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Review

Potential of using Microalgae to sequester Carbon dioxide and processing to bioproducts

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Algae are microscopic photosynthetic prokaryotic or eukaryotic organisms that can naturally grow in fresh or marine water in the presence of sunlight. Algae are capable of sequestering CO_2 and utilize nutrients like nitrates, phosphates, and other micronutrients in water to increase their body mass. In the past few decades, algal biomass has been investigated by the scientific community because of its promising applications in producing renewable food, feed, fuels, and chemicals. Additionally, microalgae's ability to fix large amounts of greenhouse gas (GHG) such as carbon dioxide (CO_2) has led researchers to investigate microalgae as an alternative way of combating climate change by sequestering flue gas containing CO_2 emitted from industries such as coal power plants, cement, steel, and petroleum refineries. This review outlines different sources of CO_2 emissions and capturing technology including microalgae. Details about the methods of cultivating and separating the algal biomass followed by thermochemical and biochemical conversion to fuels/ chemicals, microalgae-derived bioplastics, and microalgae biomass blended plastic are outlined in this review. Commercially viable continuous and semi-continuous biorefining processes such as hydrothermal flash hydrolysis and environmental benefits of using microalgae as carbon sinks and wastewater treatment are also provided.

1 Introduction

One of the biggest threats facing humanity is global warming caused by GHG emissions such as CO_2, methane (CH_4), nitrous oxides (NO_x), hydrochlorofluorocarbons, hydrofluorocarbons, and hydrogen (H_2).[1] Rise in global temperature changes weather patterns causing extreme weather events such as powerful hurricanes, melting of glaciers and rise in sea level, tornadoes, and large precipitation in some places causing flood and drought in other places leading to water shortages and wildfires. These unpredictable weather conditions bring severe damage to property, people's livelihoods, and communities. About 80% of GHG emissions are from CO_2 that is produced due to human activities. Some of these activities include agriculture, combusting fossil fuels such as coal, oil, and natural gas for producing electricity, heat, and energy for transportation and processing industries such as cement, refinery, steel, petrochemical, food, and pulp.[2] The CO_2 emissions have risen to over 35 billion metric tons per year in the last five years (Fig. 1). Over 1.5 trillion-ton CO_2 has been released into the atmosphere since the year 1751. To reduce the GHG emissions goal of limiting average temperature rise to 2°C, new regulatory policies are implemented by European Union (EU) to cap the amount of carbon emissions industry.

Similar policies are under consideration by G7 countries at the global climate summit held in Sharm el-Sheikh, Egypt in November 2022 to address the global warming threat. In 2017, the EU set up Carbon Trade Exchange (CTX), also known as cap and trade to limit the GHG emission.[3] To meet the carbon cap regulatory standard, companies are purchasing carbon credit from CTX digitally anywhere in the world in a lot of 100 tons of CO_2. Five tradable credits on CTX are: (i) Voluntary emission reduction, (ii) Certified emission reduction, (iii) Verified carbon units, (iv) EU allowance, and (v) EU aviation allowance.[4] To meet the carbon cap set by the government environmental agencies, many companies are exploring options to use carbon capture, utilization,

and storage (CCUS) technology. Many companies are exploring the option to store CO_2 in geological formations or saline aquifers (porous permeable rocks) or on the seafloor.[5,6] This review provides a comprehensive overview of the threat caused by CO_2, and how microalgae can be used to sequester CO_2 and process the algal biomass to various biofuels, bioplastics and bioproducts that could displace fossil-derived fuels and products benefiting the environment.

2 Carbon Capture, Utilization, and Storage

2.1 Flue gas cleanup and compression

Flue gas is also called exhaust gas produced while combusting fossil fuel containing particulate matter called soot, Sulfur oxides (SO_x), NO_x, Oxygen (O_2), carbon monoxide (CO), CO_2, and metal contaminants in addition to nitrogen. The composition of flue gas generated by different commercial industries varies as summarized in Table 1. Some of the common industrial carbon emissions are: (i) post-combusted flue gas containing water vapor, CO_2, particulates, heavy metals, and acidic gases; (ii) pre-combusted synthesis gas containing CO and H_2 from fuel reforming and (iii) oxy-fuel that use pure oxygen for combustion to reduce NOx formation.[7] Life cycle assessment (LCA) clearly shows the benefits of transitioning from fossil energy to renewable energy such as solar, wind, hydrothermal, geothermal, and biofuels such as bioethanol, biodiesel, and biogas that are sustainable and benefit the environment. Such a transition will help to reduce our dependence on fossil fuels.[8] However, this transition will require a larger investment and time. Several CCUS have been developed to sequester CO_2 from industrial gas pollutants and by direct air capture.[9] Fig. 2 summarizes the sources of CO_2 emissions and different CCUS used in various industries. One of the popular CCUS process to remove SO_x, NO_x, and metal ion is through ozone oxidation. In this process, the flue gas is passed through electrostatic precipitator, catalytic

oxidation reactor and wet scrubber in alkali solution to produce pure CO_2.[10] Other CCUS methods that are developed include: (i) chemical absorption using solvents like amine (mono-ethanolamine, diethanolamine, methyl-di-ethanol amine), ammonia, piperazine at temperature between 40-60^oC followed by desorption by heating the solvents at high temperature (100-140^oC);[9] (ii) physical absorption process such as Rectisol (uses methanol), Selexol (use dimethyl ethers of polyethylene glycols), Purisol (N-Methyl-2-Pyrrolidone) and ionic liquids (salt in the liquid state;[11] (iii) chemical adsorption metal oxide such as CaO followed by desorption via calcination process;[11](iv) physical adsorption using activated carbon, zeolite, and metal–organic frameworks using pressure swing or temperature swing or vacuum swing or combinations;[6] (v) membrane based separation methods either using gas separation or gas adsorption using ceramic, polymeric or hybrid membranes,[12] (vi) chemical looping where iron, magnesium, or copper metal oxides reacting with CO_2 followed reduction,[13] and (vii) biological method using phototropic organisms such as plants, microalgae, and cyanobacteria.[14]

2.2 Method of CO_2 Storage and Transportation

For the ease of utilizing CO_2 in various applications, captured clean CO_2 gases using one or a combination of CCUS are compressed between 1,500 and 2,200 psi in cylinders and transported to different locations via trucks or through pipelines (Fig. 3). In some cases, the captured CO_2 are stored as liquids (−2°C and 20 bar) or solids after subjecting to compression, expansion, separation, and cooling using liquefied natural gas or compressor. The compressed liquid or solid CO_2 is transported in cryogenic vehicles via road, railroad, and ships.[15] The cost of producing solids or liquefied CO_2 is high compared to using compressors to store in cylinders. Some of the current applications of CO_2 are: (i) enhance oil recovery, (ii) enhanced coalbed methane recovery, (iii) used as feedstock for synthesis of chemicals such as methanol (via hydrogenation), polyol, succinic

acid, $CaCO_3$, and urea,[16] (iv) fire extinguishers, (v) supercritical fluid solvent in decaffeination of coffee or extraction compounds from plant tissues, (vi) supercritical drying, (vii) heating and cooling applications, (viii) food industry (carbonation of beverages, displacing oxygen in packed foods and storage applications).

2.3 Biological method of sequestering CO_2

The rate at which CO_2 is produced is much higher than it can be consumed, hence there is an urgent need to identify ways to sequester and the carbon can be used when necessary. Phototropic organisms such as plants and microalgae are good sequesters of CO_2 in the presence of sunlight, water, and nutrients (phosphate, nitrate, and minerals). Both the organisms' body mass is comprised of biomolecules such as proteins, lipids, and carbohydrates. In recent days. there is a setback for agriculture due to climate change and unpredictable weather patterns. Plants are now cultivated by vertical farming in greenhouses by injecting CO_2 produced by industries to overcome the challenges. Producing microalgae using CO_2 as a carbon source could also produce similar biomolecules like plants which could be processed into various products such as food, feed, fuel, and chemical molecules (Table 2). In other words, producing and processing microalgae has the dual benefit of sequestering CO_2 and producing sustainable feedstock for biorefining. Microalgae cultivation needs marginal land and can be cultivated using wastewater. It is estimated that microalgae could be produced between 140 – 280 ton/ha/year depending on light-to-biomass conversion efficiency (5-10%).[17] When microalgae are cultivated indoors, biomass productivity is less affected by the seasonal cycle.[18] The algal biomass productivity is much higher when compared to land-based crops produced by plants.

There are over one million different species of microalgae and 50,000 species have been identified, many of which with unique colors, composition, and size.[19] Some of the commonly

cultivated commercial microalgae composition in comparison with plant and animal products are given in Table 2.[20] Algal cell wall comprised of cellulose, pectin, and sulfated polysaccharides, starch in plastids (20-50%), lipids, and proteins. The composition of these components depends on the species types and culture media used to produce them and growth conditions. Algae are commonly classified by size (microalgae vs. macroalgae) and color (Rhodophyta-red, Charophyceae-golden, Phaeophycean-brown, and Chlorophyta-green, etc.) (Fig. 4). These microalgal biomass are commercially produced in industries and processed into various products such as proteins, lipids, carbohydrates, natural coloring agents such as carotenoids (β-carotene, fucoxanthin, violaxanthin, zeaxanthin, lutein, antheraxanthin, canthaxanthin, astaxanthin, and echinenone) and phycobilin (phycocyanin and phycoerythrin). Commercially produced microalgae biomass is commonly sold for food, nutraceutical, and plant stimulant applications.

