Chapter 1
Biotechnological Approaches to Enhance Algae Biofuel Production

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Abstract Algae are aquatic species that may reproduce quickly and have over 3000 different breeds, making them more abrasive than terrestrial plants. They may be able to convert CO_2 from the air into oxygen and remove it from the breaking cells of algae plants, which is how they produce wonderful oil. A viable source of feedstock for biofuels, oleaginous microalgae have a number of advantages over terrestrial plants. Due to the lack of robust algal strains with increased lipid content and biomass and methodologies for economically viable oil extraction, the microalgal fuel business is still in its infancy. By carefully targeting important metabolic nodes, microalgal metabolic engineering demonstrates the huge potential to improve lipid

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accumulation without compromising cell growth. The genetic enhancement of microalgae without impairing cellular biomass remains an underutilized option for large-scale biofuel production, despite recent advances in synthetic biology. To improve microalgae as a biofuel platform for the production of biohydrogen, alcohols generated from starch, substitutes for diesel fuel, and/or alkanes, we consequently present a thorough overview of numerous biotechnological techniques in this chapter.

Keywords Biofuel · Genetic engineering · Microalgae · Nano-additive · Bio-bricks · Biorefinery

Abbreviation

ABE	Acetone-butanol-ethanol
DHA	Docosahexaenoic acid
EPA	Eicosa pentaenoic acid
EST	Expressed sequence tags
FAME	Fatty acid methyl ester
GHG	Greenhouse gases
HBV	Hepatitis B virus
LED	Light-emitting diodes
LHC	Light antenna complexes
PC	Phosphatidylcholine viation
ROS	Reactive oxygen species
SCP	Single-cell proteins
TAG	Triacylglycerols

1.1 Introduction

Algae are the aquatic species that have the fastest capability to reproduce with over 3000 different breeds, therefore more adverse than land plants. They can have the capacity to extract CO_2 from the air and turned into oxygen and have the ability to yield great oil, i.e., the breaking cells of algal plants are extracted (Adeniyi et al. 2018). Renewable fuels are bioalgal fuels derivative of algal feedstock that can be related with its capability to abundantly photosynthesize. The capability to convert all feedstock's energy into various varieties of valuable biofuels is the main advantage (Demirbas 2010). Their other application adds the creation of energy cogeneration (electricity and heat) afterward oil extraction, deduction of CO_2 gases through industrial chimney (algal bio-fixation), bio-fertilizer, animal feeds, and further food products. The important and imaginable effects that algae exist and can survive in thrilling heat, scarcity, salinity, and radiation. Therefore, ecological conditions will affect the country's crop growing ways. Freshwater and marine algae, for example, Chlorophyceae (green algae), Cyanophyceae (blue-green algae), and Pyrrophyceae (fire algae), could be cultured as expected happening in the UK (Adeniyi et al. 2018). Phaeophyceae a brown alga with synthetic cultivation techniques of photobioreactors could be genetically modified. Aimed at the creation of fatty acid methyl ester (FAME), Chlorophyceae, Cyanophyceae, and Pyrrophyceae were recommended. Because of its excessive sugar content, for ethanol production, Phaeophyceae tend to be the greatest suitable algal feedstock (Islam et al. 2015). Owing to growing response for transportation fuel, worldwide, algae have appeared as an appropriate candidate due to their sustainable and renewable characters together with financial reliability to compete by international request aimed to conveyance fuels (García-Olivares et al. 2018). The algal biofuel conversion processes, like fermentation, transesterification, and hydro treatment, are economically expensive and more complex as compared to fossil fuels. The possible earth of optimum established are sustainability for the feedstock, or the enhanced possibility of products and innovative applications because of ranges (Culaba et al. 2020). Due to algal positive characters, it tends to be construed that this feedstock is the world's one of the furthermost valuable renewable and sustainable fuel resources that could show an important role in controlling environmental contamination (Bharathiraja et al. 2018). The major threat is the emission of CO_2 from varied fossil fuels through atmosphere. Worldwide economy in the past is out of petroleum funds, whereas it became rich and switch the unfamiliar trade marketplace in light by petroleum reserves (Mohsin et al. 2019).

To all these abovementioned challenges, the only viable solution for both the worldwide economy and greenhouse gas (GHG) emission of fuel formed from various materials (plant). However, renewable energy has different bases, for example, geothermal, breeze, and solar which probably won't be economically practicable as biofuels; still these GHG play a meaningful part of resolving the concerns of global warming. Biofuels are yields of various sustainable and biodegradable feedstock that can be changed of producing opportunities for advances in agriculture as a result of direct contribution to agriculture plants (Gielen et al. 2019).

1.2 Microalgal Species for Producing Biofuels

Recent biofuel manufacturing exploiting microalga biomass is not cost-effective. To make microalga yields and by-products economically feasible, the growing research is keen to observe new microalgal candidates (Koyande et al. 2019). Various advantages of microalgal species must gain methodical concept as to present a profitable source of extreme worth of products (chemicals) like antioxidant, polysaccharides, β-carotene, natural dyes, bioactive, docosahexaenoic acid, and efficient pigment, astaxanthin, algal extracts, antioxidants, and eicosapentaenoic acid. Those classes show a fundamental function of nutraceuticals, human food, cosmeceuticals, fodder, bioremediation, and pharmaceutical aquaculture (Koyande et al. 2019).

Table 1.1 Some major microalgal species, products, and applications (Mobin and Alam 2017)

Species/groups	Products	Areas of application
Arthrospira (Spirulina) platensis	Phycocyanin, biomass	Health food, cosmetics
Arthrospira (Spirulina)	Protein, vitamin B_{12}	Antioxidant capsule, immune system
Aphanizomenon flos-aquae	Protein, essential fatty acids, β-carotene	Health food, food supplement
Chlorella sp.	Biomass, carbohydrate extract	Animal nutrition, health drinks, food supplements
Chlorella vulgaris	Biomass, carbohydrate extraction	Health food, feed, food supplement

Economically viable is to produce algal biomass, production of extraordinary worth of byproduct pull out are utmost. Normally microalgal organisms are naturally single-cell photosynthetic autotrophic microscopic located at marine and garden-fresh environments. Production of numerous multipart complexes like carbohydrates, protein, and lipids, consuming basic elements situated in their surrounding environment (Trivedi et al. 2015) (Table 1.1). Microalgae are "photosynthetic plants" like microorganisms deprived from land pland possess by particular cell and organ types. For energy production, which take carbon from surrounding airborne. By consuming organic carbon, some microalgae yield energy. Microalgae have more than 300,000 species, and around 30,000 species stay renowned. The living locals are complex or may adjust rapidly in severe environmental circumstances (e.g., heat, UV irradiation, variable salinity, and nutrients) (Ramaraj et al. 2015). However, they can yield an increased variability of interesting subordinate metabolites (biological energetic) through unique structures and biological actions which is usually not found in other candidates. The microalgae also yield certain valuable bio goods comprising polysaccharides, carotenoids (specifically β-carotene), antioxidants, docosahexaenoic acid (DHA), astaxanthin, natural dye, eicosapentaenoic acid (EPA), functional or bioactive stains, and algal extracts (Sathasivam et al. 2019) (Fig. 1.1). On a commercial level, microalgal cultivation has begun five decades ago. The global marketplace worth of the microalgae are considered around US$ 2.5 billion created via health food sector. US$ 1.5 billion are yielding DHA or then US$ 700 million by way of aquaculture. Yearly microalgal production is almost 7.5 million tons.

1.3 Promising Microalgal Species and High-Value Applications

Microalgae are divided into four main collections: (1) cyanobacteria (blue and green algae), (2) chlorophytes (green algae), (3) rhodophytes (red algae), and then (4) chromophytes (wholly remaining algae). Every set consists of more than

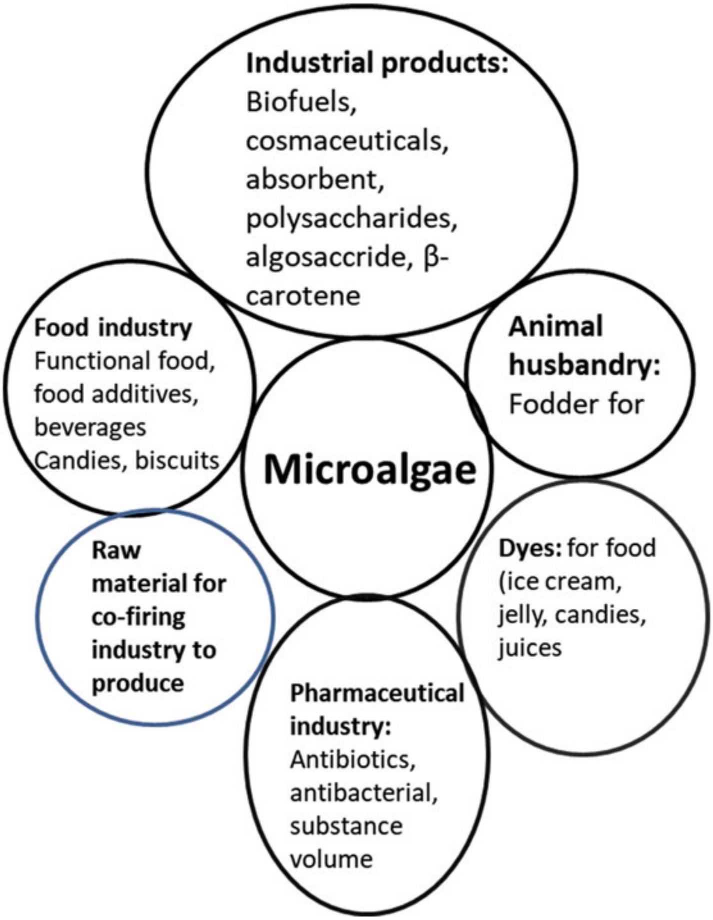

Fig. 1.1 Application of microalgae from numerous areas

hundreds of algae types, and altogether species have thousands or more of strains. For possible beneficial use, only a small variety has been studied. Bacillariophyceae (as well as diatoms), Cyanophyceae (blue-green algae), Chlorophyceae (green algae), and Chrysophyceae (including golden algae) stand generally used microalgae. Some are main microalgal types, their goods, or their application for biotechnological (Sathasivam et al. 2019).

Over the last two decades, four major microalgae are focused on biotechnological applications: (1) *Haematococcus pluvialis*, (2) *Chlorella vulgaris*, (3) *Dunaliella salina*, and (4) *Spirulina* (*Arthrospira*). The characteristics, composition, and production mechanism and, respectively, microalgal type is explained now at the following section (de Morais et al. 2015).

