is gaining popularity under the umbrella term "biorefinery" [16]. The International Energy Agency defines a biorefinery *as "the sustainable processing of biomass into a spectrum of bio-based products and bioenergy"* [17]. By generating biomass from low-cost waste, and upgrading each component into different biofuel products, the practical yield and economic returns of the biorefinery approach are far superior to those associated with the conventional lipids-focused biodiesel approach [16,18,19]. Boasting a CO_2 fixation rate that is 10-50 times greater than that of land plants [20], and responsible for fixing approximately half of all carbon dioxide in the biosphere [21], microalgae and microalgal consortia exhibit extraordinary promise for negative-emissions technologies (NETs) and eco-engineering.

2.1. Current obstacles

The myriad concerns associated with large-scale cultivation of microalgae for biomass production stem from one key issue: resources and associated costs required to facilitate the entire process, from cultivation to finished bioenergy product [18,19,22]. Depending on location, natural environment, and culture conditions, the energy and/or chemical

resource requirements for productive cultivation can completely outweigh the economic returns of using microalgae for wastewater treatment [23] or carbon sequestration [24,25], let alone the finished biofuel products [26,27]. Furthermore, many commonplace methods used for valorizing biomass to biofuel products are similarly expensive, and require the use of toxic solvents [28].

Once a major bottleneck due to economic constraints, many great strides have been made towards the goal of productive microalgal cultivation under conditions that previously required expensive engineering and energy input, such as colder environments [29], low light conditions [14], even in the presence of toxic heavy metals [30]. Microalgae can be cultivated in various types of wastewater without nutrient supplementation [31–33], which overcomes the economic hurdle of growth media preparation [34,35]. Furthermore, several studies have successfully utilized industrial flue gas as an inorganic carbon source for autotrophic microalgae bioproduction [29,34,35], and organic carbon-enriched wastewater (e.g. whey from cheese processing) for mixotrophic and heterotrophic cultivation [14,36,37]. Upgrading these waste sources into low-cost or cost-free growth media significantly reduces both financial and energetic inputs required for microalgal cultivation. The economic obstacles that remain, however, are 1) efficient and low-cost biomass harvesting, and 2) high-yield and eco-friendly upgrading of biomass to biofuels.

3. Improving biomass harvesting

The small size and low density of microalgal cells in aqueous media poses a persistent obstacle in the first stage of biomass valorization [38]. Harvest processes alone may comprise 20-30% of the total cost of biomass production [39]. Harvesting techniques must perform two tasks; condensing biomass, and separating it from liquid media (dewatering). Because of its effectiveness and technological readiness level (TRL; rated

1-9, with 9 denoting the highest readiness level), the most common industrial method for dewatering microalgal biomass is centrifugation (TRL 9) [40]. Centrifugation, apart from being highly energy-intensive [41], can impose prohibitory expense in capital cost for new microalgae startups [42,43]. Other mechanical methods, such as filtration and gravimetric sedimentation, are also considered inefficient beyond the laboratory scale [38,44].

Various compounds and materials can be employed to induce microalgal aggregation for easier harvesting. Each type of compound incurs both direct and indirect costs; the direct cost of the compound, as well as its effects on downstream processes [43]. Some compounds (e.g. ferric and aluminum salts, or synthetic acrylamide polymers [41]) can contaminate the biomass, which may interfere with subsequent chemical processes like transesterification, or biological processes like fermentation of residues to ethanol [45]. Other compounds (e.g. sodium hydroxide or calcium phosphate, [41]) produce environmentally damaging effluents, which are expensive to treat. These considerations must be taken into account when balancing the energetic requirements and final costs of the biorefinery system with its revenue [41]. Several lower-cost and lower-energy methods for biomass harvesting are reviewed in the following sections, and compared in Table 1.

3.1. Gravimetric sedimentation

Most free-living microalgae species require agitation (aeration, stirring, shaking, etc.) to remain suspended in liquid media, and will settle naturally once the agitation ceases. Gravimetric sedimentation, while energetically favorable, is a slow process highly dependent on cell size and culture density [44]. For systems using microalgae ≥70 μm and dense culture conditions, the energy savings associated with gravimetric

sedimentation may be worthwhile; however, most commercial microalgae species are smaller (2-20 μm) [41] and are too easily re-dispersed in the medium for efficient sedimentation [38]. Furthermore, the time required for gravimetric sedimentation allows cell death and subsequent proliferation of harmful bacteria and other microorganisms, which generate unwanted compounds (e.g. hydrogen sulfide) and degrade microalgal biomass [42].

3.2. Coagulation

Microalgal cells are negatively charged, which helps them remain in suspension with a mild repulsion effect, but also forms the basis for most chemical and electro-assisted coagulation methods. Coagulation actively neutralizes the negative charge on microalgal cells, mitigating the repulsion effect and causing them to aggregate, eventually facilitating gravitational sedimentation. Generally, coagulation methods involve the simple addition of coagulant compounds or an electric field to microalgal suspensions with gentle agitation, and then waiting for the biomass to settle to the bottom of the bioreactor.

3.2.1. Chemical coagulation

Chemical coagulants are ionic compounds added to the media to quench the cells' negative charge. Strongly alkaline metal salts, such as ferric or aluminum chloride, readily dissociate in water, and the resulting cations neutralize the negatively-charged cells, mitigating the repulsion effect [41]. While chemical coagulants are readily available, often inexpensive, and generally effective, most are toxic and/or corrosive, and pose significant environmental concerns. Toxic chemical coagulants may also contaminate the biomass during harvesting, and complicate downstream processing, especially if any of the biomass fractions are used for food, feed, or fertilizer [41]. Although multivalent ferric and aluminum salts are commonly used in low doses for

municipal water treatment, the quantities required to coagulate microalgal biomass are problematic [46]. Other trivalent metal sulfates (including chromium) are particularly toxic when used in high doses, and the use of these chemical coagulants generates high volumes of toxic sludge [42]. Milder anionic and non-ionic coagulants have been investigated, without much success.

3.2.2. Electrocoagulation

Electrocoagulation operates upon the same principle as chemical coagulation, using an electric field to neutralize negative charge. Electro-methods are advantageous because no chemical additives are needed, and biomass recovery is high [40]. Electrocoagulation requires "sacrificial" reactive metal electrodes, which react with the medium to produce positively charged ions and induce coagulation by inactivating repulsive negative charges on the surface of microalgal cells [47]. These cations interact with hydroxyl radicals produced by the electric field to generate metal hydroxides, which adsorb to the cell surface and assist in forming flocs, which are finally lifted to the surface via hydrogen gas bubbles (created by H_2O electrolysis) [48] or inducing the cells to move across the medium and aggregate on the cathode (positively-charged electrode) [40]. Compared with chemical coagulation, electrocoagulation has the environmental benefit of requiring no harsh chemical additives [47], and can outperform centrifugation in terms of biomass recovery [40]; however, the cost of single-use electrodes combined with the energy requirement can disqualify it from most large-scale applications on both environmental and economic grounds [49]. Misra et al. [50] circumvented this issue by applying non-sacrificial carbon electrodes in a novel configuration. They reported a recovery efficiency of 83% for *Scenedesmus obliquus* at 1.5 A, pH 9, and the addition of 6 g L^{-1} NaCl; comparable to values reported for centrifugation, filtration, and chemical flocculation.

Additionally, the use of non-sacrificial electrodes prevents metallic contamination of biomass [50].

In order to address some of the shortcomings associated with electrocoagulation, a novel reactor design was recently proposed and tested by Parmentier et al. [48] using iron (Fe) and aluminum (Al) electrodes. The study reports a remarkable 100-fold cell density after electrocoagulation, and low energy consumptions associated with both electrodes; 2.0 kWh-kg and 1.1 kWh-kg for Fe and Al, respectively. The Fe cell removal efficiency was shown to be higher than that of Al (88% and 73%, respectively), although Al electrodes showed better concentrating efficiency (up to 35,200 mg L^{-1} for Al compared with 18,500 mg L^{-1} for Fe). Finally, this study demonstrated that post-electrocoagulation effluent was nutrient-rich enough to be recycled as microalgal growth medium [48]. This study gives renewed promise to electrocoagulation as a better alternative to chemical coagulation. Further, the energy requirements for electrocoagulation could be met with solar or wind energy in many places; even a downstream bioenergy product (ex. methane, via anaerobic digestion) produced from harvested microalgae.

3.3. Flocculation

Flocculation is the process of cell aggregation (called flocs) in liquid media [38,51]. The flocculant may be a chemical, a polymer, another living organism, or an electrical field. Flocculants, unlike coagulants, do not actively neutralize the negative charge on the microalgae cell, instead using the charge to attract and aggregate cells into flocs, into an organic matrix, or onto a non-reactive electrode. Similar to coagulation, however, flocculation occurs when a flocculating compound is added to the microalgal suspension in order to induce the formation of flocs, which either settle or are more easily removed via other methods, such as centrifugation or flotation (Section 3.4).

3.3.1. *Autoflocculation*

Some microalgae are self-flocculating; e.g. spontaneously aggregating and settling if the culture is not actively agitated [52]. Introducing stress conditions in the culture, like carbon limitation, can induce auto-flocculation in some microalgae species [38]. Depletion of CO_2 and subsequent changes to pH can also induce autoflocculation [51]. In these cases, stress conditions induce microalgae to exude extracellular polymeric substances (EPS), which then naturally aggregate cells [51]. Akin to gravimetric sedimentation, and despite similarly non-existent energy requirements and low cost, autoflocculation is too slow and inefficient for most large-scale algal cultivation systems [44,51]. However, EPS introduced by the addition of other organisms (such as bacteria or unicellular fungi) during harvesting may prove a more effective and controllable method of flocculation, as detailed in Section 3.3.2.

3.3.2. *Bioflocculation*

Bioflocculation utilizes either bio-based materials or other living organisms. Organic polymers like chitosan and cellulose, which have a natural positive charge [53], help to aggregate microalgal cells, especially when augmented with cationic functional groups, which cause a bridging effect via strengthened electrostatic attraction [53]. Flocculation efficacy is influenced by molecular weight of the polymer, charge density, growth medium chemistry, microalgal cell size, and culture density [41]. Bioflocculation has the added benefit of significantly reducing energy expenditure during harvesting. Salim et al. [54] explored the effects that ratios of flocculating to non-flocculating microalgae have upon biomass recovery efficiency, and observed that higher ratios of flocculating microalgae increased recovery of the non-flocculating microalgae for all four flocculating species tested, up to 30%. Moreover, combined with centrifugation, using a ratio of 0.25

flocculating to non-flocculating microalgae, Salim et al. [54] demonstrated that biomass harvesting energy can be reduced from 13.8 to 1.83 MJ kg DW^{-1}.

Other organisms cause microalgal flocculation in natural systems, as well as during industrial applications, such as wastewater treatment, where bacterial populations are high. Van Den Hende et al. [55] co-cultured a microalgal consortium with aerobic activated sludge bacteria, which resulted in the formation of microalgal-bacterial flocs. When employed as a secondary sewage effluent treatment, these flocs met European discharge standards for N, P, NO_x, SO_x, turbidity, and pH; with a hydraulic retention time of 0.67 days. Furthermore, once agitation had ceased, they aggregated and settled much more rapidly than un-flocculated microalgae of similar cell size [55].

