Renewable and Sustainable Energy Reviews 55 (2016) 909–941

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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

Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach

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ARTICLE INFO

Article history:
Received 1 March 2015
Received in revised form
16 October 2015
Accepted 13 November 2015
Available online 5 December 2015

Keywords:
Macroalgae
Microalgae
Biofuels
Biorefinery
Carotenoid
Phycobiliprotein
Biochemical Isotopes

ABSTRACT

Due to diminishing petroleum reserves and deleterious environmental consequences of exhaust gases from fossil-based fuels, research on renewable and environment friendly fuels has received a lot of impetus in the recent years. However, the availability of the non-edible crops serve as the sources for biofuel production are limited and economically not feasible. Algae are a promising alternative source to the conventional feedstocks for the third generation biofuel production. There has been a considerable discussion in the recent years about the potential of microalgae for the production of biofuels, but there may be other more readily exploitable commercial opportunities for macroalgae and microalgae. This review, briefly describes the biofuels conversion technologies for both macroalgae and microalgae. The gasification process produces combustible gases such as H_2, CH_4, CO_2 and ammonia, whereas, the product of pyrolysis is bio-oil. The fermentation product of algae is ethanol, that can be used as a direct fuel or as a gasohol. Hydrogen can be obtained from the photobiological process of algal biomass. In transesterification process, algae oil is converted into biodiesel, which is quite similar to those of conventional diesel and it can be blended with the petroleum diesel. This study, also reviewed the production of high value byproducts from macroalgae and microalgae and their commercial applications. Algae as a potential renewable resource is not only used for biofuels but also for human health, animal and aquatic nutrition, environmental applications such as CO_2 mitigation, wastewater treatment, biofertilizer, high-value compounds, synthesis of pigments and stable isotope biochemicals. This review is mainly an attempt, to investigate the biorefinery concept applied on the algal technology, for the synthesis of novel bioproducts to improve the algal biofuels as even more diversified and economically competitive.

The employment of a high-value, co-product strategy through the integrated biorefinery approach is expected to significantly enhance the overall commercial implementation of the biofuel from the algal technology.

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http://dx.doi.org/10.1016/j.rser.2015.11.026
1364-0321/

1. Introduction

1.1. Biofuels scenario

In this twenty first century, a major research emphasis is given to the development of petroleum, coal and natural gas based refinery to exploit the less expensive fossil feedstocks. These feedstocks are used in industry to produce multiple products such as fuel, fine chemicals, pharmaceuticals, detergents, synthetic fiber, plastics, pesticides, fertilizers, lubricants, solvent, waxes, coke, asphalt, etc. to meet the growing demand of the population [1,2]. Currently, the fossil resources are not regarded as sustainable and questionable from the economic, ecology and environmental point of views [3].

The burning of fossil fuels is a big contributor to increase the level of CO_2 in the atmosphere, which is directly associated with the global warming observed in very recent decades [4]. The adverse effects of greenhouse gas (GHG) emissions on the environment, together with declining petroleum reserves, have been realized. Therefore, the quest for sustainable and environmentally benign sources of energy for our industrial economies and consumer societies has become urgent in the recent years [5].

In order to reduce the carbon emissions and the dwindling reserves of crude oil, liquid fuels derived from plant material – biofuels – are an attractive source of energy [6]. Thus, the only possible solution to this crisis is to find a sustainable (renewable) and economically feasible source of alternative energy [7]. There are many alternative energy sources such as wind, solar, geothermal and biomass which fulfill the first criterion (sustainability). However, a few of these can fulfill the second criterion (economic feasibility). The best option, to fulfill both the above criteria, is biofuel, particularly that is made from a readily available biomass feedstock [8–10]. Biofuels are liquid or gaseous fuels for the transport sector that are predominantly produced from a variety of bio-feedstocks. Bio-feedstocks or biomass refers to all the vegetable matter that can be obtained from photosynthesis. They are renewable, sustainable, biodegradable, carbon neutral for the whole life cycle and environmentally friendly; they encourage green industries and agriculture and are applicable as motor fuels, without or with slight engine modifications. The great versatility of biomass as a feedstock is evident from the range of materials that can be converted into various solid, liquid and gaseous fuels using biological and thermochemical conversion processes [7].

Several biofuels, including bio-ethanol, -methanol, -diesel and -hydrogen, appear to be attractive options for the future of transport sector. The production of biofuels is expected to rise steadily in the next few decades [11]. Biomass energy is the largest renewable energy source, representing 10.4% of the world's total primary energy supply or 77.4% of global renewable energy supply [12]. At present, several countries such Brazil, the United States, Germany, Australia, Italy and Austria are already using biofuels such as bioethanol and biodiesel. It is expected that, this trend will continue to grow and more countries will use biofuels [13,14]. Global biofuel production has tripled from 4.8 billion gallons in 2000 to about 16 billion in 2007, but still accounts fall less than 3% for the global transportation fuel supply, according to US Department of Agriculture report [15].

1.2. First generation biofuels

'First generation' biofuels can offer some CO_2 benefits and can help to improve domestic energy security. However, a concern exists about the sourcing of feedstocks, including the impact, it may have on biodiversity and land use and competition with food crops. A 'first generation' biofuel (i.e. biodiesel (bioesters), bioethanol and biogas) is characterized either by its ability to be blended with petroleum-based fuels, combusted in existing internal combustion engines and distributed through existing infrastructure, or by the use in existing alternative vehicle technology like FFVs ("Flexible Fuel Vehicle") or natural gas vehicles [16]. The production of first generation biofuels is commercial today, with almost 50 billion liters produced annually. There are also other niche biofuels, such as biogas which have been derived by an anaerobic treatment of manure and other biomass materials. However, the volumes of biogas used for transportation are relatively smaller today [4].

However, the first generation biofuels seems to create some skepticism to scientists. There are concerns about environmental impacts and carbon balances, which sets limits in the increasing production of biofuels of first generation (Table 1). The main disadvantage of first generation biofuels is the food-versus-fuel dispute, one of the reasons for rising food prices is due to the increase in the production of these fuels [17]. Therefore, for the abatement of GHG, it is recommended to have more efficient alternatives based on both renewable and conventional technologies [18].

The sustainable and economic production of first generation biofuels has however come under scrutiny. Their potential to meet liquid transport fuel targets is being set by the government to help in achieving the goals of oil-product substitution, economic growth and climate change mitigation, which are limited by [19]:

1. Competition for land and water used for food and fiber production [20,21];
2. High production and processing costs that often require government subsidies in order to compete with petroleum products [22];
3. Widely varying assessments of the net greenhouse gas (GHG) reductions once land-use change is taken into account [23];

Therefore, lignocellulosic feedstock can offer the potential to provide novel biofuels, the biofuels of the 'second generation' [24].

1.3. Second generation biofuels

Second generation biofuels produced from 'plant biomass' refer largely to lignocellulosic materials, as these make up the majority of the cheap and abundant nonfood materials available from plants. However, at present, the production of such fuels is not cost effective because there are a number of technical barriers that need to be overcome before their potential can be realized [18]. Plant biomass represents one of the most abundant and underutilized biological resources on the planet and is seen as a promising source of material for fuels and raw materials. At its most basic, plant biomass can simply be burned in order to produce heat and electricity. However, there is a great potential in the use of plant biomass to produce liquid biofuels. However, biofuel production from agricultural by-products could only satisfy a proportion of the increasing demand for liquid fuels. This has generated a great interest in making use of dedicated biomass crops as feedstock for biofuel production [25]. Therefore, it is anticipated that, these second generation biofuels could significantly reduce CO_2 production, do not compete with food crops and some types can offer better engine performance (Table 1).

When commercialized, the cost of second generation biofuels has the potential to be more comparable with standard petrol, diesel and would be a cost effective route to renewable, low carbon energy for road transport [4].

1.4. Algae: renewable feedstock for biofuels

Algae are responsible for over 50% of primary photosynthetic productivity on earth and are budding sunlight factories for a wide range of potentially useful products, but are rarely used for commercial purposes [26–29].

