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REVIEW ARTICLE

Third-generation biorefineries: a sustainable platform for food, clean energy, and nutraceuticals production

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Abstract

Sustainable transformation of biomass into a wide range of valuable chemicals, fuels, and materials is the eventual goal of a biorefinery. Algal feedstock (microalgae and macroalgae) is a principal component of third-generation (3G) biorefinery empowering the bio-renewables industry. While first-generation (1G) biorefineries are commercially viable, products (fuels and commodity chemicals) from second-generation (2G) and 3G biorefinery are not yet commercially competitive due to the gross technical challenges, scalable and production cost issues. Because of the inherently diversified nature of feedstock used in 3G biorefineries, a myriad of specific bioproducts can be produced. Furthermore, stable food/feed supply, environmental concerns, climate change, and geopolitical issues have necessitated the exploration of 3G feedstocks into fuels and renewable chemicals. Considerable success has been seen in research laboratories in the last two or three decades which led to mature technical developments in algal biomass conversion. However, the scale-up issues are still posing a big challenge for the commercial exploitation of algal feedstock into fuels and chemicals. Nevertheless, various products such as nutraceuticals, pharmaceuticals, and cosmetics are successfully being produced from algal feedstock. This review paper describes the technical developments, industrial scenario, environmental issues, and range of diversified products from 3G biorefineries. Specially, we focus on the exploration of algal biomass into fuels and biochemicals via multidisciplinary technological routes.

Keywords Algae · 3G biorefinery · Biofuels · Nutraceuticals · Biochemicals · Bio-economy

1 Introduction

A continuously growing world population needs more food, energy, and materials. Today, as per UN latest data, presently, the world population is 7.3 billion, which is expected to be 9.7 billion by 2050. The whooping demand for food, materials, and energy will be fulfilled through the efficient use of natural resources. It has been predicted that by 2050, the food demand is going to be increased from 59 to 98% [1].

Currently, crude petroleum is the primary source of energy and materials, which is not renewable and the principal cause of climate change and increased greenhouse gas emissions. Therefore, this is a timely requirement to find the sustainable alternative of crude petroleum [2, 3]. Among the promising alternatives of gasoline or crude petroleum, agricultural crops and their products, lignocellulosic biomass (agro-residues, forestry wastes, energy crops, and grasses, etc.), and algal biomass have shown remarkable progress and could be promising eco-friendly base products. Biorefinery facility collates biomass conversion steps, procedural configurations, equipment/machinery, and facilities to produce fuels, power, and chemicals from a variety of biomass [3]. Undoubtedly, the concept of biorefinery can be considered analogous to conventional petroleum refineries. Biorefinery can be classified into two types: conventional biorefinery, where the production of wine, beer, vegetable oil, and paper exists for centuries. Later, innovations in food production (sugar, potato starch) in the mid-nineteenth century allowed the production of wheat

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and corn, starch-based products in the twentieth century [4]. Modern biorefineries or advanced biorefineries aim to explore the utilization of lignocellulosic biomass, algal biomass into energy, fuels, and value-added chemicals, and nutraceuticals. Advanced biorefineries are under development stage and have the potential to cater to the growing demand for food/feed, energy, and house-hold commodities in the future.

Conventionally, algae are explored for the production of amino acids, vitamins, feed, and other industrial products at a commercial scale. However, because of their inherent composition having carbohydrates and oils, they are now being considered a potential source of biofuels and renewable platform chemicals [5–7]. Algae convert carbon dioxide into renewable fuels and biochemicals; thus, they are potential candidates in third-generation biorefineries [8]. The integration of first (1G)-, second (2G)-, and third (3G)-generation biorefineries using the crop grains or juice (1G biorefinery), lignocellulosic residues (2G biorefinery), and algae biomass (3G biorefinery) is crucial to obtain high-value products such as amino acids, vitamins, biopigments, and antioxidants in conjunction with commodity chemicals and bioelectricity [7, 9]. The implication of 3G biorefinery producing bio-renewables will pave the way for the holistic development of a circular economy. In the present review, we have attempted to provide the critical developments in 3G biorefinery using microalgae as a feedstock towards harvesting and pretreatment techniques of algal biomass for the production of fuels, chemicals, and nutraceuticals.

1.1 Principle feedstock for 3G biorefinery

Algae serve as a base feedstock for 3G biorefineries as potential starting material for bio-renewables production [6, 10]. Generally, microalgae are aquatic unicellular biomass comprised of basic organic and inorganic elements [8]. Algae are primarily made up of different amounts of protein, carbohydrates, and lipids. Table 1 shows a comparison of the composition of various algae with main food/feed sources. The growth of microalgae is dependent upon the type of cultivation system employed, availability of light, levels of CO_2 and oxygen, temperature, and availability of nutrients like nitrogen and phosphorus [13]. Solar energy is utilized by these microorganisms to associate carbon dioxide and water with creating biomass. This process is much more efficient and rapid than the plants on land [7, 14].

Algal crops are known producers of nutraceuticals, animal feed and feed supplements, and many other products commercially [6]. Moreover, the increased global requirement for energy has resulted in the exploration of potential sources for the production of clean energy and platform chemicals [6]. As microalgae possess high photosynthetic conversion efficiency, they are now the most widely explored alternatives for producing fuels and chemicals [8]. Microalgae are the important reservoirs of high-value nutrients like bio-colorants, structural carbohydrates, and amino acids [15] (Fig. 1). Human consumption of microalgae as nutritional supplements leads to the evolution of microalgal production on an industrial scale [16]. Biopigmented molecules produced by microalgae have profound applications in the nutraceutical and food sector. Microalgae (*Dunaliella* sp., *Chlorella* sp., *Scenedesmus* sp., etc.) and cyanobacteria (*Spirulina* sp. and *Nostoc* sp.) are considered important sources of fine chemicals and health supplements. For instance, *Haematococcus pluvialis* is a potent natural reservoir of keto-carotenoid-astaxanthin. Astaxanthin possesses excellent antioxidant properties making it a preferred supplement of the human diet [17]. Microalgal biomass finds its applications in cosmetics, nutritious food/feed, and pharmaceuticals as value-added products [18].