1.3.1 Spirulina

Spirulina (*Arthrospira*) is multicellular, blue and green algae, symbiotic, and filamentous that use nitrogen from the atmosphere. *Spirulina* has two unique shapes; disk-like or spiral rod. Phycocyanin (blue color) is the main photosynthetic pigment in *Spirulina*. The *Spirulina* microalgae likewise hold carotenoids and chlorophyll a. The presence of Phycoerythrin makes the color of microalgae pink or red. Autotrophic contain *Spirulina* then they replicate via binary fission (Mobin and Alam 2017). In 2014 the global manufacture of *Spirulina* sp. was 86,000 tons, as indicated by a report by FAO. It is some of cells considered algae used for extensive outside culture. The high pH (9–11) is best to grow with an extraordinary bicarbonate concentration. To culture *Spirulina*, paddle wheel-driven raceway ponds are used (Mobin and Alam 2017). In the ponds, water varies from 250 to 500 mm because of the microalgae density and seasons. Depth of water pond is also dependent upon pond size, optimal light absorption, and flow velocity by the microalgal culture. Temperature plays a vital role in *Spirulina* productivity (Mobin and Alam 2017). It grows well in between 35 and 37 °C. *Spirulina* filamentous mowing is informal. The starting focus to reached using an inclined gravity screen going on vibrating screefilter (Zhang 2015). Its dewatering remains attempted through vacuum belt filter building a solid pate about 15%; further detail on *Spirulina* is that it is rich in protein, minerals, essential amino acids, vitamins, or required polyunsaturated fatty acid and pigments comprising Zeaxanthin, Myxoxanthphyl, and Phycocyanin. It carries (46–71%) proteins, (8–16%) carbohydrates, and (4–9%) lipids (dry weight). *Spirulina* vital amino acid contains leucine, valine, and also isoleucine. It contains comparatively extraordinary meditation of vitamin K, provitamin A, vitamin B_{12}, and also β-carotene. Fatty acid of *Spirulina* also includes Llinolenic and γ-Linolenic acid and $\Omega - 3$ with $\Omega - 6$ polyunsaturated fatty acids. DHA is the natural source for *Spirulina platensis* demonstrating up to 9.1% of the whole fatty acid (Hemantkumar and Rahimbhai 2019). It furthermore encloses an antioxidant-rich combination more than carotenoids. *Spirulina* mineral content hinge on water, grown and this one content of calcium, iron, and magnesium delivers great nutritional-value (Bensehaila et al. 2015). According to studies, *Spirulina* powder covers provitamin A (2.330 × 03 IU/kg), vitamin E (100 mg, 100/g), β-carotene (140 mg, 100/g), riboflavin B_2 (4.0 mg, 100/g), niacin B_3 (14.0 mg, 100/g), thiamine B_1 (3.5 mg, 100/g), vitamin K (2.2 mg, 100/g), vitamin B_6 (0.8 mg, 100/g), inositol (6 mg/g), vitamin B_{12} (0.32 mg, 100/g), biotin (0.005 mg, 100/g), folic acid (0.0 mg, 100/g), and pantothenic acid (0.1 mg, 100/g) (Ragaza et al. 2020).

1.3.2 Chlorella

Chlorella is spherical-shaped, single-cell (2–10 μm) in diameter, and phototrophic green microalgae without flagella. In its chloroplast, both chlorophyll a and chlorophyll b (green photosynthetic pigment) are present. It multiplies speedily and needs sunlight, water, CO_2, and a slight volume of minerals (Simosa 2016). *Chlorella* was grown in photobioreactor large round tanks and paddle wheel mixed open and circular-open ponds. Though making of microalgae used for aquaculture is mostly at smaller scales, or at most of situations, it is produced within (20–40 L) carboys and huge plastic bags (~1000 L in volume) (Kunjapur and Eldridge 2010). *Chlorella* is harvested by auto-flocculation or centrifugation. Following the collection of biomass sprayer exists drum dried and dust is wholesaled straightforwardly for used to proceed tablets. Chemical configuration of *Chlorella* indicates which comprises (12–28%) carbohydrate, (111–58%) protein, and (2–46%) lipids via dry weight. The usual configuration of *Chlorella* residue may be present at Borowitzka. It also encloses several vitamins like that provitamin A (55,500 IU/kg), vitamin E (1 mg 100/g), inositol (165.0 mg 100/g), thiamin B_1 (1.5 mg 100/g), biotin (191.6 mg 100/g), riboflavin B_2 (4.8 mg 100/g), vitamin B_6 (1.7 mg 100/g), vitamin B_{12} (125.9 mg 100/g), folic acid (26.9 mg 100/g), β-carotene (180 mg 100/g), and pantothenic acid (1.3 mg 100/g). *Chlorella*'s worldwide profitable market worth esteem is above a billion US dollars (Hemantkumar and Rahimbhai 2019).

1.3.3 Dunaliella

Dunaliella is edible, nutrient-rich, single-celled, flagellated, and extremophile green microalgae. *Dunaliella* is brought into being at countless fresh waters or marine water habitats. *Dunaliella salina* (*D. salina*) has gained ample care from scientists by the intense stages of antioxidant actions (Dolganyuk et al. 2020). It is the best source of β-carotenoids, which contains a high extent of β-carotene (above 14% of dry biomass) contrast with further well-known cradles. *Dunaliella* may be grown over 30% NaCl saturation though the ideal development salinity is 22% NaCl saturation. There is only a rare reported challenging type and predators for *Dunaliella*; thus, modest open pond culture is sufficient for it. *Dunaliella salina* (*D. salina*) is full-fledged at each shallow, extensive areas (5–200 ha), in light paddle wheel race way ponds and unstirred ponds which is more than 1000 m^2 in area (Dolganyuk et al. 2020). For *D. salina* using technique is the semi-continuous culture. It has been discovered that at lower salinity level is the best to grow *Dunaliella* closely (22% (w/v) NaCl) saturation however β-carotene content (nearly 33% NaCl). *Dunaliella* shows promising growth at lower salinity conditions, but there are risks of contamination by brine shrimp or even by *Artemia* or protozoa. There are several manufacturing tactics researched by Ben-Amotz for *Dunaliella*. The challenging culture was using nitrogen inadequacy, extreme salt concentration, or penetrating solar radiation which boost fruitful biomass of *Dunaliella* and then β-carotene fabrication (Pourkarimi et al. 2020). By using the centrifugation technique, harvesting of *Dunaliella* has been completed, further flocculating and employing centrifugation methods manipulating the cell membrane at hydrophobic nature. Certain complications coupled to the reaping of *D. salina* are:

1. Cells are of jaggedly to the comparable density like as culture medium.
2. Lower cell concentrations in the culture (typically less than 0.1 g dry weight per liter).
3. Cells are very faint because of no cell wall.
4. Instability of cell (uncertainty of cells are broken in the period of harvesting, so the rapidly dissolving of β-carotene) (Hosseini Tafreshi and Shariati 2009).

Immediately after being harvested, the biomass might be sprayed through drum dried, and the β-carotene may be detached straightly by means of hot oil or any additional solvents. The biochemical configuration of *Dunaliella* is protein (49–57%), lipids (6–8%), and carbohydrate (4–32%) of its total dry weight (Wu and Chang 2019).

1.3.4 Haematococcus pluvialis

Haematococcus pluvialis (*H. pluvialis*) present as unicellular bi-flagellate freshwater Chlorophyta microalga dispersed around the world. Under different stress conditions, the accumulation of a wide variety of strong antioxidant astaxanthin is renowned as *Haematococcus pluvialis* (more or less 2–3% on dry weight). A commercially producing organism is astaxanthin of *H. pluvialis* (Shah et al. 2016). Heterotrophic, photoautotrophic, or mixotrophic centered on progress state, internal or secure photobioreactors, or sweeping channel ponds are mostly recycled for *H. pluvialis* refinement. Standard photobioreactors castoff for refinement cover simmer column, tubular, plus airlift photobioreactors (Narala et al. 2016). A twice step agriculture approach tactics are generally adapted for marketable nearer efficiency. The initial step includes the mounting of algal green biomass at motile stage in a secured system (photobioreactors) and exposed skill (like that pond raceways), and the following step comprises manufacturing of astaxanthin covering aplanospores enlargement through the insufficiency nitrite or phosphate and then improved light intensity and temperature (Kim 2015). Then accretion of astaxanthin is squeezed via natural features, for example, salt concentration, nutritional stresses, light, and pH. Cellular structure of *H. pluvialis* differs remarkably among red-green phases of agriculture. Mowing is attempted by using a grouping of flaccid resolving besides following flotation and centrifugation. Mowing of biomass has got dried out by shower, chilling, sun-oriented drying, lyophilization, or cryodesiccation. To dry high-esteem *H. pluvialis* items, splash drying is the most fitting strategy (Shah et al. 2016). A widespread variety of procedures has been industrialized toward interrupting the *H. pluvialis* cell or recovering the intracellular metabolites. Then mechanical procedures (bead milling expeller pressing) are the most appropriate cell disruption technique. *H. pluvialis* cells are cuddled underneath extraordinary pressure toward breaking the dense pollen ultimately. Later in the disruption of cell walls, the biomass of *H. pluvialis* is essential to be treated within a couple of times to evade damage. However, super critical carbon dioxide (SC-CO_2) and solvent drawing out methods are well-known owing to their proficiency or compatibility. *H. pluvialis* is able to mass more or less 5% dried weight of astaxanthin and is well-thought-out as the good organic cause of the great worth of carotenoid colorant. Due to the huge cost of manufacture, synthetic astaxanthin leads the presently profitable market (yearly production of 130 tons creating a value of US$ 200 million) (Sanzo et al. 2018). Some additional microalgal species areas are as under studying for their appropriate for food or nutritional supplements bases. In the 1970s *Scenedesmus* was not found favorable for human or animal intake due to its huge cost (Ghani et al. 2020). In the 1990s, the dinoflagellate *Crypthecodinium cohnii* has been recognized as the utmost capable entrant for DHA creation. In heterotrophic culture, the cell bulks of *Crypthecodinium cohnii* have been attained up to around 40 g/L with a lipid percentage of 15–30%. The DHA value for this lipid is described to 25–30% (Santos-Sánchez et al. 2016).

1.4 Microalgae

Microalgae have gained considerable attraction worldwide currently, by their wide-spread usage potency in the renewable energy, biopharmaceutical industries, and nutraceutical. Microalgae are renewable, viable, cost-effective homes of biofuels, bioactive medicinal products, and food ingredients. Several microalgae kinds have been examined for their likely function as significant yields with extraordinary biological and pharmacological potentials (Subhadra 2010). As per a biofuel, microalgae are an entire alternative for melted remnant fuels with charge, renew-ability, and environmental interests. It has a considerable capability to change atmospheric CO_2 into valuable products such as carbohydrates, lipids, and additional bioactive metabolites (Hussain et al. 2021). For biopharmaceuticals and bioenergy, the microalga is a viable source, some challenges and problems are remaining, but they must be overcome to advance the equipment from experimental-phase to built-up level. For bioethanol production, pretreating biomass, dewatering algal culture for biomass production is the most essential and challenging feature for improving the growth rate and product synthesis (Tonnaer 2017). For biodiesel production, most microalgae species are appropriate, because of their high lipid contents of 50–70% and may reach 80% such as incase of microalgae *B. braunii*, which accumulate up to 80% of oil in its biomass. Polyunsaturated fatty acids are vital in tissue integrity and have beneficial health effects. Omega-3 and omega-6 fatty acids, in particular, are crucially essential for humans but the human body cannot produce them by itself. Many microalgae species (e.g., *Arthrospira platensis*, *Porphyridium cruentum*, and *Odontella*, *I. galbana*) have been explored for their capability to synthesize these fatty acids. *Pavlova lutheri* produces polyunsaturated fatty acids in large quantities. Microalgae are a rich source of various vitamins; *P. cruentum* produces a high quantity of vitamin C and E, as well as β-carotene (vitamin A); *Haslea ostrearia* is a rich source of vitamin E (tocopherol).