Extracellular compounds excreted by heterotrophic bacteria and cyanobacterial blooms are the most probable mechanism of flocculation, but the exact mechanism remains unclear. Fungal species can function as a living bioflocculant; positively-charged hyphae attract negatively charged microalgae and aggregate them in the hyphal matrix [39,41]. A comparative study between fungal spores and fungal pellets identified temperature, glucose addition, pH, and ratio of fungi to algae as critical parameters for fungal-mediated bioflocculation; the highest efficiency occurred after 28 h, at 40 °C, 5 g glucose L^{-1} and 1.1×10^4 cells mL^{-1} (using spores) [56]. Another study reported promising results using cationic cellulose nanocrystals (CNCs); compared with chitosan, CNCs induced flocculation at a concentration of 11 mg L^{-1}, while chitosan required a dose of 35 mg L^{-1}. The authors reported a 5% greater maximum flocculation efficiency for chitosan, however, warranting further study of alternative bio-based flocculants [57]. Another starch, cassia, modified with N-3-chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) to create cationic potential, achieved 93% biomass recovery in just 15 min [53]. Although generally slower and less efficient than chemical flocculation,

bioflocculation has the advantages of zero chemical contamination, and additional biomass harvested, which can translate into greater bioproduct yields, albeit with higher carbohydrate content.

3.4. Flotation

Flotation methods, such as dissolved air flotation (DAF), generate small bubbles by sparging liquid media with pressurized air. This process saturates the medium with air, forming micro-bubbles which carry cells to the surface [58]. DAF is, by itself, not particularly efficient, but has shown renewed promise in combination with other techniques [58,59]. The most basic of these is DAF combined with pH modulation; according to a 2020 study, the ideal pH for DAF harvesting of *Chlorella* is pH 12, achieving cell recovery between 96.5-97.9 [60]. However, *Chlorella* sp. and most other microalgae species are intolerant of alkaline conditions, and thus pH modulation would require significant pH adjustment prior to harvest, which may not be environmentally sound. A more robust and eco-friendly method is foam flotation, which can be coupled with other techniques (such as electrocoagulation) for effective microalgal harvesting without harsh chemicals.

3.4.1. Foam flotation

DAF efficacy can be greatly enhanced by adding surfactants (foam flotation, FF), which produce positively-charged bubbles that aggregate cells as they rise through the medium [59]. Cationic surfactants alone are generally more effective than anionic surfactants, due to negative cell charge; however Liu et al. [59] demonstrated a marked improvement of performance of anionic sodium dodecylsulfate (SDS) when augmented with chitosan. Foam flotation requires more sophisticated sparging equipment, such as microporous spargers or mechanical agitators, but bubble formation using FF methods is considered

more energetically efficient than DAF methods alone by creating hydrophobicity in microalgal cells, allowing the bubbles to more easily carry them to the surface of the medium [51]. A study utilizing *Chlorella* and the surfactant cetyl trimethylammonium bromide (CTAB) found that just 100 mg L^{-1} CTAB resulted in 91% biomass recovery [61]. However, other harvest parameters were less feasible, such as an optimal pH of 10; too alkaline for most eukaryotic microalgae. To avoid costly and environmentally detrimental pH adjustments, this method could be employed for alkaline-tolerant cyanobacteria, which are naturally more tolerant to alkaline conditions, and could be grown at higher pH levels [20].

3.5. Attached growth

When cultivating filamentous species, the simple addition of removable surfaces for attached growth can dramatically reduce the amount of energy required for biomass harvest. A system developed by Adey et al. [62] utilizes floating screen mesh to encourage attached growth of filamentous microalgae in natural systems, and has shown potential for pollution remediation and biomass production [63,64]. Kangas et al. [63] reported biomass recovery values between 11-18 g dry weight (DW) m^{-2} day^{-1} with a mean of 12.3 ± 1.6 g DWm^{-2} day^{-1} when an Algae Turf Scrubber (ATS) system was deployed for pollution remediation in the Susquehanna River (Pennsylvania, USA). Employing this concept in a closed bioreactor (Fig. 2), Wicker and Bhatnagar [65] achieved a 33% increase in total biomass harvested as compared to conventional liquid cultivation, using mixed consortia with both free-living and filamentous species. By coupling cultivation of easily-harvested filamentous microalgae with emergent eco-friendly harvest techniques for cells in suspension, energy costs could be significantly reduced, and biomass yields enhanced.

4. Upstream processing

While harvested biomass can be utilized directly for many applications (e.g. for use as biofertilizer [17]), generally, it must undergo pre-treatment and processing, especially before use as fuel. The first pre-treatment stage is cell lysis; e.g. the disruption of cell walls and membranes to liberate intracellular metabolites, a process which can be applied to wet or dried biomass, and may be partially achieved during harvesting [38]. Harvesting methods like centrifugation persist in the industrial realm today partly because they provide pre-treatment by mechanical shearing [66]. Conversely, depending on the targeted bioproducts, cell lysis during harvesting can release cellular compounds into the medium, making them difficult or impossible to recover [66]. Common techniques for pre-treatment and lipid extraction are summarized in Table 2.

4.1. Cellular disruption

Lysing microalgal cells prior to lipid extraction and other processes (e.g. fermentation or AD) greatly increases the efficacy of subsequent treatments [38]. Cellular disruption can be applied to wet, dewatered, or dried biomass, depending on the ultimate downstream pathway, and are often conducted simultaneously with other processes, such as solvent extraction [67]. To minimize energy expenditure, this review focuses on methods that can bypass the drying step entirely and be applied directly to dewatered biomass or slurries. Further considerations in choosing methods for cell lysis include operational costs, scale-up potential, and sensitivity of target compounds to conditions formed during cell lysis, e.g. thermal degradation of longer chain fatty acids by temperature increases associated with ultrasound or microwave treatments. However, towards the goal of bioenergy production, most raw biomass products require more, rather than less, rigorous pre-treatments.

4.1.1. Ultrasound pre-treatments

Ultrasonic waves propagated throughout liquid media generate cycles of alternating high and low pressure, resulting in the creation and collapse of microbubbles, ultimately causing cell cavitation [67]. Ultrasound probes are inserted into microalgal suspensions, where they generate ultrasonic waves with the aim of creating a biphasic solution, with liberated lipids in the organic layer. Ultrasonic pre-treatments and ultrasound-assisted extraction (UAE) methods are effective for cell lysis and biomass solubilization, although selecting an appropriate solvent system is imperative for efficiency [67,68], and generated heat can cause unwanted degradation of cellular constituents [69].

Wang et al. [70] compared a 3.2 MHz focused ultrasound with a low-frequency 20 kHz non-focused ultrasound, and demonstrated that the 3.2 MHz focused treatment was more effective in terms of cellular disruption and energy requirement than the low-frequency 20 kHz treatment. However, when both frequencies were combined in a simultaneous treatment, the relative lipid increase rate was increased further, suggesting that frequency has an effect on cell lysis during ultrasound treatments [70]. Ultrasonic lysis is more effective at low frequencies (18-40 kHz), but still requires significant energy input for generating ultrasonic waves, and for cooling the medium to minimize uncontrolled thermal degradation and denaturation of valuable intracellular components [69]. Scale-up of ultrasonic processing is further hindered by its range of efficacy; cavitation occurs most efficiently in close proximity to ultrasonic probes, and decreases significantly with distance from the source of ultrasound waves [71,72].

4.1.2. Microwave pre-treatments

Microwave pre-treatments and microwave-assisted extraction (MAE) methods apply electromagnetic waves at 0.3 to 300 GHz, which form steam within the cells and cause

lysis via thermal induction. Like sonication, thermal degradation must be considered, and the costs and energy input associated with industry-level microwave treatments make scale-up difficult [73]. However, recent developments have shown renewed promise for MAE by reducing exposure time and microwave frequency required for effective cell lysis by combining with other techniques [68]. During a comparative study, MAE was used as a pre-treatment followed by four conventional solvent systems for lipid extraction from wet marine microalgae biomass. MAE increased lipid yield in all systems, with the greatest yield obtained from the Folch method [74], increasing from 8.47% lipid yield (non-MAE treated) to 12.8% with MAE [75]. Although MAE can reduce lipid extraction times (from 15 h to a few min) and decrease solvent usage by a factor of 10 [76], the use of organic solvents remains undesirable. Because of its high TRL (7-8), however, MAE may yet serve to enhance emergent extraction techniques, detailed in Section 4.2.3.

4.1.3. Pulsed electric field treatments

The principle of pulsed electric field (PEF) pre-treatment is the same as the concept of electroporation (used to penetrate cell membranes for genetic transformation). It can be utilized with "greener" polar solvents, such as ethanol or ethyl acetate, to increase extraction efficiency [77]. Applying an intense electric field by inserting electrodes into microalgal suspensions for short periods (nanoseconds to milliseconds) results in cell membrane permeability, facilitating exchange of materials across intracellular and extracellular space [78]. Because of shorter exposure times, PEF treatments are less costly than UAE or MAE (the energy costs of which depend upon power rating). PEF treatments are advantageous because they do not contaminate or damage target compounds [72,79]. While methods like solvent extraction degrade the cell wall, PEF targets the cellular membrane, and can even be applied in cycles to the same microalgal cultures without

significant cell death [80].

Recent findings have highlighted the efficacy of PEF, especially when used with other extraction methods. Silve et al. [81] reported a 97% lipid recovery using PEF pre-treatment and a 20-h Soxhlet extraction procedure (using a water/ethanol/hexane solvent system). These results are remarkable considering the mere 10% lipid recovery from untreated biomass, the control in this study.

Previous findings show that field strength had no measurable effect upon cell disintegration when an electric field was applied to an aqueous microalgal suspension [82]. However, Buchmann et al. [80] explored the effects of PEF on living cells and the possibility of continuous PEF extraction without whole culture harvesting. While a lower strength electric field (10 kV cm^{-1}) allowed cells to repair themselves and recover at a high rate (93.8-99.5%), it did not yield significant protein. When a stronger electric field (20 kV cm^{-1}) was applied, it reduced the cells' ability to recover (29.4-46.0%) and hindered growth, but yielded higher protein. This study further examined the effect of field strength on membrane permeability, demonstrating the common theory of electropermeabilization; a critical field strength must be exceeded to open membrane pores large enough for target molecules to pass [80]. This result mirrors previous findings that show higher field strengths (exceeding 40 kV cm^{-1}) are optimal for extraction [83]. There is a trade-off effect; efficient extraction comes at the expense of cell death [80]. PEF is best applied as a harvesting method, to precede or augment other processes.