Table 1 Comparison of carbohydrate, lipid, and protein content (% dry matter) in different algal species with food sources (based on the Trivedi et al. [11]; Becker et al. [12]

Source	Protein	Carbohydrates	Lipids
Rice	8	77	2
Meat	43	1	34
Milk	26	38	28
Soybean	37	30	20
Baker's yeast	39	38	1
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Scenedesmus dimorphus	8–18	21–52	16–40
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Chlamydomonas reinhardtii	48	17	21

Rhodophyta such as *Gigartina* sp., *Chondrus* sp., *Hypnea* sp., *Furcellaran* sp., and *Eucheuma* sp. are the important feedstocks for a natural hydrophilic polysaccharide-carrageenan. Carrageenans find their utility in various industries like food and pharmaceutical [19]. The source of kappa and iota carrageenan is from *Kappaphycus alvarezii* and *Eucheuma denticulatum*, respectively (www.oilgae.com, accessed on March 14, 2020). Ingredients Solutions Inc., USA, makes various kinds of carrageenan products. These products have several applications in food products and beverages (http://www.isi.us.com/carrageenan/2016). Several brown algae such as *Macrocystis pyrifera*, *Laminaria hyperborean*, *Lessonia flavicans*, large weeds (*Lessonia nigrescens*), and kelp species (*Durvillaea antarctica*, *Durvillaea potatorum*) serve as a source for various alginate production (www.oilgae.com, accessed on March 14, 2020). Alginates are structural phycocolloid widely used in the food sector and pharmaceutical

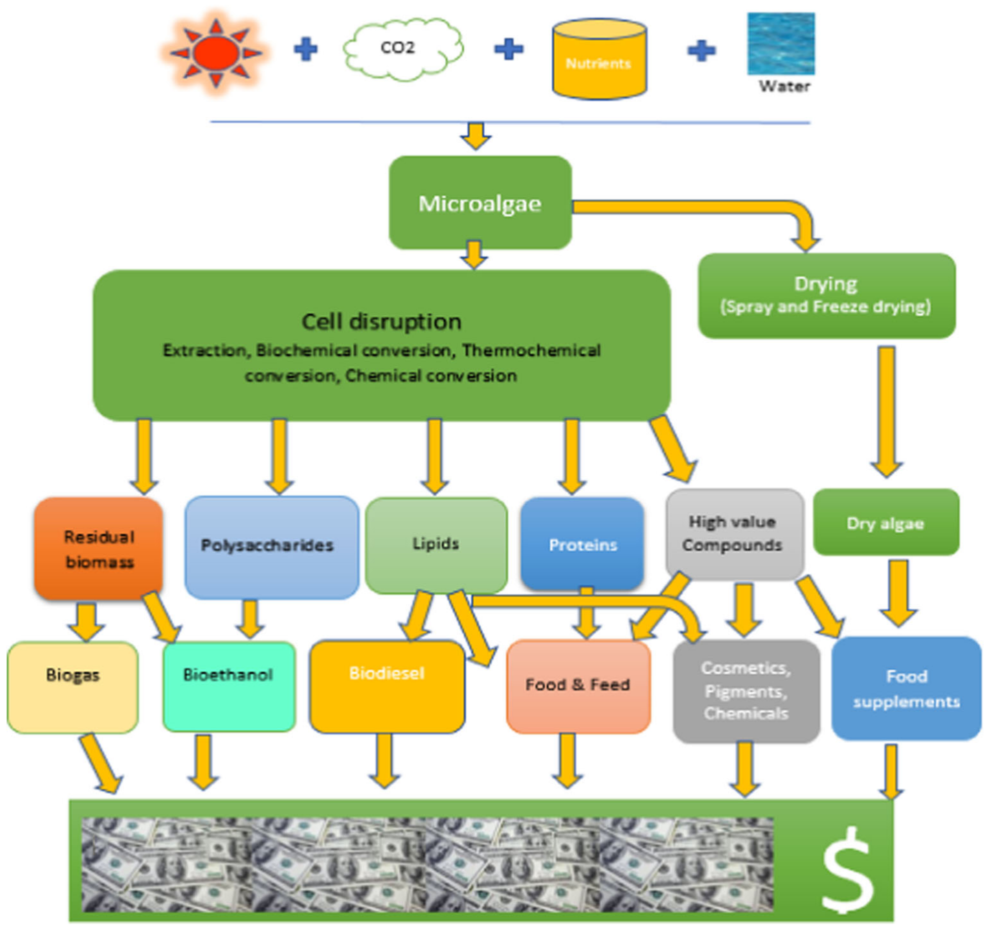

Fig. 1 Various biotechnological products from microalgal feedstock under the 3G biorefinery concept

(as alginic acid) industries. Approximately 30,000 tons/year of alginate are produced globally with a net value of around $339 million/year [20]. Table 2 shows the major global leaders for the production of algal products.

Because of health awareness, products like omega-3 fatty acids, β-carotene, and astaxanthin have high demand in society. Adding to the list, new products like lutein, zeaxanthin, biopolymers, and bioplastics from algal feedstock are paving the commercial success of 3G biorefinery. Integrated processing of microalgal-based liquid fuel production has unique advantages in a 3G biorefinery. Liquid transportation fuels (diesel, ethanol, and jet fuel) can be produced from carbohydrate- and oil-rich microalgal feedstock [21]. Biomass or algae are the sources of 3G biofuels, also referred to as drop-in biofuels, produced through different thermochemical and biochemical processes. Hughes et al. [14] reported that drop-in fuels from algae have high energy per gallon and the processes of extraction also offer a wide range of co-products supporting biorefineries to be more environmentally sustainable. The lipid content of algae determines its potential to generate biofuel. Algae have gathered substantial attention as a biodiesel producer because of high growth rates, the ability to grow in extreme conditions, possessing high lipid content (20–50%) and high growth rates [22].

Microalgae are the potent producer of biofuels due to their inherited properties of possessing a high amount of structural carbohydrates and lipids, eventually fixing carbon dioxide [23] (Fig. 1). Biodiesel production is feasible as the oil is accumulated inside the microalgal cell [24]. After the extraction of lipids, the residual biomass can be utilized for biofuel and biochemical production. *Chlamydomonas reinhardtii* and *Dunaliella salina* are some fast-growing species of algae that have been extensively explored along with various *Chlorella* sp. and *Botryococcus braunii* grows slowly but is known to accumulate enormous quantities of lipids [25, 26]. The lipid content of *Chlorella* sp. is very high (approximately 60 to 70%), making this organism a subject of intensive investigation [27]. Chen et al. [28] reported the highest productivity of 7.4 g/L/day from *C. protothecoides*. There are some geographical and technical issues associated with algal biomass. Lipid extraction becomes difficult due to the high-water content of algal biomass, so it becomes necessary to dewater it either by centrifugation or filtration. Processes of culturing and dewatering are energy-intensive. Ríos et al. [29]