Microalgae have a broad spectrum of industrial applications. Mostly wastewater purification and biofuel production have been reported. Some industrial applications are high-value food, nutraceuticals, pharmaceutical products, health food for humans, fodder additives, polysaccharides, cosmeceuticals, antioxidants, dyes, food for aquaculture, and bioplastic production. Microalgal biomass is also used as a raw material for the co-firing industry to generate power because it has a high heating value other than biomass feedstock. *Spirulina* is used for cholesterol reduction and immune system enhancement. Sulfated polysaccharides of *Spirulina* are broadly used as an antiviral agent. Its tablets are being marketed since 1975 in Japan. *Chlorella* composes of vital substances: β-1,3-glucan. This compound is a free radical scavenger that enhances immune response, also responsible for lowering lipid in the blood. It is also effective in stomach ulcers, wound healing, antitumor activity, and removing toxins from the body. *Spirulina* has a preventive effect in hypercholesterolemia. *Chlorella* is effective on low blood sugar level, enhances hemoglobin concentration, and acts as hepato-protective agent. Astaxanthin from *Haematococcus* is utilized in aquaculture for coloring fish muscles (salmon fish). The antioxidant characteristics of astaxanthin prevent the production of inflammatory compounds and also prevent protein degradation, oxidative stress, and macular degeneration. *Spirulina*, *Dunaliella*, and *Chlorella* all are widely used in the food industry. Their biomass is also utilized for forming a variety of health products including tablets and capsules. Three microalgae are used for bread, noodles, candies, bean curd, and other common food with high health values. *Chlorella vulgaris* stimulates the synthesis of skin collagen; they also help in the regeneration of fibers and make the skin surface free from wrinkles.

Extract of *Spirulina* minimizes the age effects. Purified phycobiliproteins, a product of *Spirulina*, are used in cosmetics, food (colorant), antioxidant treatment, anti-inflammatory, photodynamics of various cancers, leukemia and tumor treatment, and florescent marker production.

1.5 Macroalgae

Algae are distributed in an extreme and diverse environment. Due to their high content in compounds, they are valuable with different biological activities, including both complex organic compounds, both primary and secondary metabolites. It is significant to observe that the majority of these substances include phytopigments (carotenoids and xanthophylls), polyunsaturated fatty acids, phenolic compounds, tannins, peptides, lipids, vitamins, enzymes, terpenoids, and others. Thus algae are viable and economical biomass sources of valuable compounds with potential applications in the pharmaceutical, nutraceutical, chemical, food, and cosmetic industries due to their biological active and regenerative characteristics (Fig. 1.2). Microalgae gained more and more value due to their usage in health aspects. They are promoting properties that can reduce the risks of many chronic diseases and help to extend the life span. They are also used for wastewater treatment or as natural

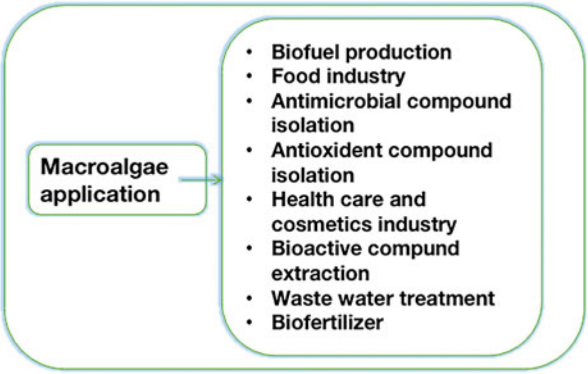

Fig. 1.2 The main application of macroalgae

fertilizer in agricultural areas, therefore improving the quality of products and reducing the need for chemical fertilizers. As a source, the potency of macroalgae of renewable energy is also of considerable interest. These aquatic organisms mitigate carbon dioxide emissions and nowadays are being used as feedstock to form “clean” or so-called third-generation biofuels.

1.6 Chemical Composition

Different marine macroalgae (seaweed) as a source of bioactive and essential compounds had the advantages to use an under-utilized sustainable natural reserve. It had been confirmed that secondary metabolites are made up of biomass (Biris-Dorhoi et al. 2020). The chemical composition has changed due to natural conditions (temperature, candlepower, sea water salinity, and growth habitat) and genetic modifications among species. The protein substance can go from 7% to 31% of total dry weight and a lipid substance varying from 2% to 13% of total dry weight. A big quantity of carbohydrates can range from 32% to 60% of dry weight (Aratboni et al. 2019). Macroalgae are considered a true natural source of vitamin A and E (tocopherol). The abundance of vitamin B_{12} advances the macroalgae created products concerning the dietary supplement for a vegetarian lifestyle taking a risk of deficiency of B_{12} (Biris-Dorhoi et al. 2020). The numerous microelements found in seaweeds are mostly supported by their mineral content; sodium, mechanism, and calcium, which present above 97%, you look after the general mineral content. Additional microelements like iron, zinc, manganese, and copper are discovered in minor quantities (ranging from 0.00 to 0.094) of seaweeds of dry weight (Arguelles 2020). Phenolic compound existence in macroalgae has taken great attraction due to their particular bio-activities and health supporting benefits incorporating antiallergic, antiproliferative, antioxidant, antimicrobial, antidiabetic, and neuro-protective properties (Santos et al. 2019). Secondary metabolite’s presence in macroalgae is encouraging the regular defense system against several injuries, diseases, and environmental aggression. For an extended time, macroalgae are encouraged for their prospective function in protective cancer rate, tumor development, and even health upturn after chemotherapy or radiotherapy treatments (Biris-Dorhoi et al. 2020). Iodine can also be used as an anticancer response, due to its capability to start apoptosis in cancer cells. Similar characteristics are often ascribed to the omega-3 fatty acid like stearidonic acid and hexadecatetraenoic acid discovered in eatable marine algae-like *Ulva* and *Undaria* along with 40% of total fatty acids (Biris-Dorhoi et al. 2020; Fitton et al. 2008).

1.7 Laminarian

Alginate, fucoidan, and lots of other seaweed polysaccharides are proven to possess antitumor activities. A great quantity of polysaccharides (~65% polysaccharides in total dry weight) are often found in many seaweeds like *Ascophyllum*, *Ulva*, *Palmaria*, and *Porphyra* (Ghosh et al. 2021). Alginate filling the intestinal system additionally aids in boosting immunity and intestinal health, reducing the risk of cancer (Eliaz et al. 2007). Laminarian and fucoidan induce apoptosis to prevent cancer, but also unidentified seaweed polysaccharides are able to exhibit straight and unintended antitumor influence. *Sargassum latifolium* reserved cytochrome P450 IA and glutathione S-transferases and prevent cell viability and stimulating apoptosis. Another study about the antiviral potential of algal against foodborne viruses is getting interested about current years; recently the accessible data are still limited (Zorofchian Moghadamtousi et al. 2014). The most compounds from algae that are proved to possess antiviral potency are sulfated polysaccharides, including sesquiterpene hydroquinone, carrageenan, sulphoglycolipids, and fucoidan (Ahmadi et al. 2015). Polysaccharides derived from marine and their lower relative molecular mass oligosaccharides derivatives are displayed to have a kind of antiviral activities and also exercise antimicrobial and antioxidant influence (Zhu et al. 2021). The algal polysaccharides can overturn the DNA replication and leave the host cell colonization by the virus. For instance, the antiviral capability of polysaccharides from brown seaweed exposed substantial inhibiting action against the hepatitis B virus (HBV) and DNA polymerase, accordingly influencing its replication. The antiviral action of those polysaccharides is exerted through suppression of virus adhesion to the host cells (*U. pinnatifida*, bladder wrack, *Cystoseira indica*). Fucoidan inhibits syncytia formation with remarkable selectivity (IS50 $>$ 2000) during the early phases of virus infectivity (0–60 min post–infection) (Gheda et al. 2016).

1.8 Algal Biofuel Production

There are an excellent revolution and challenges in biofuel production to exchange fossils fuels. The biological yield of biofuels is apprehending the world's market thanks to the restrictions of petroleum-based fuel. Researchers are more interested in the exploration of latest technologies for biofuel yield. Biomass for biofuel production is one among the alternatives thanks to its sustainability and renewability, low CO_2, fewer greenhouse gases, etc. The most issue regarding biomass usage is the efficiency to exchange complete fossil fuels (Khan et al. 2018). For biomass generation, land use causes ethical issues that are consequences in enhancing food prices. The most focus of scientists is to believe new technologies to beat energy needs, decrease the prices of fuel, and be ready to solve environmental issues too (Fribourg 2008). Photovoltaic technology systems are going to be directly employed by genetically engineered photosynthetic microorganisms of completely synthetic factories. Biofuels are considered the foremost promising within the short term as their market maturity is above other options. Global climate change concerns the buildup of greenhouse gases causing linked regarding the utilization of fossil fuels because it is the major energy source (Hannon et al. 2010). To beat the problems of global climate change, the one solution is to believe the potential of microorganisms to use renewable substrates for biofuel production, thanks to tremendous progress in several industries which are resulting in polluting the environment. The microbial technology gives the simplest solution with an environmentally friendly approach, by identifying the microbial strains, causative agents for the matter, and implementing the useful one for environmental bioremediation (Srivastava 2019). Microalgae have long been known as potential good sources for biofuel production due to their comparatively high oil level and speedy biomass production. Microalgae raise very timely as competed to continental crops; algal mass can grow on non-arable lands using non-potable saline or wastewater (Srivastava et al. 2020).