Microalgae are unicellular organisms that are present either in freshwater or marine system.[19] They are broadly classified as eukaryotic microalgae (e.g., *Chlorella vulgaris, Scenedesmus obliquus, Haematococcus pluvialis, Dunaliella salina, Porphyridium purpureum*) (size 2-100 μm) and the prokaryotic cyanobacteria, also known as blue-green microalgae or Oxyphotobacteria (e.g., *Spirulina platensis, Nostoc commune, Synechocystis sp.,* and *Oscillatoria limnetica*) (size 0.5-60 μm),[21] Different strains of microalgae are used for cultivation either in fresh or wastewater in different modes: (i) photoautotrophic mode (presence of sunlight, CO_2, and micronutrients); (ii) heterotrophic mode (uses glucose, glycerol, and others as the carbon source to meet the energy requirement in the absence of sunlight) or (iii) mixotrophic mode (combinations of both photoautotrophic and heterotrophic conditions).[22]

3 Cultivation, Harvesting, and Processing of Microalgae

3.1 Microalgae Cultivation

Microalgae cultivation faces economic feasibility due to low biomass productivity, high infrastructure, and energy requirement.[23] Identifying the right cultivation and harvesting method influences the yield and cost of producing algal biomass.[24] Three widely used microalgae cultivation methods are: (i) raceway pond, (ii) close loop photobioreactor (PBR), and (iii) revolving algal biofilm (RAB), as shown in Fig. 5. Their advantages and disadvantages of different microalgae cultivation methods are summarized in Table 3. Factors such as light intensity, light supply, CO_2 sparging, pH, salinity, water depth, and quality of nutrients play critical roles in microalgae yield. Raceway pond microalgae cultivation pilot scale systems at Arizona Center for Algae Technology and Innovation (AzCATI) and other facilities in countries like Estonia, Faroe Islands, Spain, France, Iceland, Ireland, Sweden, Netherlands, Australia, and the United Kingdom use shallow interconnected pond with motorized paddlewheels or circular pond with rotating agitator. Paddle wheels and agitators are used to facilitate aeration, ensure equal distribution of sunlight and nutrients, and avoid microalgae biofilms formation and settling down.[25] This method of microalgae cultivation is widely used in the industry due to relatively cheaper infrastructure and operating costs. Average annual productivity of some promising brackish strains *Monoraphidium minutum* (26BAM) and Scenedesmus obliquus (UTEX393), and marine strains *Tetraselmis striata* (LANL1001) and *Picochlorum celeri* (TG2) increased from 11.6 to 17.6 gm m^{-2} d^{-1} after optimization trails carried out AzCATI.[26] The U.S. Department of Energy (DOE) Bioenergy Technologies Office target remains 25 gm m^{-2} d^{-1} annual average by 2030. Nonetheless, there are drawbacks to open pond systems, such as water loss due to evaporation, light exposure limitations (cloudy days or bad weather), larger land requirements, and contamination (such as birds depositing fecal matter and air pathogens that spread through air). Since the system is subject to outdoor weather, controlling media temperature and pH can prove challenging as well. PBRs are

closed-loop systems where nutrients (nitrates, phosphates, and minerals) and contamination can be precisely controlled to produce high algal biomass.

PBRs can be either vertical or horizontal tubes operated indoors with artificial lights or outdoors (open area or greenhouse) where natural sunlight is used.[27,28] Vertical PBR uses less land space compared to horizontal PBR systems. A tubular PBR is composed of long transparent polycarbonate or glass tubes, where the microalgae are circulated by either a mechanical pump or airlift systems, allowing proper mixing of nutrients.[29] The diameter of tubular PBRs ranges from 10 to 60 mm and several hundred meters long. Some of the drawbacks of PBR are low gas exchange, low illumination surface area, high shear stress, accumulation of biofilm on the tube surface, and high energy input. Other PBR uses bubble columns, flat plates, and fermenters with motorized agitators. Due to high infrastructure cost of cultivating microalgae, only high commercial value nutraceutical products such as Poly-Unsaturated Fatty Acids (PUFAs) such as Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and Docosapentaenoic Acid (DPA), β-carotene, astaxanthin; pigments such as phycobilin and Exo-Polysaccharides (EPS) are produced.[30]

RABs were first reported in the year 1970s and were used to remove phosphates and nitrates from wastewater using aluminum disks.[31] Subsequently several innovations were made to the RAB system and tested in wastewater treatment facilities at a pilot scale.[32–34] RABs operates like raceway pond with the key difference being an installed revolving biofilm on a cylinder. About 15 different materials were evaluated as the film in the RAB system such as stainless steel, nylon, polyethylene fabric, fiberglass, polystyrene, polycarbonate, and some natural materials. Among them, cotton and jute fabric were found to be highly effective support materials for microalgal biofilms.[35] Microalgae grown on the rotating biofilm can be harvested by simply scraping the

microalgae from fabric film in a continuous mode significantly reducing the cost associated with harvesting microalgae from water. Some of the disadvantages of RAB over the conventional suspended growth methods include CO_2 mass transfer in water and lighting requirements. The average microalgae yield is 1.65 tons/million gallons of wastewater per year using the RAB pilot system at Gross Wen Technologies, Iowa, USA and there is potential to increase the yield to 5 tons/million gallons of wastewater by bubbling CO_2 which must be confirmed through experiments as mentioned in a report prepared by Pacific Northwest National Laboratory in 2022.[36]

3.2 Microalgae Harvesting

Harvesting microalgae from water is energy intensive process due to, (i) low sedimentation velocity, (ii) smaller cells size (ranging from 0.8 to 30 μm), (iii) negative charge on the cell surface that repel each other, and (iv) small concentrations of biomass in large volumes of water (from <0.5 g/L in outdoor open ponds).[37] It is estimated that 20-30% of total microalgae production costs are invested in separating microalgae from water. The least energy-intensive approach to harvesting microalgae is sedimentation.[38] This is used as an initial step of dewatering microalgae by allowing the natural force of gravity to aggregate microalgae cells by changing the pH and settling at the bottom of the culture tank. However, additional separation steps such as membrane filtration are required following sedimentation to achieve complete separation of microalgae from water. Flocculation of microalgae reduces the cost of separation by an order of magnitude. During the flocculation process cells aggregate and settle down at the bottom of the tank.[39] Some of the commonly used flocculants are, metal salts, biopolymers, fungal mycelium ultrasound, inorganic–organic hybrid polymers and magnetic coagulants.[40]

Another widely used microalgae flocculation method is electrocoagulation (EC).[41]

Cultured microalgae in water are subjected to electrolysis that produces microbubbles due to the release of hydrogen at the cathode and oxygen at the anode causing floc flotation called electro flotation. Simultaneously, the oxidation of the anode made up of iron or aluminum releases protons and electrons to the microalgae suspension which destabilize microalgae cells and facilitate coagulation. Some of the electrodes also leach out during oxidation.

Anode: $2H_2O \rightarrow O_2 + 4e^- + 4H^+ \quad E^0 = (+ 1.23 - 0.059\ pH)$ (1)

Cathode: $2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad E^0 = (- 0.83 + 0.059\ pOH)$ (2)

Like other flocculation methods, the EC system is used as a primary concentrator in algae cultivation facilities where cultured microalgae slurry with 05-0.1% dry matter can be concentrated to 1-3% dry matter. Several continuous EC systems have been developed and sold commercially to process thousands of liters/hours of cultured algal solution to produce concentrated algal slurry. Some of the advantages of the EC system are: (i) pH adjustment is not required, (ii) a low dose of coagulation addition is sufficient, (iii) flocculated microalgae float on the surface and skimmed out and algae and (iv) effective performance with high removal rates. The drawbacks include periodic replacement of anode since they leach out due to oxidation reactions, high energy consumption, and result in metal ion contamination in microalgae biomass. Subsequently, either continuous flow centrifugation or crossflow membrane filtrations are used as a secondary concentration step to produce algal slurry to 15-30%. To further increase the algal dry matter content to >90%, several drying methods such as solar drying, belt drying, drum drying, vacuum tray drying, or spray drying are used.[42] Some of the advantages and disadvantage of the different microalgae separation process is given in Table 4.

3.3 Algal Biorefinery

Algal biorefinery will process the native or genetically modified algal biomass (either dry or de-

watered 15% slurry) produced in fresh or salt water from nearby locations into various main products/co-products and produce minimal waste.[43] Some of the established methods developed at bench and pilot scale can be scaled up in a biorefinery using automated machines. The size of algal biorefinery could vary depending on the volume of microalgae processed each day. The profitability of an algal biorefinery depends on the volume of main products such as feed, biofuels, and chemicals produced from protein, lipids, and carbohydrates respectively, and co-bioproducts such as pigments/dyes, cosmetics, nutraceuticals, and even therapeutic molecules.[44] Depending on the processing methods used algal biorefinery can be classified into two types: (I) Thermochemical and (II) Biochemical processing,[45] as given in Fig. 6. Nevertheless, fuels and chemicals produced in algal biorefinery is a sustainable alternative to fossil fuel derived products that benefit the society, economy, and environment.[46]

Whole dried microalgae produced by international companies are, Earthrise nutraceuticals, USA; Cyanotech corporation, USA; Hainan DIC microalgae, China; Japan Algae Co., Ltd., Japan; Parry nutraceuticals, India; FEMICO, Taiwan; Nan Pao International Biotechnology Co., Ltd., Taiwan; Biorigin, Switzerland; TAAU Australia Ptv. Ltd., Australia; Taipei, Taiwan; Roquette Klotze, Germany; Blue Biotech, Germany; Earthrise Nutritional's, USA.[47] Many companies in Europe produce macroalgae and related products.[48] Nutraceutical products produced from microalgae by international companies are, Cognis Nutrition and Health Co. Australia; Nature Beta Technology Ltd. Isrel; Cyanotech Corp, USA; Mera Pharamecuticals Inc USA; Fuji Chemical Industries, apan; BioReal AB, Sweden; Algeternal technologies, LLC., USA; Algae Biosciences Corporation, USA; Solix Algredients, USA; TerraVia Holdings, Inc. USA; Earthwise Spirulina, USA; Nutrex-Hawaii Hawaiian' Bulk Supplements, USA; Taiwan Chlorella Manufacturing Co. Taiwan; Far East Bio-Tec Co., Ltd. Taiwan; Nutress and Zenith Nutrition,