Table 2 Leading commercial producers of algal products in the world

Product	Producers	References
Astaxanthin, Spirulina Nutrex-Hawaii	Cyanotech, Kailua-Kona, HI	https://www.cyanotech.com
Agro-chemicals	Seambiotic, Tel Aviv, Israel	http://www.seambiotic.com)
Nutraceuticals	Mera Pharmaceuticals, Kailua Kona, HI USA	(www.aquasearch.com)
Astaxanthin	Fuji Chemical, Toyama-Pref. Japan	www.fujichemical.co.jp
Carrageenans	Ingredients Solutions Inc. (USA),	www.isi.us.com
	Ceamsa (Spain)	www.ceamsa.com
	FMC Biopolymer (USA)	www.foodnavigator-usa.com
	KelcoApS (Denmark)	https://www.cpkelco.com
	Ina Food Industry Co. Ltd. (Japan)	www.kantenpp.co.jp
Carrageenan Pectin, Hyaluronic acid	FMC BioPolymer	www.fmcbiopolymer.com/pharma
EPA Omega-3 oil	AlgiSys LLC, Akron, OH, USA	www.algisys.com
Alginates	Kimica and Chemifa Food (Japan)	https://kimica-algin.com/products/
	Bright Moon (China)	www.bmsg.com
	Kimica (Chile)	https://kimica-algin.com/
Spirulina	Earthrise Nutritionals, LLC, CA, USA; https://www.earthrise.com/	https://meticulousblog.org/top-10-companies-in-algal-pigments-market/
Pigments (astaxanthin, phycocyanin)	BlueBioTech Int. GmbH (Germany)	https://www.bluebiotech.de/com/profil/organisation.htm
	Cyanotech Corporation (USA)	https://www.cyanotech.com/
Polysaccharides	Prasinotech (Scotland)	https://prasinotech.no/
	AlgoSource Technologies (France)	http://algosource.com/
Cosmetic for skin aging	Codif (France)	https://www.egactivecosmetics.com/codif_en.html
	Exsymol (Monaco)	https://www.exsymol.com/
	E.I.D Parry Ltd. (India)	http://www.eidparry.com/

suggested that the optimization of the general process is mandatory so that all stages in microalgal transformation are balanced. The transesterification process or hydrogenolysis helps in the turning of algal lipids to aviation fuel [30]. Microalgal biomass is a potential source of biogas and bioethanol production [31]. The production of biogas from microalgal biomass occurs by anaerobic digestion, whereas hydrolysis and subsequent microbial fermentation of sugars yield bioethanol and biochemicals [32]. The microalgal cell generates and stores biohydrogen during the photolysis of photosynthesis [33]. Table 3 presents some examples of co-generation of biohydrogen and biogas from a single microalga.

High production rates, high oil contents, and low land requirements are the beneficial points associated with various microalgal species, thereby making these species a potential feedstock for biodiesel production [41]. Despite the positive features, the actual short- and mid-term commercialization of microalgal biodiesel has been critically questioned [42]. There are wide varieties of value-added products (such as omega-3 fatty acids) that can be obtained from microalgal oil. Therefore, it would be more economically justified to employ microalgae for such purposes rather than as feedstock for biodiesel production [43].

1.2 Bio-processing of third-generation feedstock: technical aspects

Years have witnessed an acceleration in advancement for 3G biofuels as an outcome of technological advancement and knowledge gained through extensive research on cellulosic ethanol. The large-scale production of algal biomass is a cumbersome process due to the necessity of large volumes of inoculum for maintaining purity [5].

1.3 Harvesting technologies

Several techniques are considered for the harvesting of algal biomass, which include gravity sedimentation, chemical- and electro-flocculation, centrifugation, membrane filtration, foam fractionation, and ultrasonic separation [44]. Gravity sedimentation is a slow rate and less energy-intensive process. Microalgal biomass harvesting on a large scale requires more energy and incurs a high cost [45]. Strain type, cell density, and culture conditions are some of the important factors in choosing a better harvesting technique for the chosen microalgae [46]. Research is still on its move to explore an

Table 3 Co-generation of renewable fuels from some microalgae

Microalgae species	Type of renewable fuel	References
Chlamydomonas reinhardtii	Biohydrogen and biogas	Mussgnug et al. [31]
Chlorella sp.	Biohydrogen and biodiesel	Dasgupta et al. [34]
Co-culture of *Scenedesmus* sp. and anaerobic sludge in starch-rich wastewater	Biohydrogen and biodiesel	Ren et al. [35]
Chlorella sp.	Biohydrogen and biodiesel	Sengmee et al. [36]
Chlorella sp.	Biodiesel and biohydrogen	Bhuyar et al. [37]
Chlorella sp.	Biomethane	Wu et al. [38]
Dunaliella sp.	Biohydrogen	Chen et al. [39]
Dunaliella tertiolecta	Bioethanol	Varela-Bojórquez et al. [40]

efficient process of harvesting microalgae for primary and secondary metabolite production at the industrial level [15].

1.4 Algal biomass pretreatment techniques

The pretreatment is an essential step for the algal biomass conversion into different industrial commodities, e.g., lipids, pigments, carbohydrates, and proteins through various extraction protocols and chemical conversion routes [47]. Like the terrestrial biomass, algal biomass can also be pretreated by physical, chemical, physico-chemical, and biological processes [48]. The mechanical pretreatment methods primarily include collision (bead collision, shear force-based (homogenization), cavitation-based (ultrasonication, hydrodynamic cavitation), and electroporation-based (pulsed electric field) techniques. The mechanical-based pretreatment methods have an advantage of high disruption efficiency; however, they suffer from high energy, maintenance, and installation costs [49]. High temperature–based algal biomass pretreatment methods depend on the magnitude of heat and pressure (autoclaving), steam-coupled pressurization and depressurization (steam explosion), and repeated freezing-thawing cycles. The major drawbacks associated with thermal pretreatments include non-operative on large-scale applications except freeze-thaw cycles [50]. Chemical pretreatments of algal biomass include the utilization of weak acid/alkali aiming at the utilization of wet algal biomass. Nevertheless, chemical-mediated pretreatments have corrosion issues in addition to the severe process conditions with a denaturation issue of pigments and proteins [51]. Finally, the biological pretreatment methods act with the addition of algal biomass-degrading enzymes under mild reaction conditions with precise specificity. However, the cost of enzymes and longer processing times are the two major impediments of biological methods eventually affecting the viability at industrial scale [52].

The recent trends on algal biomass-pretreatment methods are primarily addressing the cumbersome problems related to the mechanical, thermal, chemical, and biological processes and introduced different emerging pretreatment techniques such as microfluidizer [47], pulsed arc technology [48], and coated membrane techniques [47]. The qualitative analysis of different algal biomass pretreatments is usually executed through different microscopic methods such as scanning electron microscopy and transmission electron microscopy. These methods have been started recently to structurally analyze the algal biomass surface changes and qualitatively through the fluorescence microscopy [53]. The quantitative analysis of different algal biomass pretreatments is determined through the data collection of cell biomass composition and sugar/lipid recovery [54].

2 Thermo and biochemical processing of algal biomass towards value-added products

Microalgal biomass is a source for various industrially valuable products that can be obtained through thermo and biochemical processes. The thermochemical conversion technologies employ gasification, pyrolysis, direct combustion, and thermochemical liquefaction processes for getting diverse bioenergy commodities [25]. The biological route utilizes the alcoholic fermentation, anaerobic digestion, and photobiological hydrogen production approaches to obtain the various biofuels and bioenergy [55].

2.1 Gasification and pyrolysis

In gasification, algal biomass gets converted into "syngas" (H_2+CO) at elevated temperatures under low-oxygen conditions. The produced syngas serves as a substrate to upgraded biofuels through FT (Fischer-Tropsch) process or newer advanced catalytic processes. Dauenhauer and co-workers [56] employed a single, small reactor to manifest catalyst-driven gasification for direct third-generation biofuel production, which replaces the usual conventional thermal gasification processes consisting of three individual reactions.