The microbial-related bioremediation process has benefited society by exploiting the metabolic abilities of microorganisms. Due to the depletion of worldwide petroleum and its value increases, biodiesel is becoming one among the simplest promising worldwide energy markets within the coming future. Growing pattern in the biofuel and high-rate biochemical yield decline the necessity for nonrenewable and artificial sources, to decrease the harmful environmental effects and advance biorefinery (Koenraad et al. 2015). It's likely that the biochemical market will increase from 2% to 20% interest by the year 2025 due to the main growing demands for bio-based products which have directed the R & D efforts to specialize in commercially oriented research styles (Cordova et al. 2020). At the present, biofuels have gotten significant potential among the financial and environmental disasters of fossil fuels. Fuel reproduction molecules as isoprenoids, fatty acid-derived molecules, hydrocarbons, and improved alcohols have a plus over their conventional complement due to engine compatibility, compatibility with present storage, higher density, and transport structure. Furthermore, biologically functional high-value substances containing isoprenoids and fatty acid-derived biomolecules are of environmental, biotechnological, pharmacological, agricultural, and also industrial significance (Adegboye et al. 2021). For the assembly of required titers for subsequent generation, there's a requirement for improvements in gene-splicing techniques that have assisted scientists to develop advanced robust strains (Bharadwaj et al. 2020). Biofuels also require starchy/sugary substrate or lipid-rich biomass for their successive conversion into advanced alcohols: butanol, isoprenoids, bioethanol, and fatty acid-derived substances (Mehmood et al. 2021). First-generation feedstock include food crops, for instance, sugarcane, barley, corn, beetroot, wheat, etc.; however it made the conversation of food deficiency (Hirani et al. 2018).

1.9 The Second-Generation Feedstock

Second generation focused intensely towards the raw and waste materials to account for the issues of first-generation feedstock, yet they're fundamentally expensive and laborious pretreatments for their decomposition into simpler components for their simpler succeeding adaptations to those products (Anto et al. 2020). Microalgae and cyanobacteria are photosynthetic microbes considered because third generation feedstock supply both higher lipid-based pieces of stuff and alcohols. Due to their biochemical composition, microalgae got a standing over lignocellulosic biomass, which contains lipids, protein contents, and carbohydrates (Abomohra and Elshobary 2019). Carbohydrate ingredients are viable to yield bioethanol and alcohols, while the lipid section is employed to supply biodiesels, isoprenoids, and extra lipid-based compounds. The restraints of cultivation, harvesting, and downstream processing are thanks to the commercial execution of third-generation feedstock (Laurens et al. 2017b). The deficiencies of the previous generations were alleged to be reported by GM microbes (designated as fourth generation) to support the growth proficiency and product yield. The earlier decade has understood a rise in extreme value biochemical productions and biofuels engaging microbial cell factories (Shuba and Kifle 2018). To reduce the greenhouse gases and to satisfy the worldwide energy burdens, technological advancement requires specialization in as follows:

1. To boost up the biochemical production, through improving the microbial cell factories.
2. For higher efficiency, optimize the existing production technologies.
3. Development of cell capability to yield biofuel-related designer molecules that advance fuel quality.

Combined methods could support in overwhelming the technological difficulties met during designing a functional, and reliable biofuel production pipeline. For the modernization of metabolic pathways, genome editing (like gene insertion and removal) has developed the reconstruction efforts because it advanced the metabolic engineering of both native and non-native hosts to yield renewable biofuels. By using of strain improvement technique, several approaches are used for the assembly of high titer, e.g., genetic modification, promoter engineering, pathways synthesis, process engineering, enzyme engineering, and competitive pathways blocking (Aro 2016).

Advancement in synthetic biology decreases the struggles, contrasting previously in developing microbial cell factories (Xia et al. 2019). Therefore, the key challenge is the partial number of existing obvious genes and their combinatorial control on metabolomics structure of the host organism. The modification consequence during a complicated phenotype-genotype results in opposing and accidental effects (Manzoni et al. 2018). The purpose for receiving developed titers from microbial cell factories is careful rebuilding and optimization of the metabolic pathway regulation in terms of organic phenomenon, enzyme activity, and metabolic influx (Lee et al. 2018). Difficulties are encountered during a different system of biology and gene-splicing methods to make fourth generation alongside the possible solutions to handle these challenges.

1.10 Heterologous Synthesis of Hydrocarbons

The international marketplace for *n*-butanol is growing at a prompt rate with a predictable market worth of 5 billion USD (Tao et al. 2014). Biological fermentation process (acetone-butanol-ethanol pathway) or petrochemical/chemical methods (for 7.0–8.0 billion USD/year) are usually used for the economic production of butanol. However, the dependence of chemicals on the price of oil makes this alternative unsuitable for the near future. Additionally, *n*-butanol synthesis is taken into account carbon neutrality and sustainability. It's produced naturally by *Clostridia* spp. through acetone-butanol-ethanol (ABE) pathway; however slow ratio of growth, complicated life cycle, complex nutrient need, and demanding genetic modifications constrain the assembly (biological) of butanol (Vogt and Richnow 2013). Furthermore, Clostridium spp. is incapable of directly using low-price substrates like hemicellulose, organic waste, and cellulose and must be dependent on the molasses and starchy materials whose accessibility has been limited by environmental dependence and competition with human food correspondingly (Kucharska et al. 2018). Special engineering strategies are adapted by using non-butanol-producing species, like *E. coli*, *B. subtilis*, *P. putida*, *S. cerevisiae*, and *Lactobacillus brevis* to extend marketable production targets of butanol. Toxicity induced by butanol is the primary major difficulty to become commercial yields. With the chaotropic action of 37.4 kJ/kg/M, butanol interrupts the macromolecular complexes of the host cell and then produces oxidative destruction which finally results in product-induced growth (Cray et al. 2015).

1.11 Production of Microalgae-Biofuels with Nano-Additives

For biofuel generation, microalgae are seen as potential feedback within the current age due to its energy-rich source, cheap culture approaches, inflated rate of growth, the prominent ability of CO_2 fixation, and O_2 accumulation to the atmosphere. Recently, research is constant regarding the improvement of microalgal biofuel technologies (Zhu et al. 2018) (Fig. 1.3). Application of nano-additives has been understand as a prominent improvement to mitigate this experience. At different stages from microalgal cultures, the nano-additive application to end-product use presented a solid possibility into the longer term (Anwar et al. 2020). Currently, the foremost complex technology is that the nanotechnology incorporation with the applications of bioenergy by the nano-energy zone possesses a durative on biofuel transformation mechanism and improvement of engine progress. This technology is described because of the design of a material or device on a nanoscale (10–9 m). To accomplish the biofuel product and develop the effectivity of biofuel consumption in diesel and petrol, nanotechnology has been introduced through nano-additives like nano-crystals, nano-droplets, nano-fibers, nano-magnets, etc. (Hossain et al. 2019).

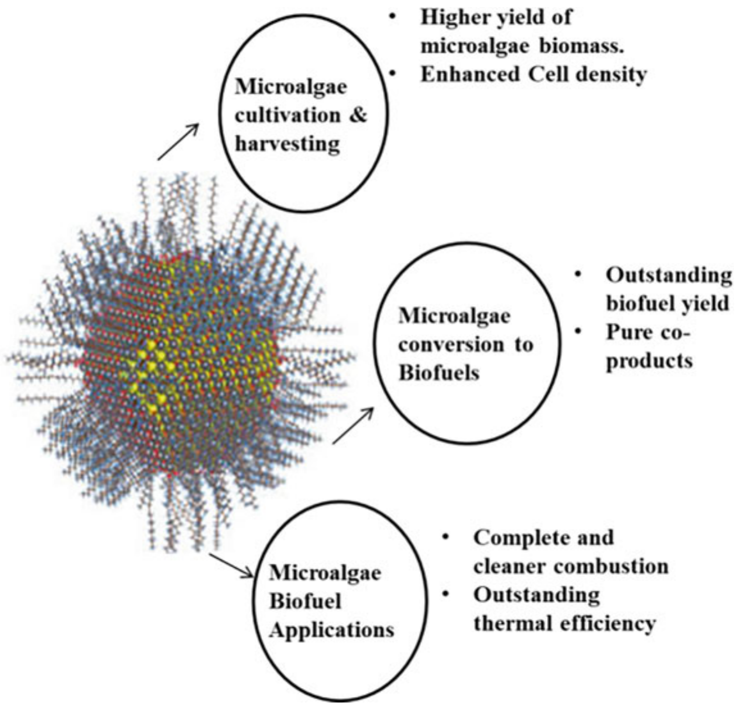

Fig. 1.3 Nano-additive application for the enhancement of microalgae cultivation to biofuel implementation

The thought of microalgae cultivation has appeared to the spotlight for biofuel production due to many positive outlooks like:

1. They don't clash with human and animal food chains.
2. Rich in proteins, carbohydrates, and oil contents.
3. Ability to sprout on aqueous media like wastewater, saline water, and water and assimilate nutrients from brackish water, highly contaminated water.
4. Low tide requirement.
5. Support the power to supply whole year naturally with sunlight existence.
6. Are often grown within the bare land (especially within the cold region), ponds, waste dump area, river, and municipal and industrial waste drainage.
7. Create a sustainable O_2 production system.
8. Use of CO_2 for photosynthesis respiration helps CO_2 elimination from atmosphere (Pal et al. 2019).

Furthermore, microalgae have a really short harvesting biological clock and yield promising biomass that generate greater yield of the specified biofuel. Interesting microalgae have an extraordinary quantity of lipids, protein, and carbohydrates, the only element of biofuel conversion. For the biofuel productions, nanotechnology applications are implemented, meanwhile when the present notorious approaches of usual microalgae culture, biofuel production includes some restrictions like uneven industrial-scale microalgae yield, increased cost harvesting and production, the energy utilization of biofuel construction from microalgae, and therefore the expansion of greenhouse emission concentration within the environment (Work 2014). For various phases of microalgae cultivation, nanotechnology applications are often entitled to microalgae-biofuel application fuel engines due to stability, durability, crystallinity, proficiency, recyclability, catalytic performance, high storing capacity, absorption, and eco-friendly characteristics (Hossain et al. 2019). Due to improvement, nanotechnology improved microalgal cultivation, the larger yield of several microalgae biofuels also as microalgae-biofuel implications in diesel engines and petrol. Numerous nano-materials, e.g., nano-tubes, nano-particles, nano-fibers, nano-sheets, and further nano-structures, are seen as active nanocatalysts in direct and indirect approaches in biofuel (e.g., bioethanol, biodiesel, biomethane, and others) for product improvement (Darwesh et al. 2021). However, magnetic nanoparticles were used as a transporter for enzyme control for biodiesel and bioethanol production effectivity. Due to potent paramagnetic properties, magnetic nanoparticles were also favored for methanogenesis to yield biomethane (Hossain et al. 2019). Bioenergy yields from lignocellulosic biomass (agriculture excesses) and waste of industries (slurry) furthermore as algae (macro and microalgae), with optimization at nanoscale that has just enforced on the instrument of nano-particles, characteristics of biomass, and various application on biomass progression (Kamla et al. 2021). The scientific assent is that by the process of photosynthesis respiration, algae convert O_2 and CO_2 and generate an enormous quantity of cellular energy contents into sugar, proteins, and lipid (Shuba and Kifle 2018). Development and industrialization threaten the present ecosystem due to the dumping of heavy metals, waste containing sulfur, nitrogen, phosphorous, etc. commonly with exhaling a high quantity of CO_2 to the free air (Hossain et al. 2019).