Several biofuel candidates were proposed to displace fossil fuels, in order to eliminate the vulnerability of energy sector. Biodiesel and bioethanol produced from terrestrial plants have attracted the attention of the world as potential substitutes. The availability of crops that serve as source for biofuel production is limited [30]. However, due to "food versus fuel" competition as well as land consumption of these biofuels, they have brought much controversy and debate on their sustainability [31].

Therefore, it is necessary to find new feedstock, suitable for biofuel production, which does not drain the edible feedstock supply. One alternative to the conventional crop is algae. Third generation technology is based on algae or cyanobacteria that contain a high oil mass fraction grown in ponds.

Biofuel production from algae is widely considered as one of the most efficient methods. They appear to represent the recent renewable source of biofuels that could meet the global demand for transport fuels [32]. Algae represent an economically and environmentally sustainable, renewable source of biomass for the production of biofuels [33]. Algae are simple aquatic organisms that photosynthesize, but there are an estimated approximately 300,000 species, whose diversity is much greater than that of the land plants [6].

Algae can convert almost all of the energy in biomass residuals and wastes to methane and hydrogen. Certain algae and cyanobacteria have high lipid contents [34]. Algae can be cultivated in farms absorbing CO_2 from the air. They contain oils that can be used as raw material for biodiesel production [35]. Algae also contain carbohydrates, which can be converted into bioethanol. They have the advantage that, they do not conflict with food production (Table 1). In addition, they have the potential to cover the global demand for transportation fuels [30].

Algae's potential as a feedstock is dramatically growing in the biofuel market. Algae have many desirable attributes as energy producers [36–38]:

1. Algae is the most promising non-food source of biofuels,
2. Algae has a simple cellular structure,
3. Algae contain lipid-rich composition (40–80% in dry weight). Microalgae produce 15–300 times more lipid for biodiesel production than the traditional crops, which do on an area basis [39]. Their lipid content could be adjusted through changing growth medium composition [40].
4. A rapid reproduction rate and high growth rate; e.g., doubling in 24 h [41]. They could be harvested more than once in a year [42].
5. Salty or wastewater could be used [42].
6. Atmospheric carbon dioxide is the carbon source for the growth of microalgae [42].
7. Algae biofuel contains no sulfur, is non-toxic and highly biodegradable [42].

From a practical point of view, they are easy to be cultivated, can grow with slight or even no attention, using water, which is unsuitable for human consumption and effortless to obtain nutrient [43].

1.5. Enhancement of economic feasibility of biofuels from algae

Algae can be used as a feedstock for biofuel production and also play a major role to control environmental pollution and provides plenty of nutritional supplements. Potential of algae for other purposes are as follows:

- Removal of carbon dioxide (CO_2) from industrial flue gases by algae bio-fixation [44], reducing the GHG emissions of a company or process while producing biodiesel. High purity CO_2 gas is not required for algae culture. It is possible that flue gas containing 2–5% CO_2 can be fed directly. This will simplify CO_2 separation from flue gas significantly [45].
- Wastewater treatment by utilizing water contaminants such as NH_4^+, NO_3^-, PO_4^{3-} can be effectively used as nutrients for microalgae [43–45].
- Oil extracted algal biomass (after oil extraction from algal biomass) can be used to produce ethanol, methane or simply burned for energy co-generation (electricity and heat). It can

Table 1
Comparison of three-generation biofuels with petroleum products.

	Petroleum products	First generation	Second generation	Third generation
Technology	Petroleum refinery	Microbial fermentation, chemical and enzymatic transesterification	Pretreatment, hydrolysis and fermentation, transesterification	Metabolic engineering for direct synthesis, fractionation of algal biomass
Feedstocks	Crude petroleum	Vegetable oils and corn sugar feedstocks	Non food, cheap and abundant plant waste biomass (Agricultural and forest residue, etc)	Algae
Products	CNG,LPG, diesel, petrol, kerosene and jet fuel	Biodiesel, corn ethanol, sugar alcohol	Hydrotreating oil, bio oil, FT oil, lignocellulose ethanol, butanol, mixed alcohols	Biodiesel, bioethanol, biohydrogen, biomethane
Benefits	1. High energy density: highly compact portable source of energy used for most forms of mechanical transportation	1. Environmental friendly 2. Economic and social security	Environmental friendlyNot competing with foodAdvance technology still under development to reduce the cost	1. Environmental friendly 2. Not competing with food and Agricultural land 3. Oil productivity is very high when compared with all other biomass 4. Algae is the most promising non-food source of biofuels 5. Algae has a simple cellular structure 6. Lipid-rich composition (40–80% in dry weight) 7. A rapid reproduction rate 8. Algae can grow in salt water and harsh conditions 9. Algae thrive on carbon dioxide from gas- and coal-fired power plants 10. Algae biofuel contains no sulfur, is non-toxic and highly biodegradable.
Problems	1. Depletion/ 2. Declining of petroleum reserve 3. Environmental pollution 4. Economic and ecological problems	1. Limited Feedstocks 2. Food vs. fuel competition 3. Blended partially with conventional fuel	1. Agricultural land consumption 2. Complicated processes	1. Low product yield at large scale 2. Less biomass production

also serve as a livestock feed, used as organic fertilizer due to its high N:P ratio [43,44].

- Combined with their potential to grow under undefined conditions and their limited nutritional requirement, they can be grown in areas which are unsuitable for agricultural purposes, independent of the seasonal weather changes, thus, not competing for arable land use and make use of wastewater as the culture medium, without the need for freshwater [43].
- Algae's high value commercial products could offset the capital and the operation costs of the process. Products are: (a) mineralized carbon for stable sequestration; and (b) compounds of highly commercial value. By selecting appropriate algae species, either one or both can be produced. Depending on the type of algae species, high value bio-active compounds can also be extracted, including a large range of fine chemicals and bulk products, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants and other fine chemicals and biomass [46,47].
- Because of this variety of high-value biological derivatives, with many possible commercial applications, algae can potentially revolutionize a large number of biotechnology areas including biofuels, nutrition and food additives, cosmetics, aquaculture, pharmaceuticals and prevention of environmental pollution [43,47].

1.5.1. *Biorefinery approach: the high-value co-product strategy*

The term "biorefinery" was coined to describe the production of biofuels as well as high value co-products from biomasses by the integration of bioprocessing and appropriate low environmental impacting chemical technologies in a cost-effective and environmentally sustainable manner [39]. Algae biorefinery approach is represented in Fig. 1 [16].

Both macroalgae and microalgae have the capacity to produce vast array of high-value bioactive compounds that can be used as pharmaceutical compounds, health foods and natural pigments [48]. Significant examples includes: Alginates, Carrageenans, Agars, Agarose [49], acetylic acids, β-carotene [50,51], vitamin B [52], ketocarotenoid astaxanthin [53], polyunsaturated fatty acids [54] and lutein [51,55]. The economical feasibility of algal biofuel production should be significantly enhanced by a high-value co-product strategy. This strategy involves sequentially, the cultivation of algae with an algal farming facility (CO_2 mitigation, wastewater treatment), extracting bioreactive products from harvested algal biomass, thermal processing (pyrolysis, liquefaction, or gasification), extracting high-value chemicals from the resulting liquid, vapor and/or solid phases and reforming/ upgrading biofuels for different applications.

In the year 2010, Naik et al. [16] discussed the integral utilization of these feedstocks for the production of value added chemicals and biofuels. They also reviewed the cost reduction technologies and the processes to convert biomass into useful liquid biofuels and bioproducts.

In accordance with the current review, Brennan and Owende [56] premeditated the technologies underpinning microalgae-to-biofuels systems, focusing on the biomass production, harvesting, conversion technologies and the extraction of useful co-products. The study also includes the synergistic coupling of microalgae propagation with the potential for mitigation of environmental impacts associated with energy conversion and utilization.