During pyrolysis, biomass undergoes thermal depolymerization at a moderate temperature under anoxygenic conditions. Pyrolysis converts biomass into three fractions viz. (1) liquid, also known as pyrolysis oil; (2) solid, also known as biochar; and (3) gaseous fractions. These fractions can be further utilized for generating fuels and chemicals. The composition of the pyrolysis product stream can be easily feasible by tuning the pyrolysis process conditions [57]. The different fractions of bio-oils produced through pyrolysis can be further upgraded to industrially important fine chemicals, bioenergy, and biofuels. Huber and co-workers developed an updated single-step pyrolysis approach utilizing catalysts for the conversion of biomass into high-octane-rating aromatics [58]. A microwave process can do economic production of third-generation biofuel with better conversion rates in short reaction times [59]. The extractions of algae get significantly improved in the microwave-assisted approach, resulting in high yields. This approach is not only more efficient but also reduces extractive-transesterification time [60]. Genetic modification of algae is a mode to overcome the problems associated with its cultivation, harvesting, and processing, thereby producing cost-competitive algal fuels [61].

2.2 Photobiological hydrogen production

H_2 is one of the most promising fuels with a replacement potential of fossil fuels in the long run [62]. H_2 can be produced biologically with the help of photosynthetic biomachinery of microalgae (aerobically) or by non-photosynthetically either (aerobically or anaerobically) [63]. Inorganic carbon, such as CO_2, is used aerobically and starch, the organic carbon source, is utilized anaerobically [36]. Metabolic reactions of microalgal cells also generate H_2. Water biophotolysis (direct or indirect) during photosynthesis generates H_2 by green microalgae. Photosystems I and II capture sunlight during oxygenic photosynthesis, thereby mediating direct biophotolysis. In direct biophotolysis, breaking down of water molecule takes place yielding H_2 with subsequent release of O_2. Carbohydrate (starch) produced during the dark reaction generates H_2 in indirect biophotolysis processes. A biological system produced the carbohydrate in the presence of water and carbon dioxide (absorbed from the atmosphere). Thus, H_2 and CO_2 were generated by the breakdown of carbohydrates. Being highly sensitive to O_2, hydrogenase works under anaerobic condition to produce H_2, whereas oxygenic photosynthesis produces O_2. Hence, an incompatibility exists between the aerobic and anaerobic processes of H_2 production [63]. The research is going on to study the H_2 production through aerobic and anaerobic conditions using *C. reinhardtii* and other algal species as model organisms. TAP medium is one of the well-known culture media for H_2 production. When freshwater algal cultures are deprived of sulfur and phosphorus, and when seawater algae cultures are deprived of phosphorus, these conditions stimulate the algae to undergo photoproduction of H_2 [36].

Nano-technology plays a crucial role in microalgal technology, starting from growth conditions to the utilization of algal fuel in engine studies. The positive features of nano-tech include its sturdiness, adsorptive capability, recyclability, reliability, catalytic efficiency and crystallinity, high storage capability, exceptional biofuel acquiesce, and eco-friendly features. The biofuel and bioenergy sector also attained better results with the utilization of nanomaterials (in the form of fibers, sheets, tubes) as catalysts. Nano-entities also serve as a successful immobilization matrix for different enzymes binding for the production of biodiesel and bioethanol. The magnetic nano-particles' high coercivity coupled with probable paramagnetic traits favors methanogenesis for biomethane production [64].

2.3 Biodiesel production from microalgal biomass

Emission of CO_2 is a major concern for which the focus has now shifted from the use of regular diesel to the use of biodiesel. Plants and microalgae have the potential to use CO_2 as an inorganic carbon source for their metabolic reactions. Production and processing of microalgae demand power, and this power can be generated when biodiesel is used as a fuel [5]. Accumulation of lipids in the form of triacylglycerides (TAG) [65] [49] takes place in microalgal cells when environmental conditions become unfavorable (stress conditions) either in the form of nutrient deficiency or the amount of light. Lack of nitrogen may significantly reduce the cell division, as the protein that is pivotal for cell wall formation becomes scarce [66]. Production of biomass is negatively affected due to the lack of phosphate in the cultivation medium, which also reduces the lipid production but favors the unsaturated fatty acid concentrations [67]. The inclusion of organic carbon sources in cultivation media favors the algal growth and lipid accumulation [68].

Triacylglycerols found in the oil extracted with the help of solvents are broken down to diglycerides and monoglycerides in consecutive steps of alcoholysis (mostly methanolysis rather than ethanolysis, propanolysis, and butanolysis). The efficiency of methanolysis can be enhanced with the utilization of acid, base, and enzyme catalysts and also through the utilization of supercritical conditions. Fatty acid alkyl esters (FAAEs, mostly FAMEs rather than FAEEs) were the main products while glycerol served as a by-product [24]. If complete solubilization of triacylglycerols does not take place by solvents, the extractive process is considered a failed attempt, which results in reduced oil extraction [69]. The oil recovery also affected by the drying temperature of biomass since the vitalization of fatty acids at higher temperatures. Hence, choosing an efficient extraction technique is a pre-requisite to attain enhanced biodiesel yields [65].

Microalgae symbolize the feedstock for third-generation biodiesel, with much higher yields than other crops. Recent years have witnessed worldwide attention towards the microalgal diesel production by focusing on the optimization of algal lipid extractions towards probable successful biofuel technology [70, 71]. The process of conversion of microalgal oil to biodiesel is similar to the procedures of the manufacture of biodiesel oil from any other oilseed. It thus can fairly possibly employ similar translation processes to produce biodiesel. The employment of biodiesel in diesel engines not only improves the engine performance to some extent but also curtails the emission of the engine except the NOx emissions. Moreover, the engine output depends on the operational conditions of the engine. Particular emphasis needs to be implemented towards more investments in the 3G-based biodiesel sector by taking care of cumbersome costs associated with the harvesting and lipid extraction processes of microalgae to compete with the existing petrodiesel price. Moreover, the performance of the engine and reduction of emission can be improved when research also focuses on the combustion of microalgae in an internal combustion engine [72].