4.1.4. Osmotic shock treatments

Osmotic shock employs a sharp increase or decrease in the salinity of the medium, which disrupts osmotic equilibrium and ruptures cell membranes. Hypersaline stress occurs when the salt concentration is greater outside the cell, causing cells to contract and release

intracellular fluids. Conversely, hyposaline stress occurs when salt concentration is higher within the cell, causing cells to draw in water, expand, and burst. Salt (NaCl) induces hypersaline conditions, while desalinating agents (such as sorbitol) are used to create hyposalinity [84]. Osmotic shock has marked advantages over physical methods like sonication and microwave, including low energy input, cost effectiveness, and ease of scale-up. However, osmotic treatments alone can be time-consuming. Their efficiency can be increased by combining with physical treatments, e.g. microwave [85]. The efficacy of osmotic treatments depends on the solubility of salts or desalination agents in the medium and permeability of microalgal cell membranes.

Yoo et al. [84] combined osmotic shock with polar and non-polar solvents for simultaneous cell lysis and lipid extraction from wet (99.4% water) biomass, intending to simulate economically reasonable conditions. The optimal salt concentration was determined to be ~60 g/L; analogous to brine effluents produced by desalination plants. While this method shows promise for efficient and cost-effective lipid extraction, the residual algal biomass cannot undergo further processing by anaerobic digestion (AD), as high salt concentrations inhibit anaerobic bacteria [86]. Furthermore, the most effective lipid recovery reported in this study resulted from cell wall deficient mutants, not wild type *Chlorella* [84]. Research has since established that higher growth rates and lipid productivity in wild type strains yield a greater net product recovery than mutants engineered to express reduced (or absent) cell walls, regardless of harvesting method [87].

4.1.5. Enzymatic treatments

Commercial enzymes such as lysozyme and cellulase selectively degrade cell walls with a low energy of activation [88]. Enzymes are advantageous for their specificity and low toxicity, because they do not form any inhibitory byproducts that can disrupt further

processing, and because enzymes operate at or near ambient temperatures, this method poses no risk of thermal degradation of sensitive compounds [69,72].

The greatest hindrance of enzymatic lysis is cost. Enzymes are produced by cultured organisms, and enzyme isolation is difficult due to denaturation. Enzymes are sensitive to temperature and pH. However, coupled with other pre-treatments, and assuming an economically viable pathway to biofuels, enzymatic treatments could yet hold promise. When developing a mechano-enzymatic pre-treatment method for fermentation of macroalgal biomass to bioethanol, Amamou et al. [89] demonstrated that vibro-ball and centrifugal milling resulted in 126% and 129% increases, respectively, in sugar liberation from biomass when coupled with enzymatic hydrolysis, compared with enzyme treatment alone. While bead and centrifugal milling are not effective for microalgal extractions on the large scale [69], simultaneous use of other methods such as microwave treatments or pulsed electric fields could increase enzymatic hydrolysis efficacy.

4.2. Lipid extraction

Extraction methods significant impact lipid content and quality. For biofuel production, important factors are carbon chain length, degree of saturation, and octane (or cetane) number of lipids extracted [79]. In many cases, cellular disruption methods (Section 4.1.) can be applied with lipid extraction simultaneously, to increase yield, minimize energy input, and target specific lipids for producing biofuels [73]. Several techniques are compared in Table 2.

4.2.1. Solvent extraction

A conventional pre-treatment preceding biodiesel production is lipid extraction using solvents. Coupled with other cell disruption methods, like sonication, solvent extraction

can effectively target and isolate desired compounds from harvested biomass [73]. Solvent extraction operates upon the basic chemistry premise “like dissolves like”; e.g. polar solvents dissolve polar lipids (mainly found in cellular membranes), whilst non-polar solvents can target neutral lipids (used as energy storage molecules, which are preferred for biodiesel production) [79]. Methods combining polar and non-polar solvents are generally the most effective, with polar solvents serving to lyse polar lipid-based cellular membranes, and dissolving target lipids in non-polar solvent. The solvent mixture is then mixed with water to form a biphasic solution, in which the aqueous layer contains polar solvent and polar lipids, whilst the hydrophobic layer consists of non-polar solvent and targeted neutral lipids [79]. Conventional solvent extraction methods such as Folch et al. [74] (chloroform/methanol, 2:1) and Bligh & Dyer [90] (chloroform/methanol, 1:2) are well established and commonplace in the laboratory, but cannot be efficiently scaled up, due to cost, corrosiveness, toxicity, and environmental impact [38]. Thus, green solvents have been targeted in recent research, aiming to fulfill the following criteria; lipid specificity, effective cell lysis, immiscibility with water, volatility, low cost, and low toxicity/corrosiveness [38,73].

Hexane is considered a “greener” solvent, especially for Soxhlet extraction. The Soxhlet procedure is continuous; i.e. solvent is continuously evaporated and re-condensed so the biomass is constantly supplied with fresh solvent [73]. Compared with methanol and chloroform, hexane is less toxic and equally selective, although hexane extraction requires drying and cell disruption [91]. Combined with relatively low energy requirements of Soxhlet extraction, hexane (and hexane blends) [73] show promise as an extraction method with high TRL, although yields per unit of energy expended need improvement [91]. Other organic solvents with better environmental profiles than chloroform/methanol are becoming cheaper and more readily accessible. Kanda et al. [91]

demonstrated that liquefied dimethyl ether (DME) can effectively extract lipids from diatoms without any pre-treatment to disrupt silica-based cell walls. Maximum lipid yields from hexane Soxhlet and Bligh-Dyer extraction methods, both of which require drying and pre-treatment, were 12.3% and 21.5%, respectively. Liquefied DME extraction from wet biomass with no pretreatment provided a maximum lipid yield of 22%. Because of its medium polarity and partial miscibility with water, DME is a promising new candidate for lipid extraction from wet biomass [91], detailed in Section 4.2.2.

4.2.2. Wet lipid extraction (WLEP)

Wet lipid extraction procedures (WLEP) reduce energy requirements of biomass processing by bypassing drying and directly applying extraction methods to dewatered biomass, e.g. post-centrifugation. During WLEP [92], biomass dewatered by centrifugation (with moisture content as much as 85%) is first subject to acid hydrolysis with 1 M sulfuric acid at 90 °C, base hydrolysis with 5 M sodium hydroxide at 90 °C, and followed by a second centrifugation. Several subsequent precipitation/centrifugation stages follow, requiring a second acid treatment and a final extraction step using hexane [92,93]. WLEP is ideally applied with pre-treatment techniques to microalgal slurries, e.g. PEF or certain types of solvent extraction. Drying biomass requires significant energy; utilizing WLEP to negate this results in overall energy gain rather than a negative energy balance [94]. The optimized WLEP defined by Sathish & Sims [93] and improved by Sathish et al. [94] allows biomass to be fractionated into three streams apart from the targeted lipid phase; residual hydrolyzed biomass, aqueous phase, and solid precipitate phase. By separating each fraction, this process allows them to be recycled, creating a zero-waste system, providing an economic advantage. Sathish et al. [94] recommend: 1)

upgrading hydrolyzed biomass via fermentative pathways (e.g. ABE fermentation, Section 6.2.1) or AD, 2) recycling the aqueous phase as growth medium for *E. coli* or other microorganisms, 3) incorporating protein-rich solid precipitates into aquaculture feed, and 4) converting extracted lipids into high-quality biodiesel via transesterification. Several drawbacks to WLEP, however, include energy requirements of multiple centrifugation steps and heat treatments, as well as potential environmental consequences of using strong acids, bases, and hexane. Future research to improve WLEP should focus on reducing the use of acids, bases, and organic solvents; ionic liquids described in the following section may offer a novel solution towards this research aim.

4.2.3. Ionic liquids

Ionic liquids (ILs) are non-aqueous salt solutions which contain only ions; typically composed of an organic cation and a polyatomic anion for the purposes of microalgal extraction [73]. They are non-volatile, thermally stable, chemically inert, and have low melting points, low vapor pressures, and good miscibility with most organic and inorganic solvents; all of which contribute to their efficacy as a solvent for microalgal extraction [73,95,96]. ILs can dissolve other cellular constituents whilst keeping microalgal lipids in suspension [79]. Although currently expensive to produce, ionic liquids are also recoverable after use as a solvent, due to their low vapor pressure. Other drawbacks related to IL use are length of extraction time, high ratio of IL to biomass, and the need for dewatering prior to cell disruption [97]. However, used in conjunction with microwave or ultrasonic cellular disruption methods, ILs show intriguing promise as a green solvent. Kim et al. [98] demonstrated an increase of 18 and 26 mg/g (dry cell weight) in total lipid yield using 1-butyl-3-methylimidazolium dimethyl sulfate ([Bmim][$MeSO_4$]) over Bligh and Dyer's and Soxhlet extraction methods, respectively. They achieved 1.6 times more lipid when [Bmim][$MeSO_4$] was combined with

ultrasound, and increased the reaction rate 2.7 times. Using [Bmim][HSO_4] and microwave, Pan et al. [96] increased the reaction rate 15 times for *Chlorella sorokiniana* and 10 times for *Galdieria sulphuraria*. A recent study tested two ILs, 1-octyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([Omim][NTf_2]) and 1-octyl-3-methylimidazolium acetate ([Omim][OAc]), in three concentrations (0.5%, 1.5%, and 2.5% *v/v*), and in conjunction with a 10 min 700 W microwave treatment. The 2.5% IL concentrations yielded a maximum 19.2% lipid extraction efficiency; almost doubling the Bligh & Dyer (2:1 methanol/chloroform) yield, which resulted 10.9% lipid recovery. This study showed the importance of hydrophobicity/hydrophilicity in solvent selectivity; hydrophobic ILs are better for targeting non-polar lipids, whilst hydrophilic ILs are more selective for polar lipids [97].

Addressing selectivity, other research has investigated the potential of "switchable" ionic liquids (S-ILs) – i.e. ILs with inducible and reversible hydrophobic-hydrophilic conversion abilities. A comparative study of novel azole-based C_6-dissopropanolamine (DIPA) liquids showed efficient separation of lipids from IL solvent by simply bubbling CO_2 gas through the extraction phase. Moreover, lipid analysis showed that the fatty acid composition of lipids extracted by S-ILs was similar to Bligh & Dyer results, indicating that they could be upgraded to quality biodiesel [99]. One such liquid, C_6-DIPA-imidazole, retained >83% of its initial extraction efficiency after 5 cycles of reuse [99]; strong evidence that S-ILs could become more economical in the future.

4.2.4. Supercritical fluids

A fluid is supercritical when it reaches a temperature and pressure beyond its critical point, where liquid and gas phases are no longer distinct [71]. Supercritical (SC) CO_2, methanol (MeOH), and ethanol (EtOH) have been popular choices for microalgal

extractions because the solvent polarity is reduced by the supercritical state; this means that they can be used to extract non-polar lipids, and separated easily by returning solvents to subcritical [79,100]. SC-CO_2 is attractive for lipid extraction compared with traditional organic solvents; above supercritical levels, CO_2 induces high permeability and diffusivity across cell membranes. SC-CO_2 is faster, non-toxic, and does not react with target lipids [100]. It demonstrates high selectivity towards biodiesel-desirable lipids [100], and, if extraction occurs at a facility that uses thermoelectrical, the CO_2 required for can be obtained from exhaust gas [71]. However, a comparative technoeconomic analysis of SC-CO_2 revealed that it is not always the most economical method. When investigating costs associated with producing biogas from microalgae via supercritical water gasification (SCWG, Section 7.3.), Albarelli et al. [101] reported that investment costs rose by 71% when wet algal biomass was subjected to SC-CO_2 extraction, compared with processing whole biomass via SCWG. Further, net energy recovery decreased when lipid extraction was considered, via both low-pressure solvent and SC-CO_2 extraction [101]. SC-CO_2 is best applied where waste CO_2 is readily available, and where infrastructure already exists to effectively concentrate it under high pressure.