1.6. *Algae: potential raw materials for commercial applications*

Algae have a great potential to produce a wide range of important biochemicals for food, medical research and other uses and many exciting and important biochemicals are yet to be discovered from microalgae [27,29,47,57–60]

Apart from biofuel production algae will serve as a potential renewable source for the following commercial applications [61]: *i.*

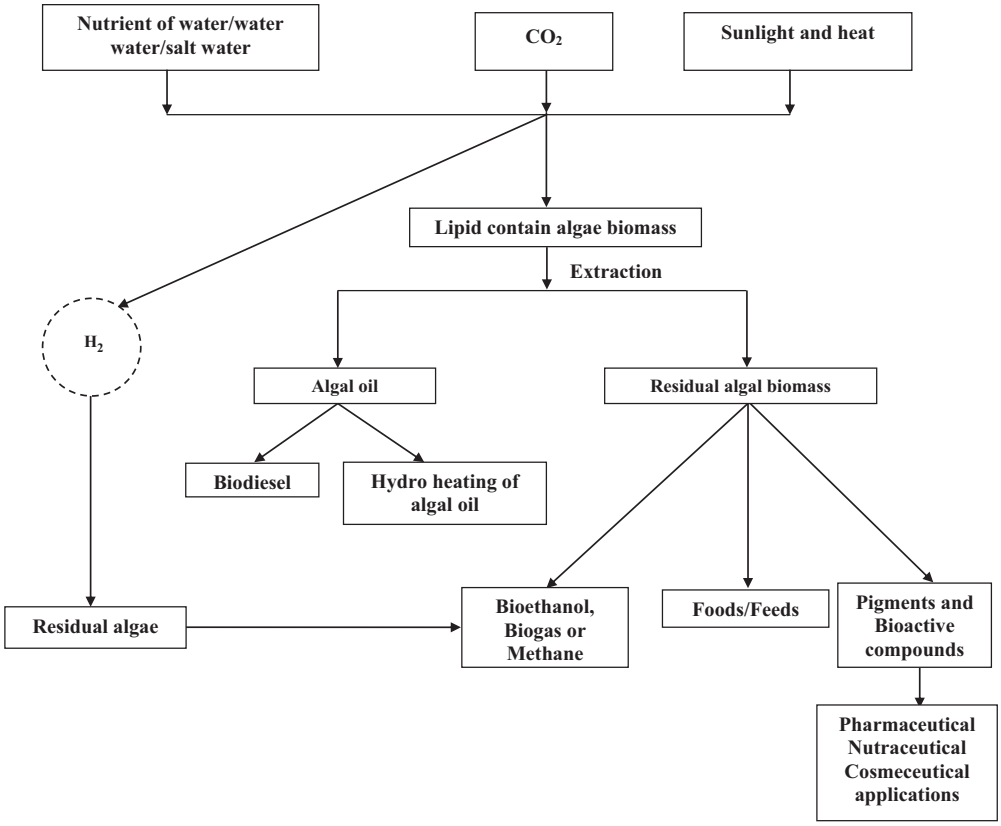

Fig. 1. Biorefinery approach for algal feedstock.

Environmental applications such as wastewater treatment and CO_2 mitigation; *ii.* Human nutrition; *iii.* Animal and aquatic feed; *iv.* Cosmetics product production; *v.* High-value molecules such as fatty acids; *vi.* Pigments synthesis such as β-carotene, astaxanthin, phycobiliproteins; *vii.* Stable isotope biochemicals; *viii.* Biofertilizer; *ix.* Drug synthesis for antimicrobial, antiviral, antibacterial and anticancer (Fig. 1).

Algae have three fundamental attributes that can be converted into technical and commercial advantages [57]:

(1) They are genetically a very diverse group of organisms with a wide range of physiological and biochemical characteristics; thus they naturally produce many different and unusual fats, sugars, bioactive compounds, etc.
(2) They can cost-effectively incorporate the stable isotopes ^{13}C, ^{15}N and ^{2}H into their biomass and thus into the various compounds they produce.
(3) They comprise a large, unexplored group of organisms and thus provide a virtually untapped source of products.

Limited reports were established for the production of biofuels from macroalgae and the commercial applications of both macroalgae and microalgae. Therefore, this deficient endeavor is taken as a main objective for this review.

This review focuses on possibilities of different types of biofuel such as bio-diesel, -ethanol, -hydrogen, -methane, -propane production from both macroalgae and microalgae and the production technologies are briefly discussed. Furthermore, this review furnishes the outline of “biorefinery concepts” to enhance the economic feasibility of biofuels from algae combined with high value co-product strategy. Commercial value assessment of macroalgae and microalgae are elaborated in detail.

This discussion aims to highlight the potential of algae, both macroalgae and microalgae for biofuel production and synthesis of novel high value compounds in commercial echelon, so as to improve the mortal life of algae. Therefore, in the first part, biology of algae and biochemical composition of macro- and micro-algae are presented. Then the conversion technologies for algal biomass into biofuels and integrated approaches of biofuel production with environmental pollution control are described in detail. Finally, commercial applications of microalgae and macroalgae are elaborated.

2. Biology of algae

Algae are photosynthetic aquatic organisms. The term “algae” refers to a polyphyletic, artificial assemblage of organisms [62]. The term is often used to refer specifically to eukaryotic organisms, thus excluding photosynthetic bacteria (such as cyanobacteria, which are also referred to as ‘blue-green algae’). They may be unicellular (microalgae) or multicellular (macroalgae). The latter category includes seaweeds. The algae are very diverse in evolutionary terms. With over 40,000 species already identified and with many more yet to be identified, algae are classified in to multiple major groupings as follows: cyanobacteria (Cyanophyceae), green algae (Chlorophyceae), diatoms (Bacillariophyceae), yellow-green algae (Xanthophyceae), golden algae (Chrysophyceae), red algae (Rhodophyceae), brown algae (Phaeophyceae), dinoflagellates (Dinophyceae) and ‘pico-plankton’ (Prasinophyceae and Eustigmatophyceae). Algae are made up of eukaryotic cells. These are cells with nuclei and organelles. All algae have plastids, the bodies with chlorophyll that carry out photosynthesis. However, the various strains of algae have different combinations of chlorophyll (chl) molecules (Table 2). Some have only chl A, some A and B, while other strains, A, C and D [63].

2.1. Macroalgae

Macroalgae or seaweed is a macroscopic, multicellular, marine algae that lives near the seabed (benthic) [64]. The term includes some members of the red, brown and green algae. A seaweed may belong to one of the several groups of multicellular algae: the red algae, green algae and brown algae. As these three groups are not thought to have a common multicellular ancestor, the seaweeds are a polyphyletic group. In addition, some tuft-forming bluegreen algae (cyanobacteria) are sometimes considered to be seaweeds. Seaweeds' appearance resembles non-arboreal terrestrial plants.

Macroalgae contain the following parts:

- Thallus: the algal body
  Lamina or Blade: a flattened structure that is somewhat leaf-like
1. Sorus: a spore cluster
2. On *Fucus*, air bladder: a floatation-assisting organ on the blade
3. On kelp, float: a floatation-assisting organ between the lamina and stipe

Stipe: a stem-like structure, may be absent
Holdfast: a specialized basal structure providing attachment to a surface, often a rock or another alga
Haptera: a finger-like extension of the holdfast anchoring to a benthic substrate

The stipe and blade are collectively known as the frond.

2.2. Microalgae

Microphytes or microalgae are microscopic algae, typically found in freshwater and marine systems [6]. They are unicellular species, which exist individually, or in chains or groups. Depending on the species, their sizes can range from a few micrometers (μm) to a few hundreds of micrometers. Unlike higher plants, microalgae do not have roots, stems and leaves. Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and simultaneously use the greenhouse gas carbon dioxide to grow photo autotrophically.

The biodiversity of microalgae is enormous and they represent an almost untapped resource. It has been estimated that about 20,000–800,000 species exist of which about 40–50,000 species are described [65,66].

2.3. Biochemical composition of algae

Algal biomass contains three main components: proteins, carbohydrates and lipid. The chemical compositions of various micro- and macro-algae are shown in Table 3 [57,67]. While the percentages vary with the types of algae, which comprise upto 40% of their overall mass of fatty acids [57,68]. This fatty acids (oil) can be extracted and converted into biodiesel.

3. Algal biofuels

Diverse varieties of biofuels such as bio-oil, -diesel, -ethanol, -methane, -hydrogen, syngas and charcoal can be derived from algal biomass using multidisciplinary conversion technology. The following section of possible conversion process and end products are presented briefly.

Table 2
Properties of major algal taxonomic groups.