2.4 Bioethanol production from microalgal biomass

Bioethanol is generated as an outcome of the fermentation of sugars derived from lignocellulosic material. Bioethanol production is considered a green process as it is produced from renewable sources. In comparison with gasoline, ethanol has high octane content and superior flammability characteristics, with extended compression ratio and lower burning rate. Combustion of ethanol is improved as oxygen is present in molecular structure, as a result of which emission of carbon monoxide, hydrocarbon, and particulate is reduced [73]. Hernández et al. [74] pointed about the microalgal biomass composition with higher lipid and protein fractions and lower carbohydrate fractions, which invade bioethanol production from microalgae which will not be a promising venture due to less carbohydrate content. Production of biodiesel from microalgae was extensively studied rather than ethanol. Vegetable biomass has extensively researched for ethanol production over the years [75]. For ethanol production from microalgae, it is very important to explore the organism that possesses a significant quantity of polysaccharides (cellulose, glycoproteins, hemicellulose, pectin, alginate, and agar) in their cell wall along with its ability to accumulate starch [76]. In this context, the algal species with high carbohydrate accumulation potential are the ideal choice for converting carbohydrate-turned sugar into ethanol with the possible supplementation. The accumulated/reserved carbohydrate polymers need to be hydrolyzed for monomers for successful conversion into bioethanol by efficient microbes [77]. Despite not having the lignin in the microalgal feedstock, a viable pretreatment is still required to access the renewable sugars [2, 78]. Pretreatment facilitates the ease of access to reserve carbohydrates/sugars, which eventually leads to enhanced ethanol yields [79].

The 3G-biorefineries sector needs to utilize advanced techniques [80, 81] with efficient algal strains and needs to take measures to invest more funds to research and development sectors to reduce the production cost to compete with the petroleum fuels. More research focus has to pragmatize towards the co-product stream of 3G-biorefineries for the production of valuable industrial components, healthrelated products, animal feed, and bioenergy/electricity production, which pave the way for integrated, sustainable 3G-biorefineries to become a reality in the real world. The product chain includes ranging from different chemical commodities [7, 82–84].

2.5 Co-products' diversification from third-generation biorefinery

The primary aim of the biorefinery is to produce biofuel energy along with high-value products, minimizing waste and increasing the profit. Third-generation biorefinery has many advantages than conventional biorefinery as it can provide biofuel, energy, and valuable industrial products, i.e., carotenoids, pigments, antioxidants, and PUFAs (polyunsaturated fatty acids). Secondly, algal biomass utilizes CO_2 from the environment, making the process overall eco-friendly and green. Another benefit of using microalgae is its ability to grow very fast within a shorter period. Figure 1 shows the different products and by-products associated with third-generation biorefinery.

2.6 Microalgae as a food and feed product

Microalgae are an important source of food and feed products. In fact, microalgae are being used by humans for centuries. The use of microalgae as a functional food is getting increasing interest in the last two or three decades. Microalgae as functional food showed beneficial effects on human overall health, low risk of diseases, and increased life expectancy [85]. They are a rich source of proteins, minerals, vitamins, carotenoids, antioxidants, etc.; for example, Spirulina is already used as a food supplement and sold by many companies. Microalgae are commonly rich in water- and fat-soluble vitamins and have proved their efficiency as a viable food supplement. They are natural reservoirs of important vitamins and minerals essential for human health and vitality [86].

Microalgae are a known source of protein which is possible for human consumption. Microalgae can be a successful source of good protein for an increased human population. Along with proteins, microalgae are an abundant source for beta-carotene, chlorophyll, protein vitamins (C-phycocyanin, A-phycocyanin), and fatty acids (oleic, palmitic, stearic, linoleic acid, and c-linolenic acids) too which possibly replace the chemical-based compounds [87]. Biopigments from algae have anticarcinogenic, anti-oxidative, and anti-hypertensive properties [88]. Microalgal proteins have all the essential amino acids required for holistic human health. The protein content in microalgae is higher than the commonly used protein for example poultry, meat, and milk. Microalgae are also a rich source of antioxidant-rich fat-soluble vitamins and vitamin E. Microalgae are found to synthesize omega-6 and its family members such as linoleic acid, γ-linolenic acid (GLA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These omega-6 fatty acids have pronounced effects on human wellbeing reducing arthritis, hypertension, and cardiovascular diseases [85].

The extraction technique plays a vital role in the isolation of these natural bioactive compounds in their bioactive form. Bioactive compounds from microalgae should be extracted in such a way that they should not lose the activity of bioactive compounds and this is one of the technical problems that needs to be solved [89]. When bioactive compounds are extracted by solvent, care should be taken about solvent toxicity issues for probable application in food industries. Factors that affect the extraction process are temperatures, solvent systems, and nature of the targeted bioactive molecule. Despite having numerous health properties, microalga-based food formulations have not achieved the desired success. This is primarily due to the high cost and unawareness in the society [88]. In addition to human food, microalgae have also been increasingly used in aquaculture. Because of the cost incurred in downstream processing to concentrate, storage, and collection, the commercial viability of algal food and feed is still a big concern. For more acceptability of microalgae as food/feed, prebiotic properties have to be studied in more detail [85]. Given the aforementioned health benefits of microalgae, this is sure that alga-derived health products are going to thrive in the market with a large acceptance in society. However, techno-economic hurdles are required to be addressed properly. Besides this, quality assurance, safety measurements, regulatory issues are the key drivers which need to be taken care of in a tangible manner for the mass use of microalgae as food and feed.

2.7 Algal lipids

Algal lipids contain eicosapentaenoic acid (EPA), linolenic acid, arachidonic acid, and docosahexaenoic acid (DHA) and have applications in pharma and food industries [41, 90]. Algae can store a decent amount of lipid content up to 50% under optimized growth conditions [91, 92]. Algal lipids contain polyunsaturated fatty acids (PUFAs), which are utilized for biodiesel production and as a food supplement [93]. Moreover, omega-3 fatty acids of PUFAs have profound applications in cardiovascular disease treatment. The enhanced microalgal PUFA yields are possible through the tuning of algal growth conditions and the utilization of advanced culture systems and adopting genetic modifications [11]. Various solvent-, extraction-, microwave-, ultrasonic-, electroporation-based techniques have been practiced for lipid extraction from microalgae [94]. The proposed techniques utilize a great extent of temperature, energy, and solvent volumes for the execution of algal lipid extraction. The disadvantages associated with the existing lipid extraction techniques can overcome by utilizing eco-friendly solvent-free extraction techniques such as osmotic pressure-, enzyme-mediated, and $scCO_2$ (supercritical carbon dioxide)-, and isotonic-based extractions [80]. The residue after extraction can be utilized as animal feed or can convert into liquid fuel.

2.8 Algal polysaccharides

Algal carbohydrates comprise glucose, cellulose, and starch, including different polysaccharides, which are the main components of algal carbohydrates. The percentage of polysaccharides present in microalgae varies with the microalgal genera and species [95]. For example, green algae contain 25–50%, red algae 30–60%, and brown algae 30–50% of carbohydrate. The various algal species are reported for its polysaccharide production potential, which include *Ascophyllum* (42–70%) [12], *Porphyra* (40–76%) [96], *Palmaria* (38–74%) [97], *U. lactuca* (55–60%) [98], and *C. reinhardtii* (UTEX 90) (60.0%) [99]. Alginate polysaccharides derived from brown algae are used as a stabilizer in the pharma industries. It also has applications in photography, textile, and paper industries [100].