1.12 Nano-Additives for Microalgae Biomass Conversion to Biofuels

Among all microalgae biofuel, biodiesel got high importance because of the commercial and admired biofuel within the oil market. For the production of biodiesel, applications of basic and acidic nano-catalyst spheres can substitute chemical compounds as sodium methoxide by reacting with the oil and free fatty acids. The constructive effect of this nano-catalyst is that reaction occurs with pressure and coldness also as advance reduces the environmental bome by sodium methoxide (Hossain et al. 2019). Another study about biodiesel at industrial level confirmed that commercial CaO-NPs offered 91% biodiesel conversion efficacy throughout scaled-up catalytic transesterification. Microalgal development with sphere-shaped nano-particles constituted of calcium, and sand silica compounds shown that cellular growth of microalgae boosted significantly exclusive of destroying harvesting also as biofuel production from oil (Akia et al. 2014). The research study about mesoporous silica nano-catalyst Ti-loaded SBA-15 signified ten times advanced free carboxylic acid (FCA) and water tolerance level than another catalyst for production of biodiesel from oil also as this nano-catalyst accomplished three times improved than other effective nano-catalysts titanium silicate-1 (TS-1) and titanium oxide silicate (TiO_2-S) (Chen et al. 2013). Furthermore, Ti-loaded Santa Barbara Amorphous-15 (SBA-15) nano-catalyst application reduced the chemical (alkaline catalyst, NaOH) cost of transesterification process for biodiesel production by recycling the nano-catalyst also as this method is more environment friendly (Hossain et al. 2019). Another study show that sulfate incorporated Ti-SBA also performed as a biocatalyst to vary oil to 100% esterified bio-lubricant. Bio-oil of microalgae with the aid of nano-particle aided to source of bio-lubricant from bio-oil. Another research reported that Niobia (N_2O_5) incorporated with SBA-5 applications on biodiesel production from biomass via esterification characterized a big development for microalgae-biodiesel yield (Chen et al. 2020). A kind of nano-particle, zeolite (an alumina silicate mineral), has been used as a billboard absorbent through transesterification process. Zeolites are able to absorb the unfavorable moisture content (4–6%) and generate pure glycerine as a co-product furthermore of biodiesel (Tran 2018).

1.13 Genetically Engineered Algae for Biofuels

For several years due to the revolution of the global level energy crisis, microalgae have been developed and are using as a replacement of feedstock for the yield of biofuels. Furthermore, for producing key chemicals, microalgae consider having huge strength for bio cell factories like recombinant enzymes, alcohol, hydrogen lipids, and protein (Jagadevan et al. 2018). Microalgae-dependent renewable energy is considered very economic due to the concept of high-value products such as the algal biorefinery approach. Genetic engineering is a new discipline that combine in a sequence of engineering and science to aid support construct and design novel-biological systems and to gain themes that are formulated rationally (Jagadevan et al. 2018). The tools and resources used for artificial gene network construction, nuclear manipulation, and reproduction of microalgae of genome-scale are limited. Microalgae of genetic engineering (GE) are appearing to be a biofuel synthesis commercial release without such ecological studies or public information to inspect the possible risks (Kumar et al. 2020). Green eukaryotic algae and cyanobacteria a blue-green algae are likely to spread-from ponds which are uncluttered and on the subordinate scale with smaller possibilities from unopened photo bioreactors. Cyanobacteria are especially problematic to detect due to the danger of horizontal gene exchange with discrete microbes (Day and Stanley 2012). Before novel, genetically engineered algae transfer to the environment; environmental and major biosafety dangers should usually be addressed by experts' teams like ecologists. For the synthesis of biofuels, biologists assumed that microalgae will be reformed by using a vision or perception from artificial biology and the innovative process of producing biofuels in the form of genetically engineered organisms (GEO) (Ancillotti et al. 2016) (Fig. 1.4). High-throughput sequencing of hybridization, DNA sequencing, metagenomics, and accelerated evolution. Genetic engineering is currently expanding, becoming more accessible, and becoming more effective. Nemours strains of algae have been selected worldwide and collected for raw genetic material (Park and Kim 2016). The pathways and related genes are vital for the biosynthesis of biodiesel in *Dunaliella tertiolecta*, a non-model aquatic flagellate (Rismani-Yazdi et al. 2011). In the microalgae, the composition of DNA which enhances the rate of growth, and Boston the nitrogen efficiency uses, have been studied for Nemours patents. In photobioreactors and in open ponds, the growing algae are very expensive. By research, it is summarized that there is still much need to do innovation including genetically engineered microalgae cultivation (Barry et al. 2016). The novel characters can be accomplished by methods of non-transgenic therefore value assessment, to increase the performance of wild strains, recombinant DNA is used. To introduce microalgae at the commercial level, Sapphire Energy Corporation works together with Monsanto company to investigate innovative genes which converse extra growth and additional positive traits (Snow and Smith 2012). There are spin-off applications in crop plant. The company Joule Unlimited has patented a mix of foreign microbe genes and genetically engineered cyanobacteria that makes more ethanol. Novel GEOs are

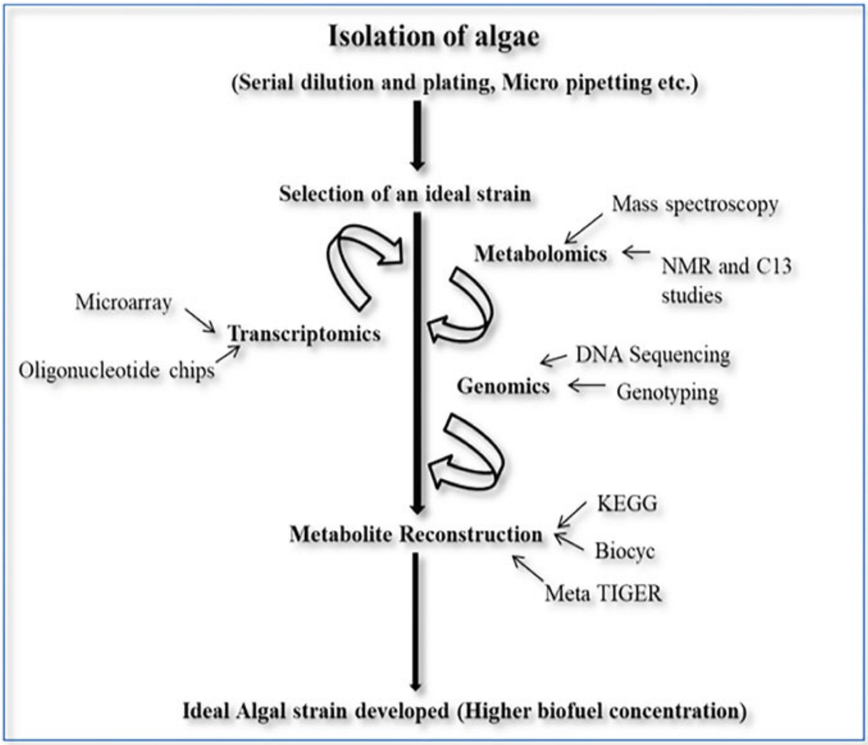

Fig. 1.4 Pictorial representation of the overall process toward biofuel production in microalgae using synthetic biology approach selection of an ideal strain redirecting metabolism to maximize the synthesis of the targeted biofuel

represented within the atmosphere. Moreover, major biosafety dangers should be addressed to invest cause of harm and to the potential stage of exposure (Snow and Smith 2012). To GE algae endurance outside of open lakes or encased bioreactors is essential standard source for hazard investment. The problem has arisen that genetically engineered algae discover lab-created strains must unable to subsist within the rough specially if tamed or kept forming huge volumes of industrial type of by-products. If an unproposed release era to prissiest, the concerned GE algae choose that their developing descendants may have that would allow them to survive and continue in natural surroundings (Henley et al. 2013). Such engineered traits, for example, resistance to severe temperatures or improved growth, may help GE algae survive and develop in a suitable ecosystem.

Significantly more had to think about the condition of lab than their regular habitats (Joutey et al. 2013).