S. no.	Taxonomic group	Nuc	Chlorophyll	Carotenoids	Bilo proteins	Storage products	Flagellation & cell structure
1.	Bacillariophyta (Diatoms)	Eu	a,c	β-Carotenefucoxanthin.	No	Chrysolaminarin, oils	1 Apical flagellum in male gametes: cell in two halves with elaborate markings.
2.	Chloro phycophyta (green algae)	Eu	a,b	β-Carotene, rarely lycopene, lutein.	No	Amylose (starch), oils	1, 2, 4 to many, equal, apical or subapical flagella.
3.	Chrysophycophyta (golden algae)	Eu	a,c	β-Carotene, fucoxanthin	No	Chrysolaminarin, oils	1 or 2 unequal, apical flagella, in some, cell surface covered by characteristic scales.
4.	Cyanobacteria (blue green algae)	Pro	a,c	β-Carotene, phycobilins	Phycoerythrin, phycobilins	Glycogen	Non-flagellated forms
5.	Phaeco phycophyta (brown algae)	Eu	a,c	β-Carotene, fucoxanthin, violaxanthin	No	Laminarin, soluble carbohydrates, oils	2 Lateral flagella
6.	Dinophyta (Dinoflagellates)	Meso	a,c	β-Carotene, peridinin, neoperididnin dinoxanthin, neodinoxanthin.	No	Starch, oils	2 Lateral, 1 trailing,1 girdling flagellum, in most, there is a longitudinal and transverse furrow and angular plates.
7.	Rhodo phycophyta (red algae)	Eu	a, rarely d	β-Carotene, zeaxanthin	Phycoerythrin, Phycocyanin	Floridean starch Oils, Glycogen	Flagella absent
8.	Euglenoids	Meso	a,b	Phytoene, phytofluene, c-carotene, 8-zeacarotene, and f-carotene	No	Paramylon	Euglenoids have two flagella rooted in basal bodies located in a small reservoir at the front of the cell. In Euglena, one flagellum is very short, and does not protrude from the cell, while the other is relatively long, and often easily visible with light microscopy.
9	Cryptophyta	Eu	a,c_2	Alloxanthin	Phycoerythrin	Amylose	Two slightly unequal flagella
10	Haptophyta	Eu	a,c_1,c_2	Hexanoyloxyfucoxanthin, fucoxanthin, and zeaxanthin	No	Chrysolaminarin	Two slightly unequal flagella
11	Xanthophyta (yellow green algae)	Eu	a,c	β-Carotene, diadinoxanthin	No	Chrysolaminarin	1 Flagella
12	Raphidiophyta (Chloromonads)	Eu	a,c	β-Carotene, diadinoxanthin zeaxanthin, violaxanthin and an auroxanthin	No	Oils	Pair of flagella

Nuc=nuclear characteristics (Prokaryote, Mesokaryote, or Eukaryote).

Table 3
Biochemical composition of macroalgae and microalgae expressed on a dry matter basis (% dry weight) [57,67].

Algae	Protein	Carbohydrates	Lipid
Macroalgae			
Hypnea valentiae	11.8–12.6	11.8–13.0	9.6–11.6
Acanthophora spicifera	12.0–13.2	11.6–13.2	10.0–12.0
Laurencia papillosa	11.8–12.9	12.0–13.3	8.9–10.8
Ulva lactuca	11.4–12.6	11.6–13.2	9.6–11.4
Caulerpa racemosa	11.8–12.5	16.0	9.0–10.5
Ulva reticulate	12.83	16.88	8.50
Enteromorpha compressa	7.26	24.75	11.45
Chaetomorpha aerea	10.13	31.50	8.50
Chaetomorpha antennina	10.13	27.00	11.45
Chaetomorpha linoides	9.45	27.00	12.00
Cladophora fascicularis	15.53	49.50	15.70
Microdictyon agardhianum	20.93	27.00	9.40
Boergesenia forbesii	7.43	21.38	11.42
Valoniopsis pachynema	8.78	31.50	9.09
Dictyosphaeria cavernosa	6.00	42.75	10.51
Caulerpa cupressoides	7.43	51.75	10.97
Caulerpa peltata	6.41	45.00	11.42
Caulerpa laetevirens	8.78	56.25	8.80
Caulerpa racemosa	8.78	33.75	10.63
Caulerpa fergusonii	7.76	23.63	7.15
Caulerpa sertularioides	9.11	49.50	6.99
Halimeda macroloba	5.40	32.63	9.89
Codium adhaerens	7.26	40.50	7.40
Codium decorticatum	6.08	50.63	9.00
Codium tomentosum	5.06	29.25	7.15
Microalgae			
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	–	1.9
Scenedesmus dimorphus	8–18	21–52	16–40
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51–58	12–17	14–22
Chlorella pyrenoidosa	57	26	2
Spirogyra sp.	6–20	33–64	11–21
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Euglena gracilis	39–61	14–18	14–20
Prymnesium parvum	28–45	25–33	22–39
Tetraselmis maculata	52	15	3
Porphyridium cruentum	28–39	40–57	9–14
Spirulina platensis	46–63	8–14	4–9
Spirulina maxima	60–71	13–16	6–7
Synechoccus sp.	63	15	11
Anabaena cylindrica	43–56	25–30	4–7

3.1. Conversion technologies for algal biofuels

The technically feasible conversion options for algal biomass and end-use of derived energy or energy carriers (liquid or gaseous fuels) are discussed in this section. The conversion of algal biomass-to-energy encompasses the different processes, which depend, to a large extent, on the types and sources of biomass, conservation options and endues [69]. The conversion technologies applied for algae biomass can be divided into two basic categories. They are thermochemical and biochemical conversion (Fig. 2) [16,70,71]. Factors that influence choice of conversion process include: the type and quantity of biomass feedstock; the desired form of the energy; economic consideration; project specific; and the desired end form of the product [72].

3.1.1. Thermochemical conversion

Thermochemical conversion is the thermal decomposition of organic components in biomass to yield fuel products [70]. The thermochemical conversion process includes direct combustion, gasification, liquefaction and pyrolysis as shown in Fig. 2. When biomass is heated under oxygen deficient conditions, it generates synthesis gas, or syngas, which consists primarily of hydrogen and carbon monoxide. This syngas can be directly burned or further processed for other gaseous or liquid products. In this sense, thermal or chemical conversion of biomass is very similar to that of coal [73].

3.1.1.1. Direct combustion for energy production. Combustion is the chemical reaction between a fuel and oxygen which usually takes place in the presence of air and is more commonly known as burning. The products are carbon dioxide and water with the release of heat. Biomass is burnt in the presence of air to convert the stored chemical energy in biomass into hot gases [74]. Sulfur emissions (0.05–0.2 wt%) are much lower and the formation of particulate can be controlled at the source [75]. Combustion is usually performed in a furnace, a boiler, or a steam turbine at temperatures above 800 °C. It is possible to burn any type of biomass, but combustion is only feasible for biomass with moisture content $<50\%$, dry weight [69]. Net energy conversion efficiencies for biomass combustion power plants range from 20% to 40%, with higher efficiencies obtained in larger systems (>100 MW) or when biomass is co-combusted in coal fired power plants [72].

Only a very few reports are available for technically viable utilization of algal biomass in direct combustion in literature, but a life cycle assessment (LCA) of coal-algae co-firing [77] recommended that coal-algae co-firing could lead to lower GHG emissions and air pollution. Macroalgae species from the British Isles: *Fucus vesiculosus*, *Chorda filum*, *Laminaria digitata*, *Fucus serratus*, *Laminaria hyperborea* and *Macrocystis pyrifera* from South America, has been studied by Ross et al. [78]. They demonstrated macroalgae as fuel by investigating the combustion behaviors of macroalgae. Combustion behavior was investigated using TGA in an oxidizing atmosphere and pyrolysis products were analyzed by gas chromatography–mass spectrometer (GC–MS) [78].