Another polysaccharide carrageenan from red algae has applications in the pharmaceutical, food, and textile industry. It has antioxidant and antiviral activity. Red alga–based agar has application as a stabilizer and gelling agent. Sulfated polysaccharide ulvan obtained from *Ulva lactuca* has antitumor properties and has applications in the production of fine chemicals. Another polysaccharide which boots our immune system is laminarin. It also provides a protection agent for severe irradiation and bacterial infection.

2.9 Pigments and vitamins

Carotenoids, chlorophylls, and phycobiliproteins are three important natural pigments found in microalgae. Carotenoids are considered color-imparting components that are responsible for giving color to different parts of plants [28]. These pigments have widespread applications in different sectors such as pharmaceutical industries, cosmetics industries as a vitamin precursor, as a food coloring agent, nutraceutical, and food and feed supply industries. Many carotenoids were known for anti-inflammatory activity, anti-aging activity, UV-radiation protective activity, immune system boosting effects, and anticancer activity. For example, astaxanthin obtained from *Dunaliella salina* has many biological activities. The estimated global market of astaxanthin is about $257 million, and the cost per kilogram of astaxanthin is about $7150 [101]. β-Carotene and astaxanthin are also known to prevent certain human eye diseases such as cataracts. β-Carotene has many applications in pharmaceutical industries, cosmetic industries, and feed and food industries.

Another natural pigment phycobiliproteins present in microalgae has anticancer, antiviral, anti-allergic, antioxidant, and neuroprotective properties and anti-inflammatory activities which makes it a unique product in the health care sector [102]. Other applications of phycobiliproteins in the food industry and pharmaceutical industries are natural dyes. Microalgae are also a rich source of vitamins which are mostly fat-soluble vitamins and vitamin E with antioxidant capabilities. Vitamin production in microalgae depends on (i) the nitrogen (N) availability and (ii) nitrogen concentration and its source in the culture medium [103]. The lesser production of vitamin B_{12} per cell by cyanobacteria has been reported with the lower nitrogen concentration in the media. Riboflavin is another vitamin found in microalgae essential for humans. Lutein is another biopigment obtained from the microalgae. The global lutein market is expected to reach USD 396.4 million by 2024 (https://www.globenewswire.com accessed on 24 December 2019). Dark and green leafy vegetables, corn, and egg yolk are the conventional sources of lutein, while petal of marigold flowers is a current organic source of lutein. Microalga-derived lutein are good alternative over plant-derived lutein because most of the plant-derived lutein are found in esterified form and purification needs chemical saponification. In contrast, in microalgae, lutein exists in the free non-esterified form [104]. Lutein production from microalgae was studied by different researchers, for example, *Chlorella protothecoides* [105, 106], *Dunaliella salina* [107], *Scenedesmus almeriensis* [108], and *Galdieria sulphuraria* [109]. *Chlorella protothecoides*, *C. zofingiensis*, *Muriellopsis* sp., and *Scenedesmus* sp. are the microalgal species that contain a high content of lutein [110, 111]. The use of genetic engineering can help to improve lutein content in microalgae. Lutein derived from microalgae can be used in soft drinks, dairy products, salads, and confectionery.

2.10 Proteins

Microalgal biomass is also extensively used as a high-protein-rich supplement in different nutraceutical and aquaculture industries [112]. Protein content in microalgae varies from strain to strain. Some examples of microalgae with high protein content are *Spirulina maxima* (60–71%), *Anabaena cylindrica* (43–56%), *Chlorella vulgaris* (41–48%), and *Synechoccus* sp. (63%). Due to the high nutritive value and rich protein content of some microalgae species, some algal species are used as a human food source. Examples of such microalgae include *Dunaliella salina*, *Aphanizomenon flosaquae*, *D. tertiolecta*, and *Spirulina plantensis*. Also, some reports suggested that algae can substitute many conventional protein sources—fish meal and soybean [113].

The extraction of proteins from microalgal biomass mainly depends on the pH, ionic strength, and bulk aqueous phase salt type [114]. Spirulina is a rich source of different vitamins and amino acids and phytopigments with high protein content can serve as a well-versed food supplement [115]. Different countries of the world are engaged in the production of Spirulina and Spirulina-based products. China is the largest producer of Spirulina while the USA ranks first in Spirulina-based products [113]. Algae can be used in place of poultry feed as sources of protein [113]. A 10% decrease of cholesterol was reported in color-turned (due to the presence of high-carotenoid content) egg yolks on poultry feeding with *Porphyridium* sp. [116].

Genetic modification is becoming popular in different fields of science, including 3G biorefinery. By genetic engineering, the superior strains can be developed which can help in improving the photosynthetic efficiency of algae that eventually increased algal biomass production within the economic matrices [84]. Besides, the genetic modification can also make algae more resistant towards abiotic factors and can reduce the production of unfavorable by-products [117]. Protein synthesis of the algae can also be enriched by genetic engineering. Microalgae have eukaryotic nature, which permits them to produce glycosylated proteins. Presently, microalgae-based recombinant proteins are not commercialized, but it offers economical production of commercially important recombinant proteins [118]. According to Song et al. (2018) [119], there was a 31.8% increase in protein content and an 11.6% increase in biomass production by using transgenic *Chlorella pyrenoidosa* (K05).

2.11 Cosmetics

Cosmetic market is growing rapidly, and most people prefer natural products rather than chemical-based products. Microalgae serve as a rich source for different cosmetology products [12]. Glycerol, obtained as a byproduct of algal biorefinery, has many applications in the cosmetic and pharmaceutical sectors [120]. Phycocyanin (blue colorant), a Spirulina's product, has a use in the cosmetics and food industry as an ingredient for lipsticks and eyeliners [121]. β-Carotene has many applications in the cosmetic industries, along with other industrial applications. A carotenoids' lycopene is reported to be an effective antioxidant and can neutralize free radical derived from oxygen. Its antioxidant activity is more than that of the carotene, tocopherol, and lutein with an application as a sun-screen agent [122]. Compounds mycosporine and scytonemin derived from algae can absorb UV radiation which makes them suitable to use in the pharmaceutical industry and cosmetic industry [91]. According to

Laurens [123], around 20 industries are actively engaged in the microalgae-based cosmetic business sector, for example, Fitoplancton Marino (a European company), which produces nutraceuticals, cosmetics, and health products from marine microalgae.

2.12 The industrial scenario of algal technologies

In recent years, a large number of industries, government organization, and research institutes are actively working to make algal technologies cheap and sustainable. A good number of firms are in the business of algal-based products. Three US-based companies, i.e., Sapphire Energy Inc., Algenol (USA), and Solazyme Inc., produce biofuel, biodiesel, and biojetfuel [124]. Astaxanthin, an alga, is sold by different companies like Alga technologies (Israel), BioReal (USA), Mera Pharmaceuticals Inc., and Solix Algadrients Inc. (USA) [125]. According to Hejazi and Wijffel [126], the annual global production of astaxanthin was 200 million US$ (2500 US$/kg) and estimated projection of astaxanthin sales was US$ 555.4 million in 2016 (https://www.grandviewresearch.com/industry-analysis/global-astaxanthin-market accessed on 18 July 2019). The Australian company Muradel Pvt. Ltd. is engaged in the production of different microalgal product chains of biofuels, animal feed, oleochemicals, building materials, and biofertilizers. Biorizon Biotech, Spain, is working in a business of microalga-based amino acids and biofertilizers. Sabana project started in 2016, aimed at developing microalgae-based biofertilizer, biopesticide, and feed additives with a demo plant facility of 300 tons/year capacity [127].

