Renewable and Sustainable Energy Reviews 47 (2015) 295–307

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Algae based biorefinery—How to make sense?

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ARTICLE INFO

Article history:
Received 16 June 2014
Received in revised form
5 February 2015
Accepted 8 March 2015
Available online 30 March 2015

Keywords:
Algae
Biofuels
Biojet
Bioremediation
Biorefinery

ABSTRACT

The interest in algae based biofuels and chemicals has increased over the past few years because of their potential to reduce the dependence on petroleum-based fuels and chemicals. Algae is touted to be the most suitable and sustainable feedstock for producing green energy as the whole process is carbon—neutral in nature and can also be utilized for environment cleaning applications. This review article mainly focuses on how algae can be used as an efficient and economically viable biorefinery feedstock. An effective biorefinery using algae can only be constructed through its integration with other industries. To make sense of the algal biorefinery concept, there is a need to establish a proper connection between the various input and output streams of the products, as well as the services to be provided by the participating industries. Also highlighted in this article, is the entire spectrum of energy and non energy products that can be obtained using algal biomass as the raw material.

Contents

1. Introduction

The first generation of bioenergy strategies involved biofuel production based on sugar, starch, vegetable or animal oils using conventional technology [1], but these methods have been globally

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http://dx.doi.org/10.1016/j.rser.2015.03.052
1364-0321/

criticized because they competitively consume food resources [2]. To circumvent this problem, the second generation of bioenergy uses non-edible or waste vegetable oils and agricultural wastes such as lumber, straw and leaves; however, the availability of these was less [3]. Also, terrestrial bioenergy production systems are now facing issues related to indirect emission and carbon debt from land clearance and hence are becoming a sustainability hurdle for further expansion [4–7]. Therefore, a more sustainable feedstock had to be evolved to overcome these limitations.

Microalgae have been recognized as an alternative, so-called third generation feedstock not only because they remove carbon dioxide from the atmosphere, but also because they contain a much higher lipid content per biomass (Table 1) than other plants [9–11]. Marine microalgae species growing in seawater can also reduce fresh water consumption [12]. In addition, it can be grown with wastewater which indicates a high environmental sustainability of this feedstock [13].

Environmental factors, such as temperature, salinity, illumination, pH-value, mineral content, CO_2 supply, population density, growth phase and physiological status can greatly modify the chemical composition of algal biomass. Under conditions of high light intensity and nitrogen limitation, the flow of carbon fixed in photosynthesis is diverted from the path of protein synthesis to that leading to lipid and/or carbohydrate synthesis [14]. A detailed physicochemical characterization of the microalgae is essential, as it will allow determining which algae are best suited for different applications and purposes [15].

Table 1
Typical oil yields from the various biomasses [8].

S.N.	Crop	Oil yield (l/ha)
1	Rubber seed	80–120
2	Corn	172
3	Soybean	446
4	Safflower	779
5	Chinese tallow	907
6	Camelina	915
7	Sunflower	952
8	Peanut	1,059
9	Canola	1,190
10	Rapeseed	1,190
11	Castor	1,413
12	Jatropha	1,892
13	Karanj	2,590
14	Coconut	2,689
15	Oil palm	5,950
16	Microalgae (30% oil by wt)	58,700
17	Microalgae (70% oil by wt.)	136,900

Table 2
General composition of different algae (% of dry matter) [24,28].

Alga	Protein	Carbohydrates	Lipids
Anabaena cylindrica	43–56	25–30	4–7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Cholrella pyrenoidosa	57	26	2
Cholrella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Dunaliella bioculata	49	4	8
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Scenedesmus dimorphus	8–18	21–52	16–40
Spirogyra sp.	6–20	33–64	11–21
Arthrospira maxima	60–71	13–16	6–7
Spirulina platensis	46–63	8–14	4–9
Spirulina maxima	60–71	13–16	6–7
Synechococcus sp.	63	15	11
Chlorella vulgaris	51–58	12–17	14–22
Prymnesium parvum	28–45	25–33	22–38
Tetraselmis maculate	52	15	3
Porphyridium cruentum	8–39	40–57	9–14

Hence, the use of microalgae as feedstock for the production of biofuels offers many opportunities if challenges in large-scale cultivation, harvesting and conversion to useful fuels can be overcome [16]. Generally, centrifugation, flocculation and membrane filtration techniques have been proposed to concentrate algae from their growth medium. Among the various harvesting techniques, membrane filtrations offer several advantages because they do not require additive or coagulants and are able to function at moderate temperature and pressure and reduce the formation of undesired products, which further simplifies the subsequent purification of specific metabolite and the use of the residual biomass [17].

To find the appropriate application of algal lipid at industrial level, the fatty acid profile analysis is an important task. Recently, there has been an increased interest in the development of alternative methods that improve fatty acid profile analysis. These methods involve mainly three criteria: (1) direct trans-methylation of lipids, (2) elimination of the need for preliminary extraction steps, and (3) using a single-step derivatization procedure for generating fatty acid methyl esters (FAMEs) to denature the protein fraction [18].

Microalgae have the potential for co-production of valuable products like carbohydrates, lipids, and proteins, starch, cellulose and polyunsaturated FAs (PUFAs), pigments, antioxidants, pharmaceuticals, fertilizer, energy crops [19–21], natural colorants and also as biomass that can be used as animal feed after oil extraction. The most widely used biofuel is bioethanol, which is produced from sugar-based (sugar beets, sugarcane) and starch based (corn, wheat, barley, etc.) feed stocks [22], while technology leading to conversion of lignocellulosic materials (bagasse, corn stover, rice straw, switch grass, and so on) into ethanol is still under development [12].

Microalgae are currently being used to commercially produce carotenoids, for example, *Haematococcus pluvialis* for astaxanthin and *Dunaliella salina* for β-carotene. Several reports have stated that the unusual cell membrane of *Dunaliella* allows its cells to maintain high concentrations of intracellular glycerol without leakage to the external medium under normal conditions despite the sharp concentration gradient across the membrane [23]. This microalga is able to withstand temperatures over 50 °C for more than 8 h and produces pigments including astaxanthin, lutein, canthaxanthin after the cells are stressed for a period of time. *Chlorella* is widely produced and marketed as a health food supplement in many countries, including China, Japan, Europe and the US, with an estimated total production around 2000 t/year [15]. Nutritionally important fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are commercially obtained from various marine fishes and microalgae [24]. Microalgae are a potential source of bio-active compounds with pharmaceutical, biomedical and nutraceutical prospects [25]. Therefore, microalgae could play important role in producing biofuels and bio-based chemicals based on both their natural components and refined (or fermented) products [26].

Fundamental principles of biochemistry show that the maximum theoretical energy conversion of the full sunlight spectrum into organic matter lies around 10%. So the yields of the products have a stoichiometric and thermodynamic constraint on them. The yields with outdoor cultures is one third to one tenth of the theoretical yield and therefore to make the whole process more profitable, the real challenge is to improve the efficiency of the photosynthetic system [27].