1.14 Algal Biorefinery Products

There is no comparison between the economic suitability of microalgae biofuels with existing technologies but no focus on the innovation of high-value co-products from microalgae to develop the foundation of economy on biorefinery (Patil et al. 2008). Biorefinery is an organization where high-rate products and energy fuel are produced, for example, lipids, pigments, protein, antioxidants, vitamins, and carbohydrates are formed through different processes by biomass (Chew et al. 2017). Lipids, carbohydrates, and proteins are abundant in microalgae, and the absolute amounts of these biological compounds varied among microalgal strains. It has been described in research that *C. reinhardtii*, cyanobacteria, *A. variabilis*, and *Anabaena cylindrica* green algae strains of algal are famous to produce hydrogen in presence of sunlight (Aratboni et al. 2019). The organisms listed above can extract protons and electrons from water and hydrogen. This is done with the help of the chloroplast (Hyd A2 and A1) hydrogenase, which speeds up the process. Research shows that the metabolism of C. reinhardtii's respiratory system was changed by finishing strength competition with hydrogenase for an electron. This made the plant's natural rate of biohydrogen production faster (Jagadevan et al. 2018). Moreover, the hexose uptake protein expression in a production of hydrogen *C. reinhardtii* mutant (Stm6) shows 150% enhancement in the ability to formats H_2 (Doebbe et al. 2007). Because of the upgrading research which is investigating the fourth generation of biofuels, numerous studies on the production of bioethanol by implementing algal strains like *Desmodesmus* sp., *Tetraselmis suecica*, and *Porphyridium cruentum* have been stated. Through recombination system of double homologous, the strain of algal PCC 6803 *Synechocystis* sp. formed, and this is able to do CO_2 photoautotrophically changing to bioethanol, by means of improved theoretical growth of ethanol per gram of 0.696 as in contrast to glucose ethanol per gram 0.51 through *S. cerevisiae* (Doebbe et al. 2007). There is a need to do concentration on the research of the production of bioethanol which is derived from algae to make this method economically feasible. Various researches directed the biobutanol production from specific microalgae like its strain which is full of carbohydrates. CL-MI, *Neochloris aquatica* acid is a pretreated biomass of biodiesel residues of microalgae and JSC-6 *Chlorella vulgaris* biomass (Behera and Varma 2016; Doebbe et al. 2007). Iso-butyralaldehyde with iso-butanol production a strain of algal *Synechococcus elongatus* was studied for the production of butanol. A higher ratio of iso-butyraldehyde produce with the GE *S. elongates* than those researches from lipid, hydrogen, or ethanol production with algae or cyanobacteria through the upregulation of ribulose bisphosphate oxygenase/carboxylase (Rodriguez and Atsumi 2014). The major difficulties to introduce biobutanol's large product at the commercial level are the other by-products that impurify the production of the yield of pure butanol. Biodiesel has similar chemical attributes as fossil fuels and consider a good alternative. Various algal species like *Dunaliella*, *Chaetoceros*, *Nannochloropsis*, *Scenedesmus*, *Pseudochlorococcum*, and *Botryococcus* are famous to concentrate a high ratio of native lipids (Kolesinska et al. 2019). Algal strains' metabolic pathways can format 16–20.5% fatty acids *n*-Carbon as precursors for biodiesel growth. Formation of fatty acid for the synthesis of biodiesel has happened through *Nannochloropsis* sp. Carotenoids and biohydrogen are other by-products. Seventy percent of pigments and 45 g/100 g lipids dry biomass were extracted when supercritical CO_2 fluid extraction is used. For the production of biodiesel, another research was performed to investigate on a high lipid-forming microalgae (*Euglena sanguinea*) (Kolesinska et al. 2019). It was confirmed that this biodiesel will not cause any disturbance in the engine because of unsaturated availability in fatty acids of C: 18:1 along with SFA (C24:0-C22:0, -C16:0, -C18: 0) in it. Hence, it could be used in automobiles without any change in engine structure. Flux analysis, in particular, can give an effective technique of prediction in biology systems by measuring carbon flux during accumulation, growth, and carbon fixation altogether (Nagy and Tiuca 2017). Calvin cycle's coefficient data is controlled by enzyme flux; it helps in the growth and fixation of carbon. Metabolic subsequent map and metabolic ^{13}C flux data derived from algae can originate the targets and steps which are involved in lipid metabolism (Behera et al. 2021). These data show that pathways and enzymes which they modified are exert significant and its rate-limiting control on the high metabolism. In the present, researchers are investigating on the pathways of metabolic engineering for enlargement of fatty acid chain length (Park and Nicaud 2020). This modification will help to enhance the growth of algal biodiesel production in the future. Nemours researches have been done on the genes of microalgal encoding enzymes that are involved in the preparation of high-value carotenoids like *C. reinhardtii* type of microalgae but cannot prepare keto-carotenoid, and gene of β-caroteneketolase through In *C. Haematococcus pluvialis* was discovered. *C. reinhardtii* is working on developing a novel ketocarotenoid. From *P. tricornutum* of *C. vulgaris* of nitrate reductase gene promoter and transformed terminator and the recombinant strain was shown to be capable of producing high-value proteins (Aratboni et al. 2019). For biopolymer production, microalgae can be used as expression systems, for example, poly-3-hydroxybutyrate which is a key precursor for biodegradable plastic production. Over the original strain, the strain that was designed with a facility for revocable opening to high flux of carbon was able to produce much higher levels of 3-hydroxybutyrate (Jagadevan et al. 2018). As a protective matrix for hydrated biofilms, most microalgae generate extracellular polymeric compounds in their immediate habitats. Anti-coagulant, anti-viral, drag reducers, antioxidants, and biolubricant are examples of high-value applications for these compounds. Consumed biomass of microalgal and lipid-extracted algae (LEA) is a true replacement of the manufacturing of different products as it comprises 30–60% carbon residual which is present as fermentable sugar (Fanesi et al. 2019).

Lipid-extracted alga has been usually utilized through an anaerobic mechanism as methanation's substrate. Another research which was conducted on product-extracted algal samples like protein and lipid extracted enhancement was noted in biogas production. Pre-treated algal has higher production while using cumulative methane. The yield of methane increases while using anaerobic digestion of lipid-excused biomass than the exhausted biomass of non-lipid (Rabii et al. 2019). It is also used in the fermentation of butanol. It is also used in the fermentation of butanol. Lipid extracted algal may also be efficiently transformed into liquid fuel, most of the alkanes through hydrothermal upgrading and liquefaction processes like by hydrocracking and hydrotreating. 69.5% of energy efficiency is noted overall at a higher heating value (Ramirez et al. 2015). In another research on *Scenedesmus acutus*, it was observed that it can assimilate nitrogen from lipid extracted algal residues and was capable of conversing nitrate within media of culturing media, therefore adding recovering of nutrients. The phosphorous/nitrogen limit in microalgae hints at a discrepancy in the productivity of liquid, and the acetyl-CoA carboxylase gene expression was used to verify this (Mahmud et al. 2021).

1.15 Microalgae Biofuel Engineering

Because of the infected technique of harvesting and low yield because of less effective design of photobioreactor and inadequate efficiency of photosynthetic, it is compulsory to maintain balance between liquid production and biomass content (Xu et al. 2020). The genetic engineering tool's aim is to increase the structure of enzymes involved in storage and reduction of lipid catabolism mechanisms as well as the synthesis of lipid, which has huge potential for engineering vital metabolisms (Mahmud et al. 2021). The interactions between the many enzymes that algae uses to produce lipids. In recent years, ACCase acetyl-CoA carboxylase overexpression, gene knockout of acyl-CoA synthase, oxidation of fatty acid (acyl Co oxidase), camitineacyl transferase, fatty acyl-CoA dehydrogenase, and acyl-CoA synthase, and acyl-CoA synthase have become more popular. Genetic manipulation is difficult since editing techniques are species specific and cannot be used interchangeably due to strategies including defensive-like restriction and methylation enzymes, codon usage, nucleic acid uptake, and cell porosity.

There are just a few completely annotated microalgal genomes accessible, if used with advancements in technology of sequencing. In the future, there is predicted to be a substantial increase in this type of data (Lv et al. 2013).

1.16 In Microalgae: "The Synthetic Biology"

The core of artificial biology is capable to build synthetic monitoring circuits that can resist cellular performance depending upon use defined (input/signal/stimuli) which produce the most wanted output like as biofuel chemicals and proteins in the creation of necessary changes in metabolism (Mukherji and Van Oudenaarden 2009). The most focus is being devoted to the use of large-scale microalgae through sustainable or strong energy from microalgal biotechnology, where feedback is given at (a) During metabolic engineering, improve their photosynthetic efficiency to make more oil and improve mass cultures by increasing the rate at which carbon is taken up. (b) Using carbon flux as a fuel source, energy-rich molecules are produced. (c) Strong and committed algal cells are spreading out which can hold out low cost with large-scale farming causing in minor carbon footprint of the produced chemicals and lower operational cost (Jagadevan et al. 2018). The function of mock biology via the building artificial biological schemes by a blending of engineering policy through biotechnological utensils, grounded on genetic, metabolic, or regulatory statistics collected by trials. It is a field of biology that concentrated on re-engineering or rebuilding the metabolic alleyways by means of starter of genetic units then detected such as “biological circuits.” Then crucial purpose is redesign microalgae that obtain the original task. The rebuilding of applicable biochemical lanes (metabolite products are vital) addicted to incomplete and complete copied replacements necessity of a steady assemblage beside toward assimilation of heterologous DNA segments into masses and chassis-strains (Konig et al. 2013). The diverse says gene that can be contrived and manipulated for renewal are (a) digestion, ligation of DNA wreckages, (b) in vivo homologous recombination, and (c) in vitro homologous recombination of fragments (Finnigan and Thorner 2015).

1.17 “Bio-bricks” or Circuits as the Synthetic Elements

Definite challenges require to be essential to commercialize transgenic microalgae. For genetic engineering, there are poor molecular apparatus, and there is a minimum level of heterologous gene faces from the nucleus. By the emergence of this narrative genomic instrument, synthetic biology is increasing rapidly, and the concept of "biobricks" in microalgal systems may be established (Moses et al. 2017). Uniform DNA segments of DNA having a common interface are known as bio-bricks that can be collected into biological methods. Such types of portions are exchangeable units like the organizers, ribosomes binding sites (RBS), terminators, trans-elements, or several directing molecules which are utilized to stimulate the genetics of microalgae and eventually their metabolism (Røkke et al. 2014). The genetic tool omics method play a vital role in configuring corridor and re-construction at microalgae by supporting to recognize the metabolism inside the entire structure, i.e., stimulated via response onward and backward coils which disturb the output. Get the high quantity of omics data liquid-inducing conditions (stress stimulus) are channelized therefore prominent the effective instruction of metabolisms through the application of mock biology not only mere overexpression of enzyme coding genes but also pointing the regulatory networks (Warner and Suggett 2016).

1.18 Single-Cell Protein

Single-cell proteins (SCP) are termed as bio proteins, biomass, and microbial proteins, which is a substance of the dried cell. SCP is formed via microorganisms, for example, yeast, bacteria, fungi, and algae. Therefore fungi and bacteria are the main manufacturers of the protein. Increasing efficiency of proteins by that foundation was primarily owing to their speedy progress level, and comparatively their chemical environment has complex protein level. More classes of algae are recycled as they are cultivated in the water environment (Warner and Suggett 2016). Furthermore, with the high content of protein, single-cell protein also has high contents of vitamins, nucleic acids, fats, minerals, and carbohydrates. Moreover, single-cell protein has high levels of amino acids, which include lysine, threonine, and methionine. These proteins are the best resource of algae than other exclusive options, e.g., soybean meals and fish. The conclusion has appeared that single-cell protein (SCP) could be merely a substitute for other traditional and exclusive (plants and animals) protein bases inside humans (Warner and Suggett 2016). Protein foods found on microbial algal biomass have the energy to fulfill this necessity, and these microbes are thought out as protein-rich supplements and ingredients in animals and human diets. This is also a good substitute because it doesn't require a vast area for growth and proliferation (Wells et al. 2017). Contrasting plant productions are also free of atmosphere and seasonal deviation, and it must be created all through the year. Furthermore, it couldn't produce greenhouse gases in surroundings as per plants' protein sources (Wells et al. 2017) (Table 1.2). The most crucial thing to decrease the production of single-cell protein is to choose the economical and applicable substrates or biodegradable agro-industrial by-products for the microorganisms as a nutrient basis to yield and grow tons of proteins (Spalviņš 2020). Due to this, in the past diverse substrates were used. Few common substrates are yam peel, apple pomace papaya waste, potato peel, pineapple waste, etc. It's important to select the surplus products for propagation of single-cell protein-forming microorganisms (Spalviņš 2020). For industrial purposes and study, the accessibility of microorganisms is not a big problem, and numerous strains of bacteria, fungi, algae, and in the lab yeast can be cultured in different ways. The center is now algal sources for

Table 1.2 List of substrates for various algal species (substrate for algae to produce SCP)

Algae	Substrate
Spirulina species	Carbon dioxide
Chlorella salina	Alkaline waste effluent
Caulerpa racemosa	Carbon dioxide
Spirulina maxima	Sunlight and carbon dioxide
Chlorella species	Carbon dioxide
Sargassum	Carbon dioxide and sunlight
Dunaliella	Carbon dioxide and sunlight
Laminaria	Carbon dioxide and sunlight
Diatoms and *Chlorella*	Carbon dioxide and sunlight

single-cell protein (SCP). Most kinds of microalgae are nurtured for human and animal utilization or generally have strong protein substances raised up to 70%. The protein from algae is primarily source of fats like chlorophyll, mineral salts vitamins, fatty acids, and omega-3. They hold less concentration of nucleic acid 3–8%. African and Mexican natives near texcoco harvested an algal species *Spirulina* (Yaakob et al. 2014). When it got dried, then it was used in human diets. As a feed source, *Scenedesmus* and *Chlorella* biomass have been used. Improved protein content, simple cultivation, fast growth, and appropriate utilization of solar energy have vital subsidy in which they are broadly recognized as feed ingredients all through the world. Green algae are noble antioxidant (Mobin et al. 2019). The interest looks more motivated due to high crude protein content, by the potential production of docosahexaenoic acid (DHA) and omega-3 fatty acid (i.e., eicosapentaenoic acid (EPA)), and carotenoids termed as astaxanthin an antioxidant, and pink pigment for salmon and shrimp and immune stimulant, e.g., by the strain of *Aurantiochytrium* (Matos 2017).