2.13 Impact of third-generation biorefinery in bio-economy

The economy in which the raw material for chemical and energy is obtained from the natural and renewable biological resources is known as bio-economy. Third-generation biorefineries are the basis for building a bio-based economy because they provide an equal chance for growth in all economic sectors. The bio-based economy consists of different sectors such as bioenergy, biofuels, material, pharmaceutical, and chemical sectors. According to the IREA (International Renewable Energy Agency), the global renewable energy sector is the employment creating sector and in the year 2017, 10.34 million people got employment in this sector [128]. The commercial production of *Chlorella* was dated back from the 1960s in Japan [129]. After that, the commercial production of microalgae was increasing and reached 7.5 million tons. One estimation that revenue of about US$ 1.25×10^9 each year can generate by processing dry algal biomass (5000 metric tons) as the by-product [130]. The estimated world market demand for microalgae is about $6.5 billion in which 2.5 billion is towards dietary supplements, $1.5 billion accounts for medicinal- and nutritional-based microalgal products, and $700 million for aquaculture and animal feed products. According to a report by Persistence Market Research, worldwide trades of microalgae are projected to exceed US$ 75 Mn by 2026-end (https://www.persistencemarketresearch.com/market-research/microalgae-market.asp accessed on 24 July 2019).

Different studies are reported on the techno-economic assessment of microalga-based products. According to Brownbridge et al. [131], a survey on the sensitive production parameter of algal biodiesel production and concluded that the production costs primarily rely on oil content, production capacity, annual productivity per unit area, and increased carbon price rate. Another report on closed microalgae cultivation system by Batan et al. [22] showed that 63% of the total cost was utilized as operating cost, investment cost was about 30% while 7% was utilized for land purchase. The study showcased by US DOE, the production cost of ton dry algal biomass/ton, was estimated to be around $1227 in 2015 [132]. It was found that producing 1 ton of dry algal biomass in an open raceway pond was about 87% of the overall cost used, and the remaining costs wereassociated with the harvesting (10%) and other purposes (facility and storage, 3%). While analyzing the production cost of algal oil from algal biomass as a substrate for biodiesel production seems to be very high compared with other sources, the production cost of algal biodiesel is possible by expanding the by-product chain streams to compensate for the high cost associated with the algal-based biofuels.

2.14 Environmental considerations and SWOT analysis

Sustainable development is a development that fulfills the present needs, along with the supply chains to the demands of future generations [133]. The impact of biorefinery on the environment, social and economic, needs to be considered while evaluating any process by considering the entire bio-based product chain. Figure 2 shows all three fundamental pillars of the sustainability of algal feedstock for biofuel and biochemical production. 3G biorefineries have the potential to make biofuels and biochemicals commercially competitive. Presently, cellulosic ethanol fulfills the specific demand of the globe with value-added side-chain biorefinery. Cellulosic ethanol can significantly curtail fossil fuel consumption and CO_2 emissions. Food-based biofuels do not considerably reduce CO_2 emissions. Food crops should not be chosen as a raw material for biofuels and for replacing fossil fuels. On the contrary, algal-derived biofuels or biochemicals significantly cut down the GHG emissions and have comparatively lower carbon footprints. For biorefineries aiming the production of biofuels/biochemicals, lipids and carbohydrates are the major building blocks in 3G biorefineries. The cost of biofuel (biodiesel and bioethanol) production from microalgae is

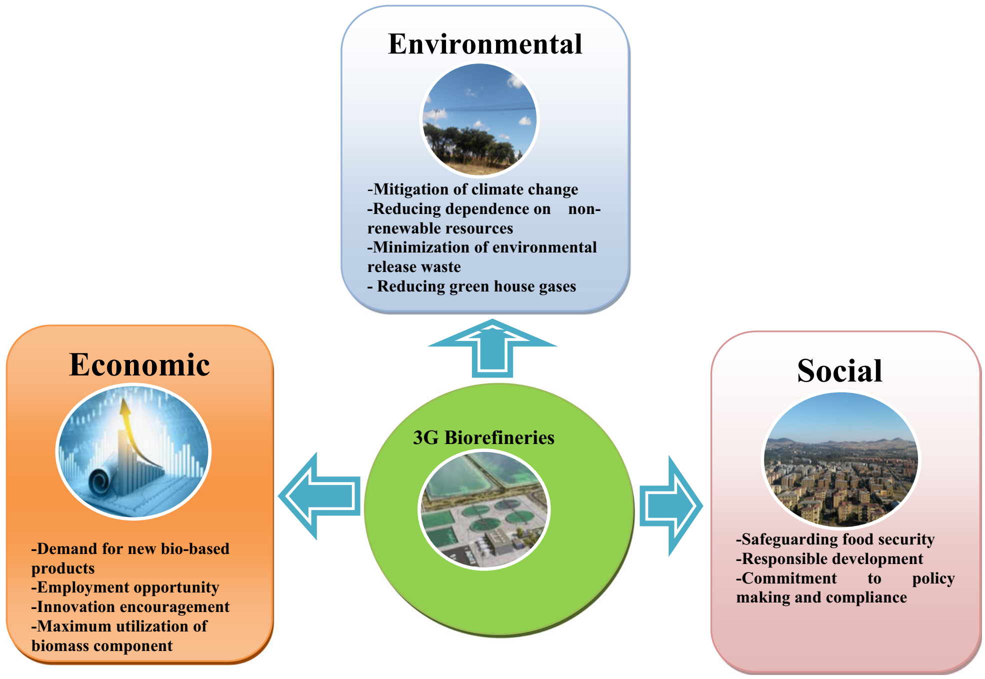

Fig. 2 Environmental, economic, and social benefits of algal biorefinery towards sustainable technology development

comparatively higher than conventional gasoline and first-generation ethanol. Cost comparative analysis of 3G biofuels has been studied rationally less than 1G and 2G ethanol. Alga-based biofuels (biodiesel and bioethanol) can significantly lower carbon emissions, i.e., CO_2, NO_2, S, and particulate matters. Besides this, algae can be grown on wastewaters, leachate, industrial wastes, etc., thus mitigating pollution from the environment efficiently (Fig. 3).