4.3. Residual biomass

Following lipid extraction, a solid carbohydrate- and protein-rich residue remains. Most downstream processes applicable to residual biomass require pre-treatment, which is most commonly acid hydrolysis, used to break down complex carbohydrates into smaller glucose subunits that are more bioavailable to yeast or bacteria. In many cases, this breakdown process is achieved during cell lysis and lipid extraction; the general aim of pre-treatment is to reduce the degree of polymerization of carbohydrates, so that they are more effectively utilized by other organisms during subsequent biological conversion

processes (Section 6). Valorizing residual biomass is imperative for attaining economic viability of targeted biofuels; all side streams must provide revenue. Depending on culture conditions and harvesting methods, residual biomass may contain pigments valued as health supplements, proteins useful in animal feed, and other metabolites that can be isolated and refined. No fraction should go unused in a microalgal biorefinery.

5. Downstream processing – lipids

Following separation of target compounds from raw biomass, various chemical processing methods are applied to refine these constituents into usable fuels. Two major sectors that require fungible biofuel replacements are the transport and heating/power generation sectors, which largely rely upon oil. To date, liquid fuels are needed throughout the entire transport sector, from motorcycles to cargo ships. State-of-the-art refinery pathways towards bio-based oils are reviewed below, while target end-products are summarized in Table 3.

5.1. Transesterification

Transesterification is the most well-known and widely-implemented method for producing plant-based biodiesel. During transesterification, alkyl groups in plant-based triacylglycerols (TAGs; or triglycerides, TGs) are exchanged with alkyl groups of an acyl acceptor (an alcohol or ester), resulting in fatty acid methyl esters (FAMEs), the primary constituent of biodiesel [72,79,102]. Transesterification requires a catalyst and an acyl acceptor (Fig. 3a), and most pathways produce glycerol as a by-product [103]. Because it produces a liquid fuel with high combustion efficiency, transesterification remains the most popular of several lipid processing methods. It mimics gasoline in viscosity and combustion properties, and is a leading candidate for petroleum blending towards the aim of phasing out fossils altogether [72,79]. Biodiesel can be produced from almost any lipid

source; plant-based [104,105], animal-based [106], microbial [103], or even sewage sludge [107]. The biggest obstacle in renewable biodiesels is the economic hurdle; the cost of production still outweighs the price of the fuel, compared with petroleum prices (especially considering the COVID-caused drop in oil prices [3]), although emergent biodiesel production pathways are under development to minimize these costs.

5.1.1. Catalysis

Catalysts for transesterification are divided into two categories; homogenous (same phase as the reactants, e.g. liquid), and heterogenous (different phase than the reactants, e.g. solid or gaseous) [103]. Conventional homogenous catalysts are strong acids and bases. Base catalysis removes a proton from the acyl acceptor, making it a potent nucleophile [104,108]. Strong bases saponify free fatty acids (FFAs, present in all natural lipids), which causes emulsification of FAMEs with glycerol, complicating separation and compounding expense [106]. Strong acids serve as a proton donor to carbonyl groups in the fatty acid chain, rendering them strongly electrophilic [108]. Acid pre-treatment to reduce FFA content >2.5% (wt.) of the extracted lipid is an effective measure against saponification [109], but both acids and bases produce environmentally damaging effluents which require costly neutralization before they can be safely discharged [110]. Solid heterogeneous catalysts include alkaline mineral compounds like calcium oxide or zeolite particles, and acidic compounds like zinc oxide and porous carbon materials. A breakthrough thermochemical conversion method designed by Kwon et al. [105] demonstrated non-catalytic transesterification of animal fats using porous charcoal at ambient pressure, and found that the process was enhanced by the presence of CO_2. Moreover, applying this method for 1 min at 350-500 °C combines esterification of free fatty acids and transesterification of triglycerides into a single reaction, with 98.5%

conversion efficiency to biodiesel. Methanol was used as an acyl acceptor, but a later study from the same group reported similar success using silica gel in place of charcoal and EtOH as an acyl acceptor to valorize sewage sludge lipids to biodiesel via non-catalytic transesterification [107].

Transesterification may also be catalyzed by enzymes, notably lipase, which is unique in driving a non-reversible reaction [111]. Enzymatic esterification is an eco-friendly reaction suitable for oils with high FFA. However, enzymes are highly sensitive to temperature and pH, and are prone to denaturation. This shortcoming has been partially mitigated by whole-cell biocatalysis, during which whole cells containing intracellular lipases are immobilized for use in transesterification. Immobilizing enzymes increases their potential for recovery and reuse, thereby reducing the process costs by allowing enzymes to be repeatedly recycled; Su et al. [110] reported ~80% efficacy after five uses and acetone washes of immobilized lipase Novozym435. The major disadvantage to this approach is inefficient mass transfer of the enzyme, although the potential for cost reduction and environmental feasibility warrants future research [112].

5.1.2. Acyl acceptors

Methanol remains the most common acyl acceptor for transesterification; economically problematic due to widespread use and demand in other industries. Furthermore, methanol is highly toxic, and produces waste streams that are environmentally detrimental [104]. Other short-chain alcohols, such as EtOH, propanol, butanol, and amyl alcohol can be used, but are more expensive than methanol, and react less effectively with TAGs during base-catalyzed transesterification [109].

An alternative non-alcohol acyl acceptor is dimethyl carbonate (DMC). When converting other plant-based oils (cottonseed, soybean, and rapeseed), Su et al. [110] demonstrated FAME yields 2-3 times higher using DMC than MeOH. Additionally, this particular

study coupled DMC to lipase catalysis, and showed that DMC is non-inhibitory to sensitive lipases [111]. To optimize DMC as an acyl acceptor, Jung et al. [105] investigated transesterification of avocado oil to biodiesel. They demonstrated a maximum FAME yield of 92.96% (compared with a maximum 61.19% yield using MeOH) at a reaction temperature of 380 °C; an improvement attributed to the miscibility of DMC with avocado oil. These results are promising for developing greener transesterification pathways; DMC is neutral and odorless, non-corrosive, and non-toxic, inexpensive, and an effective solvent [111]. Further advances in non-catalytic transesterification were made by Mani Rathnam et al. [102]; comparing supercritical ethanol and ethyl acetate for the transesterification of dry microalgal biomass. They reported conversion rates of >60% for supercritical ethyl acetate, and a remarkable >95% for supercritical ethanol [102]. Nanomaterials are also gaining ground for transesterification catalysis, especially in terms of cost efficiency; nanocatalysts are reusable. Teo et al. [113] used a synthesized nano-calcium methoxide ($Ca(OCH_3)_2$) to achieve a FAME yield of 96% after five consecutive cycles.

5.1.3. Conventional vs. direct transesterification

Conventional transesterification occurs in two-stages. Lipids are extracted using a non-polar solvent (e.g.) hexane, and then reacted with a catalyst and acyl acceptor. This mechanism is water-sensitive due to saponification and potential hydrolysis of TAGs to FFAs, and is best applied to dried and pre-treated biomass [109]. Biodiesel yield and quality from conventional transesterification are superior to those from other methods, but, in many cases, the high cost of pre-treatment can comprise as much as 88% of the total production cost, which cannot yet be offset by the revenue returned by the finished product. Moreover, the energy requirements and extensive use of solvents make conventional transesterification environmentally questionable [28].

Direct transesterification takes advantage of WLEP (Section 4.2.2.). Also known as *in-situ* transesterification, it involves simultaneous extraction of lipids from dewatered (not dried) biomass and reaction with excess methanol. Due to water content in wet biomass, an acid pre-treatment may be required, followed by base-catalyzed transesterification [93]. A comparative study showed that a reaction temperature of 90 °C was ineffective for biodiesel production with water content >20%, but could yield 100% conversion at a temperature of 150 °C [114]. The optimal conditions reported were 4 mL methanol, 8 mL n-hexane, 0.5 M H_2SO_4 per 100 mg dry weight equivalent of wet biomass to achieve a 92.5% biodiesel yield with FAME content of 93.2%. The conversion efficiency is impressive, but toxic solvents and strong acids pose environmental and scalability problems. The use of these reactants for *in-situ* transesterification can be reduced; however, Nguyen et al. [115] demonstrated a 33% reduction in methanol requirement by adding 0.1 mol (per 1 mol MeOH) of either *n*-butanol or tetrahydrofuran [115]. Park et al. [116] achieved a maximum 97.1% biodiesel yield using ethyl acetate and ethanol in the presence of sulfuric acid as a catalyst, foregoing methanol entirely.

Finally, supercritical fluids are gaining momentum for *in-situ* transesterification of wet biomass. Tobar and Núñez (2018) compared different concentrations of CO_2 with ethanol and methanol at reaction temperatures of 200 and 300 °C, and reported increases of biodiesel yield by 23% at 200 °C and 26% at 300 °C when CO_2 concentration was increased from 0.0005 to 0.003 g CO_2/g alcohol. A recent study using supercritical methanol reported a maximum FAME yield of 96.9% with methanol loading of 115 mL g^{-1} biomass, and biomass lipid and water content values of 52% (w/w), 5.75 mL g^{-1}, respectively [118]. These recent improvements in *in-situ* transesterification of wet biomass show promise for significantly reducing the production costs of microalgal biodiesel.

6. Downstream processing – residues

Lipid extraction conveniently pre-treats residual algal biomass for subsequent conversion. The high costs and energy requirements of lipid conversion techniques have motivated studies examining energy balances of biogas production. Biogases include CH_4, H_2, and syngas (a mixture of CH_4 and CO, which may also include CO_2 and H_2). Of these, CH_4 and H_2 can be derived via biological processes (bacterial fermentation or AD), while syngas is generated via *Clostridia* fermentation or as a product of pyrolytic gasification (Section 7). Depending on major components remaining in the residue, processes should be selected to maximize biofuel product yield and minimize energy cost. For example, residues dense in complex polysaccharides require additional pre-treatment for fermentative pathways, and are better suited to AD, which foregoes extensive pre-treatment [119]. Conversely, high-nitrogen residues are unfit for AD due to ammonium inhibition [86], and should alternatively be upgraded by fermentation. Carbon to nitrogen (C/N) ratios can vary between species and culture conditions, and are a strong determining factor when choosing a processing pathway for residual biomass. Both AD and fermentation have the advantage of high TRL (9), and have been implemented on the industrial scale for decades. They have much lower energy requirements compared with more technologically sophisticated upgrading techniques, such as hydrothermal gasification or pyrolysis, although fermentation requires more chemical processing to isolate the volatile compounds produced (e.g. distillation). Both of these technologies also share a common drawback; they evolve greenhouse gases (both CO_2 and CH_4 from AD, and CO_2 from fermentation), valuable as energy products, but which must be carefully controlled and effectively utilized.