The biorefinery concept has been identified as the most promising way for the creation of a biomass-based industry. If the goal of biorefineries is to transform biomass into biofuels and high value-added products, the existing and emerging technologies for these

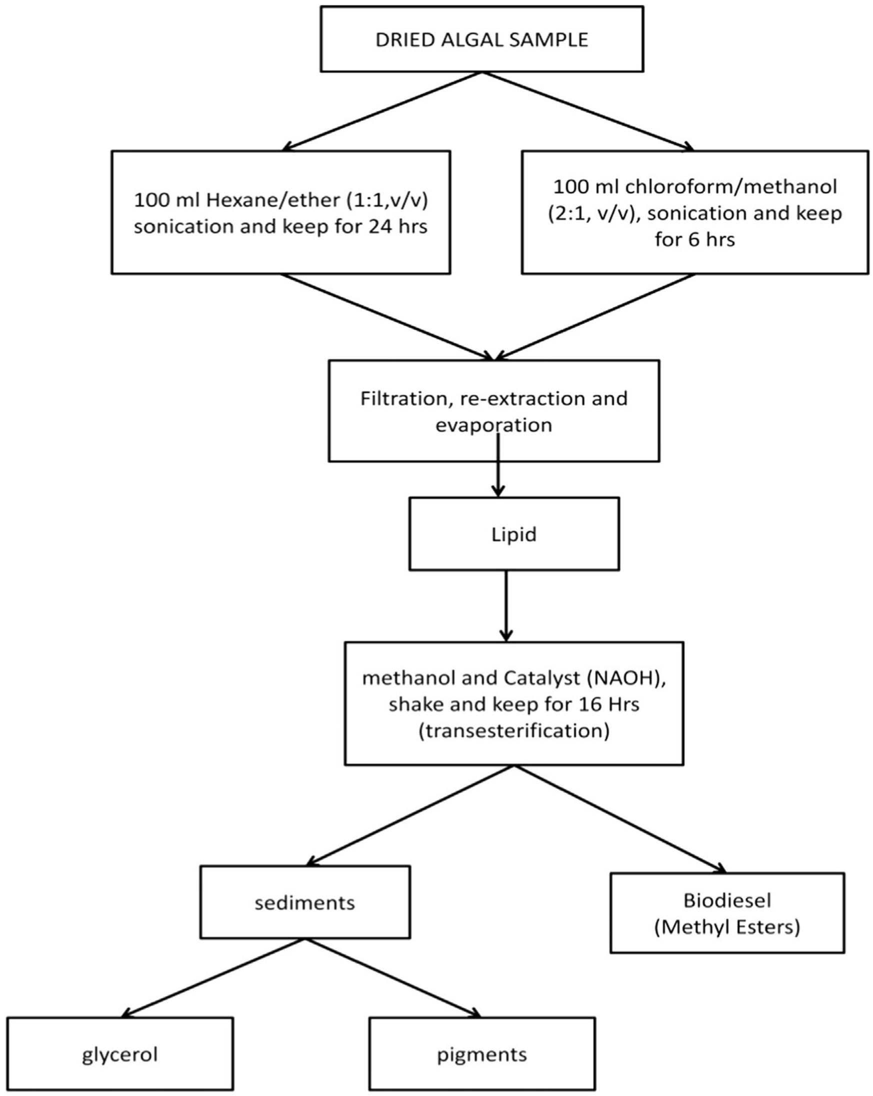

Fig. 1. Procedure for biodiesel production from algal systems using two solvent systems [34].

Table 3
Comparison between alkali-catalysis and lipase-catalysis methods for biodiesel fuel production [37].

	Alkali-catalysis process	Lipase-catalysis process
Reaction temperature	60–70 °C	30–40 °C
Free fatty acids in raw materials	Saponified products	Methyl esters
Water in raw materials	Interference with the reaction	No influence
Yield of methyl esters	Normal	Higher
Recovery of glycerol	Difficult	Easy
Purification of methyl esters	Repeated washing	None
Production cost of catalyst	cheap	Relatively expensive

transformations have to be reviewed, because in a biorefinery, these technologies must be applied together. In fact, the various co-products derived from the algal biorefinery can also be fed into various industries.

2. Algae applications

Algae can be used as a feedstock for obtaining a number of products. These products can be divided into energy and non energy based on their potential usage. Application of algal feedstock for environment cleaning purpose has also been described.

2.1. Energy products from algae

2.1.1. Biodiesel

Algal biodiesel is a carbon-neutral fuel, which means it assimilates about as much CO_2 during algal growth as it releases upon fuel combustion [8]. For this reason, algae-based fuels are said to be the most effective and sustainable response to climate change [13]. Biodiesel production from these requires release of lipids from their intracellular location, which should be done in the most energy-efficient and economical ways. Table 2 shows the general composition of different algal strains.

Micro-emulsification, pyrolysis or catalytic cracking, are cost intensive and produce a low quality biodiesel. Transesterification is the most usual method to convert oil into biodiesel [29]. Transesterification converts raw and viscous microalgal lipid (triacylglycerols/free fatty acids) to lower molecular weight fatty acid alkyl esters [30]. The alkoxy group of an ester compound is exchanged by an alcohol (alcoholysis), carboxylic acids (acidolysis) [31] or an ester (interesterification). Some of the catalysts used for transesterification are: (1) alkaline catalyst (potassium hydroxide, sodium hydroxide and sodium methoxide); (2) acid catalyst (hydrochloric acid, sulfuric acid and sulfonic acid phosphoric acid) (3) enzymatic catalyst include lipases (4) inorganic heterogeneous catalyst (solid phase catalyst). The process of transesterification is affected by the mode of reaction conditions, molar ratio of alcohol to oil, type of alcohol, type and amount of catalysts, reaction time, temperature and purity of reactants [32]. An alkali catalyst can be problematic when free fatty acid content in the oil is above 3%, yielding it unsuitable for direct biodiesel production from unrefined oils [33]. Fig. 1 gives detailed steps for biodiesel production from algal biomass using alkaline catalyst.

The current trend of carrying out transesterification reactions is through the enzymatic route. Lipase enzymes can be used for transesterification purpose. These enzymatic biocatalysts are of two types extracellular and intracellular lipases [35]. Particular attention has been dedicated to the use of lipases as biocatalysts for biodiesel production due to their favorable conversion rate obtained in gentle conditions and relatively simple downstream processing steps for the purification of biodiesel and by-products. However, in comparison to conventional chemical processes, the major obstacles for enzymatic production of biodiesel are the cost of lipase, the relatively slower reaction rate and lipase inactivation caused by methanol and glycerol [36]. Table 3 gives a comparison of alkali-catalysis and lipase-catalysis methods for biodiesel fuel production.

Algal biodiesel has also been found to meet the International Biodiesel Standard for Vehicles (EN14214). Selection of species for biodiesel production depends on fuel properties and oil content along with engine performance and emission characteristics [38]. In brief, key parameters defining biodiesel quality and properties, such as, Cetane Number (CN), Iodine Value (IV), Cloud Point (CP) and Cold Filter Plugging Point (CFPP) are estimated based on fatty acid methyl ester (FAME) profiling. A comparison of typical properties of fossil oil with the bio-oil obtained from microalgae indicated that bio-oil from microalgae has a lower heating value, lower viscosity and higher density compared to fossil oil [39].

Glycerol is obtained as a by-product during the transesterification process. Crude glycerol obtained from the synthesis of biodiesel could be used as a carbon source and converted to valuable metabolic products (viz. organic acids, microbial biomass, single cell oil, mannitol) by using eukaryotic microorganisms such as yeast and fungi.