3.1.1.2. Gasification for syngas production. Gasification is a term that describes a chemical process by which carbonaceous materials (hydrocarbon) are converted to combustible gas mixture or synthesis gas (syngas). It is a partial oxidation of biomass with air, oxygen and/or steam at high temperatures, typically in the range 800–1000 °C [79]. A flow diagram of algae system for fuel production by low temperature catalytic gasification of biomass is shown in Fig. 3 [70]. Syngas can be produced from biomass by two routes namely catalytic and non-catalytic gasification. Non-catalytic process requires a very high temperature for operation, of about 1300 °C, whereas catalytic process can be operated at significantly lower temperature. In advances, the temperature requirement is expected to go downward further from the current value of about 900 °C [73]. The gasification step involves the reaction of biomass with air, oxygen, or steam to produce a gaseous mixture of CO, CO_2, H_2, CH_4 and N_2 either known as producer gas or syngas, depending on the relative proportions of the component gases [76,80]. Producer gas is primarily useful as a fuel for stationary power generation, whereas syngas is presently used to make a range of fuels and chemical intermediates. Syngas is a low calorific gas (typical 4–6 MJ m^{-3}), that can be burnt directly or used as a fuel for gas engines or gas turbines [81]. For transportation fuels, the main syngas derived routes to fuels are as follows: 1. hydrogen by water-gas-shift reaction (WGS) [82] and 2. hydrocarbons by Fischer–Tropsch (F–T) synthesis or methanol synthesis followed by further reaction to produce hydrocarbon or oxygenated liquid fuels [83]. The WGS reaction uses CO, H_2O to give H_2 and CO_2. It can be used to upgrade producer gas to syngas by enriching the H_2 content or to produce H_2 as a product [16].

Several studies have been conducted on gasification characteristics of microalgae biomass. Hirano et al. [84] partially oxidized *Spirulina* at temperature ranging from 850 to 1000 °C and

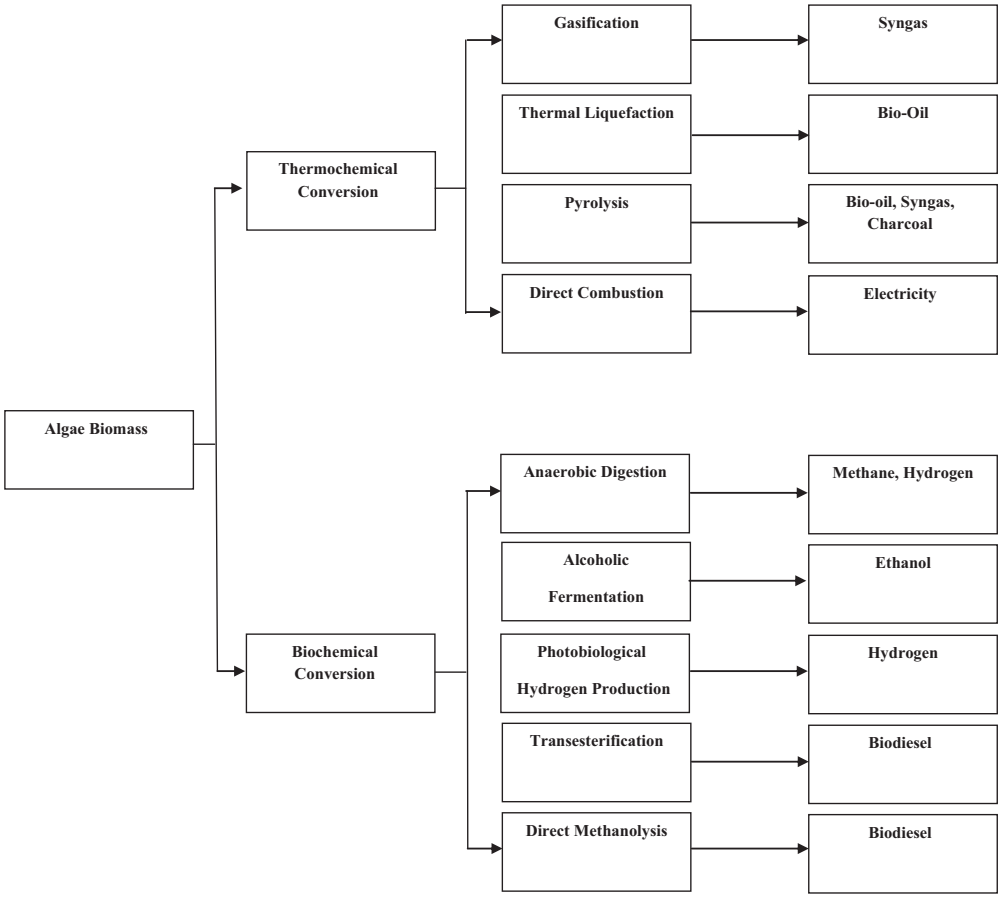

Fig. 2. Potential algal biomass conversion processes modified from Naik et al. [16].

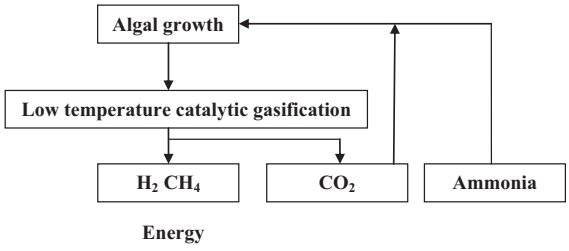

Fig. 3. Flow diagram of algal system for fuel production by gasification [70].

determined the gas composition required to generate theoretical yield of methanol. They estimated that algae biomass gasification at 1000 °C produced the highest theoretical yield of 0.64 g methanol from 1 g of biomass. Minowa and Sawayama [85] gasified the microalgae *Chlorella vulgaris* in a novel system with nitrogen cycling to obtain a methane-rich fuel. All nitrogen in the microalgae was converted into ammonia during the gasification, and the recovered solution, in which ammonia was dissolved, could be used as a nitrogen nutrient. A novel energy production system with nitrogen cycling combined with low temperature catalytic gasification of the algae has been reported [85]. Elliot and Sealock [86] have also developed a low temperature catalytic gasification of biomass with a high moisture content. Biomass with a high moisture is gasified directly to a methane rich fuel gas without drying. In addition, nitrogen in the biomass is converted to ammonia during the reaction [85].

The LCA study on utilization of macroalgae for enhanced CO_2 fixation and biofuel production was performed by Aresta et al. [87]. They demonstrated that, there is a potential energy benefit associated to recycle carbon by enhanced fixation of CO_2 by macroalgae. In the best case considered so far, macroalgae can generate a net energy in the order of 11,000 MJ t^{-1} dry algae compared to 9500 MJ t^{-1} than a microalgae gasification can do.

3.1.1.3. Thermochemical liquefaction for liquid biofuel production. Thermochemical liquefaction is a process that can be employed to convert a wet algal biomass material into a liquid fuel [88]. Algal cell precipitates derived from centrifugation, which are of high moisture content, are thus good raw materials for liquefaction [89]. This process is a low-temperature (300–350 °C), high-pressure (5–20 MPa) process aided by a catalyst in the presence of hydrogen to yield bio-oil [74]. Liquefaction usually produces water insoluble oils of high viscosity and usually requires solvents, reducing gases such as CO or H_2 and/or catalysts to be present in addition to biomass [80]. The liquefaction is performed in an aqueous solution of alkali glycerin, propanol, butanol or direct liquefaction [90] or alkaline earth salt at about 300 °C and 10 MPa without a reducing gas such as hydrogen and/or carbon monoxide [91]. The process utilizes the high water activity in sub-critical conditions to decompose biomass materials down to shorter and smaller molecular materials with a high energy density [88]. Alkali salts such as sodium carbonate and potassium carbonate, can act as catalyst for hydrolysis of macromolecules such as cellulose and hemicellulose into smaller fragments. The separation scheme is presented in Fig. 4 [89,91–93].