India and Qualitas, USA.[49] Biofuels producing companies are: A2BE, USA; Accelergy Corporation, USA; Aleor, France; Algae Floating Systems, Inc, USA; Algaewheel, USA; Algal Scientific, USA; Algenol Biofuels, USA; NTX (formerly Aquaflow Bionomic Corporation), New Zealand; Aquatic Energy LLC, USA; Aurora Algae, AXI International, USA; BioAlgene, Inc., USA; Bio Architecture Lab, USA; Bionavitas, Netherlands; Biovantage Engineering, USA; Blue Marble Energy, USA; Cellana, USA; Circle Biodiesel and Ethanol Corporation, USA; Diversified Energy Company plc, USA; Eldorado Biofuels, USA; Genifuel, USA; Green Star Products, Inc., USA; Joule Biotechnologies, USA; Kai Bioenergy Corporation, USA; Kent BioEnergy Corporation, USA; LakeMaster Corporation, Canada; LiveFuels Inc., USA; OriginOil, USA; PetroAlgae, USA; PetroSun, USA; ReactWell, USA; Sapphire Energy, USA; Scipio Biofuels, USA; Solix Biofuels, USA; Viridos (previously known as Synthetic Genomics), USA; UOP, USA.[50]

3.3.1 Thermochemical algal biorefinery

During the thermochemical process, water helps to carry out hydrolysis, enhanced decarboxylation, and hydrodeoxygenation reactions. The thermochemical process can be sub-classified into three categories (i) Hydrothermal, (ii) Pyrolysis, and (iii) Gasification. The processing conditions are outlined below.

(i) Hydrothermal process: The Hydrothermal process can be further classified into three types: (a) Hydrothermal Carbonization (HTC) carried out at 180–250 °C, 10–40 bar where hydro char/biochar is the predominant product; (b) Hydrothermal Liquefaction (HTL) carried out at 250–350 °C, 40–165 bar where bio-oil/biocrude is the predominant product and (c) Hydrothermal Gasification (HTG) carried out at 400–800 °C, >200 bar where bio-syngas comprising of CO, CO_2, CH_4, and H_2 are predominant products.

(ii) Pyrolysis process: Dry algal biomass is heated between 400–600°C in the absence of oxygen. Depending on the heating rate, different products are produced. (a) when heated at a rate of 5–10 °C min^{-1} and a longer reaction time of 10–30 s, biochar is produced; (b) when heated for a short reaction time of 1–2 s biocrude/bio-oil, biochar, and water are produced.[51] Fractionating the protein and pyrolyzing lipid-rich solid produce bio-oil with less nitrogen content. The highest bio-oil was reported between 37.5–47.4 wt.% at 500 °C with a 22.6 MJ/kg high heating value for *Porphyra tenera* and results were comparable to agricultural residues. Several organic molecules have been reported by catalytic fast pyrolysis of microalgae which include, acetonitrile,[52] ethylene, propylene, and butene,[53] aromatics such as indole, pyrroles, amides and nitriles,[54] and hydrocarbons.[54,55]

(iii) Gasification process: Dry algal biomass is heated to high temperature (700–1000 °C) under controlled amounts of steam, oxygen, carbon dioxide, and/or air to produce bio-syngas comprising of CO, CO_2, H_2, CH_4, alkanes, and aromatics. It was discovered that syngas production was inversely proportional to heating rate.[56]

3.3.2 Biochemical Processing

The internal cell wall is comprised of pectin, agar, alginate, and algaenan polymer; while the external cell wall is comprised of pectin, furans, hemicellulose, and glycoproteins in a microfibrils matrix of cellulose, and small fraction of fucose, xylose, rhamnose, arabinose, and galactose.[57] Starch is one of the major energy reserves in microalgae present plastids with semi-crystalline granulose particles composed of high molecular weight amylose polymers and highly branched amylopectin.[46] Some microalgae are harder to lyse such as *Chlorella sp.,* due to their complex cell wall matrix when compared to *Scenedesmus sp.*, and cyanobacteria *Arthrospira sp.* which have a simple cell wall matrix. During the biochemical processing microalgae cells are disrupted or lysed

to extract intracellular biomolecules such as protein, carbohydrates, and lipids. The optimization of a cell lysing process also called pretreatment is a key step in maximizing product yield and allowing microalgae-based products to be economically sustainable. Common pretreatment methods include chemical, thermal, mechanical, and biological treatment methods. Pretreatment helps to open the algal cell's external cell wall comprising of polysaccharide matrix like pectin (Poly galacturonic acid), and sulfated polysaccharides that can absorb inorganic salts such as calcium, silica, and magnesium. Some of the pretreatment methods help to gelatinize starch to facilitate the hydrolysis to produce glucose when using acid or enzymes.

Some chemical pretreatment methods are carried out either using concentrated acid (HCl, H_2SO_4, Perchloric acid) or alkali (NaOH, KOH) or peroxides in the presence of $FeSO_4$.[58] Thermal pretreatment such as hydrothermal where microalgae slurry is heated 60–230 °C and short reaction times 1–60 minutes.[59] Other thermal pretreatment includes thermochemical where microalgae are heated at temperatures between 60–180 °C in the presence of acid or base with varying concentrations of 1–10%, steam explosion, and freeze-thaw cycles.[60] Some of the mechanical pretreatment methods use high-pressure homogenization, hydrodynamic cavitation, and bead and ball milling.[61] The algal cell wall gets disrupted due to fluid shear, turbulence, shock velocity, and cavitation. Other mechanical pretreatment methods include ultrasound,[62] microwave,[63] glass beads,[61,64] and electric pulse.[65] Biological pretreatments are carried out using individual or combination of commercial carbohydrate-active enzymes (CAZymes) such as cellulases (endo-exo-glucanases, β-glucosidase); hemicellulase (xylanase, pectinase); amylases, amyloglucosidase and other enzymes (chitinase and sulfatase) produced using fungus and bacteria in varying concentration.[66,67] In some cases, using neutral (lysozyme) and alkaline proteases (alkalase) help to hydrolyze glycoproteins present in the algal cell wall into peptides.[66] Though enzyme

pretreatment has several advantages such as being environment friendly, needing mild processing conditions and does not require expensive equipment, being very specific, and does not produce degradation products that can affect downstream processing and produce high sugar yield. However, the cost of CAZymes and protease is one of the bottlenecks. Using an immobilized whole-cell catalyst should help to reduce the cost of enzymes.[68]

Among the different pretreatment methods mentioned above, acid and hydrothermal pretreatment can be continuously operated saving time and is a scalable process. A continuous algal biomass acid pretreatment process followed by fraction and conversion to various products has been developed by National Renewable Energy Laboratory and detailed processing steps have been reported.[69] However, acid pretreatment has several drawbacks such as the formation of degradation products such as hydroxy-methyl-furfural under strong acidic conditions which affect downstream processing, loss of fermentable sugar, destruction of pigments or proteins, requirement of expensive Hastelloy stainless steel reactors, and additional neutralization steps creating salts.[70]

3.4 Flash Hydrolysis

Flash hydrolysis (FH) is one of the leading hydrothermal pretreatment processes that pumps the algal slurry (10-15%) to a plug flow reactor in the temperature range of 240-280˚C under subcritical water conditions for a very short residence time (10-12 s) and resulting hydrolysate is immediately cooled down to room temperature by using a chiller unit to prevent degradation of hydrolyzed biomolecules/products, as seen in Fig. 7. Since the residence time is very short it requires rapid heating. The temperature is raised using a high heating rate furnace which allows microalgae slurry to come to the process temperature within minutes of heating time when the FH process is started. It is important to note here that biopolymer hydrolysis in subcritical water is

very sensitive to residence time. Hydrolysis products are intermediate products, and they convert further to various degradation products (phenols, tar, and other nitrogenous aromatic compounds) if allowed a longer residence time. To minimize the degradation of product formation, the FH process should be carried out for a very short residence time (in seconds) and the slurry should be immediately cooled down. Pressure in the FH process is maintained using a back pressure regulator.

Aside from chemical or biological microalgae cell disruption techniques, FH serves as an an alternative method to effectively lyse microalgae cells. The microalgae cell wall is composed of polysaccharides, glycoproteins, and phospholipids which can be broken down under high-pressure environments. The FH process uses high moisture content of algal biomass advantageously for hydrolyzing proteins to peptides and amino acids. This process results in a hydrolysate slurry composed of a liquid and a solid stream, containing the disrupted cells. The liquid portion or the liquid hydrolysate is primarily composed of soluble peptides and carbohydrates which can be isolated with further processing. On the other hand, the solid fraction is primarily composed of lipid-rich cell debris, which is referred to as the Biofuel Intermediate (BI), and some insoluble proteins and carbohydrates.[71,72] Conventional methods of proteins and carbohydrate hydrolysis from biomass involve the use of acid/alkali treatment. However, the use of chemicals is not required for the FH process to accomplish the hydrolysis of biopolymeric components. In the FH process water becomes increasingly more ionized and facilitates the hydrolysis of biopolymers.

Under conditions of FH, inorganic elements in microalgae biomass (P, S, K, Na, Ca, Mg, and others) are also solubilized, making them available for nutrient recycling since they are recovered in the liquid product.[73] Besides the extraction of nutrients and hydrolysis of protein, FH

has other benefits: (i) water is sterilized and can be safely recycled back to the algal pond after FH, (ii) the formation of tar, phenols, oxygenated hydrocarbons, and aromatic compounds produced in conventional HTL process is avoided, (iii) solid product (energy-rich macromolecules/biofuel intermediate) becomes enriched in carbon and depleted in nitrogen content leading to an energy-rich feedstock for biofuels production, and (iv) solid products becomes non-perishable and hence it can be stored for a longer period. FH process capitalizes on the difference in reaction kinetics of microalgae components (carbohydrates, proteins, and lipids) to selectively fractionate them in liquid and solid phases in very short residence times (a few seconds) in continuous flow reactors. FH process has some drawbacks, (i) energy intensive process and (ii) processing conditions will vary depending on the algal species used.