2.1.2. Biogas

The macroalgae exhibit higher methane production rates than the land-based biomass. Biogas production from macroalgae is more technically-viable than other fuels. Biogas production is not yet economically-feasible due to the high cost of macroalgae feed stocks, which needs to be reduced by 75% of the present level [40].

Microalgal biomass after lipid extraction comprises of proteins and carbohydrates that can be digested via anaerobic means to generate biogas, a renewable fuel. Biogas contains a mixture of gases; mainly carbondioxide (CO_2) and methane (CH_4). There are four main stages in the biogas production are: hydrolysis, acetogenesis, acidogenesis and methanogenesis [41]. The direct energy recovery during biogas production via anaerobic digestion could be more profitable when the algal lipid content in microalgae is lower than 40% [42].

Another method to produce biogas is through gasification technique. Gasification involves the partial oxidation of biomass into a combustible gas mixture at high temperatures (800–1000 °C) [43]. Biomass reacts with oxygen and steam to produce mixture of gases known as syngas. Syngas consists of gases like methane, hydrogen, carbon dioxide and nitrogen. Syngas can be either directly burnt to produce energy or can be used as a fuel to run diesel or gas turbine engines [6]. It can also be used as a feedstock in the production of chemicals such as methanol.

2.1.3. Bioethanol

Bioethanol can be fermented from all kinds of macroalgae by converting their polysaccharides to simple sugars and by employing appropriate microorganisms. Since macroalgae have various carbohydrates such as starch, cellulose, laminarin, mannitol, and agar, carbohydrate conversion to sugars and the choice of appropriate microorganisms are pivotal for successful bioethanol fermentation. Some of the species used for ethanol production are *Cholorococcum*, *Chlamydomonas* and *Chlorella*. Fermentative ethanol production from microalgae like *Chlorococcum* and *Chlorella vulgaris* result in better conversion rates than that of other species. Brown algae is a principal feedstock for bioethanol production because they have high carbohydrate contents and can be readily mass-cultivated with the current farming technology [44–49]. Biobutanol can also be produced from macroalgae through the acetone–butanol (AB) fermentation using solventogenic anaerobic bacteria such as *Clostridium* sp. [50].

2.1.4. Biojet fuel

One of the biggest challenges in front of the airline industry is the increase in air travel demands. The airline industry consumes over 5 million barrels of oil per day worldwide. The energy intensity in terms of Btu per passenger is lowest for railways followed by airlines [51]. Upon combustion, the aircraft jet fuel produces carbon dioxide (CO_2), water vapour (H_2O), nitrogen oxides (NO_x), carbon monoxide (CO), oxides of sulfur (SO_x), unburned or partially combusted hydrocarbons, particulates, and other trace compounds. These factors jointly create a challenge for the aviation industry to ensure the security of fuel supplies and to minimize the unwanted harm to the environment. Aviation alters the composition of the atmosphere globally and can thus drive climate change and ozone depletion [52]. The aviation industry is concerned about reducing its carbon footprint by using an eco-friendly fuel for air transport.

Renewable jet fuel for the aviation industry, also termed bio-jet fuels could reduce flight-related greenhouse-gas emissions by 60–80% compared to fossil fuel based jet fuel. Jet fuel can be categorized into Civil/Commercial Jet fuel and Military Jet Fuel. Civil/Commercial jet fuel is further categorized into three types: Jet A-1, Jet A, Jet B. Military Jet fuel is categorized into: JP-4, JP-5, and JP-8. The Jet A-1 and Jet A, both are kerosene type fuels. Jet B is a blend of gasoline and kerosene (a wide cut kerosene) but it is rarely used except in very cold climates. JP-5 and JP-8 are chemically enhanced fuels with antioxidants, dispersants, and/or corrosion inhibitors to meet the requirements of specific applications. Green bio-jet fuel is made by blending microalgae bio-fuels with conventional petroleum-derived jet fuel to provide the necessary specification properties [53].

The oil of microalgae can be converted into jet fuel by hydrotreatment (hydrotreated fatty acids and esters, HEFA). This process is also certified according to ASTM standard D7566. The resulting

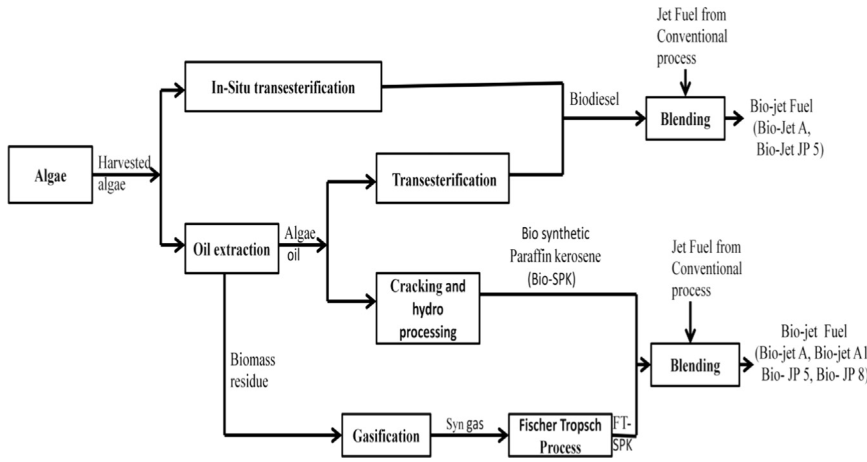

Fig. 2. Proposed production of bio-jet fuel through different routes.

fuel can therefore be used commercially in blends containing a minimum of 50% conventional jet fuel. This fuel is also referred as Hydrotreated Vegetable Oil (HVO), Hydrotreated Renewable Jet (HRJ) or Bio-derived Synthetic Paraffinic Kerosene (Bio-SPK).

In the process to synthesize Bio-SPK/HEFA, the oil is first cleaned to remove impurities using standard oil cleaning procedures. The oil is then converted to the shorter chain diesel-range paraffins, by removing oxygen molecules from the oil and converting any olefins to paraffins by reaction with hydrogen. The removal of the oxygen atoms raise the heat of combustion of the fuel and the removal of olefins increase the thermal and oxidative stability of the fuel. A second reaction, then isomerizes and cracks the diesel range paraffins, to paraffins with carbon numbers in the jet range. The end product is a fuel that contains the same types of molecules that are typically found in conventional petroleum-based jet fuel [54].

The other method to produce jet fuel is through Fischer–Tropsch process. Synthetic Fischer–Tropsch fuels are high-quality fuels that can be derived from natural gas, coal or biomass *via* the Fischer–Tropsch process. Liquid fuels can be produced from algal biomass *via* a gasification step, through the formation of synthesis gas (mainly CO and H_2) and its conversion to liquid hydrocarbon fuel via Fischer–Tropsch (F–T) process [54]. The proposed flowchart of the jet fuel production process using algal feedstock via both the above mentioned processes is shown is Fig. 2.

As safety is a paramount issue in aviation fueling, very specific needs and requirements have to be met. Currently, plenty of research is going-on to produce biofuel from microalgal source, in companies of global importance such as Shell and UOP. Two flights in the past have been tested successfully on the jetfuel made from algae oil. Continental 737–800 made a 1.5 h flight fueled with 50:50 blended biofuel of UOP biojet (made from 47.5% jatropha + 2.5% algae) and JetA-1. It was tested successfully on 7 January 2009. JAL 747-300 made a 1.5 h flight fueled with 50:50 blend biojet: Jet-A (feedstocks camelina 84%, jatropha $<16\%$ and algae $<1\%$, UOP processed). It was tested successfully on 30 January 2009 [55].