Many investigations were carried out on the characteristics of algal biomass as a feedstock (Table 4) [56]. Dote et al. [94] successfully used thermochemical liquefaction at 300 °C on *Botryococcus braunii* to achieve a maximum yield of 64% dry wt. based on oil with HHV of 45.9 MJ kg^{-1} and also declared a positive energy

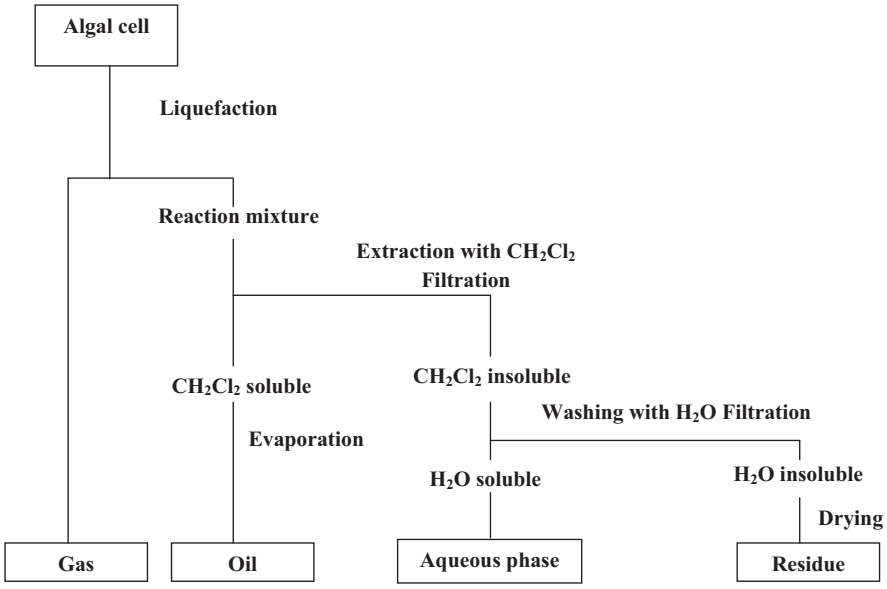

Fig. 4. Thermochemical liquefaction algal cells [103].

balance for the process (output/input ratio of 6.67:1). In a similar study, an oil yield of 42% dry wt was obtained from *Dunaliella tertiolecta* giving a HHV of 34.9 MJ kg^{-1} and positive energy balance of 2.94:1 [91].

Zhou et al. [95] investigated the possibility of using macroalgae *Enteromorpha prolifera* for bio-oil production by hydrothermal liquefaction. Effects of the temperature, reaction time and alkali catalyst on product yields were studied and the characters of liquid and solid products were analyzed using multiple analysis methods, such as, elemental analysis, Fourier Transform Infrared (FTIR) spectroscopy, GC–MS and ^{1}H Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR).

3.1.1.4. Pyrolysis for bio-oil production. Pyrolysis is a thermal degradation of biomass by heat in the absence of oxygen, which results in the production of charcoal (solid), bio-oil (liquid) and fuel gaseous products at medium to high temperatures (350–700 °C) [69,96] or by heating in the presence of a catalyst [97], at high heating rate (10^3–10^4 K/s) and with a short gas residence time to crack into short chain molecules and then being cooled to liquid rapidly [98]. The pyrolysis of biomass is targeted to recover a biofuel with a medium-low calorific power [90]. For biomass-to-liquid fuel conversion, it is deemed to have the potential for large scale production of biofuels that could replace petroleum based liquid fuel [2]. Table 5 outlines the characteristics and expected yields of different modes of pyrolysis [56,99].

Depending on the operating conditions, the pyrolysis process can be divided into three subclasses: (a) conventional pyrolysis, (b) fast pyrolysis and (c) flash pyrolysis [16].

3.1.1.4.1. Conventional pyrolysis. Conventional pyrolysis occurs under a slow heating rate (0.1–1 K/s) and residence time is 45–550 s. In the first stage of biomass decomposition which occurs in between 550 and 950 K is called pre-pyrolysis. During this stage, some internal rearrangement such as water elimination, bond breakage, appearance of free radicals, formation of carbonyl, carboxyl and hydroperoxide group take place [100]. The second stage of solid decomposition corresponds to the main pyrolysis process. It proceeds with a high rate and leads to the formation of pyrolysis products. During the third stage, the char decomposes at a very slow rate and it forms carbon rich solid residues.

3.1.1.4.2. Fast pyrolysis. It occurs in the high temperature range of 850–1250 K with fast heating rate (10–200 K/s), short solid residence time (0.5–10 s) and fine particle (<1 mm). The fast pyrolysis is recommended for the production of liquid and/or gaseous products. In fast pyrolysis process, the biomass decomposes to generate vapors, aerosol and some charcoal like char. After cooling and condensation of vapors and aerosol a dark brown mobile liquid is formed that has heating value, that is half of that of conventional fuel oil. Fast pyrolysis produced 60–75% of bio-oil, 15–25% solid char and 10–20% non-condensed gases [100]. The advantage of fast pyrolysis is that, it can directly produce a liquid fuel [101].

3.1.1.4.3. Flash pyrolysis. It differs strongly from that of conventional pyrolysis, which is performed in the temperature range of 1050–1300 K, fast heating rate (>1000 K/s), short residence time (<0.5 s) and very fine particle (<0.2 mm). Bio-oil production from biomass pyrolysis is typically carried out via flash pyrolysis [90]. The produced oil can be mixed with the char to produce bioslurry. Bioslurry can be more easily fed to the gasifier (gasifier condition: 26 bar; 927–1227 K) for efficient conversion to syngas. The conversion of biomass to crude oil can have an efficiency of up to 70–80% for flash pyrolysis process. The so called bio-crude can be used in engines and turbines [90,102]. A conceptual fluidized bed fast pyrolysis system is shown in Fig. 5 [101,103]. However, there are technical challenges as pyrolysis oils are acidic, unstable, viscous and contain solids and chemically dissolved water [104]. Therefore, the process oil will require upgrading hydrogenation and catalytic cracking to lower oxygen content and removes alkalis [76].

Since algae usually have high moisture content, a drying process requires much heating energy [92]. Algae are subjected to pyrolysis in the fluid bed reactor. The result of the reaction then flows to a cyclone and is separated into char, biofuel and gas. The resultant gas can be used for heating, for drying the raw material or for heating for the pyrolysis process [103].

Compared to other conversion technologies, research on pyrolysis of algal biomass is quite extensive and has achieved reliable and promising outcomes that could lead to commercial exploitation [56]. Miao and Wu [105] used fast pyrolysis to enhance oil yield from microalgae *Chlorella prothothecoides*. The recorded oil yield of 57.9% dry wt (HHV of 41 MJ kg^{-1}) and the results suggest that pyrolysis has potential in algal biomass-to liquid conversion. Miao et al. [98] achieved bio-oil yields of 18% (HHV of 30 MJ kg^{-1}) and 24% (HHV of 29 MJ kg^{-1}) with fast pyrolysis of *C. prothothecoides* and *Microcystis aeruginosa* grown phototrophically, respectively. Demirbas [106]

Table 4
Thermochemical conversion technologies applied on algal biomass [56].

Conversion process	Microalgae	Production	Temp (°C)	Pressure (MPa)	Liquid content (% dry wt.)	HHV (MJ kg^{-1})	Gas Content (% dry wt)	Solid content (% dry wt)	Reference
Gasification	*Spirulina*	N/A	1000	0.101	–	–	64	–	[84]
Thermochemical liquefaction	*Botryococcus braunii*	N/A	300	3	64	45.9	–	–	[94]
Thermochemical liquefaction	*Dunaliella tertiolecta*	N/A	300	3	42	34.9	–	–	[93]
Pyrolysis	*Chlorella prothothecoides*	Heterotrophic	450	0.101	57.9	41	32	10.1	[105]
Pyrolysis	*Chlorella prothothecoides*	Phototrophic	450	0.101	16.6	30	–	–	[105]
Pyrolysis	*Chlorella prothothecoides*	Phototrophic	500	0.101	18	30	–	–	[96]
Pyrolysis	*Chlorella prothothecoides*	N/A	502	0.101	55.3	39.7	36.3	8.4	[106]
Pyrolysis	*Microcystis aeruginosa*	Phototrophic	500	0.101	24	29	–	–	[96]

Table 5
Different modes of pyrolysis [99].