The resulting Flash Hydrolyzed slurry will undergo a solid/liquid separation step via a vacuum filter or centrifugation. This separation step allows for the isolation of the BI, which is reported to have a recovery of 24-52 wt.% of solid residue, depending on which strain is used.[73] In addition, the BI following FH retains >90% of the microalgae lipids in *Scenedesmus sp.* or *Nannochlorapsis*. The lipids profile of the recovered BI does not change as compared to raw microalgae due to the FH process. The BI can undergo additional processing steps to produce various products. On the other hand, the liquid portion containing soluble protein can be subject to precipitation using chemicals such as HCl or ammonium sulfate, resulting in the precipitation of microalgal proteins. The precipitated proteins are capable of being converted into various products, such as high-value protein feed with applications in the aquaculture industry.

Some of the key benefits of performing the FH process are: (i) *Continuous process:* Algal slurry (10-15%) could be continuously hydrolyzed using the FH process with a short residence time without any chemicals and is scalable. (ii) *Hydrolyze proteins:* Protein in microalgae is

hydrolyzed to soluble peptides and amino acids that could be separated and used for bio-foam production. (iii) *Hydrolyzed carbohydrates:* Insoluble complex carbohydrates are hydrolyzed to soluble oligosaccharides and (iv) *BI:* The insoluble residue left behind after FH has rich lipid content and low nitrogen content which makes it a good substrate for producing sustainable aviation fuel. The downstream processing steps to produce a sustainable microalgal protein aquaculture feed will be discussed in detail in the following sections. Finally, the remaining soluble sugars (primarily long-chain oligosaccharides) can undergo acid or enzymatic hydrolysis producing fermentable monomeric sugars for producing fuels and chemicals, as given in Fig. 8.

3.5 Processing carbohydrates

In general, microalgae contain carbohydrates in the range of 10–25 wt.% on a dry basis. If carbohydrates can be hydrolyzed in the form of monomeric and oligomeric sugars they can be fermented by using microorganisms and chemical catalysts to various products ethanol, butanol, bioplastics, furans, succinic acid, and food and feed additives.[74] Among different sugars present in microalgae, starch is a very important sugar and is essential in the food industry. Starch is used in the food industry as a thickener, emulsifier, gelling agent, and stabilizer. The three most common methods of hydrolyzing/extracting carbohydrates involve (i) the use of acid (H_2SO_4, HCl, H_3PO_4), (ii) the use of alkali (NaOH, and ammonia), and (iii) enzymatic hydrolysis. Additionally, microwave-assisted extraction, ultrasonic-assisted extraction, and ionic liquids have been used to extract sugars from microalgae. The FH process has been used to selectively hydrolyze sugars by tuning the process temperature. Most of the hydrolyzed sugar by the FH process are water-soluble oligomers. These oligomeric sugars can be hydrolyzed to monomers using mild acid treatment or enzymatic hydrolysis. Aqueous two-phase systems are biocompatible and efficient liquid-liquid extraction methods for the fractionation and purification of sugars and other biomolecules.[74]

Glucose was found as the major fraction (70% w/w of all sugars) followed by other monosaccharides such as mannose, galactose, xylose, and arabinose when *Scenedesmus obliquus* was subjected to FH process.[75] The utilization of algal sugar to produce valuable chemicals is an important area that needs to be explored. The hydrolysis is typically conducted in the presence of acidic catalysts such as Brønsted acid, metal chlorides, HZSM-5, and Sn-Beta. Table 5 outlines the various fuels and chemicals that can be derived from carbohydrates, as well as proteins and lipids. Some of the organic acids that could be produced from algal carbohydrates are listed as the top 10 chemicals by US DOE used for producing biopolymers.

Algae contain a significant amount of carbohydrates (25–60%), consisting of monosaccharides and polysaccharides including glucose, fructose, galactose, mannose, starch, and cellulose. The type and concentration of carbohydrates varies among different species. Being a rich and inexpensive source of renewable carbon and low lignin content, algal biomass demonstrates the tremendous potential to be used as feedstock for the fermentation industry. The complex polysaccharides are hydrolyzed into fermentable sugars using chemical and/or enzymatic methods. The extracted sugars could be transformed into a variety of products via microbial routes including bacteria, yeast, and fungi and are classified as third-generation biorefineries.[76] Ethanol has been the most researched product obtained using sugars from algal biomass.[77,78] The ethanol yield achieved (>0.40 g/g) on algal biomass has been comparable to first-generation feedstocks, however, the titer remains too low to be commercialized. For example, the microalgal strain *Scenedesmus obliquus* achieved a carbohydrate content of 51.8%, mainly composed of glucose (~80%).[79] The acid hydrolysis of wet microalgal biomass followed by fermentation of glucose-rich hydrolysate using *Zymomonas mobilis* resulted in an ethanol titer of 8.55 g/L with a yield (99.8%) close to the theoretical one. Similarly cellulosic pulp from macroalgal strain *Gracilaria*

verrucosa (red seaweed) which is known for agar production.[80] The fermentation of enzymatic hydrolysate (0.87 g sugars/g cellulose) by *Saccharomyces cerevisiae* produced an ethanol concentration of 14.9 g/L with a conversion yield of 0.43 g/g after 16 h. Besides ethanol, algal biomass has been employed for acetone-butanol-ethanol (ABE) fermentation where n-butanol is the product of maximum interest due to its attractive features to serve as biofuels, even better than ethanol.[81] Sequential alkali and acid pretreatment for extraction of sugars from carbohydrate fraction of *Chlorella vulgaris* followed by ABE fermentation by *Clostridium acetobutylicum*.[82] The total ABE production was 19.9 g/L whereas n-butanol was 13.1 g/L with a conversion yield of 0.24 g/g and productivity of 0.66 g/L. h.

The carbohydrate-rich biomass has also been employed for fermentative production of other platforms and industrially relevant chemicals. Lactic acid (LA) is a top platform chemical by US DOE with wider applications in the food, pharmaceutical, chem,ical and textile industries. There have been many reports on fermentative LA production using algal biomass made use of acid hydrolysate of microalga *Chlorella vulgaris* as feedstock for LA production.[83] The LA titer, yield, and productivity achieved batch and continuous fermentation using immobilized cells of *Lactobacillus plantarum* were 39-43 g/L, 0.93-0.99 g/g, and 7.0-10.0 g/L. h, respectively. Like LA, succinic acid (SA) is a platform chemical and versatile industrial molecule. The bioproduction of SA has been attempted from an algal mass. An integrated approach to recover lipids from green alga *Scenedesmus acutus* and the use of carbohydrate fraction for SA accumulation by *Actinobacillus succinogenes* has been reported.[84] The continuous fermentation led to SA titer, yield, and productivity of 30.5 g/L, 0.70 g/g, and 1.1 g/L. h, respectively. The downstream processing of SA resulted in a recovery of 60% with purity of 98.4%. They also recovered lipids from the flocculated cake at 83% which were to a renewable diesel blend stock through

deoxygenation and hydroisomerization. This work demonstrates that algal mass can be used to generate multiple products with a biorefining approach. 2,3-Butanediol (BDO) is a C4 chemical with vast commercial potential. Extracted sugars (glucose and galactose) from *Chlorella pyrenoidosa* which were used for BDO production by *Klebsiella aerogenes* and the results were compared with pure sugars.[85] The results were comparable in terms of titer (4.0-5.0 g/L) and yield (90-95%), however, BDO production time on hydrolysate was longer (20 h versus 10 h) which could be due to the presence of inhibitors. These results show the efficacy of extracted algal sugar is as good as chemical sugars. Algae are known as lipid-accumulating cell factories with prime applications in the biofuel industry. The co-production of valuable chemicals from carbohydrate fraction would certainly add significantly towards enhancing the commercial viability of third-generation biorefineries. Currently, algal-based production of biofuels and biochemicals is in the R&D stage and commercial production is restricted by high cost and energy, therefore, innovative research and investment are required to bring it to mass-scale production.

3.6 Processing lipids

Both polar (e.g., glycerophospholipids) and nonpolar (e.g., triacylglycerols (TAGs)) lipids are produced by microalgae. These lipids play an important role in cell structure and energy storage.[86] Structural lipids (polar lipids) are long chains of fatty acids which could be transformed to obtain PUFAs such as EPA, DHA, and DPA. Besides, biofuels, microalgae lipid is a plant-based source of EPA and DHA (two omega-3 fatty acids) that are essential for health. PUFAs precursors to second messengers (EPA, DHA) are considered one of the promising sources of the human diet that are known to regulate inflammation, immunity, blood vessels, platelets, synaptic plasticity, cellular growth, pain, sleep, and they can be a viable alternative to fish oil.[87] Algal oils are better than fish oil in terms of consistency of composition, sensory properties, and ease of production.

The use of algal oils in food supplements is expected to grow as demand for EPA and DHA increases. Triacylglycerols (TAGs) play a fundamental role in energy storage within the microalgae cell. TAGs from microalgae are used for biodiesel (fatty acid methyl ester) production. Polar lipids (phospholipids and glycolipids) are deleterious for biodiesel production since they cause emulsification and catalyst depletion. The first- and second-generation biodiesel derived from edible and non-edible plant oil, while the microbial oils are primarily used to produce the third-generation or advanced biodiesel. Currently, biodiesel is produced from plant oil by transesterification with an alcohol (methanol or ethanol), for the reason of cost, methanol is used most frequently, in the presence of a base or acid or an enzyme as a catalyst. Algal biodiesel is produced by transesterification TAGs extracted from dried microbial biomass and reacted with methanol.