2.2. Non energy products from algae

2.2.1. Carbohydrates

The accumulation of carbohydrates in microalgae is due to CO_2 fixation during the photosynthetic process. Photosynthesis is a biological process utilizing ATP/NADPH to fix and convert CO_2 captured from the air to produce glucose and other sugars through a metabolic pathway known as the Calvin cycle [56]. These carbohydrates are either accumulated in the plastids as reserve materials (e.g., starch), or become the main component of cell walls.

Some studies demonstrated that there was a competition between lipid and starch synthesis because the major precursor for triacylglycerols (TAG) synthesis is glycerol-3-phosphate (G3P), which is produced via catabolism of glucose (glycolysis) [57,58]. Thus, to enhance biofuels production from microalgae-based carbohydrates, it is vital to understand and manipulate the related metabolisms to achieve higher microalgal carbohydrate accumulation via strategies like increasing glucan storage and decreasing starch degradation. The carbohydrate content of microalgae could be enhanced by the use of various cultivation strategies, such as irradiance, nitrogen depletion, temperature variation, pH shift, and CO_2 supplement [59–62].

Information about the cell wall composition is necessary to effectively utilize them as carbon sources for bioenergy and as chemical sources for biomaterial and bioproducts. Composition of microalgal cell wall and storage products is given in Table 4. The algal carbohydrates are mainly composed of starch, glucose, cellulose/hemicellulose, and various kinds of polysaccharides. Of these, algal starch/glucose is conventionally used for biofuel production, especially bioethanol [63] and hydrogen production. Except the starch in plastids, microalgal extracellular coverings (e.g., cell wall) are another carbohydrate rich part which could be transformed to biofuel. The major polysaccharide constituents of red algae are galactans such as carrageenan and agar [64,65]. Carrageenans can be readily obtained by extracting red seaweeds or dissolving them into an aqueous solution. Major sugars, present in the brown macroalgae are glucan, mannitol, and alginate. Alginic acid (i.e., alginate), accounts for up to 40% dry wt. as a principal material of the cell wall [66].

Currently, algal polysaccharides represent a class of high-value compounds with many downstream applications in food, cosmetics, textiles, stabilizers, emulsifiers, lubricants, thickening agents and clinical drugs. Algal sulfated polysaccharide exhibit a wide range of pharmacological activity, including acting as antioxidant, antitumor, anticoagulant, anti-inflammatory, antiviral and immunomodulating agents. The sulfated polysaccharides derived from *Porphyridium* sp. have a significant potential for use in anti-inflammatory skin treatments because of their ability to inhibit the migration and adhesion of polymorphonuclear leukocytes [19].

2.2.2. Pigments

Microalgae contain a multitude of pigments associated with light incidence. Besides chlorophyll, the most relevant are phycobiliproteins, which are helpful in improving the efficiency of light energy

Table 4
Composition of microalgal cell wall and storage products [12].

Division	Cell wall	Storage products
Cyanophyta	Lipopolysaccharides, Peptidoglycan	Cyanophycean Starch
Chlorophyta	Cellulose, hemicelluloses	Starch/lipid
Dinophyta	Absence or contain few cellulose	Starch
Cryptophyta	Periplast	Starch
Euglenophyta	Absence	Paramylum/ lipid
Rhodophyta	Agar, carrageenan, cellulose, calcium carbonate	Floridean starch
Heterokontophyta	Naked or covered by scales or with large quantities of silica	Leucosin/lipid

Table 5
Some high-value bioproducts extracted from microalgae [72].

Product group	Applications	Examples (producer)
Phycobiliproteins carotenoids	Pigments, cosmetics, pro vitamins, pigments	Phycocyanin (*Spirulina platensis*) β Carotene (*Dunaliella salina*) Astaxanthin and leutin (*Haematococcus pluvialis*)
Polyunsaturated fatty acids (PUFAs)	Food-additive, Nutraceutics	Eicosapentaenoic acid (EPA) (*Chlorella minutissima*) Docosahexanoic acid (DHA) (*Schizochytrium* sp.) Arachidonic acid (AA) (*Parietochloris incisa*)
Vitamins	Nutrition	Biotin (*Euglena gracillis*) α-Tocopherol (vitamin E) (*Euglena gracillis*) Ascorbic acid (vitamin C) (*Prototheca moriformis, Chlorella* sp.)

utilization and carotenoids which serve as photo-protectors against the photo-oxidative damage resulting from excess energy captured by light-harvesting antenna.

Another pigment obtained from algae is astaxanthin, a kind of ketocarotenoid, known for its powerful antioxidant properties. Astaxanthin has many benefits in the prevention and treatment of various conditions, such as chronic inflammatory diseases, eye diseases, skin diseases, cardiovascular diseases, cancers, neurodegenerative diseases, liver diseases, metabolic syndrome, diabetes, diabetic nephropathy and gastrointestinal diseases. *H. pluvialis* has been identified as an organism that can accumulate the highest level of astaxanthin in nature (1.5–3.0% dry weight), which is currently the prime natural source of astaxanthin for commercial exploitation [15].

Other algal pigments like lutein, zeaxantin and canthaxantin are used for chicken skin coloration and pharmaceutical purposes. Also, phycobiliproteins, phycocyanin and phycoerythrin are already being used for food and cosmetics applications. Carotene is currently used in health foods as a vitamin A precursor and also for its anti-oxidant effect. Anti-oxidants function as free radical scavengers, which give them an anti-cancer property. Many pigments from algae can also be used as natural food colorants, for instance, in orange juice, chewing gum, ice sorbets, candies, soft drinks and dairy products [67].

2.2.3. Protein

Proteins are of major importance in human nutrition and lack of them is one of the biggest factors in malnutrition. Some algae contains up to 60% of protein. Proteins can be used for different purposes such as animal/fish feeds, fertilizers, industrial enzymes, bioplastics, and surfactants.

A well-known alga that is currently cultivated for its protein content is the cyanobacterium species *Athrospira*, better known as *Spirulina. Spirulina* is reported to contain not only around 60% raw protein, but also vitamins, minerals and many biologically active substances. Its cell wall consists of polysaccharides, has a digestibility of 86%, and can be easily absorbed by the human body. *Spirulina* is used as a dietary supplement as well as a whole food; and is available in tablet, flake and powder form. It is also used as a food supplement in the aquaculture, aquarium and poultry industries [68].

Other algae species known to have high protein content are *Anabeana*, *Chlorella*, *Dunaliella* and *Euglena. Anabaena flos-aquae*, a N-fixing and photosynthesizing blue-green algae has been described as a good protein source in [69]. However, due to high production cost as well as technical difficulties to incorporate the algal material into palatable food preparations, the propagation of algal protein is still in its infancy.

2.2.4. Biomaterials and bioproducts

Since macroalgae have respective characteristics for gel-forming and water-dissolving, many of them are used for industrial uses. Agar is a by-product of macroalgal biorefinery because glutamic acid can be a source of valuable bio-based chemicals, i.e., as *N*-methylpyrrolidone, *N*-vinylpyrrolidone. The use of some microalgal species, especially *Arthrospira* and *Chlorella,* are well established in the skin care market and some cosmeticians have even invested in their own microalgal production system. Their extracts are found in anti-aging creams, refreshing or regenerating care products, emollient, as an anti-irritant in peelers and also in sun protection and hair care products [65].