Mode	Conditions	Liquid (%)	Char (%)	Gas (%)
Flash pyrolysis	Moderate temperature (500 °C), short hot vapor residence time (about 1 s)	75	2	13
Fast pyrolysis	Moderate temperature (500 °C), moderate hot vapor residence time (about 10–20 s)	50	20	30
Slow pyrolysis	Low temperature (400 °C), very long solids residence time	30	35	35

experimenting with *C. prothothecoides*, showed that the bio-oil yield increased in line with temperature, that increased up to a point and then decreased at higher temperatures. For example, the yield rose from 5.7% to 55.3% with an increase from 254 to 502 °C and subsequently decreased to 51.8% at 602 °C. They recorded a HHV from microalgae of 39.7 MJ kg^{-1}obtained at temperatures ranging from 502 to 552 °C. Results indicate that bio-oils from microalgae (Table 6) are of a higher quality than those extracted from lignocellulosic materials [96,105].

Zhao et al. [107] carried out the production of bio-oil from marine macroalgae *E. prolifera* by thermochemical processing methods, such as, pyrolysis using free-fall Reactor. Rowbotham et al. [108] performed thermal pyrolysis to convert macroalgae *Laminaria digitata* into fuel and commodity chemicals.

3.1.1.5. Hydrogenation. Hydrogenation is a reductive chemical reaction, that results in an addition to hydrogen (H_2), usually to saturate organic compounds. The process consists of the addition of hydrogen atoms to the double bonds of a molecule by the use of a catalyst. Algal hydrogenation is performed by using an autoclave under high temperature and pressure conditions in the presence of a catalyst and a solvent [103]. Algal hydrogenation is a three-phase operation in which contact must be established between the gaseous phase (hydrogen and hydrocarbon phase), liquid phase (mixture of solvent and liquid product) and solid particle phase (algal and catalyst) in order to achieve algal conversion and to promote the transfer of momentum, heat and mass [89,103]. In general, higher temperatures and longer reaction time increase the degree of conversion and decrease the asphaltene yield in the overall hydrogenation of algae. The oil yield and the degree of conversion increases proportionally with the maximum hydrogen pressure of about 8.2 MPa [103].

3.1.2. Biochemical conversion

The biological process of energy conversion of biomass into fuels includes anaerobic digestion, alcoholic fermentation, photo-biological hydrogen production, transestrification and in-situ transesterification [109].

3.1.2.1. Anaerobic digestion for biogas (methane) production. Anaerobic digestion (AD) is the conversion of organic wastes converted into a bio-gas, which consists of primarily methane (CH_4) and carbon dioxide, with traces of other gases such as hydrogen sulfide (Fig. 6) [16,110]. It involves the breakdown of organic matter, to produce a gas with an energy content of about 20–40% of the lower heating value of the biomass. Anaerobic digestion process is appropriate for high moisture content (80–90% moisture) organic wastes [69], which can be useful for wet algal biomass. The AD process occurs in three sequential stages of hydrolysis, fermentation and methanogenesis. In hydrolysis, the complex compounds are broken down into soluble sugars. Then,

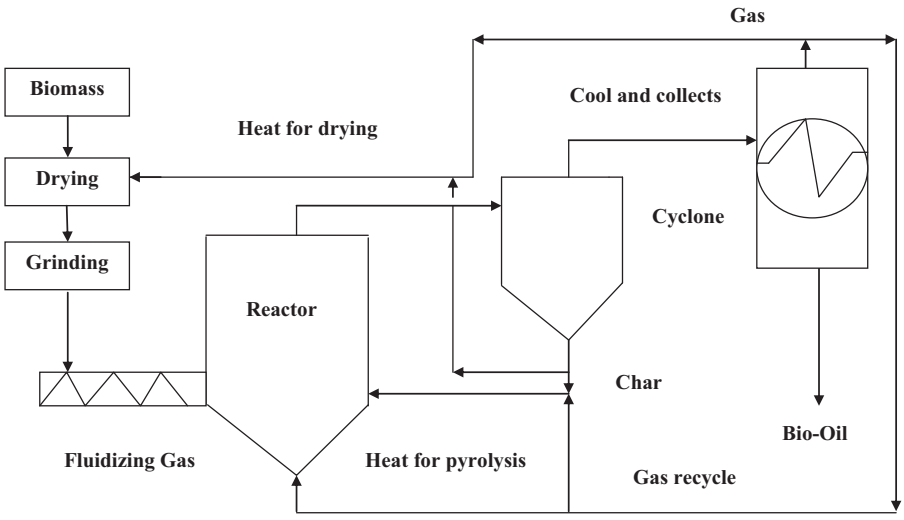

Fig. 5. Fast pyrolysis process principles [16].

Table 6
Comparison of typical properties of petroleum oil and bio-oils from fast pyrolysis of wood and microalgae [96,105].

Properties	Typical values		
	Bio-oils		Petroleum oil
	Wood	Microalgae	
C (%)	56.4	62.07	83.0–87.0
H (%)	6.2	8.76	10.0–14.0
O (%)	37.3	11.24	0.05–1.5
N (%)	0.1	9.74	0.01–0.7
Density (kg l^{-1})	1.2	1.06	0.75–1.0
Viscosity (Pa s)	0.04–0.20 (at 40 °C)	0.10 (at 40 °C)	2–1000
HHV (MJ kg^{-1})	21	29–45.9	42

fermentative bacteria convert these into alcohols, acetic acid, volatile fatty acids (VFAs) and a gas containing H_2 and CO_2, which is metabolized into primarily CH_4 (60–70%) and CO_2 (30–40%) by methanogens [111]. The gas has a heating value of 650–750 Btu/ft^3 [16]. It has been projected that, the conversion of algal biomass into methane could recover as much energy as obtained, from the extraction of cell lipids [112], while leaving a nutrient rich waste product, that can be recycled into a new algal growth medium [113,114]. Microalgae can have a high proportion of proteins, that result in low C/N ratios (ca. 10) which can affect the performance of the anaerobic digester. This problem may be resolved by co-digestion with a high C/N ratio product (e.g. waste paper). Yen and Brune [115] achieved a significant increase in methane production with the addition of waste paper to algal biomass. They obtained double the methane production rate (1.17 ml l^{-1} per day vs. 0.57 ml l^{-1} per day) from 50/50 waste paper/algal biomass blend compared to anaerobic digestion of pure algal biomass. Due to increase in the cost of energy, the anaerobic digestion of biomass is an attractive, alternative for production of fuel and biofertilizer for organic cultivation [16].

3.1.2.2. Alcoholic fermentation for bioethanol production. The term fermentation can generally be defined as the metabolic process, in which, an organic substrate goes under chemical changes due to activities of enzymes, secreted by the micro-organisms. Alcoholic fermentation is the conversion of biomass materials, which contain sugars, starch or cellulose into ethanol [69]. The sugar is converted into ethanol by yeast. There are two basic types of fermentation, (a) aerobic and (b) anaerobic depending upon oxygen, required in the process or not. Algal starch requires additional processing before fermentation [76].

Production of ethanol by using algae as raw material, can be performed, in the following procedure. The biomass is ground down and the starch is converted to sugars, which is then mixed with water and *Saccharomycess cerevisiae* yeast and kept warm in large tanks called fermenters to begin fermentation [76]. The yeast breaks down the sugar and converts it into ethanol as shown in Eq. (1) (Fig. 7).

$$\begin{array}{l} C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \\ \text{MW (180)} \quad (2\times 46) \quad (2\times 44) \end{array} \tag{1}$$

The ethanol is drained from the tank and pumped to a holding tank, to be fed to a distillation unit. A purification process (distillation) is required to remove the water and other impurities in the diluted alcohol product (10–15% ethanol) [69]. The concentrated ethanol (95% volume for one distillation) is drawn off and condensed into a liquid form, which can be used as a supplement or a substitute for petrol in cars [72,99]. The solid residue from the process can be used for animal feed or for gasification process [69]. This helps offset feedstock costs which typically make up 55–80% of the final alcohol selling price [56].

Microalgae such as *C. vulgaris* are a good source of ethanol, due to the high starch content (ca. 37% dry wt) and for which up to 65% ethanol conversion efficiency has been recorded [116]. Ueno et al. [117] investigated ethanol production by dark fermentation in the marine green alga *Chlorococcum littorale*. Under dark anaerobic conditions, 27% of the cellular starch was consumed within 24 h at 25 °C, the cellular starch decomposition being accelerated at higher temperature. Ethanol, acetate, hydrogen and carbon dioxide were obtained as fermentation products (Fig. 7) [103]. The maximum productivity of ethanol was 450 μmol/g of dry weights at 30 °C.