Since lipids can contain molecules other than TAGs such as phosphorous and sulfur-containing compounds, the fuel quality may reduce. Hence during catalytic upgradation, purified extracted lipids are preferred for producing drop-in biofuels. There have been several studies on the catalytic upgradation of whole microalgae or extracted lipids to liquid hydrocarbons (drop-in biofuels.[88] Most of these processes involve the HTL of microalgae biomass and then catalytic hydrotreatment of biocrude produced via HTL. One of the challenges in catalytic upgradation is due to the complexity of biocrude composition due to the presence of several different compounds including hydrocarbons, aromatics, organic oxygenates, and nitrogenous compounds. The high oxygen contents of biocrude make it unsuitable for direct application as a fuel.

As discussed earlier, the lipids recovered from whole microalgae via solvent extraction of the FH process contain fatty acids fraction of palmitic, palmitoleic acid, stearic acid, and oleic acid. These microalgae-derived lipids can be hydrotreated in the presence of heterogeneous

catalysts to fuel-like hydrocarbons. Conventional catalysts such as Ni/Mo sulfide are used in the hydrocracking of microalgae lipids into fuel-range liquid hydrocarbons. The hydrocracking of algal oil over Ni/Mo/Al_{13}-Mont gave aviation fuel-ranged hydrocarbons (C_{10}-C_{15}) a yield of 52%.[89] The hydrotreating processes are generally conducted in the temperature range of 270-350°C in the presence of a heterogeneous catalyst. One of the studies reported biofuels with higher heating values of 46.25 MJ/kg with oxygen and nitrogen content below 0.3% and 0.007%, respectively.[88] Noble metal catalysts such as Pt, Pd, and Ru have shown high activity in the hydrodeoxygenation process of algal lipids to produce high-grade biofuel used in jet engines.

3.7 Processing proteins

Population growth combined with limited resources has resulted in a need for alternative protein sources. The world will need an extra 265 million tons of protein by the middle of the century. In addition to oils and sugars to produce biofuels, proteins, which can reach 70% by weight in microalgae, can be used for the preparation of feed and foods and/or as supplementary ingredients. Proteins recovered from microalgae have comparable or superior functionality as other commercial protein preparations. It has been estimated that single-cell microorganisms hold the potential to meet up to 20% of conventional crop-based fish, poultry, and ruminant animal feed protein demand by 2050. Microalgae is an example of an under-exploited “crop” for producing protein.[90] There are numerous ways to extract proteins from microalgae which include enzymatic hydrolysis, using chemicals such as ammonia or sodium hydroxide, ultrasound-assisted extraction, pulsed electric field, and microwave-assisted extraction. However, the FH process as discussed earlier seems to be a sustainable approach since it is a chemical-free process, does not require drying of microalgae biomass, and is a rapid method that can be conducted in a continuous flow reactor. Microalgae protein production has several benefits over traditional high-protein crop use

in terms of productivity and nutritional value. Its essential amino acid composition meets Food and Agriculture Organization requirements, and it is on par with other commercial protein sources.

The protein co-product produced by processing microalgae using the FH process could be fermented to mixed alcohols or polyols or to produce ammonia. Additionally, there is a global demand for protein sources for people who may be protein deficient due to cultural dietary restrictions or the lack of accessible, affordable protein sources. The protein co-product (peptides and amino acids) separated using FH has the potential to help in meeting these demands. Aqueous fraction after FH of microalgae contains more than 65% of proteins in the form of soluble protein (peptides and free amino acids). The soluble protein can easily be separated from soluble carbohydrates by acidic precipitation at low pH. Protein precipitation has been optimized by adjusting pH with HCl in a range of 3.3–4.5, obtaining the higher protein recovery at pH 3.5.[91]

The concentration of extracted proteins is one of the challenges because of their unknown physicochemical properties. The selection of the method depends on the final application of the product and the scale of production. Major protein concentration methods include precipitation, using membrane filtration, and ion exchange chromatography followed by characterization using ion chromatography or mass spectrometry. For proteins purification, microfiltration could be used to remove the cell wall components, ultrafiltration could be used to isolate proteins with a molecular weight between 1 and 200 kDa, and nanofiltration could be used to remove monovalent salts and reverse osmosis to reduce the final volume.

3.8 Environmental benefits of using microalgae as a carbon sink and producing various products.

LCA for microalgae-based fuel and product systems was reviewed in 2015.[92] In this review, the assumptions made regarding algae growth modeling were cited as heavily affecting LCA results.

Research facility located on the equator could achieve a maximum theoretical algal lipid productivity of 350 m^3 $ha^{-1}yr^{-1}$, though actual algal-lipid productivity ranges were reported to be much lower, 2.3 m^3 $ha^{-1}yr^{-1}$ to 136.9 m^3 $ha^{-1}yr^{-1}$. These ranges were attributed to distance from the equator and outdoor vs. enclosed cultivation. Solvent lipid extraction, supercritical water recovery, and hydrothermal liquefaction to remove lipids from algae have been reported. After removing the lipid products, solvent extraction techniques commonly resulted in algae biomass that was processed by anaerobic digestion to make methane, which when combusted, produces on-site heat and power. When using hydrothermal liquefaction, well-to-pump greenhouse gas emissions are negative, owing to the photosynthetic sequestration of carbon dioxide during algae growth. GHG emissions for facility construction were usually ignored found they can be significant at low yields.

3.8.1 Products from Microalgae

Many products have been examined from microalgal production systems. Microalgae used to sequester phosphorous from a meat processing wastewater treatment plant were examined and compared to two pathways to make granulated triple superphosphate fertilizer.[93] Results suggest that the current method for manufacturing triple superphosphate fertilizer from phosphate rock is environmentally preferred vs. microalgae to remove phosphorous from wastewater, owing to subsequent water removal and energy consumption. Stacking improvements by using photovoltaic electricity, harvesting algae without sodium hydroxide, and using solar drying, did improve the microalgae scenario, though the use of phosphoric acid to make triple superphosphate remained less environmentally impactful. The authors concluded that pursuing higher-value products was preferable to making fertilizers when using algae.

When algae produce omega-3 fatty acids, both algal-based food and feed values increase. Animal feed production was very important for improving profitability and reducing environmental impacts from the wet lipid extraction process (a.k.a. OpenAlgae).[94] Environmental benefits result as existing animal feed operations with high environmental burdens are displaced when animal feeds are made from omega-3 enriched algal cells. Microalgal production of EPA and DHA was evaluated using heterotrophic or photoautotrophic conditions, and from farmed fish.[95] Algal pathways to these essential unsaturated fatty acids are needed, as the amount extracted from fish is not meeting the demand. Increase consumption of EPA and DHA may lead to overfishing, which results in habitat destruction and increased pressure on fisheries. Because of low algae biomass concentration and productivity, heterotrophic growth is often considered vs. photoautotrophic growth. Brewery or dairy effluent, rich in sugars, are cited as options for obtaining low-cost feedstocks for heterotrophic operation. When compared to farmed fish, heterotrophic cultivation of algae appears promising, potentially offering an alternative to fisheries to make EPA and DHA.

LCA of two scenarios was calculated.[96] The first scenario is a traditional algae oil approach using lipid extraction, solvent recovery, and transesterification, followed by biodiesel and glycerol purification, and the second scenario is an integrated approach that hydrolyzes and ferments algal sugars, distills ethanol, subjects the stillage to lipid extraction with solvent recovery, and then upgrades the algal oils by hydrotreating to make diesel and naphtha. The sale of co-products from the second approach lowers the minimum fuel selling price because of co-product credits. GHG emissions and fossil energy usage are also lower for the second scenario owing primarily to reduced hexane use during lipid extraction.

Converting glycerol into higher value products, hydrogen, propylene glycol, glycerol-tert-butyl ether, and poly-3-hydroxybutyrate, was assessed.[97] This group used a bi-criteria mixed-integer nonlinear programming method to optimize environmental and economic metrics. When all bioproducts are included as credits, favorable biodiesel economics were obtained with reduced GHG emissions relative to petroleum-based fuels and products.

3.8.2 Carbon capture and sequestration (CCS) with microalgal carbon utilization

The synergies between geological carbon CCS and microalgal carbon utilization have been reported.[98] In this study, power plants located in Texas were connected to geological sinks via supercritical CO_2 pipelines. Algal biorefineries were integrated into this supply chain to make either biodiesel by transesterification or renewable diesel by HTL. These liquid fuels were then sold at biofuel terminals that were included in the supply chain. This system was subjected to life cycle optimization to optimize both economic and environmental metrics. A Pareto curve was plotted on a cost vs. GHG emission avoided diagram to reveal optimal solutions, a suboptimal regime, and an infeasible regime. Co-producing liquid fuels using microalgae resulted in lower CO_2 reduction costs and reduced CO_2 emissions. Interestingly, renewable diesel from HTL had a larger cost reduction, while biodiesel had a greater effect on lowering GHG emissions.

3.8.3 ***Bioplastic production using microalgae***

Petroleum-derived plastic has become part and parcel of everyone's life and is widely used in packaging, consumer goods, construction, and medical devices.[99] Bioplastics are produced using plant or algal biomass-derived sugars via microbial fermentation to make precursor molecules that are then polymerized (e.g., polylactic acid (PLA), Polyvinyl alcohol (PVA), polypropylene (PP), polyvinyl chloride (PVC)).[100] Some bioplastics are directly produced using microorganisms such as algae (e.g., polyhydroxyalkenoates (PHA), polyhydroxy butyrate (PHB)).[101–104] Bioplastics

made using PHA and PHB produce in cyanobacteria are biodegradable when composted and are beneficial to the environment. Bio-based plastics are hybrid composite materials produced by combining petroleum-derived non-biodegradable plastic with a certain percentage of bioderived polymers (e.g., starch, cellulose, lignin) or algal biomass.[105,106] Though bio-based plastic is not completely combustible, they are produced to reduce carbon footprint and to conserve fossil resources. Blending different ratios of microalgae with petroleum-derived non-biodegradable plastic produces composite materials with good thermal and mechanical properties.[107,108] Bioplastics are made by blending microalgae with biodegradable polymers polybutylene succinate (PBS), polybutylene adipate terephthalate (PBAT), and PLA.[109–111] New types of bioplastics are made by blending microalgae with biomass such as starch[112] or wheat gluten.[113] LCA and TEA analysis of producing bioplastics using microalgae from different growth methods as feedstock has been reported.[114] They concluded that the GHG emission might be reduced by 67-116% when compared to petroleum-derived plastics.