In *Chlorella* species, the most important compound from the medical point of view is 1,3-glucan, an active immunostimulator, a free radical scavenger and a blood lipid reducer. The efficacy of this compound against gastric ulcers, wounds and constipation, preventive action against atherosclerosis and hypercholesterolemia, and antitumor action has also been reported [67]. Microalgae also represent a valuable source of almost all essential vitamins such as A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid [70].

Carrageenan, which can be obtained from algae, is a water soluble group of polysaccharides that are more widely used as emulsifiers and stabilizers in numerous food items. Carrageenans are especially used in chocolate milk, ice cream, evaporated milk, puddings, jellies, jams, salad dressings, dessert gels, meat products and pet foods, due to their thickening and suspension properties. Several potential pharmaceutical uses of carrageenans like antitumor, antiviral, anticoagulant and immunomodulation activities are also mentioned in the literature [71]. Summary of high value bioproducts obtained from algae is given in Table 5.

2.3. Environmental applications

The integrated approach of using microalgae for production of various products is achieved when it is combined with different environment cleaning approaches. For making the overall process more environmentally sustainable and economically viable, the algae growth is achieved using waste water and flue gas treatment. This serves a two way purpose of cleaning the environment together with producing high quantities of biomass, which can be further processed to obtain different high value products.

2.3.1. Bio-mitigation of CO_2 emissions using microalgae

Biological CO_2 mitigation has attracted much attention in the last few years. A large volume of CO_2 is emitted from the power plants and industries into the environment. Therefore, the use of flue gas emissions from an industrial process unit, as a source of CO_2 for microalgae growth, provides a very promising alternative to current GHG emissions mitigation strategies.

Microalgae can fix carbon dioxide from different sources, which can be categorized as (1) CO_2 from the atmosphere, (2) CO_2 from industrial exhaust gases (e.g., flue gas and flaring gas), and (3) fixed CO_2 in the form of soluble carbonates (e.g., $NaHCO_3$ and Na_2CO_3) [73].

Chlorococcum littorale, a marine alga, showed exceptional tolerance to high CO_2 concentration of up to 40%. It was also reported that *Scenedesmus obliquus* and *Spirulina* sp. showed good capacities to fix carbon dioxide when they were cultivated at 30 °C in a temperature-controlled three-stage serial tubular photobioreactor. For *S. obliquus*, the corresponding maximum growth rate and maximum productivity were 0.22 per day and 0.14 g l^{-1} per day, respectively. For *Spirulina* sp., the maximum specific growth rate and maximum productivity were 0.44 per day and 0.22 g l^{-1} per day, with both 6% (v/v) carbon dioxide and 12% (v/v) carbon dioxide, respectively, while the maximum cell concentration was 3.50 g dry cell l^{-1} with both CO_2 concentrations [74–76].

Microalgae *Selenastrum* sp. can efficiently utilize both bicarbonate salt and carbon dioxide gas as carbon source in culture media [76,77]. Microalgal species have a high extracellular carbonic anhydrase activity which is responsible for the conversion of carbonate to free CO_2, to facilitate CO_2 assimilation [78,79].

2.3.2. Bio-remediation of waste water and polluted soil using microalgae

Use of algae for bioremediation of wastewater was first investigated in the 1950s by Oswald and Gotaas [80]. Algae utilize the nutrients present in the wastewater for its growth. The wastewater discharged into the water bodies is hazardous to the environment and can cause various health problems in human beings. One of the benefits of using algae in wastewater treatment is that algae produces O_2 during photosynthesis, which promotes aerobic bacterial degradation of the organic components. Bacterial degradation in turn, produces CO_2, which promotes photosynthesis and the algal uptake of inorganic nutrients [81]. Algae can be used in wastewater treatment for a range of purposes, like removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals [82].

Effect of immobilization on the growth and nutrient removal of *S. obliquus* and *C. vulgaris* in artificial and urban wastewater was analyzed by Sriram and Seenivasan [83]. In case of nitrogen uptake, immobilized microalgae had higher nitrogen uptake than the free cell in both the types of wastewater, but in phosphorus removal efficiency, immobilized cultures removed more phosphorus in artificial wastewater than in urban wastewater.

Nutrient-rich wastewater from municipal sources, the dairy industry, poultry industry and other agricultural practices, which could otherwise lead to nutrient pollution (i.e., eutrophication) of water resources, can be fed into an algal production system, yielding significant pollution control benefits, with no competing land use for food production and sustainable use of underutilized resources for food and fuel production [84].

Interest in the use of microalgae will continue to grow as rural cities and developing countries look for sustainable and affordable ways to clean domestic wastewater. Rahman et al. have suggested a process for bioremediation of domestic wastewater and simultaneous production of bioproducts from microalgae using waste stabilization ponds [85].

Microalgae can also act as a potential sink for removal of toxic and harmful substances from the soil. Microalgae can help in bioremediation of heavy metal ions like iron and chromium. The three algal species, *hydrodictyon* sp., *Oedogonium* sp. and *Rhizoclonium* sp. were used for the bioremediation of heavy metals (Cadmium and zinc) present in the wastewater derived from coal-fired power generation [86]. Algae have the capability to sequester, adsorb, or metabolize these noxious elements into substantial level [87]. Microalgae possess different molecular mechanisms that allow them to discriminate between non-essential heavy metals from those essential ones for their growth [88].

3. Product estimation and characterization

Fatty acid analysis is essential to a broad range of applications, including those associated with the algal biofuel and algal bioproduct industries. Table 6 shows the fatty acid composition of some algal strains. Most recommended methods for the assay of fatty acids begin with the extraction of the targeted product from an organic matrix with a non-polar solvent such as hexane, followed by a two-step transmethylation that converts the acids to fatty acid methyl esters (FAME) [91,92].

AOAC (Association of analytical communities) has listed standard procedures for quantification of lipids and fatty acids which has been applied to the algal biomass also [93]. Bigelow et al. [89] have reported the development of a rapid, microscale, single-step, in-situ protocol for GC–MS lipid analysis that requires only 250 μg dry mass per sample.

Table 6
Fatty acid composition of selected algal strains [89,90].