1.19 Strategies to Improve the Yield of Microalgal Biomass and Lipids for Biofuel Feedstock

The yielding of biofuels, related to further sources of microalgae, has massive feedstock, because of little effect on food security, low eco-friendly, and algal genuine yield. Hence commercializing the algal biofuels needed more profits (Rawat et al. 2013). Enhancing the economics of consuming microalgae as biofuels is life-threatening to the achievement of tactics of biomass and lipid production. Spending of genetic engineering and mutagenesis are the necessary methodologies for the collection of appropriate algal strains (Mondal et al. 2017). Approaches are added to improve lipid production and biomass of microalgae like co-cultivation and genetic of microalgae with yeast and bacteria and also phytochromes and metabolic engineering. The best technique to stable the lipid production and algal biomass is to improve culture system and hybrids (combination of photobioreactors and open ponds). The usage of discarded water of agri-industrial to sowing microalgae is cooler method to decrease in production cost. These types of methods help in bio elimination of waster CO_2 and bioremediation of undesirable water. To meet the source lodging, current and future energy anxieties have triggered researchers to discover other energy sources for proliferation in fossil fuels. It is expected that the present supply of fossil fuels would be sufficient until 2030. In 107 years, 35 years, and 37 years, respectively, fossil fuels such as oil, coal, and gas will be depleted. The People's Republic of China, India, Malaysia, Indonesia, the Philippines, or Thailand is major biofuel producers. Conventional feedstock such as cooking oil, oil crops, and animal fats are utilized but not enough to meet demands to produce biofuels. Algal biofuels are an attractive aspect just toward healthier ecological act, mainly concerning lower greenhouse gas (GHG) emissions but not in food completion. The revenue of oil from microalgae is 300 times greater than soya bean and 23 times greater than oil of palm per hectare of cultivation. Fresh water for human consumption wouldn't be attained by fully fledged microalgae in waste and saline water. The waste remunerations are the main approaches to take deep attention in the manufacturing of algal biofuels by integration of expertise that includes in by-product industries leveraging environmental protection. Driven of renewable fuels from microalgae comprising biohydrogen generated via photo biological process, biodiesel resulting after microalgal oil, or methane derived from anaerobic ingestion of the algal-biomass. Energy assets in microalgae, i.e., triacylglycerols (TAG), are mined from algal cells and transformed to biodiesel via transesterification. After that great construction cost required to commercialize the algal biofuels is the key barrier for standby fossil fuels. Before consuming microalgae as a thriftily practicable feedstock for biofuels, some procedural matters must be stunned such as low biomass production, less lipid content, and relaxed development rate which are required. Fabricating lipids, to develop straight plans first to boost impressive nutrients stress as well as operating environmental influence like light, salinity, and temperature. Fresh policies are co-culturing of microalgae by new microorganisms with toting of phytohormones (plant regulators) too; chemical additives are capable tactics. For the perfection of strains in algae meant for biofuel production, metabolic and genetic engineering are potent gears.

1.19.1 Improvement of Microalgal Strains for Biofuel Production

There are 44,000–72,500 species of microalgae that have been found (Guiry 2012). Phycoprospecting and repeating of microalgae are desirable characteristics based on growth rate, and lipid production is thriving in robust strains of biofuel feedstock. Thirty-three tropical isolates of thraustochytrids were isolated from mangrove leaves by the Malaysian team for lipid production and fatty acid profiles. The peak of insulates of genus *Aurantiochytrium* and their yields of lipid oscillated as 0.27–70.86 mg/L/day with projecting fatty acid of 16:0 (Aratboni et al. 2019). Alienated strains of species *Desmodesmus* from Taiwan are curtain for lipid output and thermo-tolerance. The growing of required traits of algae in salinities (Chu 2017). To tolerate a wide variety of aptitude of 12 traits of *Tetraselmis* which is detached by natural saline in western Australia. Little strains of *Tetraselmis* were subjected to euryhaline, which resulted in increased biomass and a high level of lipid efficiency, therefore strength for biofuel efficiency (D'Adamo et al. 2014). For yielding biodiesel efficiency, whatever water is used, so it is economically and environmentally feasible to use sea water or inland saline ground water. Besides, there is less chance of adulteration in algal culture in cultured saline medium with the next organisms (Hirooka et al. 2020). The possible platform is the mutagenesis for the strain of microalgae to improve lipid and biomass efficiency. For lipid yielding, there is the usage of UV-mutagenized strain of *Scenedesmus* species, after oxidation exposes the H_2O_2. Catalases and a major enzyme in fatty acid biosynthesis' activity that is acetyl-CoA carboxylase boost expressively in the modified mutant (Sivaramakrishnan and Incharoensakdi 2017). Enlarging the biomass and lipid productivity by enlarging the mutant process of *Chlorella pyrenoidosa* is positively useful in the atmospheric temperature plasma mutagenesis process, and it is on working for yielding biomass and lipid production (Arora et al. 2020). The 33 °C temperature and pH 9.0 has kept then mutant (II-H6) can show great inborn stability and grow optimally. Through adaptive laboratory evolution development are usage for favorable microalgal strains by a screening of exact cultural conditions (Chu 2017). *Crypthecodinium cohnii* is a glucose-tolerant strain paralleled with wild strain that exhibits lipid growth by accomplished laboratory progress and also increases outputs in *Chlamydomonas reinhardtii* which is mutants of low starch (Sun et al. 2018). There is the intense spreading of extremophilic microalgae for lipid yield. In impartial lipids, the collection of polar algae fatty acyl chains, specifically C16 polyunsaturated series of fatty acids, C18:1ω9 with C118:3ω3. The six microalgae were mostly selected from the topsoil of semi-arid areas in northwest China for lipid and biomass output. *Chlorella* species 11 and *Monoraphidium dybowskii Y2* are two microalgae mined from the soil crust of biological desert, which cause high efficiency of lipid and biomass when grown in outdoors at 140-1 PBR (Aratboni et al. 2019) (Chu 2017).

1.19.2 Manipulation of Nutrients with Environmental Factors

The foremost purpose of microalgae for feedstock is to harmonize biomass and lipid efficiency.

To increase the lipid and biomass productivity is utilized to manipulated nutrient levels (e.g., phosphorous and nitrogen), and ecological features like light, salinity, and temperature are conventional edges (Yaakob et al. 2021).

1.19.3 Nutrient Stress

The foremost purpose of microalgae for feedstock is to harmonize biomass and lipid efficiency. To increase the lipid and biomass productivity is utilized to manipulated nutrient levels (e.g., phosphorous and nitrogen), and ecological features like light, salinity, and temperature are conventional edges (Remmers et al. 2018). Utilization of nitrogen acclimation inhibitor compounds to provide an alternate method to recreate the similar circumstances as *C. reinhardtii* have the state of nitrogen deficiency. Reproduction of neutral lipid composition *C. reinhardtii* caused by exposure to inhibitors (methionine sulfoximine) subsequent behavior connected to nitrogen deficiency that was only active for three days (Bono Jr et al. 2013). After the starch synthesis, most of the nitrogen limits were suppressed by the bounding of TAG (triacylglycerol) that appeared in *T. obliquus*. As shown in *Nannochloropsis oceanica*, nitrogen starvation is connected with degradation of lipid membrane that gathered phospholipids and TAG (Goncalves et al. 2016). A proteomic-based method is used to illuminate metabolic changes in nitrogen scarcity which is found in tropical *Chlorella* species. Another tactic is found to increase lipid and biomass productivity by combining high glucose with low nitrogen level in microalgae. Cultured under heterotrophic conditions increase the lipid content of *Chlorella sorokiniana* by low $NaNO_3$ and high glucose levels. To simulate lipid buildup in microalgae is the tactic to limit the culture of nutrients like sulfur (S) and phosphorous (P). For example, under phosphorous constraint (0.1 mg^{-1}), *Scenedesmus* sp. LXI could accumulate lipids up to 53% of its biomass; the lipid productivity/unit volume of culture was not increased (Yun et al. 2020). Now *C. Reinhardtii*, sulfur deficiency continued to be the primary cause of TAG accumulation by directing metabolic carbon flow away from protein synthesis and toward TAG synthesis (Shahid et al. 2020). Malnourishment of all elements can be accomplished instantly, to pretend lipid binding is the method for microalgae in nutrient-deficiency medium. By increasing, lipid content in microalgae is achieved by stress to the nutrient (Sun et al. 2018). The key control is the low biomass production as it distresses the overall lipid efficiency. Some approaches are used for lipid outputs, first stage, to yield the maximum amount of biomass by the flourished to microalgae under optimal conditions (Sajjadi et al. 2018). Some approaches are used for lipid outputs, first stage, to yield the maximum amount of biomass by the flourished to microalgae under optimal conditions. In the second stage, cultures will be exposed to stress conditions. Anxiety circumstances will be endangered when simulating growth and lipid fusion. These two stages were active to control lipid outputs in *Chlorella vulgaris*. In the initial phase, when nitrogen stress was provided, it changed the circumstances of grown microalgae which grow under nutrient-rich medium (Cui et al. 2017). Under such manipulation, at least twofold increased the lipid synthesis. Lipid throughput of *Ankistrodesmus falcatus* better by 36.5–45.5% under two-phase cultivation conditions are provided (Álvarez-Díaz et al. 2014). Beforehand being transported to nitrate and phosphate limited medium, first algae were cultivated in glucose-supplemented condition, which enhanced tenfold lipid yield of *T. obliquus* found by the studies. For high algal biomass production, it must confirm that the CO_2 level is not low enough in ambient air. 1–5% of CO_2 is frequently supported for maximal microalgal growth, and 5–15% of CO_2 is mostly aerated for laboratory cultures (Panahi et al. 2019). Most inventions are that high in CO_2 cause high in lipid efficiency. Besides, high CO_2 levels (30–50%) could stimulate the growth of PUFA and lipids in *T. obliquus* SJTU-3 and *C. pyrenoidosa* SJTU-2. Research finds that the quantity of highest CO_2 bio-fixation rate and biomass concentration was achieved once 100% CO_2 was found for microalgae developments (Show et al. 2017).