3.9 Microalgae used to treat wastewater.

Microalgae have found applications in nutrient recovery within wastewater treatment plants (WWTPs), often accompanied by beneficial fuel and electricity co-production. By cultivation of microalgae using wastewater collected from drying sludge in a secondary wastewater plant, fossil fuel use, greenhouse gas emissions, eutrophication, and water use are all reduced when including wastewater displacement credits.[115] Wet lipid extraction, combustion, hydrothermal liquefaction, and pyrolysis were examined as routes to energy products, with combustion producing the most transport energy with the lowest fossil fuel use and eutrophication. In a study wet lipid extraction using membranes and HTL achieved energy return on investments greater than 3.[94] Further, these two scenarios resulted in the lowest minimum fuel selling prices, while also being the most

environmentally beneficial to climate change, ecosystem health, and human health damage categories. Using a recycled center that has supported algae cultivation reduces the energy consumption in the activated sludge process while producing bioenergy.[115] High-rate algal ponds (HRAP) are raceway ponds where algae assimilate nutrients and make oxygen that is used by bacteria to oxidize organic substrates, thus cleaning wastewater. When biogas and biofertilizer are produced after HRAP, several environmental impacts, including climate change, are lower than a traditional activated sludge system.[116,117] Negative greenhouse gas emissions are realized when hydrothermal liquefaction is used to convert wet algae from WWTPs into renewable diesel fuel for vehicular use, though CO_2 emissions from vehicular combustion were excluded.[118] This result was supported by a manuscript that compared microwave pyrolysis, hydrothermal liquefaction, and lipid extraction of algae cells grown in wastewater, finding that hydrothermal liquefaction resulted in the lowest GHG emissions.[119]

4 Conclusion

Carbon sequestration through the bioconversion of CO_2-rich flue gas in microalgae cultivation serves not only as a solution to combat GHG emissions from industry, but also provides a source of foundational biopolymers that can be fractionated and further processed to fuels, feeds, and chemicals to meet the growing demand. Achieving the algal biomass yield set by U.S. DOE at 25 gm m^{-2} d^{-1} annual average by 2030 is important. Applying an integrated carbon sequestration and biorefinery practice in the industry would be an innovative approach toward achieving carbon-neutral bioproducts by 2050. Introducing policies and guidelines to use microalgae to sequester CO_2 from the atmosphere in WWTP will help to meet this goal. Continuous methods of lysing algal biomass using the hydrothermal FH process will help future biorefineries. The method of

fractionating these biomolecules from hydrolyzed algal slurry and further processing them to various fuels and chemicals is a sustainable and renewable alternative to fossil fuel. Either producing bioplastic using algae or blending algal biomass as a filler to produce bio composite with non-biodegradable plastic derived from petroleum or biodegradable plastic derived from plant-derived biopolymer has environmental benefits when compared to petroleum-derived plastics. Future work should be performed to optimize the cultivation, harvesting, and processing of microalgal biomass to ensure the economic feasibility of converting microalgae to bioproducts that are cost-competitive with petroleum products.

Abbreviations

ABE	Acetone, butanol, and Ethanol
BDO	2,3 - Butanediol
BI	Biofuel Intermediate
CAZymes	Carbohydrate active enzymes
CO_2	Carbon dioxide
CO	Carbon monoxide
CTX	Carbon trade exchange
CCS	Carbon capture and sequestration
CCUS	Carbon capture, utilization, and storage
CH_4	Methane
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DPA	Eicosapentaenoic acid
EC	Electrocoagulation
EPS	Exo-polysaccharides
EU	European Union
FH	Flash hydrolysis
GHG	Greenhouse gases
HRAP	High rate algal ponds
HTC	Hydrothermal Carbonization
HTL	Hydrothermal Liquefaction
HTG	Hydrothermal Gasification
H_2	Hydrogen
LA	Lactic acid
LCA	Life cycle assessment
NO_x	Nitrous oxides
O_2	Oxygen

PBR	Photobioreactor
PBS	Polybutylene succinate
PBAT	Polybutylene adipate terephthalate
PLA	Poly Lactic acid
PHA	Polyhydroxy alkenoates
PHB	Polyhydroxy butyrate
PP	Polypropylene
PUFA	Poly-Unsaturated Fatty Acids
PVA	Polyvinyl alcohol,
PVC	polyvinyl chloride
RAB	Rotating algal biofilm
SO_x	Sulfur oxides
TAG	Triacyl glyceride
WWTP	Wastewater treatment plant

Author contributions

Conceptualization: VB, SK; methodology: JP, VK, CS; visualization and drawing: HH, JP; Writing – original draft: VB; Writing - review and editing: SK, CS, VK; funding acquisition: VB.

Conflicts of interest

There are no conflicts to declare.

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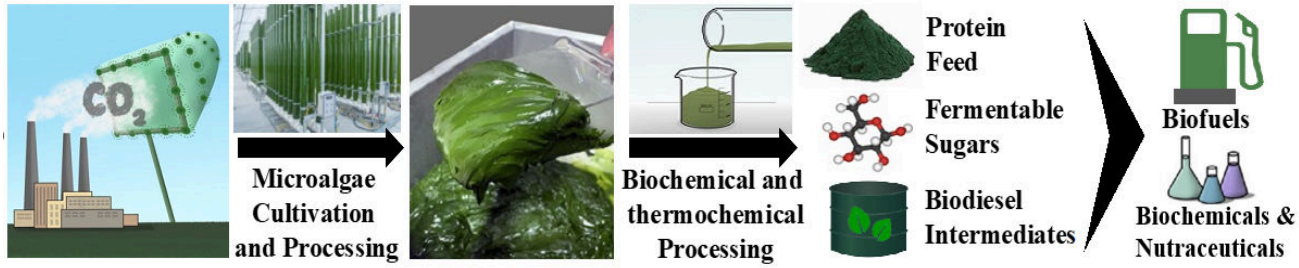
Figures

Concept Figure



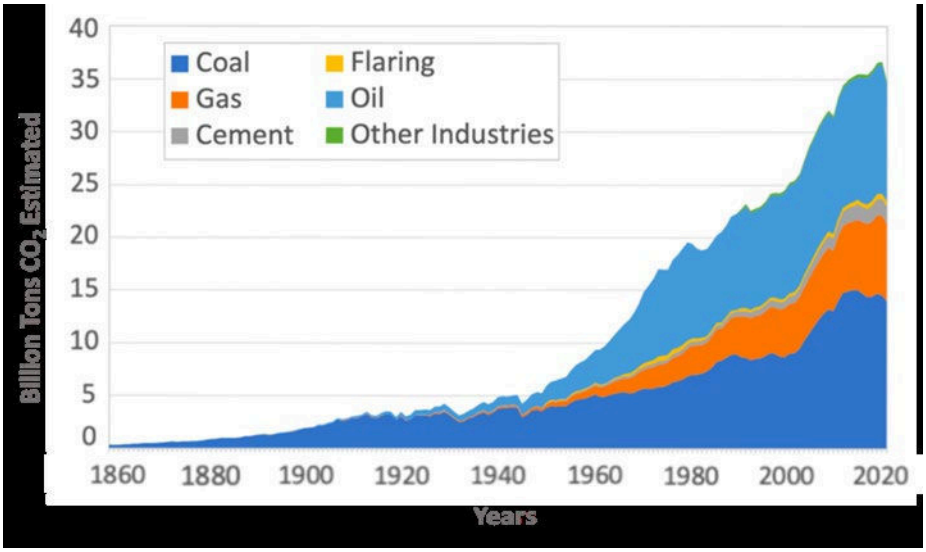
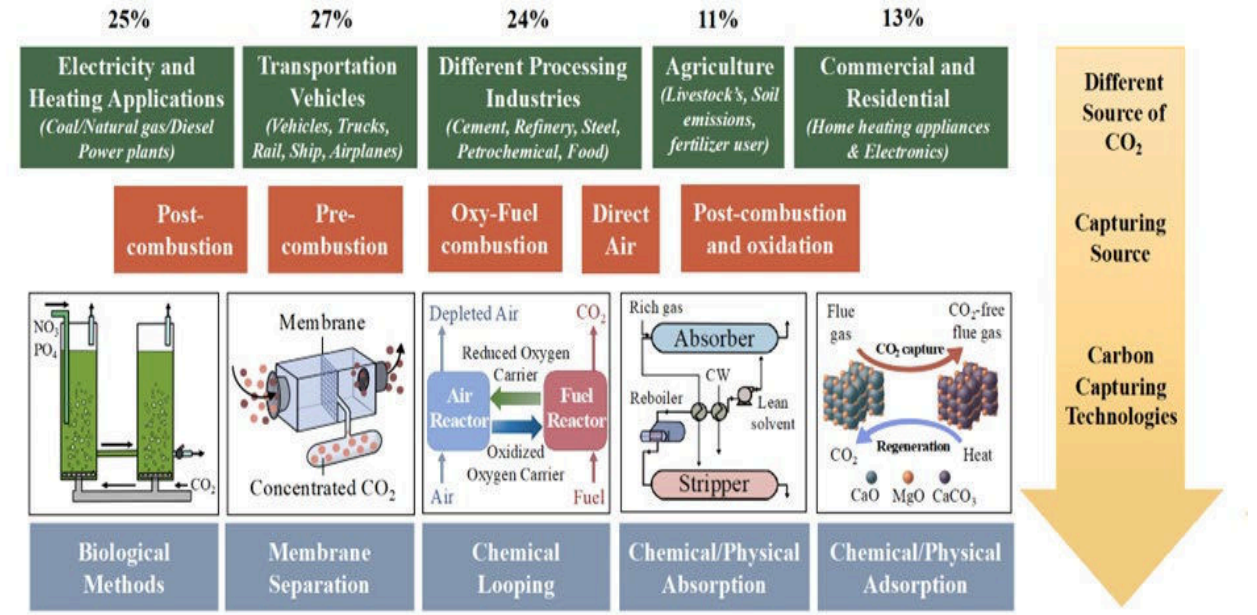


Fig. 1 GHG emissions by global industries. Here, the volume of estimated CO_2 generated by different industrial activities over several decades are given.