Lignocellulose biorefineries, so-called 2G biorefinery, contribute to a reduction of environmental impacts. Being a potent source of renewable chemicals, material and sustainable energy, lignocellulose biorefineries also help in managing climate change by curtailing the demand on fossil fuel energy. Lignocellulose biorefineries also contribute to curtailing greenhouse gas emissions. However, the land and water usage of lignocellulose biorefineries brought water pollution leading to eutrophication with possible environmental damage. Biodiversity gets adversely affected, thereby also affecting ecosystem services. 2G biorefineries have product limitations compared with 3G biorefineries. Because of the inherent nature of diverse feedstock, 3G biorefineries may offer a vast range of high value-based pharmaceuticals and nutraceuticals than 1G or 2G biorefineries with lower carbon footprints. However, a

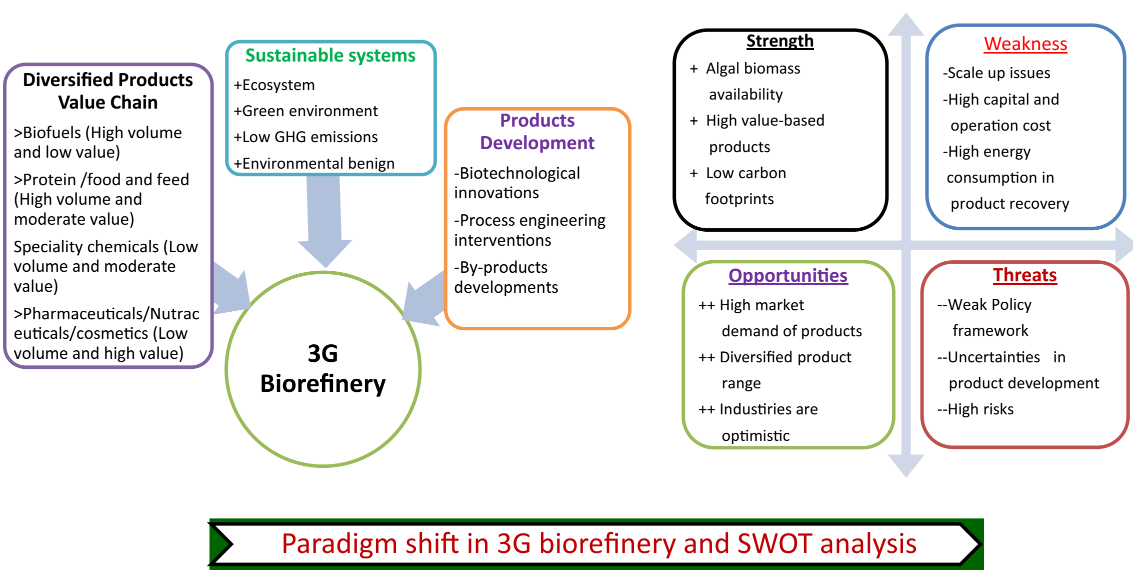

Fig. 3 Key parameters governing the viability of 3G biorefinery and SWOT (strength, weakness, opportunities, and threat) analysis

deep life cycle assessment of 3G biorefinery needs to be done by undertaking input and output flow, production chain, acquisition of raw material, generating products, and shelf life of products and co-product [134]. The International Organization of Standardization has a standardized life cycle assessment methodology in its ISO14040 series which can be a pivotal source of information for assessing life cycle analysis of 3G biorefineries [135]. Data are available from various kinds of literature dealing with impacts of biorefineries on the environment concluding that the lignocellulose biorefineries system is a potent option to mitigate changes in climate and curtailing the fossil fuel dependency. These biorefineries depend on the local availability of renewable resources, thereby revitalizing rural areas [136]. Sustainable practices of biomass acquisition are the focal point to safeguard a renewable energy supply to biorefineries. There are many categories where the impact of 3G biorefineries needs attention to carefully evaluate environmental effects. These categories have a great impact on biodiversity, water quality, land fertility, carbon stocks of soil, and ecosystem services. Evaluation of toxicological risks and energy efficiency is also mandatory for complete assessment [4]. Hence, it is a complex process to determine the overall impact on the environment and there are many chances of prevailing a certain degree of uncertainty in the final results. Figure 3 also shows some important pros and cons of 3G biorefinery through SWOT (strength, weakness, opportunities, and threats) analysis. These critical factors could be vital to researchers, industrialists, and policy-makers to gauge the merits and demerits of 3G biorefineries.

2.15 Technical challenges and future prospects

The optimized 3G biorefinery development towards viable industrial commodities requires robust algal strain selection, reasonable capital and operation cost for cultivation, pretreatment, and extraction with the desired process sustainability [137]. The major challenge in microalgal cultivation is the availability of sunlight and pH maintenance of inorganic salts. The sunlight availability can be enhanced by fabricating the system with low-cost acrylic type material. The lack of sunlight during the night hours can be overcome by providing an artificial illumination, which will ultimately enhance the overall productivity of the cultivation system. Supplying of gaseous CO_2 through sparging avoids the uptake of HCO_3- ions by algal cells, which helps in maintaining the constant pH in the algal system [138]. The energy-intensive nature of existing algal biomass pretreatment techniques can be fixed through a pretreatment technique that suits the different morphological diversified microalgae sustainably with green solvents [48, 80]. The alga-based food products for human consumption have to be formulated by adhering to the food and drug administration safety guidelines with the proper product stability information. Valorization of algal biomass towards each product line should evaluate for economic and sustainable issues through the life cycle assessment studies [139]. The scale-up of the 3G biorefinery system is more challenging. Towards addressing this issue, the researchers need to work on the issues related to sunlight availability for autotrophs and the suitability of flue gasses and industrial gaseous streams for aerobic chemoautotrophs for successful scale-up operations. Algal species in 3G biorefinery has the potential to work as a potent microbial cell factory by transforming the atmospheric CO_2 into a myriad of carbon-neutral fuels and chemicals eventually decarbonizing the economy in a substantial manner [139, 140]. The overall successful sustainable 3G biorefinery requires the integration of multiple technologies, chemical, material sciences, and biological systems with the help of advent technologies of genetic engineering, nano-, and microfluidic technologies [140].

3 Conclusion

Biorefinery remains to receive an impetus towards extending the research trends towards sustainable bio-based products. The industrial-scale biorefineries mainly differ from the available feedstock resource. Among different biorefineries, algal biorefinery occupies a prominent place as a future technology with a production potential of biofuels along with the bio-based product chain with a possible alternative to the existing/depleting petro-chemical biorefinery. The prominent stage under 3G-based algal biorefineries towards sustainable products lies in the initial fractionation of algal biomass with minimum tunable parameters. To date, the advancements in the fractionating algal biomass towards different industrial products are in infancy with forecasting inevitable research in 3G alga-based biorefinery. The integration of biorefineries could offer fuels and chemicals at affordable prices rather than stand-alone facilities. Taking into the global considerations such as food/feed demand in future, climate change and political instabilities in major oil-producing countries, this ishigh time that 3G-biorefineries should develop the scalable technologies for the large-scale production of fuels, food, and renewable chemical commodities to reduce dependency over the petroleum products and contribute to the establishment of a sustainable society.

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