Strains	Fatty acids (%)					
	16:00	16:01	18:00	18:01	18:02	18:03	20:01
Ankistrodesmus sp.	16.24 ± 0.5	3.06 ± 0.8	7.18 ± 0.7	17.66 ± 0.8	8.48 ± 0.8	28.68 ± 0.5	2.55 ± 0.1
Chlamydomonas reinhardtii	23.77 ± 0.9	1.94 ± 0.6	4.41 ± 2.5	19.73 ± 0.6	6.58 ± 0.9	25.49 ± 0.9	1.21 ± 0.1
Dunaliella sp. *(Persian Gulf)*	9.19 ± 1.5	0.80 ± 0.3	4.27 ± 1.2	22.51 ± 0.7	3.84 ± 0.5	44.31 ± 2.4	1.42 ± 0.3
Dunaliella salina (Shariati)	12.02 ± 1.5	4.45 ± 0.2	1.90 ± 0.6	23.67 ± 2.1	2.28 ± 0.6	40.36 ± 1.9	1.40 ± 0.1
Dunaliella salina (UTEX)	16.33 ± 0.4	1.03 ± 0.2	6.43 ± 0.7	19.57 ± 1.1	6.76 ± 2.5	27.70 ± 2.1	2.28 ± 0.8
Scenedesmus sp.	15.62 ± 0.5	4.06 ± 0.7	2.97 ± 0.9	15.23 ± 0.8	7.00 ± 0.9	22.99 ± 0.5	7.49 ± 2.2
Chlorella emersonii	14.75 ± 0.6	NA	9.80 ± 0.9	17.01 ± 0.3	9.04 ± 1.5	29.32 ± 1.5	2.74 ± 1.4
Chlorella protothecoides	16.15 ± 0.8	NA	6.63 ± 1.4	19.23 ± 0.6	7.02 ± 0.4	29.17 ± 1.9	2.35 ± 0.9
Chlorella salina	21.50 ± 0.8	2.62 ± 1.3	7.83 ± 0.7	14.39 ± 0.5	10.88 ± 0.7	29.75 ± 1.1	1.50 ± 0.5
Chlorella vulgaris	14.55 ± 0.9	1.183 ± 2.5	10.51 ± 1.6	23.62 ± 1.9	13.80 ± 0.5	32.10 ± 1.7	NA
Amphora sp.(Persian Gulf)	28.61 ± 1.1	38.16 ± 0.9	12.66 ± 2.3	ND	3.86 ± 1.5	4.55 ± 0.5	NA
Mallomonas splendens	7.0 ± 0.2	NA	1.1 ± 0.1	9.8 ± 1.8	12.4 ± 0.4	12.9 ± 0.9	NA
Nanochloropsis oculata	10.4 ± 0.3	NA	0.4 ± 0.0	1.7 ± 0.3	1.7 ± 0.1	0.5 ± 0.0	NA
Chrysochromulina sp.	24 ± 0.6	NA	4.6 ± 0.2	3.7 ± 0.5	25.7 ± 0.4	2.5 ± 0.1	NA
Emiliania huxleyi	4.1 0.1	NA	0.3 ± 0.0	6.6 ± 1.2	1.4 ± 0.0	2.5 ± 0.2	NA
Rhodomonas sp.	3.5 0.1	NA	0.2 ± 0.0	0.7 ± 0.1	1.3 ± 0.0	3.6 ± 0.2	NA
Prorocentrum	14.7 0.4	NA	0.6 ± 0.0	1.2 ± 0.2	2.4 ± 0.1	1.2 ± 0.1	NA

Table 7
Overview of conversion routes of plant material to biofuels [101].

Plant material	Conversion route	Primarily product	Treatment	Products
Ligno-cellulosic biomass	Flash pyrolysis	Bio-oil	Hydrotreating and refining	C_xH_x, diesel fuel, chemicals, oxygenates, hydrogen Hydrogen
	Gasification	Syn-gas	Water gas shift + separation Catalysed synthesis	Methanol, dimethyl ether, FT diesel, C_xH_x, SNG (CH4) Bio-ethanol
	Hydrolysis	Sugar	Fermentation	C_xH_x, diesel fuel, Chemicals
	Hydrothermal liquefaction	Bio-oil	Hydrotreating and refining purification	SNG (CH_4)
	Anaerobic digestion	Biogas		
Sugar and starch crops	Milling and hydrolysis	Sugar	Fermentation	Bio-ethanol
Oil plants	Pressing or extraction	Vegetable oil	Esterification Pyrolysis	Biodiesel bio-oil, diesel fuel, gasoline

Lipid extraction based on gravimetric solvent recovery is inherently variable as well as inaccurate due to the extraction of non-fatty acid based compounds such as proteins and pigments, making the quantitative determination of lipids in algal biomass very difficult. Laurens et al. [94] have described a robust whole-biomass in situ transesterification procedure for quantification of algal lipids (as fatty acid methyl esters, FAMEs) which is more accurate and reliable than the traditional solvent based lipid extraction procedures.

A simple yet sensitive spectrophotometric method to indirectly measure the lipids in microalgae is found by measuring the fatty acids (FA) after saponification. The fatty acids were reacted using triethanolamine-copper salts (TEA-Cu) and the ternary TEA-Cu-FA complex was detected at 260 nm using UV–visible spectrometer [95].

Determination of total carbohydrates in algal biomass is explained in Laboratory Analytical Procedure developed by NREL. Portions of this procedure are substantially similar to ASTM E1758 [96]. This procedure involves two-step sulfuric acid hydrolysis to hydrolyze the polymeric forms of carbohydrates in algal biomass into monomeric subunits. The monomers are then quantified by either high-performance liquid chromatography (HPLC) or a suitable spectrophotometric method. An optimized hydrolysis procedure is expected to yield complete hydrolysis of all polymeric structural and storage carbohydrates into monomeric sugars. A range of sequential and optimized inorganic acid hydrolysis conditions with respective hydrolysis liquor collection and analysis should be carried out for algal biomass.

Quantification of chlorophylls and pigments is done after extracting them in solvents like methanol, ethanol and acetone. Absorbance of the extract is determined spectrophotometrically and the pigment content is detected. The separation and quantification of individual carotenoids can be achieved using HPLC equipped with absorption or florescence detector [97]. Different chlorophylls i.e., Chl *a*, Chl *b* and Chl *c* absorb in the wavelength range 450–475 nm, carotenoids in the range 400–550 nm and phycobilins in the range 500–650 nm. Methods for the determination and quantification using high-performance liquid chromatography (HPLC) and several spectrophotometric methods for the quantification of chlorophylls a and b are described in [98]. Total nitrogen for protein determination is generally determined by Kjeldhal method after acid digestion, ammonium distillation under steam current, and titration with 0.1 N HCl [77].

4. Algal biorefinery concept

The concept of biorefining is similar to the petroleum refineries in which multiple fuels and chemicals are derived using crude oil as the starting material. Similarly, biorefining is sustainable biomass processing to obtain energy, biofuels and high value products through processes and equipment for biomass transformation [99]. A more specific and comprehensive definition of a biorefinery has been given by IEA Bioenergy Task 42 document which states, “the sustainable processing of biomass into a spectrum of marketable products and