25%
27%
24%
11%
13%
Electricity and Heating Applications
(Coal/Natural gas/Diesel Power plants)
Transportation Vehicles
(Vehicles, Trucks, Rail, Ship, Airplanes)
Different Processing Industries
(Cement, Refinery, Steel, Petrochemical, Food)
Agriculture
(Livestock's, Soil emissions, fertilizer user)
Commercial and Residential
(Home heating appliances & Electronics)
Different Source of CO_2
Post-combustion
Pre-combustion
Oxy-Fuel combustion
Direct Air
Post-combustion and oxidation
Capturing Source
NO_2
PO_4
CO_2
Membrane
Concentrated CO_2
Depleted Air
CO_2
Reduced Oxygen Carrier
Air Reactor
Fuel Reactor
Oxidized Oxygen Carrier
Air
Fuel
Rich gas
Absorber
CW
Reboiler
Lean solvent
Stripper
Flue gas
CO_2-free flue gas
CO_2 capture
Regeneration
CO_2
Heat
CaO MgO $CaCO_3$
Carbon Capturing Technologies
Biological Methods
Membrane Separation
Chemical Looping
Chemical/Physical Absorption
Chemical/Physical Adsorption

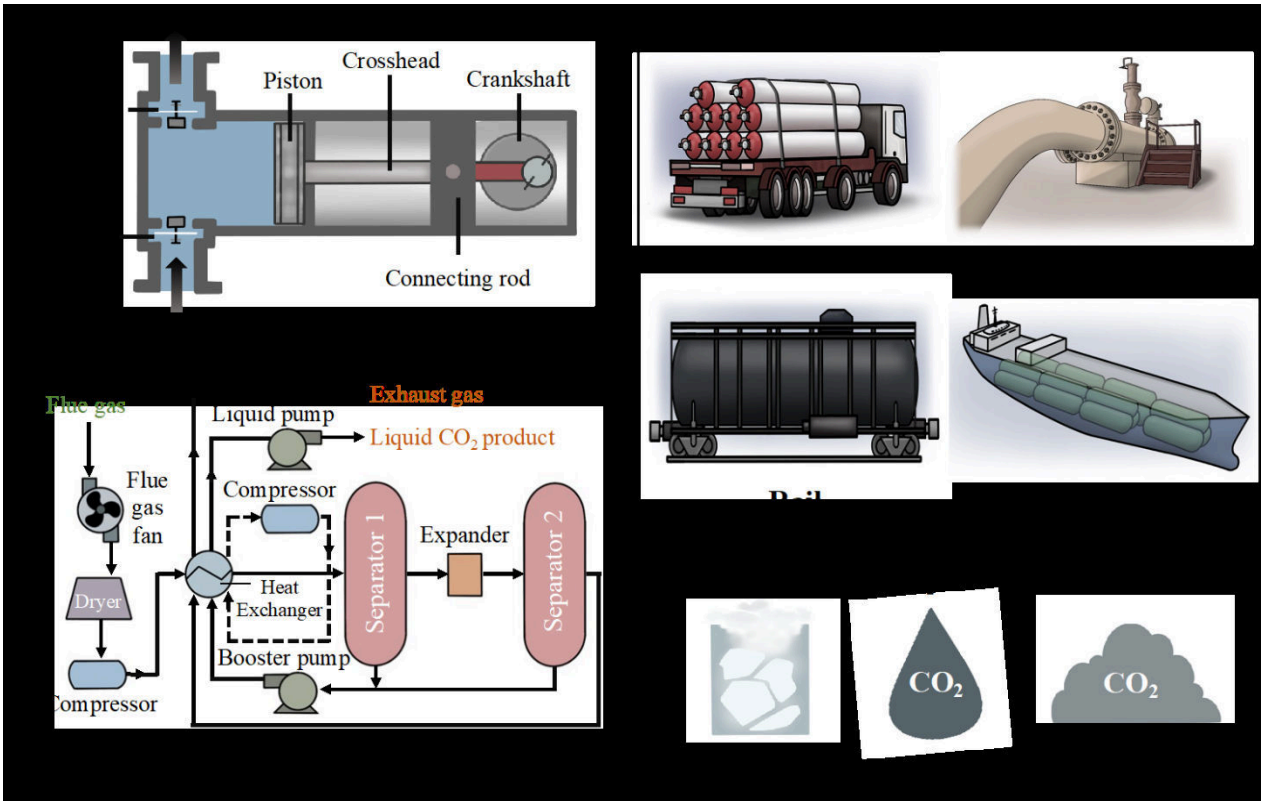


Fig. 3 Method of processing CO_2 and transportation. Here, (a) method of compressing the CO_2 into cylinders and producing liquefied CO_2 are shown; (b) The compressed gas and liquefied CO_2 are transported by road, pipelines, rail, and ship to distant locations and (c) different phases of CO_2.

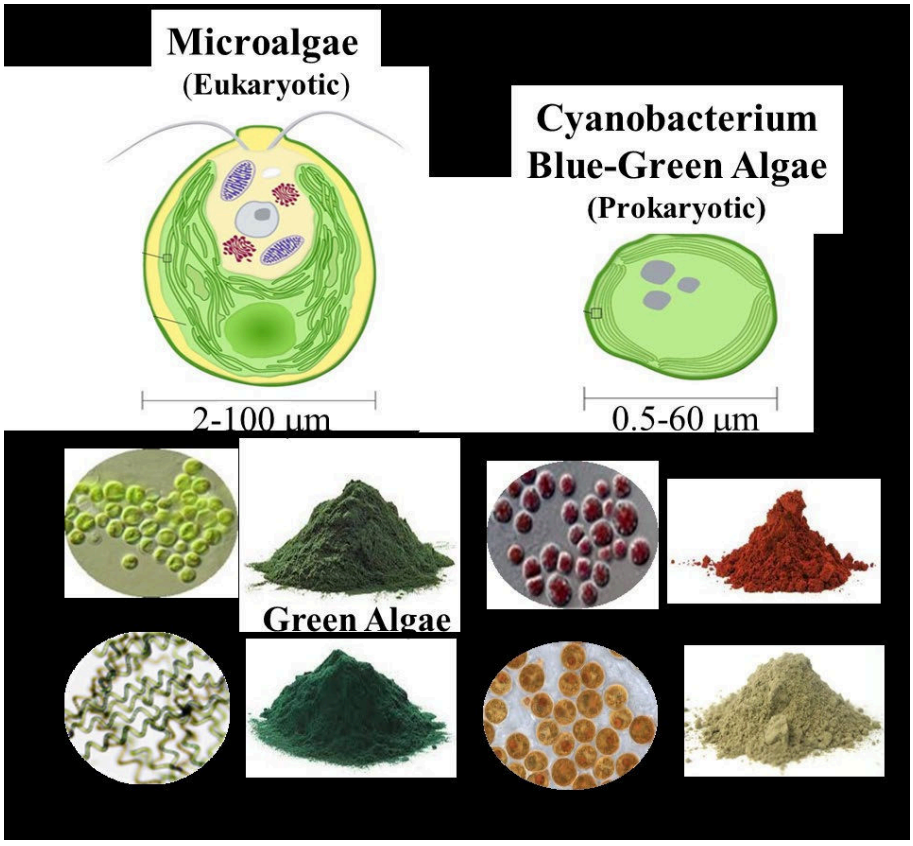


Fig. 4 Different types of microalgae. Here, the top figure shows eukaryotic microalgae and prokaryotic cyanobacteria ad the bottom figure shows different alga types: Rhodophyta (red), Phaeophycean (brown), Chlorophyta (green), spirulina (blue green) are shown. The color variation in algae is due to the type of pigments found in them.