6.1. Anaerobic digestion

Anaerobic digestion (AD) is a well-established and practical biochemical conversion process for waste treatment [120], using bacterial/archaeal consortia to convert organic material directly into mixed biogases (e.g. CH_4, CO_2, H_2, etc.) under anoxia [86,119]. The steps of AD, conducted by different consortia members, are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, hydrogen gas is evolved, but ultimately consumed during the final methanogenesis step. The major end-product constituents are carbon dioxide and methane, with small amounts of hydrogen sulfide. Biogas is upgradable to syngas, or directly combustible [121]. AD also produces two nutrient-rich digestate streams, solid and liquid. Solid digestate can be used directly as biofertilizer [122], while liquid digestate can be recycled into microalgal growth media [14,65].

Methane is a desirable fuel because its combustion generates less CO_2 and more heat per unit mass than other, more complex hydrocarbons. AD can utilize substrates with high moisture contents and variable organic compositions (e.g. different protein/carbohydrate/lipid ratios affect AD less than other pathways, e.g. fermentation), and thus biomass requires less pre-treatment [64]. It can be produced from whole microalgal biomass or lipid-extracted residues, requiring low energy input, making CH_4 economically favorable [119]. Witarsa et al. [64] compared batch, semi-continuous, and pilot-scale AD modes of operation to explore the feasibility of producing CH_4 from algae grown using the ATS system deployed in a eutrophic river. The first batch culture experiment digesting mixed microalgal culture with 93% moisture produced 158 L CH_4 per kg of volatile solids (VS), and the follow-up semi-continuous mode provided 144 L CH_4/kg VS, with 60%–62% CH_4 in the final biogas. Scale-up to pilot-level digestion yielded 107 L CH_4/kg VS, and biogas with a maximum 66.4% CH_4 [64].

A two-stage AD process converting lipid-extracted *Scenedesmus* residue to hydrogen and methane gas was demonstrated by Yang et al. [123]. The first stage involved heat treatment of dewatered microalgae sludge (to select for hydrogen-producing bacteria, Section 6.2.) for bacterial fermentation to hydrogen. The effluent produced by the hydrogen stage was then digested with untreated microalgal sludge to produce methane. By separating hydrolysis from methanogenesis, biogas yields are maximized, while waste is drastically reduced. AD is one of the most practiced conversion technologies, and should be more broadly utilized with integrated microalgal components. Microalgae can provide both a substrate (lipid-extracted or carbohydrate-rich biomass) and a treatment pathway for the resulting sludge, fitting effortlessly into a circular biorefinery scheme.

6.2. Fermentation

Fermenting carbohydrate-rich microalgal residues into alcohols (e.g. ethanol, butanol, hexanol), biogases, and other volatile organic compounds (VOCs), e.g. acetone is attractive compared to conventional agro-waste fermentation because microalgae do not produce complex carbon compounds like lignin. Despite lacking complex carbon, microalgal carbohydrates (cellulose and various starches) are not immediately accessible to fermenting microorganisms [124,125]. Fermentation is the biological conversion of simple carbohydrates by yeast or bacteria under limited oxygen, and generally requires pre-treatment and hydrolysis. However, because lipid extraction lyses cells and degrades cell walls, fermentation pre-treatment processes may be minimized, or forgone entirely.

6.2.1. Bacterial fermentation

High-nitrogen residues inhibit AD, but organic nitrogen is an essential nutrient for anaerobic production of diatomic hydrogen (H_2). Biological H_2 production relies on the enzyme hydrogenase to catalyze oxidation of H_2. Although current biological routes to

efficient H_2 production require optimization, hydrogen gas is a potential high-value by-product within some biorefinery schemes that utilize bacterial fermentation [123]. Dark fermentation of residual biomass by *Clostridium* and other bacteria is a potential source of biohydrogen. Hydrogenases are common amongst anaerobic bacteria, such as *Clostridia*, as well as facultative anaerobes like *Escherichia* and *Enterobacter sp*. Hydrogenases are, however, inactivated by oxygen, underscoring the importance of anoxia for efficient hydrogen production. Hydrogen-producing bacteria are often thermophilic, and heat-treating fermentative cultures can select for H_2 producers over other anaerobes (e.g. nitrifiers, sulfur bacteria, methanogens) [126].

Apart from hydrogen, dark fermentation produces acetic, butyric, and propionic acids. Photo-fermentation is a process by which anaerobic photo-heterotrophic bacteria, such as *Rhodobacter*, convert organic acids to H_2 and CO_2. Photo-fermentation could serve as a logical secondary step following dark fermentation. Nitrogenases are responsible for hydrogen production during bacterial photo-fermentation, and, like hydrogenases, they are inactivated by oxygen. Most photo-heterotrophic bacteria can undergo oxygenic photosynthesis, which can be inhibited by maintaining a steady feed of organic acids and minimizing CO_2 and other inorganic carbon concentrations [126]. Other fermentation products of *Clostridia* are acetone, butanol, and ethanol (ABE fermentation). Ellis et al. [127] showed that a single species could convert acid-treated mixed microalgal biomass to ABE, that yields were increased from 2.74 g/L total ABE to 7.27 g/L when supplemented with glucose, and 9.74 g/L when the hydrolytic enzymes xylanase and cellulase were added. More work is needed to optimize bacterial fermentation of microalgal biomass, especially if hydrogen is the target. Biological hydrogen production is, as yet, too inefficient to provide a substantive renewable energy solution.

6.2.2. *Yeast fermentation*

Yeast fermentation by *Saccharomyces cerevisiae* is a well-established method of converting starch- and sugar-dense biomass into EtOH and CO_2. This conversion process may take place sequentially, in separate hydrolysis and fermentation steps, or as simultaneous saccharification and fermentation [128]. Shokrkar et al. [124] showed highly effective EtOH conversion using enzymatic hydrolysis as the only pre-treatment before *S. cerevisiae* fermentation; up to 92% theoretical yield. Another study sought to identify novel carbohydrate-rich strains native to nutrient-replete local conditions. Two strains, *Desmodesmus* sp. FG and the unidentified strain SP2-3 were selected for high carbohydrate production (57% and 70% dry weight, respectively), and because *Desmodesmus* produces high amounts of fermentable sugars [125]. After dilute acid hydrolysis, *S. cerevisiae* fermentation of biomass from these strains yielded up to 0.24 g EtOH per g of biomass, which corresponds with 87.4% of maximum theoretical yield [125]. Yeast fermentation is best suited for maximizing ethanol as the target product, especially if facilities exist to recycle evolved CO_2 into microalgal growth media, creating a closed-loop system. Furthermore, fermentation residues can be gasified [121], minimizing waste and increasing economic potential. A truly ancient biotechnology, ethanol fermentation has one of the highest TRLs of any biofuel pathway, and requires low energy input; integration within microalgal biorefinery schemes holds enormous promise for maximizing economic and energy yields. Ethanol is highly valuable as a biofuel and industrial solvent, and CO_2 produced can be recycled back into the cultivation system for microalgal growth; another way that microalgal bioenergy can fit into a circular biorefinery scheme.

7. Downstream processing – whole biomass

Thermochemical conversion techniques apply high temperature and pressure over varying time intervals to produce a liquid fuel such as biocrude oil or biokerosene from whole biomass. When comparing fuels (fossil-derived or renewable), the performance of the fuel is the target, not necessarily specific chemical composition. Fuels can vary significantly in carbon chain lengths and structures, flash and freezing points, sulfur content, etc., while providing the same results in terms of specific energy, energy density, and viscosity. The best strategy towards replacing fossil fuels on the industrial scale is to define optimum blends of biofuel components that impart the targeted physicochemical properties of the end product. While biodiesel and bio-alcohols are important as gasoline replacements, biocrude should be pursued as an alternative to heavy fuel oil, the primary fuel used in aviation and large watercraft.

Currently, although biocrude yields are continuously increasing, catalytic procedures to upgrade biocrude to transportation fuel (similar to biodiesel) are too costly to be economically competitive with fossils. However, this economic disparity is shrinking as upgrading technologies improve, and the value of biocrude side streams increases. Two renewable biofuels under development to replace aviation fuel are Bio-Derived Synthetic Paraffinic Kerosene (Bio-SPK, produced via classical transesterification followed by hydroprocessing for the generation of alkanes) and Fischer-Tropsch Synthetic Paraffinic Kerosene, generated via pyrolysis to syngas, Fischer-Tropsch synthesis of alkanes, and hydroprocessing [129]. Hydroprocessing pathways are time-tested and well-established from their use in petroleum upgrading [130], and can be similarly applied to biologically-derived oils using existing infrastructures previously used for petroleum refining. Hydroprocessing generally involves hydrodeoxygenation (Fig. 3b), hydroisomerization (Fig. 3c), and hydrothermal cracking (Fig. 3d). Hydrodeoxygenation removes oxygen

molecules, which can negatively contribute to acidity, corrosivity, and viscosity of the final biofuel product, while hydroisomerization and hydrocracking convert carbon chains to desired lengths, important for favorable cold-flow properties [130]. These processes are costly, requiring excess hydrogen and often a metal catalyst (such as Pt) [130]. These technologies are well-established in the petroleum industry, but not yet robust in terms of biomass upgrading, and require further study and innovation.

Three thermochemical conversion techniques dominate microalgal biocrude production – pyrolysis, liquefaction, and gasification, each with their own advantages and drawbacks. Pyrolysis requires biomass to be dried, which can be energetically expensive, while liquefaction and gasification can each be performed on wet biomass. Both pyrolysis and liquefaction can produce a high biocrude yield, as well as other phases with possible economic value (solid and liquid side streams during pyrolysis, and a liquid aqueous side stream during liquefaction), while gasification primarily produces syngas. Each of these processes and their respective products is detailed in the following sections.

7.1. Pyrolysis

Pyrolytic conversion of raw biomass to liquid, solid, and gaseous fractions is achieved by heating biomass to 300-700 °C (or greater) in the absence of oxygen [131]. Of pyrolysis product fractions, the liquid fraction is of highest value in terms of bioenergy [121,132]. Pyrolysis also generates solids (biochar) and low-value pyrogas in different ratios, depending on the type of pyrolysis [133]. Pyrolytic processes are either “slow” or “fast”. Slow pyrolysis applies lower heating rates (5-80 °C/min) and longer vapor residence times (5-60 min), and occurs at a maximum 600 °C. Slow pyrolysis is better suited to the production of biochar rather than bio-oil [131,134]. Fast (or flash) pyrolysis occurs at heating rates between 600 and 1000 °C/s, and vapor residence times between 0.5 and 5

seconds [134]. The typical product ratios of fast pyrolysis (by % weight) are approximately 60-75% liquid bio-oil, 15-25% solid biochar, and 10-20% pyrogas [134]. Pyrolytic temperature and residence times can significantly impact the quality of bio-products, especially bio-oil, by facilitating secondary cracking reactions. Different microalgal species with variable biomass qualities will necessitate temperature and residence time optimization to maximize oil yield [135].