1.19.4 Light Intensity and Wavelength

For biofuel fabrication, it is necessary to enhance the production of lipids and biomass in microalgae. The highly attractive approach is painted, light-emitting diodes (LED) and the use of dyes among the modern approaches. Showing the microalgal culture in blue (450–475 nm) and red (630–675 nm) light increases the absorption of chlorophyll colorants and improves the functionality of photosystems I and II (Ra et al. 2016). By increasing knowledge, it comes to know that lipid addition in *T. obliquus* increases, when LED light exposure at various frequencies and also *Cyclotella cryptica* development at 450 nm (yellow wavelength) causes lipid content spread up to fourfold (Choi et al. 2015). Microalgal cells are protected from photoinhibition and photooxidation through filters made from nanoscale covering, by IR and UV rays that cause oxidative pressure (Michael 2015). The light strainer expressively boosted algal biomass up to 13–34% and biomass efficiency up to 70–100%. The filters increase the microalgal growth, but the high rate of manufacturing of filters arises an issue (Michael 2015). The additional strategy is the organic dyes which enhance the outputs in microalgae bounding, and by adding the organic dyes, it increases growth two- to threefold in lipid production of *Chlorella vulgaris* (Ramanna et al. 2017). To modifying the solar spectrum and fascinating the surplus, solar radiation is useful by using fluorescent paint solutions. Red paint accomplished the highest biomass yield (1.7 g/L) although blue paint solution is consuming to yield high lipid content (30% dry weight) (Seo et al. 2015).

1.19.5 Temperature Fluctuations

Temperature disturbs not only lipid yield but also fatty acid profiles in microalgae. Fatty acid unsaturation distresses by variation in temperature. Balancing membrane fluidity in cell development required affinity to increase fatty acid un-\saturation at a lower temperature (Morales et al. 2021). Instantly at low-temperature unsaturated fatty acid content becomes doubled in *Chlorella ellipsoidea.* Furthermore, it results in greater unsaturation in its phosphoglycerides due to low-temperature strain of microalgal confined complex amounts of α-linoleic acid (Aratboni et al. 2019). When it is imperiled to boost culture temperature, it enhances lipid yield and growth amount understudy of *Nannochloropsis* saline. Temperature variation can change the lipid profile in algae development; features of algal biodiesel are predictable to be modified under diverse seasons and climates (Bounnit et al. 2020).

1.19.6 Addition of Phytohormones

Lipid efficiency in *Monoraphidium* species rises by adding melatonin (phytohormone) coupled with photoinduction. Reactive oxygen species (ROS) levels and lipid biosynthesis-related enzyme activities are linked and affect the rise of lipid bounding (Cui et al. 2021). According to research phytohormones belongings on metabolism in algae, mainly about lipid biosynthesis. The attractive method is to surge biomass, and lipid yield is influenced by a number of phytohormones which are very little and less in cost (Chu 2017). Adding fluvic acid encouraged lipid bounding in *Monoraphidium* species through gene expression, intracellular ROS yield, activities of acetyl-CoA carboxylase, malic enzyme, and phosphoenolpyruvate carboxylase (Che et al. 2017).

1.19.7 Metabolic and Genetic Engineering

Using a molecular strategy to increase biomass and lipid accumulation, makes biofuel. Expressed sequence tag (EST) databases established for few microalgae, nuclear, mitochondrial, and chloroplast genomes of numerous species have been sequenced (Chu 2017). The genome sequence of microalgae by biofuel feedstock is possible, like *Chlorella vulgaris*, *Nannochloropsis* sp., and *Phaeodactylum tricornutum* are not present. The growth of synthetic biology tools, named "bio-bricks" such as transcriptional terminators, ribosomes binding sites, and organizers, has progressed the development in genetic engineering of microalgae. *Chlamydomonas reinhardtii* is the model organism of choice for molecular engineering aimed at biofuel throughputs as its genome has been sequenced and then availability of tool set for microalgae (Young et al. 2020). Particle bombardment, glass bead agitation, micro injection, electroporation, and Agrobacterium tumefaciens-mediated transformation are a few techniques that can be used to change algae. Successful conversion was demonstrated on *C. reinhardtii* (Doron et al. 2016). In the occurrence of DNA, glass beads, and polyethyleneglycol, the cell wall-free microalga was agitated. Interaction between the nuclear and chloroplast genomes of *P. tricornutum* and *C. sorokiniana*, extremely active cell after DNA-coated metal particle bombardment (Che et al. 2017). To adjust saturation and length of fatty acids done are arose for lipid metabolism by adding lipid biosynthesis and catabolism which is targeted by altering the microalgal for biofuel outputs (Radakovits et al. 2010). Sequenced microalgal genomes contain homologous genes that regulate lipid metabolism. Transgenic techniques were used on higher plants and algae. Possible approach for increasing lipid production in microalgae by overexpressing important enzymes involved in the processing of fatty acids. With further research, instead of lipid pathways, the carbohydrate has focused by overexpressing the glucose-6-phosphate dehydrogenase (G6PD) from the pentose phosphate pathway; the output of NADPH was increased that run to make greater the production of lipids up to 55.7% dry weight in *P. tricornutum* (Blatti et al. 2013). Chloroplast contain G6PD and also presence of increasing in amount and size of lipid bodies, representing the buildup of neutral lipids. Additional skills are the destruction of lipid catabolism for lipid bounding of microalgae (Chu 2017). In an order to targeted knock-down of a multifunctional phospholipase/lipase/acyltransferase enzymes superior lipid production deprived of disturbing the growth of *T. pseudonana* (Aratboni et al. 2019). Hence, the reserved lipid catabolism might disturb biomass efficiency and proliferation. In particular varieties of microalgae, for cell division, the catabolic passageways are vital in providing the energy substrates. Through inducible agents, lipid synthesis genes are controlled in their expression. During the static phase, microalgal cultures must acquire high density, while promoters must be inspired to improve the expression of lipid synthesis genes. Microalgae recognized some pretend promoters, comprising one with copper-responsive elements in *C. reinhardtii* and other promoter responsive to nitrate in diatoms (De Bhowmick et al. 2015). To increase lipid content yield in microalgae is to delaying metabolic pathways which binds the energy-rich storage compound like starch (Ge et al. 2017). For instance, the starch-less mutant of *C. pyrenoidosa* was originated to integrate higher levels of lipids through nitrogen deficiency while showing high progress rate and biomass yield than wild type. The starch-deficient strains in *C. reinhardtii* appeared when disturbing the genes which code for ADP-glucose pyrophosphorylase or isoamylase (Radakovits et al. 2010). Microalgal fatty acids require a chain length between 14 and 20, mainly 16:0, 16:1, and 18:1. Chain lengths of numerous fatty acids are acyl-ACP thioesterases which regulate enzymes that hold some forms of variation of organisms, precise for certain fatty acid lengths (Glencross 2009).

1.19.8 Photosynthetic Efficiency

Designed to enhance algal biomass by capturing the most light, its necessary process increases biofuel production. To advance photosynthetic proficiency is to greater the fascinated level of photosynthetically active pigments. *Acaryochloris marina* has been revealed to absorb light in the nearby infrared region for chlorophyll, demonstrating the strength of transmission of microalgae by the ability for biofuel production (Carvalho et al. 2011). Due to non-photochemical reduction by diminishing the size of antenna in photosystem to lessen the chance of damages energy in microalgae. Microalgae developed inside the lab to have light antenna complexes (LHC) that catch the lights in dim lighting conditions (Borowitzka 2016). Somehow, microalgae once grown in height irradiance maximum of photons immersed will be lost as fluorescence and heat to protect against photodamage. Total of 80–90% of energy loss from the system by the inefficient fluorescence and heat loss (Mussgnug et al. 2007).

1.20 Conclusion

The first stage is to increase enough biomass by photosynthesis for good growth of biomass of algae needing CO_2 sufficiency, nutrients, optimal temperature, as well as the high-performance strains that affect the enzyme kinetics. Research of these factors yet depends on cultures of laboratory-scale (Zhou et al. 2014). Various engineering aspects of the culture system must be tuned in addition to photosynthetic and biological optimal output. *C. reinhardtii* was employed at bench level to systematically investigate the conditions which improve the production of lipid and its growth using experimental approaches with modified integrated computational. By this approach, different parameters are set for the availability of carbon and nitrogen substrate. Following this model, it was discovered that using 0.0742 g/L nitrogen, 0.005–2.1906 g/L acetate, beginning biomass inoculum, a 32.85% increase in algal oil productivity could be achieved, using artificial intelligence (AI), namely, evolutionary and statistical learning, as well as neural learning-based methodologies in an innovative method to improving and managing productivity and costs in algal biofuel production (Boyle et al. 2017). Bayesian clustering and Nave models, closest neighbor, and hidden Markov models are some of the statistical approaches that can be used in AI. One study found that yeast cells may be induced to release TAG into the medium via random mutagenesis (González-Díaz et al. 2008). The use of ABC transporters, which are involved in the mediating of wax release in plants, is another technique to promote lipid secretion from microalgae. Before metabolic engineering may be used to increase the synthesis of lipids in microalgae, the principles of metabolism of lipid in microalgae must be addressed. Analysis of lipidomic using advanced technologies like UPLC/Q-TRAP will help researchers learn more about the assimilation of TAG and lipid metabolism in microalgae (Radakovits et al. 2010). This method was used to do an extensive profile of polar glycerolipids in *Nannochloropsis oceanica*, involving 112 species, to investigate the changes of such lipids in response to N shortage. Through oleic acyl desaturation, phosphatidylcholine (PC) was used as a linoleic and linolenic acyl donor. Through oleic acyl desaturation, phosphatidylcholine (PC) served as a linoleic and linolenic acyl donor and PC converted acyl 16:0 and 18:1 to TAG under N stress conditions (Servaes et al. 2015). The valorization of microalgal products is dependent on a biorefinery idea, which should be the way ahead to enhance algal biofuel economics production. The biorefinery system's main goal is to produce a wide range of products with little or no waste creation (Laurens et al. 2017a). An intriguing technique would be to boost the yield of TAG, a high-value lipophilic compound like carotenoids and LC-PUFA, in prospective microalgae in real time. Such yields share not only storage sinks and biosynthetic precursors but also the stimulation of the molecules' maker, which is frequently dependent on common environmental influences (Schüler et al. 2017). Methods with a high throughput, such as triggered fluorescence sorting of cells, will be useful in separating producers triple capable of simultaneously collecting large amounts of TAG, LCPUFA, and carotenoids (Han et al. 2020). Furthermore, transcriptomics comparisons between wild type and triple producers could also be used to recognize products of gene involved in the biomolecules stimulation. To boost production of lipid and algal biomass, as well as to improve the economics of biofuels whose productivity is dependent on microalgae, multi-pronged techniques including many methodologies should be used. As a result, optimal scale pilot plant testing is needed to confirm the outcomes of laboratory-scale experiments.

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