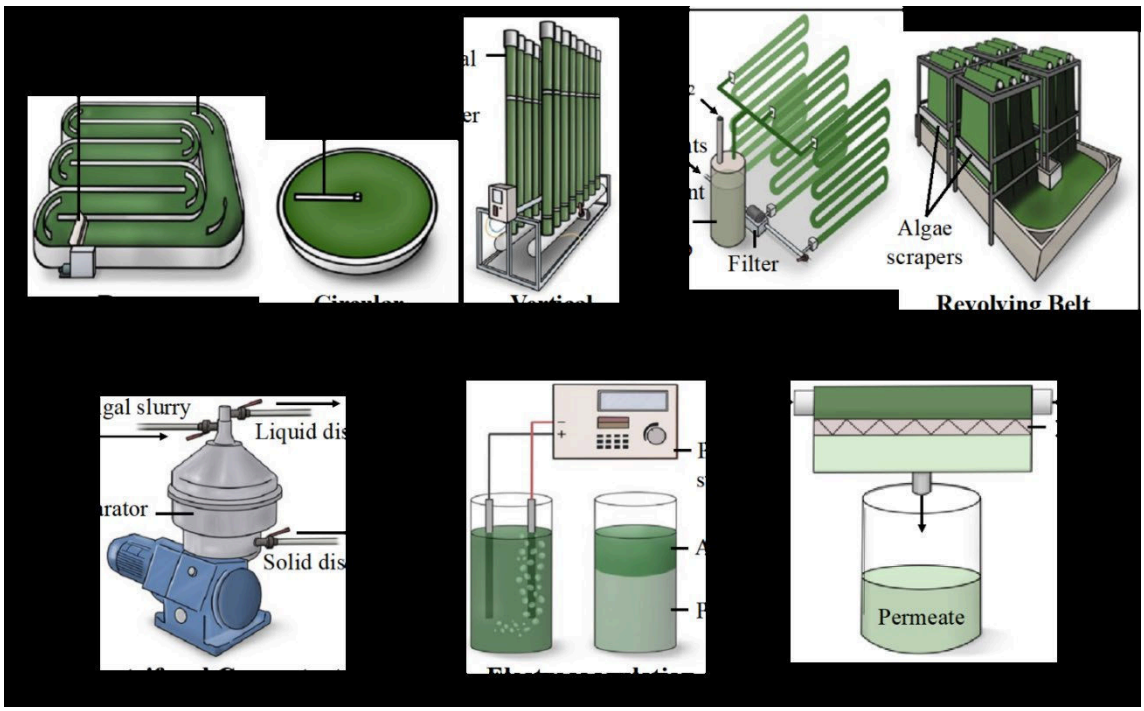
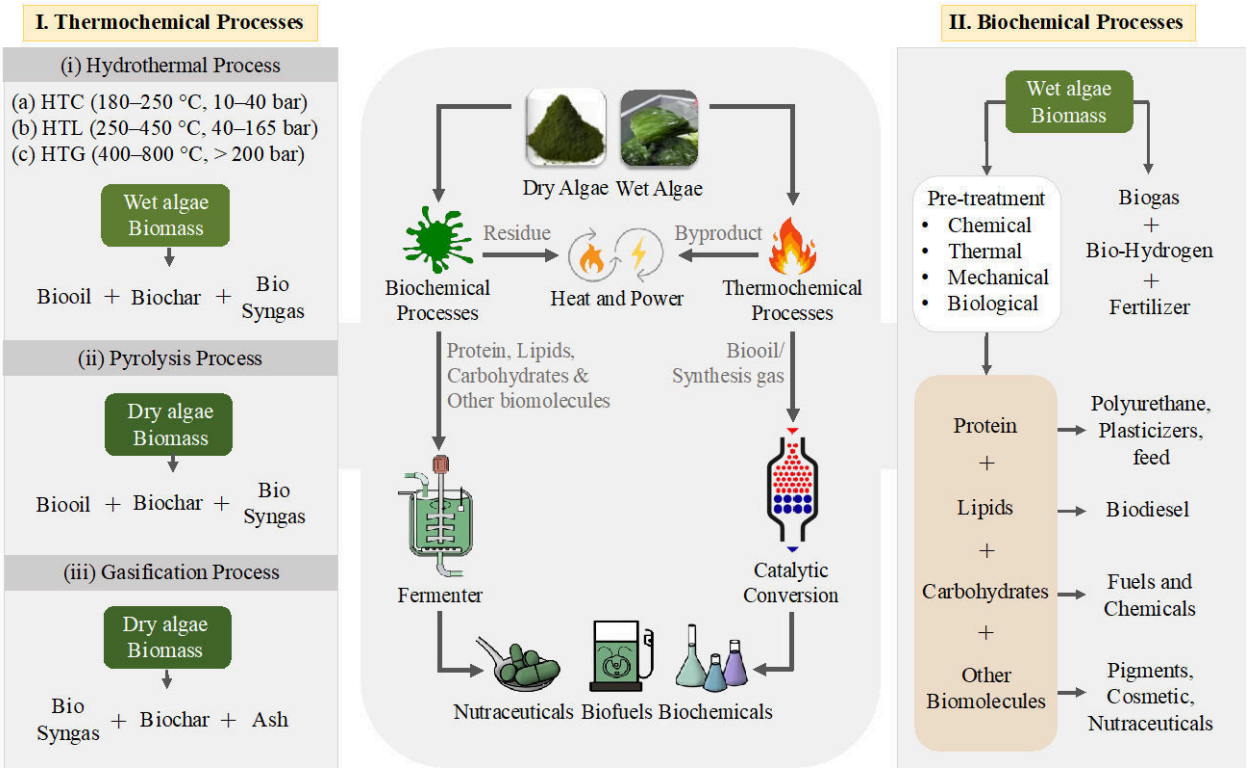


Fig. 5 Different methods of cultivating and separating algal biomass from water. Here, (a) different commercially used methods of cultivating algae and (b) different commonly used methods to separate algal biomass from water.


I. Thermochemical Processes
(i) Hydrothermal Process
(a) HTC (180–250 °C, 10–40 bar)
(b) HTL (250–450 °C, 40–165 bar)
(c) HTG (400–800 °C, > 200 bar)
Wet algae Biomass
Biooil + Biochar + Bio Syngas
(ii) Pyrolysis Process
Dry algae Biomass
Biooil + Biochar + Bio Syngas
(iii) Gasification Process
Dry algae Biomass
Bio Syngas + Biochar + Ash
Dry Algae
Wet Algae
Residue
Byproduct
Biochemical Processes
Heat and Power
Thermochemical Processes
Protein, Lipids, Carbohydrates & Other biomolecules
Biooil/ Synthesis gas
Fermenter
Catalytic Conversion
Nutraceuticals
Biofuels
Biochemicals
II. Biochemical Processes
Wet algae Biomass
Pre-treatment
• Chemical
• Thermal
• Mechanical
• Biological
Biogas + Bio-Hydrogen + Fertilizer
Protein + Lipids + Carbohydrates + Other Biomolecules
Polyurethane, Plasticizers, feed
Biodiesel
Fuels and Chemicals
Pigments, Cosmetic, Nutraceuticals

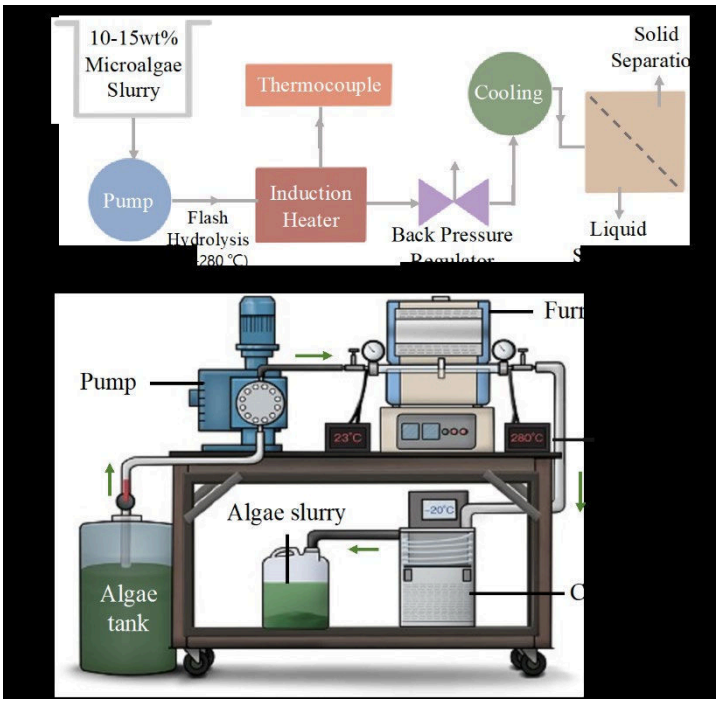
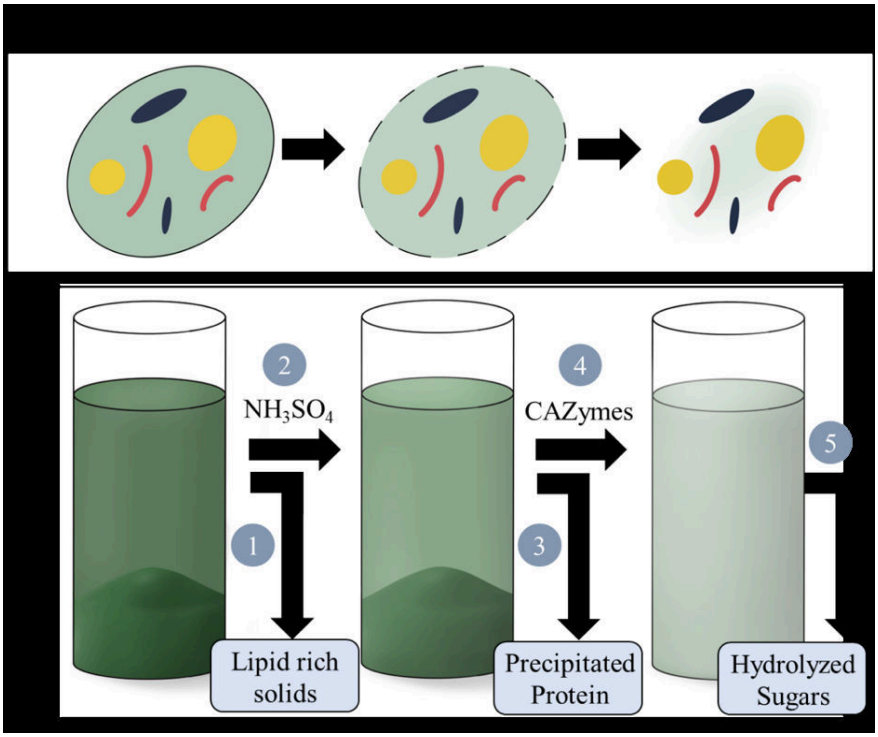


Fig. 7 Graphic illustration of flash hydrolysis skid mount unit.


2
NH_3SO_4
4
CAZymes
5
1
3
Lipid rich solids
Precipitated Protein
Hydrolyzed Sugars

Potential of using microalgae to sequester carbon dioxide and processing to bioproducts

Balan, Venkatesh

2023-09-15

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