The primary drawback of pyrolysis is the need for thorough dewatering, drying, and finely powdering biomass. However, if pyrolysis follows an efficient sequence of pretreatment steps, the economic and environmental value of microalgal pyrolysis to bio-oil may prove worthwhile. Considering the feasibility of using microalgae for effective nutrient recovery from wastewater under a variety of culture conditions [19,31,36,136], one of the most promising techniques for carbon-conscious nutrient recycling is the utilization of biochar as a soil amendment. Apart from the benefit of carbon sequestration by incorporating biochar into soils, these treatments provide other agricultural advantages, such as improved soil pH, conductivity, and controlled release of nitrogen and phosphorus due to the complexity of biochar molecular structure; all of which can increase agricultural yields [131,137]. Taken together, the economic returns of bio-oil and biochar produced from microalgae cultivated in wastewater warrant consideration.

7.2. Liquefaction

Hydrothermal liquefaction (HTL) applies high temperature (250–350 °C) and pressure (5–25 MPa) to wet algal biomass. Because water is used as the reaction medium, HTL is well-suited for upgrading wet biomass into biocrude, and offers the possibility of co-liquefaction with other sources of wet biological lipid, such as sewage sludge [138]. HTL converts all biomass fractions (carbohydrate, protein, and lipid) into bio-crude oil [28]

using pressure and temperature to decompose and reform biomolecules in different phases (gaseous, aqueous, and biocrude), which naturally partition once the mixture is returned to ambient conditions [139]. HTL generates a higher biocrude yield than either catalytic hydrothermal gasification (CGH) or pyrolysis, because, in addition to lipids, proteins and carbohydrates are also converted into biocrude [140]. However, resulting biocrude has higher water content, viscosity, and heteroatom content, compared to biocrude derived from other methods [141]. However, a catalyst-free method for liquefaction of low-lipid cyanobacteria using ethanol has shown potential for upgrading carbohydrate/protein rich microalgal biomass, as well as lipid-extracted residues. Zhou et al. [142] compared several processing techniques, including traditional methods such as Soxhlet extraction, and concluded that 1) hydrothermal treatment was effective for extracting proteins and saccharides, and 2) ethanol liquefaction protein/saccharide extracted residues provide biocrude with higher selectivity, low nitrogen content, and satisfactory energy density. Finally, the post-liquefaction aqueous phase can be fermented [142] to produce the ethanol necessary for further ethanol liquefaction under a zero-waste biorefinery scheme.

Conventional hydrothermal liquefaction does have some disadvantages. The energy required to create high temperatures and pressures may result in a negative energy balance in a poorly-designed system. When applied to whole biomass, protein and carbohydrate contents can affect biocrude quality by contributing excessive nitrogen and oxygen [143]. Recent findings have demonstrated that waste effluent from HTL processes can be recycled as microalgal growth media, however. McGinn et al. [144] tested nitrogen and phosphorus recovery from HTL effluent by *Scenedesmus*, achieving removal efficiencies of >99% and 68%, respectively, when nitrogen was recovered as ammonia and phosphorus was precipitated as struvite prior to microalgal use. These strides in HTL

technologies and techniques highlight its potential value in a circular biorefinery.

7.3. Gasification

The conversion of biomass into a mixture of combustible gases via partial oxidation at high temperatures (800–900 °C) is known as gasification. Biomass can be gasified in a gaseous reaction medium or via supercritical water gasification (SCWG) which facilitates effective hydrolysis [145]. The bio-syngas produced by direct microalgal gasification has low calorific value, and is best suited as gas engine/turbine fuel [121]. A newer gasification technique, chemical looping gasification (CLG), allows tighter control over syngas composition by using an oxygen carrier particle as the gasifying agent. Recently, CLG has increased the quality of microalgal syngas while reducing production [146].

Most microalgal biomass is a poor feedstock for gasification, although gasification of waste residues from other downstream processes could prove worthwhile. A specific type of gasification using lower temperature and pressure (typically ~350 °C and 21 MPa) is capable of producing methane and carbon dioxide from otherwise unusable residues. Applied with a metal catalyst, this method, (catalytic hydrothermal gasification, CHG), can effectively upgrade carbohydrate- or protein-biomass or residues to biogas [147]. A comparative study found that SCWG of non-lipid-extracted biomass yielded 8.3% more energy (MJ) as compared with SCWG of biomass that had undergone conventional solvent extraction [101].

From a biorefinery perspective, CHG is a sensible second stage following HTL (Section 7.2.). HTL functions as a lipid extraction and conversion method, producing two streams, lipid and aqueous. Gasification of the aqueous stream utilizes a waste product and valorizes it to another valuable bioenergy product (CH_4), and holds potential for nutrient recycling by capturing nitrogen, potassium, and micronutrients in a sterilized aqueous

stream, as well as precipitating phosphorus species [40]. These nutrients can be recycled back into a microalgal cultivation medium, and have high economic value for agriculture [122].

8. Conclusions and future perspectives

Circumstances (old and new) are forcing radical change in the global energy economy. As different parts of the world are experiencing COVID-related economic contractions, especially in the transport sector, strong efforts should be made to fund and develop resilient bioenergy systems. Synergistic combinations of upstream and downstream techniques are necessary to realize economic viability of microalgal biorefinery systems, and should be specifically tailored to local conditions, in terms of regional climate, economics, infrastructure, and available resources. Some of the most promising emergent upstream techniques are electricity-based, such as electrocoagulation for biomass harvesting, and electro-assisted lipid extraction. These electro-methods are easily powered by renewable energy sources, and, because they do not degrade or react with microalgal biomass, are highly suited to a biorefinery scheme that seeks to efficiently upgrade all biomass fractions. Fermentation and AD are low energy options minimal capital and operational costs, and can generate valuable bio-alcohols, biogas, and other industrially relevant compounds such as acetone. More sophisticated technologies for producing drop-in transport fuels are under development and beginning to close economic gaps, but require further study and optimization. Cleaner fuels like bio-kerosene or butanol may never be competitive with fossil equivalents as standalone products, but they can become competitive if all production side-streams are valorized into saleable products to offset conversion costs. Fungibility of bioenergy products, especially in terms of transport fuels, is imperative.

A model biorefinery scheme prioritizing low energy requirement and environmental practices is illustrated by Figure 1. In terms of harvest/dewatering, three options with different tradeoffs are listed. For centrifugation, if infrastructure exists already (as it is common in waste treatment and other industrial processes) there is no reason to discard it as an option, considering its efficacy. In order to make centrifugation environmentally sound, centrifuges can be retrofitted to run on renewable energy (e.g. solar panels in a climate like Sub-Saharan Africa) or linked to a renewable grid (e.g. extensive wind grids found in Norway and the UK). If 1) centrifuges are not already available or 2) not easily powered by renewables, options like foam flotation and bioflocculation are effective, non-toxic, scalable, and easily applicable in almost every microalgal cultivation system. Cellular disruption could just as easily be bypassed if the microalgal feedstock is high in carbohydrate (in this case, whole biomass processed in an anaerobic digester or fermenter). For higher lipid content, or other VAPs, the two most eco-friendly and industrially viable extraction options are PEF-assisted and MAE. Pulsed electric fields are appropriate for extracting delicate compounds, which could be damaged by heat during microwave treatments. However, microwaves are already used on the industrial scale in various applications, and may provide a fast and effective method for lipid recovery. Both extraction techniques are most efficient when used with a solvent; the most promising green solvent reviewed in this review is ethyl acetate (compared to ionic liquids, which remain prohibitively expensive to synthesize, compromising scalability). Ethyl acetate can be recycled as an acyl acceptor in subsequent transesterification. Dimethyl carbonate (DMC) is another green acyl acceptor, with demonstrated efficacy in non-catalytic transesterification. Additionally, hydrothermal processing has been used in petroleum refineries since the second World War for hydrocarbon upgrading.

The same processes (deoxygenation, followed by cracking and/or isomerization; Fig. 3b-d) can be applied to microalgal lipids to produce high quality fuels. The expense of this pathway, however, can only be justified where the infrastructure and hydrogen supply exist already (e.g. refinery cities like Newark, USA), and for oil-rich feedstocks (>70% lipid content). Finally, two of the most time-tested biological conversion methods reviewed herein are AD and yeast fermentation. Each have a TRL of 9, and the mechanisms that drive them are well-constrained. Lipid-extracted microalgal biomass is ideal for either pathway; although, high nitrogen content inhibits AD. The side streams generated by each pathway (e.g. digestate, CO_2) are easily recycled into microalgal growth medium, and the products are widely used and can be further refined (e.g. biogas into syngas).

Different circumstances exist across different regional climates and societal infrastructures, and not every part of this biorefinery model may fit every circumstance. With the abundance of technological possibilities described, and, notably, adequate funding to develop infrastructure, zero-waste biorefineries can be customized to suit local conditions and used to repurpose aging technologies. The world has seen remarkable change and detrimental economic contraction across transportation industries this year. The post-COVID economy will require extraordinary stimulus, best facilitated by shifting towards renewable energy. This global energy market flux provides unprecedented opportunities for job creation and novel infrastructures, aimed at engineering a climate-friendly and sustainable energy future without sacrificing luxuries such as air travel and globalized trading.