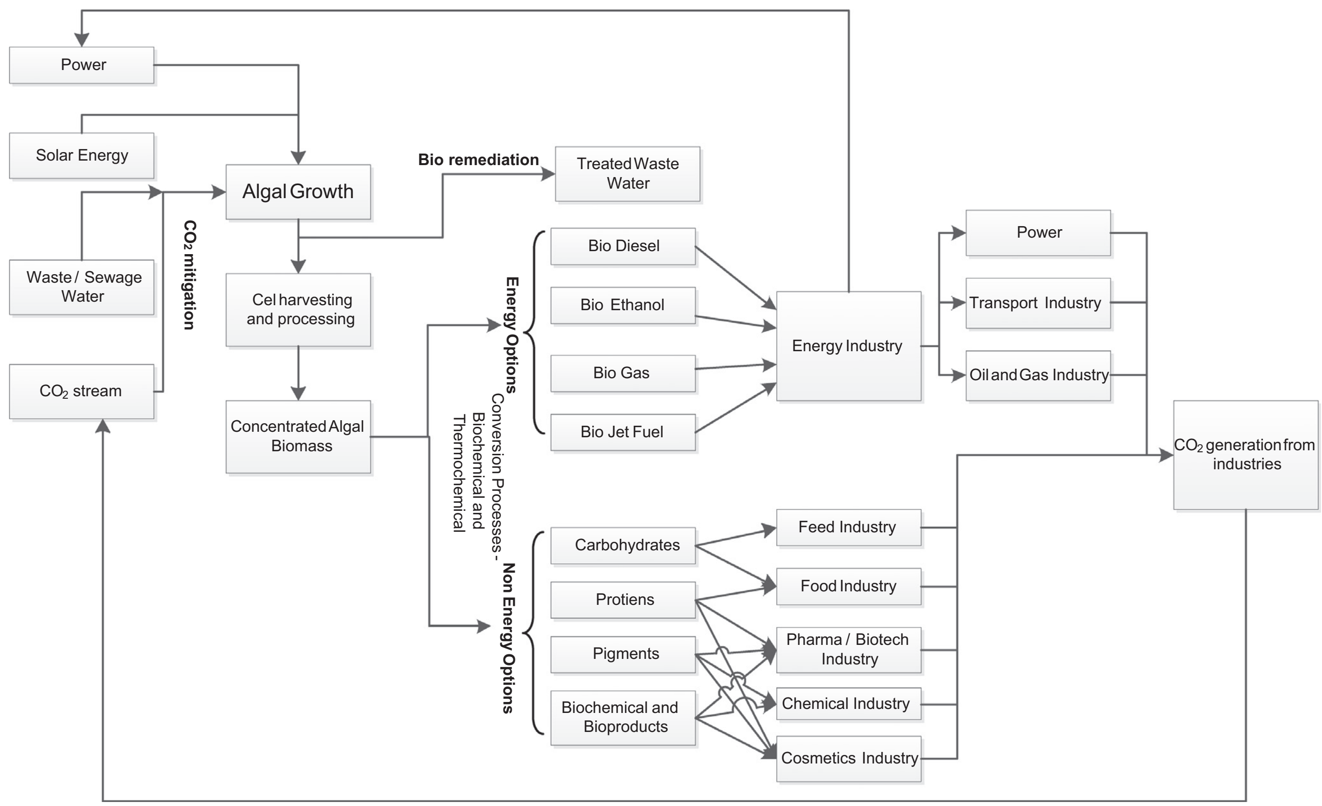

Fig. 3. Proposed schematic flow sheet for a microalgae biorefinery.

energy" [100]. The biorefinery concept has been identified as the most promising way to create a biomass-based industry.

There are four main types of biorefineries: biosyngas-based refinery, pyrolysis-based refinery, hydrothermal upgrading-based refinery, and fermentation-based refinery. Table 7 gives an overview of the different conversion routes of plant material to biofuels and the products obtained. Biosyngas is a multifunctional intermediate for the production of materials, chemicals, transportation fuels, power and/or heat from biomass. Thermochemical and biochemical conversion products from biomass are upgraded before ultimate refining processes. Biorefinery includes fractionation for separation of primary refinery products. The main goal of the biorefinery is to integrate the production of higher value chemicals and commodities, as well as fuels and energy, and to optimize the use of resources, maximize profitability and benefits and minimize wastes [101].

Microalgae are considered to be futuristic raw material for establishing a biorefinery because of their potential to produce multiple products. A biorefinery can take advantage of the differences in biomass components and aims to maximize the value derived from the biomass feedstock [102]. The biorefinery concept can bring many environmental deliverables while mitigating several sustainability-related issues with respect to greenhouse gas emissions, fossil fuel usage, land use change for fuel production and future food insufficiency. A new biorefinery-based integrated industrial ecology encompasses the different value chain of products, co-products, and services from the biorefinery industries. Cross-feeding of products, co-products and power of the algal biofuel industry into the allied industries is desirable for improving resource management and minimization of the ecological footprint of the entire system. The biomass, after the oil has been extracted from it, can be used as animal feed, converted to fertilizer and for power generation. The power generated can then be put back to producing more biomass. The CO_2 released by the power generation plant can be used again for the production of algal biomass, thus reducing CO_2 in the atmosphere [103,104]. The integrated industrial ecology will definitely lead to infrastructure development and regional economic sustainability of rural communities. Bio-refineries can produce energy in the form of heat or by producing biofuels, molecules for fine chemistry, cosmetics or medicinal applications, materials such as plastics and sources of human food and animal feed. Fig. 3 illustrates the proposed schematic flow sheet of the algae based biorefinery.

Integration of the algal sector with the dairy industry could co-produce non-fossil based methanol for biodiesel production. Integration of the algal fuel sector with aquaculture offers a new inland-based animal production system to meet the world's growing protein demands [84]. Process integration is very important to ensure that water is recycled and used efficiently wherever possible as biorefineries use large amounts of water for the purification and separation processes. Economics can be highly improved if the fresh water consumption is reduced without the addition of any extra technologies such as heat exchangers. This can be achieved by using microalgae, which does not require fresh water, such as brackish or marine species [41]. The biorefinery framework can also yield value added by-products which can be used directly or indirectly in the food production sector [105,106]. For example, it is estimated that 100 million tons of protein will be additionally produced from biomass-based biofuel feedstock as a by-product in the future [107]. Integration with the lignocellulosic industry could produce cellulase/hemicellulase like enzyme for hydrolysis processes, thereby increasing the commercial viability of both sectors. Some algal strains such as *Chlamydomonas* and *Dunaliella* which have been genetically modified to express cellulases and hemicellulases opens the door for integrating enzyme production as a by-product from the algal biofuel sector, which in turn can be fed to the enzymatic hydrolysis step in cellulose-based feedstock [84].

Several reports in the recent past have discussed about the development of algal biorefinery. Microalgae strain *Amphiprora* sp. was used for obtaining high value pigments and phycobiliproteins, fermentable sugars, crude algae oil and biodiesel in order to develop a topology of microalgae-based biorefinery [108].

Selected species of microalgae (freshwater algae, saltwater algae and cyanobacteria) were used as a substrate for fermentative biogas production in a combined biorefinery. Anaerobic fermentation has been considered as the final step in future microalgae-based biorefinery concept [109]. Gouveia [110] has discussed in detail, different production routes in the biorefineries based on different algal strains. *Nannochloropsis* sp. biorefinery has the potential for the production of oil, high value pigments and biohydrogen. However, economically the most favorable biorefinery was the one producing oil, pigments and H_2 via Supercritical Fluid Extraction (SFE). *Anabaena* sp. biorefinery included H_2 production through autotrophic route as well as by dark fermentation through *Enterobacter aerogenes*. The biorefinery stated by Campenni et al. [111] used *Chlorella protothecoides* as a source of lipids and carotenoids. *Chlorella* was grown autotrophically under high salinity and luminosity stress conditions. The leftover biomass can be used for hydrogen or bioethanol production in a biorefinery approach, as the residue still contains sugar, taking advantage of all the *C. protothecoides* gross composition. Olguin [112] highlighted that the biorefinery strategy offers new opportunities for a cost-effective and competitive production of biofuels along with non-fuel compounds. Author studied an integrated system where the production of biogas, biodiesel, hydrogen and other valuable products (e.g. PUFAs, phycocyanin, and fish feed) could be possible. Pacheco et al. [113] pointed a biorefinery using *Spirogyra* sp., a sugar-rich microalga, for biohydrogen and pigments production. Pigment production was necessary to improve the economic benefits of the biorefinery, but it was mandatory to reduce its extraction energy requirements that are demanding 62% of the overall energy. Budarin et al. [114] have focused on the development of a microalgae biorefinery concept based on hydrothermal microwave pyrolysis, which will not need water removal and therefore could be more energy efficient.