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Highlights

- COVID-related changes in global energy demand have spurred growth of renewables
- Microalgal biorefinery schemes can help achieve bioenergy resiliency
- Upstream and downstream methods for microalgal bioenergy production were reviewed
- Technologies were critically analyzed to minimize energetic and monetary costs
- A pathway is proposed to realize the economic viability of microalgal biorefineries

Graphical abstract

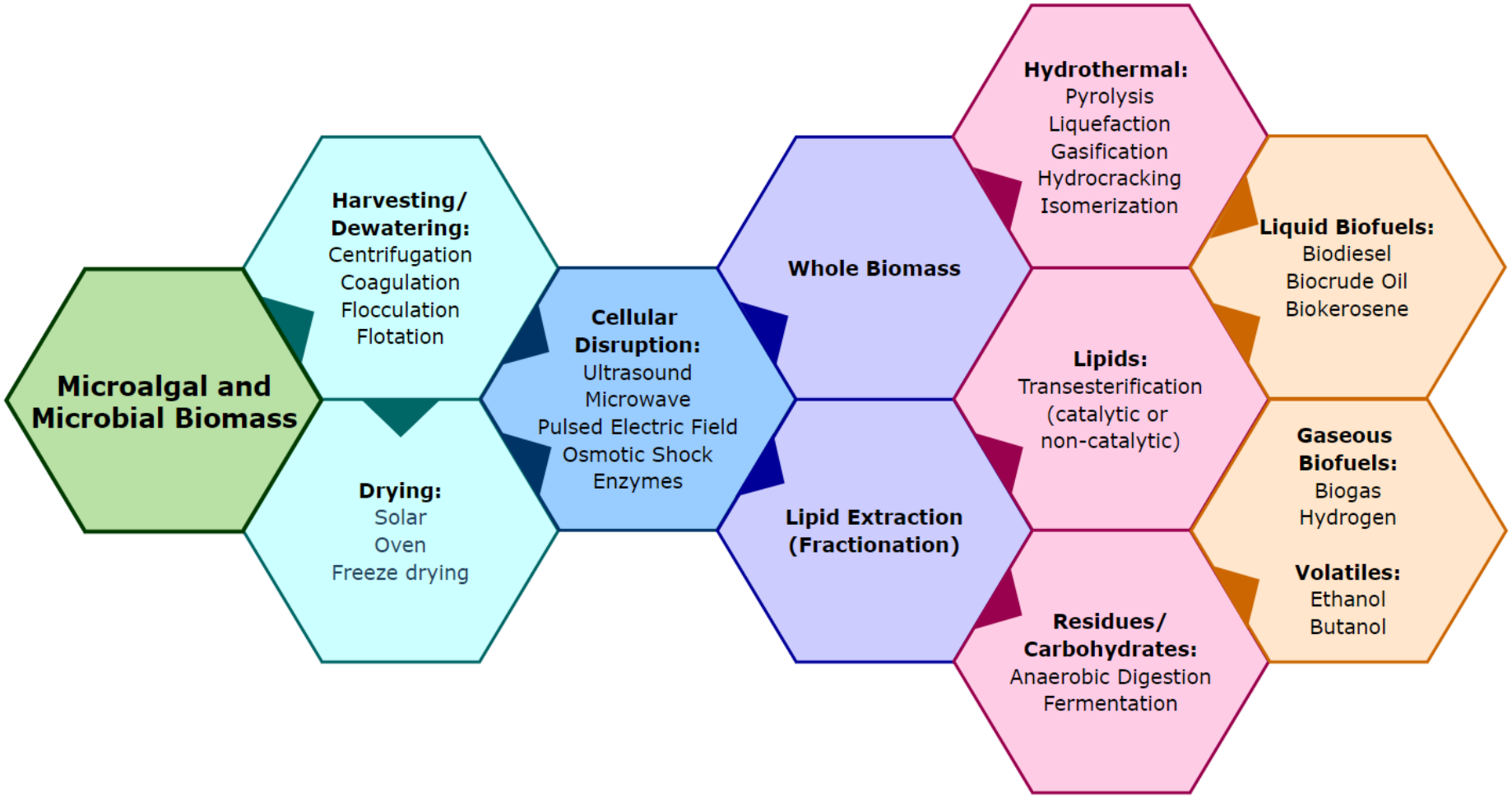


Table 1: Comparison of harvesting methods for microalgae in liquid media (SDS: Sodium dodecyl sulfate; CTAB: N-cetyl- N-N-N-trimethylammonium bromide)

Method	**Species**	**Initial biomass concentration**	**Condition**	**Recovery (%)**	**Effects on biomass and processing**	**References**
Centrifugation	*Chlorella* sp.	4.86×10^9 cells/mL	4000 rpm, 10 min	100	Cells prone to physical shear, some intracellular compounds may be lost; lipids may also be emulsified	[43]
	Scenedesmus obliquus	n/a	8000 rpm, 10 min	~100		[45]
Gravimetric sedimentation	*Chaetoceros calcitrans*	n/a	27 °C, illuminated, 8 d	91	Long settling time can promote bacterial growth which degrades biomass	[52]
		n/a	4 °C, dark, 8 d	70		
Chemical coagulation	*Chlorella minutissima*	220×10^6 cells/mL	$Al_2(SO_4)_3$ 1 g L^{-1}, 1.5 h	60	Al and Fe ions contaminate biomass, making it unfit for consumption, and may inhibit downstream bioprocesses	[148]
		220×10^6 cells/mL	$Al_2(SO_4)_3$ 0.75 g L^{-1}, 5 h	90		
		220×10^6 cells/mL	$Al_2(SO_4)_3$ 0.25 g L^{-1}, 5 h	38		

		220×10^6 cells/mL	$FeCl_3$ 0.75 g L^{-1}, 5 h	65		
		220×10^6 cells/mL	$FeCl_3$ 0.25 g L^{-1}, 5 h	57		
Electrocoagulation	*Microcystis aeruginosa*	1.2×10^9–1.4×10^9 cells/L	Sacrificial iron electrode, pH 7, current 1 mA cm^{-2}, 45 m	78	Al and Fe ions contaminate biomass, making it unfit for consumption, and may inhibit downstream bioprocesses	[47]
		1.2×10^9–1.4×10^9 cells/L	Sacrificial aluminum electrode, pH 7, current 5 mA cm^{-2}, 25 m	100		
	Scenedesmus obliquus FR75119.1	2.4 ± 0.01 g/L	Non-sacrificial carbon electrode, pH 5	73	Minimal effect upon biomass or downstream processes	[50]
		2.4 ± 0.01 g/L	Non-sacrificial carbon electrode, pH 7	65		
		2.4 ± 0.01 g/L	Non-sacrificial carbon electrode,	66		

			pH 9			
		2.4 ± 0.01 g/L	Non-sacrificial carbon electrode, pH 9, addition of 6 g L^{-1} NaCl	83	NaCl can inhibit downstream anaerobic digestion processes	
Autoflocculation	*Chaetoceros calcitrans*	n/a	pH adjustment to 10.2; NaOH 5 M, 4 h	98	Requires pH correction before downstream processing	[52]
	Chlamydomonas sp. CRP7	n/a	Cationic cassia gum, 15 m	93	Possible increase in carbohydrate content	[53]
Bioflocculation	Chlorella vulgaris	$OD_{750} = 1$	*Ankistrodesmus falcatus*, 3 h	50	Minimal effect of microalgal bioflocculant upon biomass or downstream processes	[54]
	Neochloris oleoabundans	$OD_{750} = 1$	*Tetraselmis suecica*, 3 h	72		
	Chlorella vulgaris	$OD_{680} \approx 1$	Filamentous fungi *Aspergillus oryzae*, pH 4-5, no glucose addition, 3 d	63	Minimal effect upon downstream processes, fungi may increase biomass quantity and carbohydrate content	[39]
		$OD_{680} \approx 1$	Filamentous fungi *Aspergillus*	93		

			oryzae, pH 4-5, glucose 10 mg L^{-1}, 2 d			
	Microalgal consortium containing *Chlorella* sp., *Pediastrum* sp., *Phormidium* sp., and *Scenedesmus* sp.	n/a	Aerobic activated sludge bacterial inoculum	98	Use of sewage sludge inoculum renders microalgal products unfit for consumption, but increased lipid content of total harvested biomass	[55]
Flotation	*Chlorella* sp.	6.8×10^5 cells/mL	Dispersed air, chitosan 10 mg L^{-1}, SDS 20 mg L^{-1}	90	Minimal effect of chitosan or surfactants upon biomass or downstream processes	[59]
		6.8×10^5 cells/mL	Dissolved air, CTAB 40 mg L^{-1}, pH 7	87-92		
	Dunaliella salina	n/a	Dissolved air, $Al_2(SO_4)_3$ 0.15 g	95	Al and Fe contaminate ions biomass, making it unfit for	[58]

			L^{-1}, pH 5		consumption, and may inhibit downstream bioprocesses	
		n/a	Dissolved air, $FeCl_3$, 0.15 g L^{-1}, pH 5	99		

Table 2: Comparison of common methods and solvent systems used for simultaneous cell disruption and lipid extraction from microalgae (RT, room temperature; FR, flow rate; [Bmim], 1-Butyl-3-methylimidazolium).

Method	**Species**	**Conditions**	**Solvents / other compounds**	**Lipid yield (%)**	**Advantages**	**Drawbacks**	**References**
Conventional solvent	*Nannochloropsis gaditana*	60 °C, 45 min	Methanol	38.3	Fast, effective	Toxicity, wastewater generation	[68]
	Chlorella sorokiniana	120 °C, 60 min	Chloroform/methanol 1:1	1			[96]
	Chlorella vulgaris	65 °C, 2 h	Chloroform/methanol 2:1	2.9			[98]
	Chlorococcum sp.	800 rpm agitation, RT, 7.5 h	Hexane/isopropanol 3:2	4.8			[100]
Ultrasound	*Chlorella vulgaris*	40 kHz, 2.68 W/m^2, 25 °C, 60 min	Chloroform/methanol 1:2	52.5	Fast, effective in combination with solvents	Loses efficacy with distance from sonication point,	[67]
		40 kHz, 2.68 W/m^2, 25 °C, 30 min	Dichloromethane/methanol 1:2	10.9			

		40 kHz, 2.68 W/m^2, 25 °C, 30 min	Hexane/isopropanol 3:2	2.2		energetically expensive, may degrade sensitive compounds	
	Nannochloropsis gaditana	21.5 kHz, 100 W, 60 °C, 20 min	Methanol	38.1			[68]
	Scenedesmus sp.	10 kHz, 5 min	None	7			[85]
	Chlorella vulgaris			5.5			
Microwave	*Nannochloropsis gaditana*	2.45 GHz, 25-30 W, 90 °C, 10 min	Methanol	40	Very fast, easily scaled up	Energetically expensive, may degrade delicate compounds	[68]
	Scenedesmus sp.	2.45 GHz, 100 °C, 5 min	None	10			[85]
	Chlorella vulgaris			10			
Pulsed electric field	*Auxenochlorella protothecoides*	4 MV/m, 3 Hz, 1 µs pulse	EtOH/hexane/H_2O 1:18:7.3 (post-PEF, 20 h extraction)	97	Increases solvent	Difficult scaleup,	[81]

	Ankistrodesmus falcatus	45 kV, 360 ns pulse	Ethyl acetate/methanol/ H_2O 15:5:9 (post-PEF)	88	efficacy when used as a pre-treatment	requires specialized technology	[77]
Osmotic shock	*Chlorella vulgaris*	1 min vortex, 48 h incubation	10% NaCl	8	Inexpensive, relatively non-toxic	Slow, inefficient	[85]
	Scenedesmus sp.			7			
Enzymatic extraction	*Chlorella vulgaris*	37 °C, pH 4.8, 2 h	Snailase	6.8	Eco-friendly, selective, low energy requirement	Expensive, enzymes are difficult to isolate due to pH and temperature sensitivity	[88]
		55 °C, pH 4.8, 10 h	Lysozyme	24			
		55 °C, pH 4.8, 10 h	Cellulase	22			
Ionic liquids	*Chlorella vulgaris*	65 °C, 18 h	[Bmim][CF_3SO_3]/methanol 1:1	19	Selective, non-toxic	Difficult and expensive to synthesize	[95]
	Chlorella vulgaris	60 °C, combined with ultrasound	[Bmim][$MeSO_4$]	7.4			[98]

	Chlorella sorokiniana	120 °C, 60 min, combined with 120 W ultrasound treatment	[Bmim][HSO_4]	16			[96]
		120 °C, 60 min, combined with 800 W microwave treatment	[Bmim][HSO_4]	23			
Supercritical CO_2	*Chlorococcum* sp.	FR 400 mL/min, 10-50 MPa, 60 °C, 60 min	CO_2	5.8	Non-toxic, utilizes waste gas	Energetically expensive, requires high pressures	[100]
	Chlorella vulgaris	36 MPa, 60 °C, with bead milling	CO_2	17.9			[95]

Table 3: Biofuels derived from microalgal biomass and their respective processing pathways. Abbreviations: TE, transesterification; PYR, pyrolysis; HTL, hydrothermal liquefaction; G, gasification; AD, anaerobic digestion; VS, volatile solids; ATS, algal turf scrubber; PHP, photobiological hydrogen production; F, fermentation; ABE, acetone, butanol, ethanol; RT, retention time.

Fuel	**Feedstock**	**Process**	**Parameters**	**Temp.**	**RT**	**Energy recovery/yield**	**References**
Biodiesel	*Monoraphidium* sp. KMC4	TE	H_2SO_4 and NaOH catalysis	70 °C	3 h	78%	[16]
	Nannochloropsis sp.	TE	Nano $Ca(OCH_3)_2$ catalysis	80 °C	3 h	99%	[113]
	Chlorella sp. FC2 IITG	TE	Direct TE using supercritical methanol	255 °C	25 min	96.9%	[118]
Biocrude oil	*Chlorella vulgaris*	PYR	Ni-loaded zeolite catalyst	500 °C	30 min	10.4%	[135]
	Microcystis sp.	PYR	Nitrogen carrier	500 °C	15 min	55%	[132]
	Nannochloropsis sp.	HTL	Nano Ni/SiO_2 catalyst	250 °C	60 min	30 wt%	[140]
	Chlorella sp., sewage sludge	HTL	H_2 0.3 MPa	340 °C	30 min	57.9%	[138]
Syngas	*Chlorella vulgaris*	G	Oxygen carrier	800 °C	20 min	93.9%	[146]

Methane	*Chlorella* sp., septic tank sludge	AD	Mesophilic, batch digestion	35 °C	30 d	300 mL g^{-1} VS	[120]
	Mixed-species ATS consortia biomass	AD	Mesophilic, semi-continuous	35 °C	20 d	144 L CH_4 kg^{-1} VS	[64]
Hydrogen	*Chlamydomonas reinhardtii* and bacterium *Thiomonas intermedia*	PHP	$Na_2S_2O_3$ supplementation	25-30 °C	n.a.	255.52 µmol H_2 mg^{-1} Chl	[149]
	Lyngbya sp.	PHP	Benzoate supplementation	32 °C	n.a.	17.05 µmol H_2 g Chl a^{-1} h^{-1}	[150]
Ethanol	*Chlamydomonas mexicana*	F	Simultaneous saccharification fermentation by *S. cerevisiae*	30 °C	3 d	10.5 g L^{-1}	[128]
	Enriched mixed-species microalgal	F	Separate hydrolysis fermentation by *S. cerevisiae*	30 °C	24 h	6.41 g L^{-1}	[124]

	consortia						
	Mixed-species biomass from municipal wastewater	F	ABE fermentation by *Clostridium saccharoperbutylacetonicum* N1–4	35 °C	96 h	0.53 g L^{-1}	[127]
Butanol	Mixed-species biomass from municipal wastewater	F	ABE fermentation by *Clostridium saccharoperbutylacetonicum* N1–4	35 °C	96 h	7.79 g L^{-1}	[127]

Table of Abbreviations

TRL	Technological readiness level
NETs	Negative-emissions technologies
EPS	Extracellular polymeric substances
CNCs	Cationic cellulose nanocrystals
CHPTAC	N-3-chloro-2-hydroxypropyl trimethyl ammonium chloride
DAF	Dissolved air flotation
SDS	Sodium dodecylsulfate
FF	Foam flotation
CTAB	Cetyl trimethylammonium bromide
UAE	Ultrasound-assisted extraction
MAE	Microwave-assisted extraction
PEF	Pulsed-electric field
DME	Dimethyl ether
WLEP	Wet lipid extraction procedure
ILs	Ionic liquids
Bmim	1-butyl-3-methylimidazolium
Omim	1-octyl-3-methylimidazolium
S-ILs	Switchable ionic liquids
DIPA	C_6-dissopropanolamine
SC	Supercritical
MeOH	Methanol
EtOH	Ethanol
SCWG	Supercritical water gasification
TAGs	Triacylglycerols
TGs	Triglycerides
FAMEs	Fatty acid methyl esters
FFAs	Free fatty acids
DMC	Dimethyl carbonate
AD	Anaerobic digestion
VOCs	Volatile organic compounds
ABE	Acetone, butanol, ethanol
HTL	Hydrothermal liquefaction
CGH	Catalytic hydrothermal gasification
VAPs	Value-added products

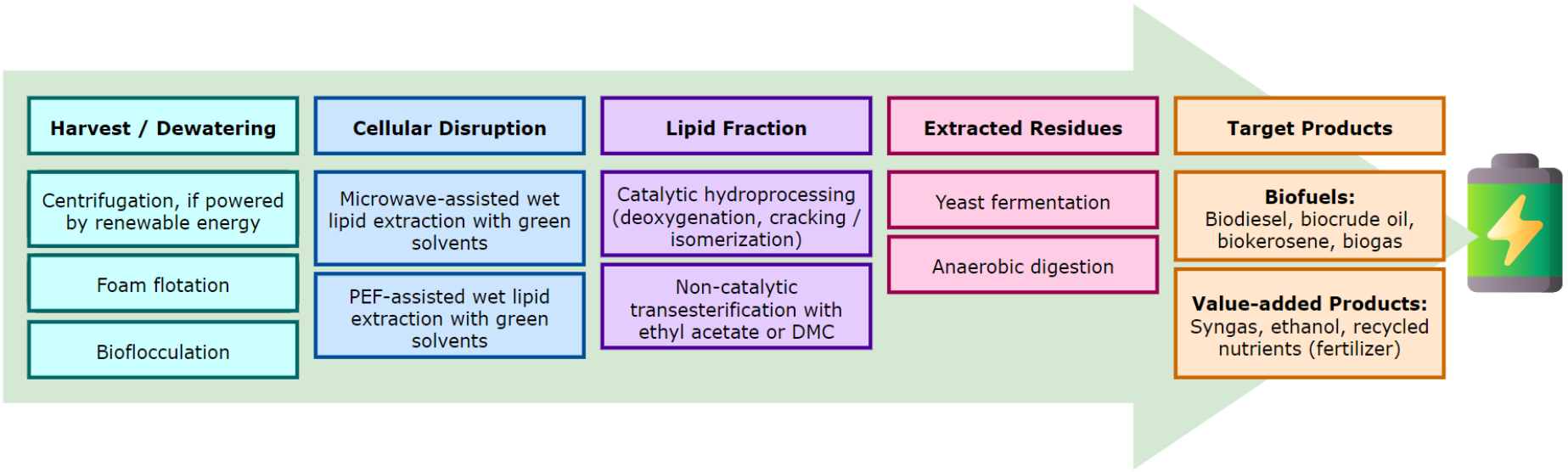
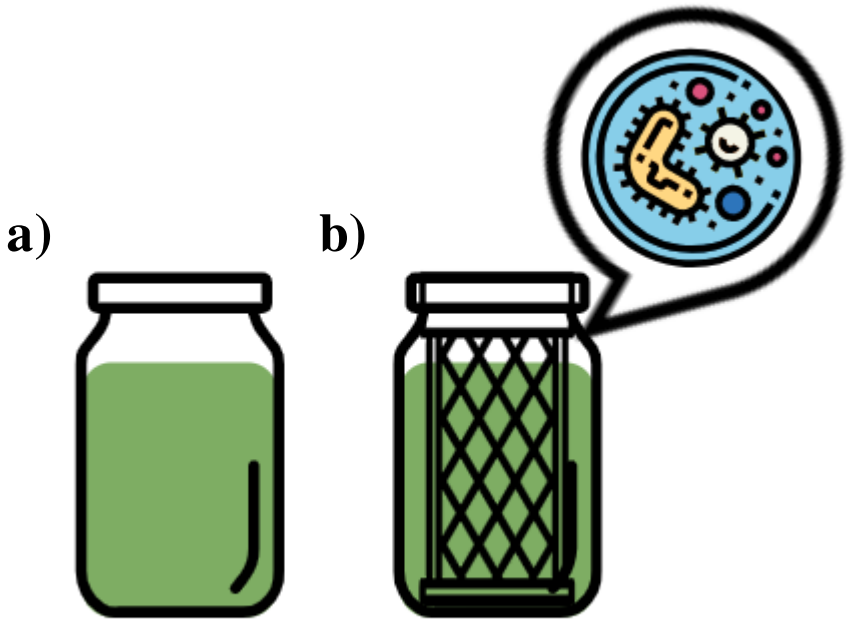


Fig. 1: A model biorefinery process chain prioritizing lower cost, lower energy, green approaches.

Fig. 2. a) Traditional liquid cultivation, b) attached growth on reusable plastic scaffolding, c) concept applied at the laboratory scale [65].



c)

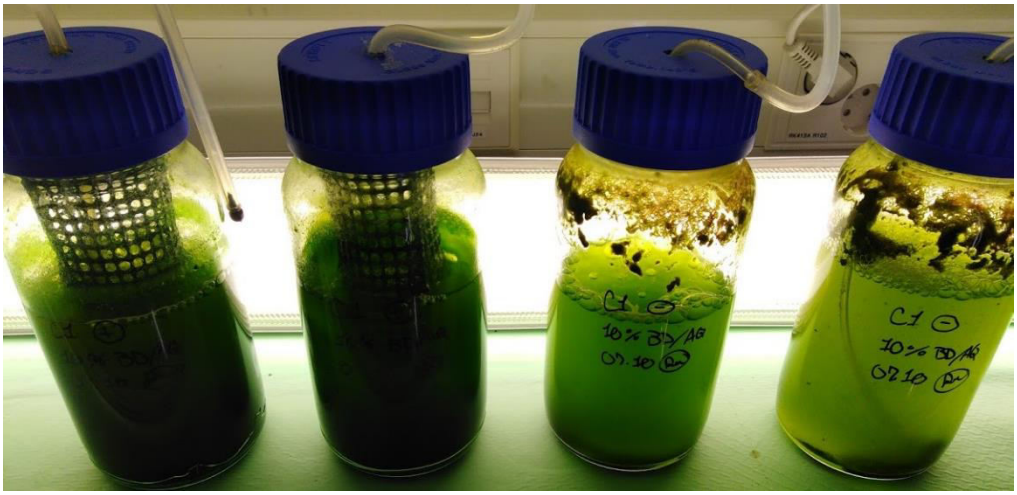


Fig. 3. Chemical conversions commonly used to produce liquid biofuels; a) transesterification [108], b) hydrodeoxygenation, c) isomerization, d) hydrocracking [130].