The main bottleneck of the biorefinery approach is to separate the different fractions without damaging one or more of the product fractions. There is a need for mild, inexpensive and low energy consumption separation techniques to overcome these bottlenecks [110].

However, a multisector restructuring for a new industrial ecology needs to be developed to encompass the value chain of different products, by-products and services from the booming algal biorefinery industries.

5. Techno-economic feasibility of the algal based options

Oil from algae is likely to be an important energy feedstock of the future. A detailed review of algae-to-fuels research, development, and commercialization would not be complete without an investigation of the potential costs of the technology. There is great uncertainty with respect to the economics of future commercial scale algal production. Economic considerations and principles of green design suggest that if algae-to-fuel technology is to be successful, biofuels must be produced simultaneously with value-added co-products.

The properties of the biodiesel obtained is dependent on the fatty acid profile of the algae oil. The fatty acid profile of algal oils tends to both extremes, namely high content of saturated FAs (Fatty acids) and high content of PUFAs (Poly unsaturated fatty acids). Overall, palmitic acid is the most commonly occurring fatty acid in algal oils. Myristic acid also appears to be the second most commonly found fatty acid in algae oils. However, the presence of saturated FAs and PUFAs in the algae oil leads to poor cold flow properties and poor oxidative stability simultaneously. At the same time cetane number of the algal biodiesel may meet the requirements in the standards, due to the presence of high cetane number compounds like methyl meristate and methyl palmitate. Kinematic viscosity of the algal biodiesel also fits in the limits set by the standards [115]. However, the favorable properties exhibited by the algal biodiesel are beneficial only when coupled with high production rates and consistency in the production process.

Many algal species are highly sensitive to the factors like temperature, light intensity and nutrients. Any deviation in these factors may cause a change in the product profile. Therefore the process parameters and conditions should be highly controlled to obtain reproducible results. Peter et al. in their publication report showed that the biochemical composition of the biomass influences the economics; in particular, increased lipid content reduces other valuable compounds in the biomass [27]. It also mentions that the hardest problems in assessing the economics are the cost of the CO_2 supply and the uncertain nature of the downstream processing.

Although significant literature and reports are available on microalgae growth, more work needs to be undertaken. Photobioreactors are expensive to scale, so direct secretion of fuel molecules from algae cells has attracted interest as a way of reducing the cost of harvesting. Use of algal strains which naturally secrete lipids or algae engineered to secrete lipids from the direct photosynthetic conversion can help in decreasing the downstream processing costs [116]. Both primary and secondary harvesting procedures are energy-intensive and expensive. Improving this aspect of algal processing will reduce costs associated with biogas and biodiesel production. Also, the development of more efficient and environmental friendly lipid extraction/transesterification processes is needed to improve the sustainability of algal biodiesel [117]. At large scale applications the use of artificial media is not profitable or viable, therefore the use of wastewater and flue gas (or other waste streams) would be highly recommended for microalgae cultivation [118]. The combination of wastewater treatment and other waste streams combined with anaerobic digestion of the microalgae after valuable lipids have been removed, improves the profitability of the plant considerably [119].

Algal biodiesel production is currently 2.5 times as energy intensive as conventional diesel, but co-production and decarbonisation of the electricity utilized in the production process will make algal biodiesel a financially and environmentally viable option for future transport energy infrastructure [120].

According to Rajkumar et al., higher net value could be achieved by using a combined operation in which algal lipids are converted to diesel fuel and cellulosic part of the biomass (after lipid extraction) is enzymatically converted to glucose, which is fermented to produce bioethanol and other by-products [121].

Life cycle analysis (LCA) of any product is an important aspect and should be kept in consideration for its feasibility in usage. According to ISO 10440, LCA is a "compilation and evaluation of the inputs and outputs and the potential environmental impacts of a product system throughout its life cycle". [122]. Thus, LCA gives us an overall picture of the superior quality of energy dynamics, reliability and environmental impacts [118]. Esra and Mustafa have calculated the exergetic efficiency of the environment friendly algal biodiesel production process. The renewability indicator is found to be positive, thereby showing that algae-biodiesel-carbon dioxide cycle is indeed renewable [123]. Also, simulation studies incorporating fluid dynamics and algae growth models will be very useful in predicting the reactor design efficiency and life cycle analysis. This way viability of options for large scale culturing can be determined [124].

Summing up all the above aspects, the economic feasibility of algal biofuel production will depend on lowering the costs and/or increasing the efficiency of the following: (i) culturing systems, water, nutrient and CO_2 requirements, (ii) modification of the photosynthetic capability and productivity, (iii) the method of the cell harvest (iv) the method of cell rupture and/or subsequent lipid extraction, (v) cost of the bio/green diesel production from the crude lipid fraction and (vi) market potential and value of the by-products and/or energetic value of the 'waste' fraction [124].

Dozens of startup companies are attempting to commercialize algal fuels. According to 2011 editorial survey of many current top-level research endeavors into algal biofuels, producing biofuels from algae at smaller scales is already well established, but the current production methods require significant improvement [125]. Thus, technological advances and highly optimized production systems are required are required for making algal biofuels more economically sound [126]. Specifically, upstream advancements regarding genetic modification of algal species and downstream upgrades to the separation and extraction technologies are necessary for commercial viability of biofuels produced from algae [127]. Most importantly, following an integrated biorefinery approach with cross feeding of products can increase the profitability to a huge extent and can change the whole perspective with which the algal biofuel industry is seen at present.

6. Conclusions and perspective

The percentage of lipids, carbohydrates, proteins or any other product obtained from the algae depends on the nature of the algal strain and the conditions provided during its growth. Also, the yield of the quantities varies with the parameters set-up during the various stages of growth. Thus, an intelligent choice of the algal strain and the growth conditions has to be made for obtaining the desirable products. Also, significant cost reductions may be achieved if CO_2, nutrients and water can be obtained at low cost. A major R&D initiative is required to enhance the yield of the products and at the same time reduce the overall operating cost.

Hence, if someone talks of algal biorefinery, one should bear in mind the marketing potential of the product chosen and its integration with various industries. To make the whole system more appropriate and logical, a sound analysis of the market driving forces is very necessary.

Algae possesses a huge potential for use as a raw material in biorefinery as it is capable of producing a range of products. Therefore, the "only biofuel" production approach will not be commercially viable and the economics of other options will play the key role. So, in place of the single product line conventional approach, a matrix approach leading to numerous options is desirable for successful operation of algal biorefinery. To sum up, if it is possible to restructure the industry in the above proposed fashion, the algal biorefinery approach will make complete sense, and it will prove to be the most beneficial feedstock for production of fuels and chemicals in the future.

Acknowledgements

The authors are grateful to the Director, CSIR-Indian Institute of Petroleum for his never ending support and also for providing the necessary facilities for the execution of algae project CSC 0116/03.

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