3.1.2.3. Photobiological process for hydrogen production. Hydrogen (H_2) is a clean and an efficient energy carrier [79]. Algae possess the necessary genetic, metabolic and enzymatic characteristics to photo produce H_2 gas [118]. Under anaerobic conditions hydrogen is produced from algae, as an electron donor in the CO_2 fixation process and is developed in both light and dark [119]. During photosynthesis, algae convert water molecules into hydrogen ions (H^+) and oxygen; the hydrogen ions are then subsequently converted by hydrogenase enzymes into H_2 under anaerobic

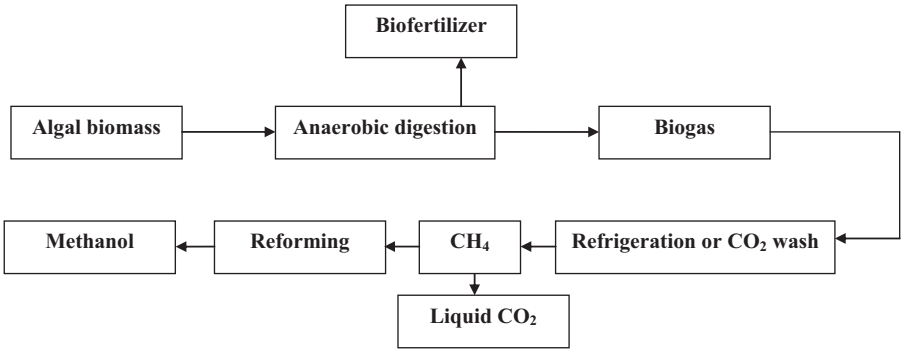

Fig. 6. Anaerobic digestion of algal biomass.

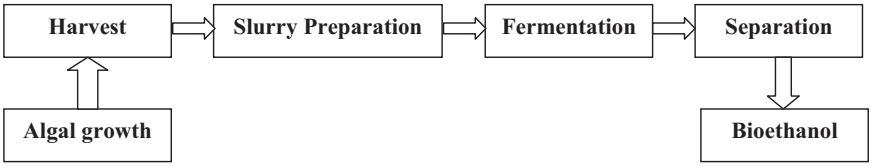

Fig. 7. Fermentation process of algae feedstock [103].

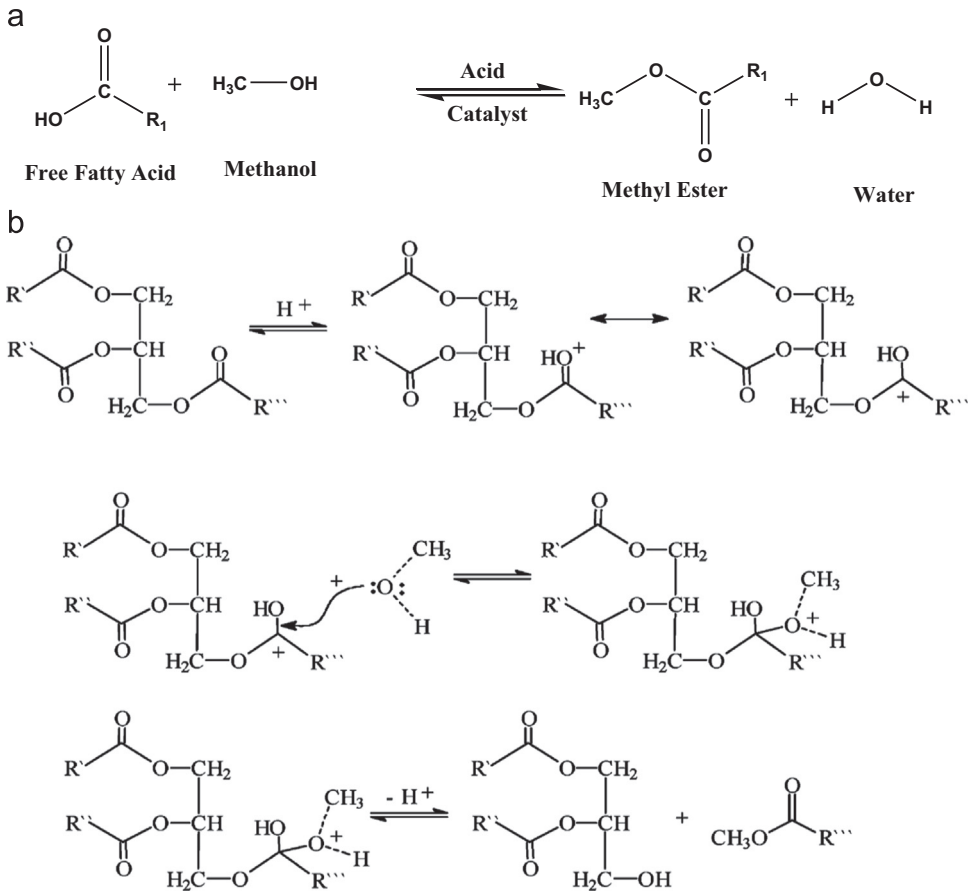

Fig. 8. Acid catalyzed transesterification. (a) Reaction. (b) Mechanism [97,126].

conditions [111]. Due to reversibility in the nature of the reaction, hydrogen is either produced or consumed by the simple conversion of protons to hydrogen [79]. The photosynthetic oxygen produced, inhibits the key enzyme hydrogenase and the photosynthetic hydrogen production process is hindered [111,120–122]. Therefore, algae cultures for hydrogen production must be subjected to anaerobic conditions. There are two fundamental approaches for photosynthetic H_2 production from water. The first is the H_2 production process, which is a two stage photosynthesis process, where photosynthetic oxygen production and H_2 gas generation are spatially separated [118].

In the first stage, algae are grown photosynthetically in normal conditions. During the second stage, the algae are deprived of sulfur, thereby inducing anaerobic conditions and stimulating consistent

hydrogen production [122]. This production process becomes limited with time, as hydrogen yield will begin to level off after 60 h of production. The use of this production system does not generate toxic or environmentally harmful products but could give value added products as a result of biomass cultivation [123].

The second approach involves the simultaneous production of photosynthetic oxygen and H_2 gas. In this approach, electrons that are released upon photosynthetic H_2O oxidation are fed directly into the hydrogenase-mediated H_2-evolution process [118]. The H_2 productivity is theoretically superior to the two-stage photosynthetic process. Melis and Happe [122] found that using the two-stage photosynthesis process and H_2 production, a theoretical maximum yield of hydrogen by green algae could be about 198 kg H_2 ha^{-1} per day.

3.1.2.4. Transesterification for biodiesel production. Transesterification is a reaction, in which, the glycerol backbone of the triglycerides (TG) is replaced by methanol which esterifies fatty acids, the side chains of TG, into methyl esters (biodiesel). The general equation for transesterification is shown in Fig. 9 [124]. Specifically, a TG molecule (primary compound in algal oils) reacts with a low molecular weight alcohol, yielding a mono alkyl ester and a byproduct glycerin, which is used in the pharmaceutical and cosmetic industries. The transesterification reaction proceeds in 3 steps: 1. TG reacts with methanol in the presence of a catalyst to produce diglycerides (DG) 2. DG reacts with methanol to generate monoglycerides (MG) 3. Finally, MG reacts with methanol to produce ME and glycerol. One mole of ME is generated from per mole of methanol reacted at each step, in all 3 mol of ME are produced [125]. The reactions are often catalyzed by an acid or a base (Figs. 8 and 9) [97], using a homogeneous or heterogeneous catalytic process [126]. This is the most predominant and significant method used to produce biodiesel for commercial scale.

Miao and Wu [32] performed transesterification reaction with *C. protothecoides* for the production of biodiesel. The best combination of factors was 100% catalyst quantity (based on oil weight) with 56:1 M ratio of methanol to oil at temperature of 30 °C. Biodiesel production efficiency of *C. vulgaris*, *Rhizoclonium hieroglyphicum* and mixed algae culture was measured by transesterification process.

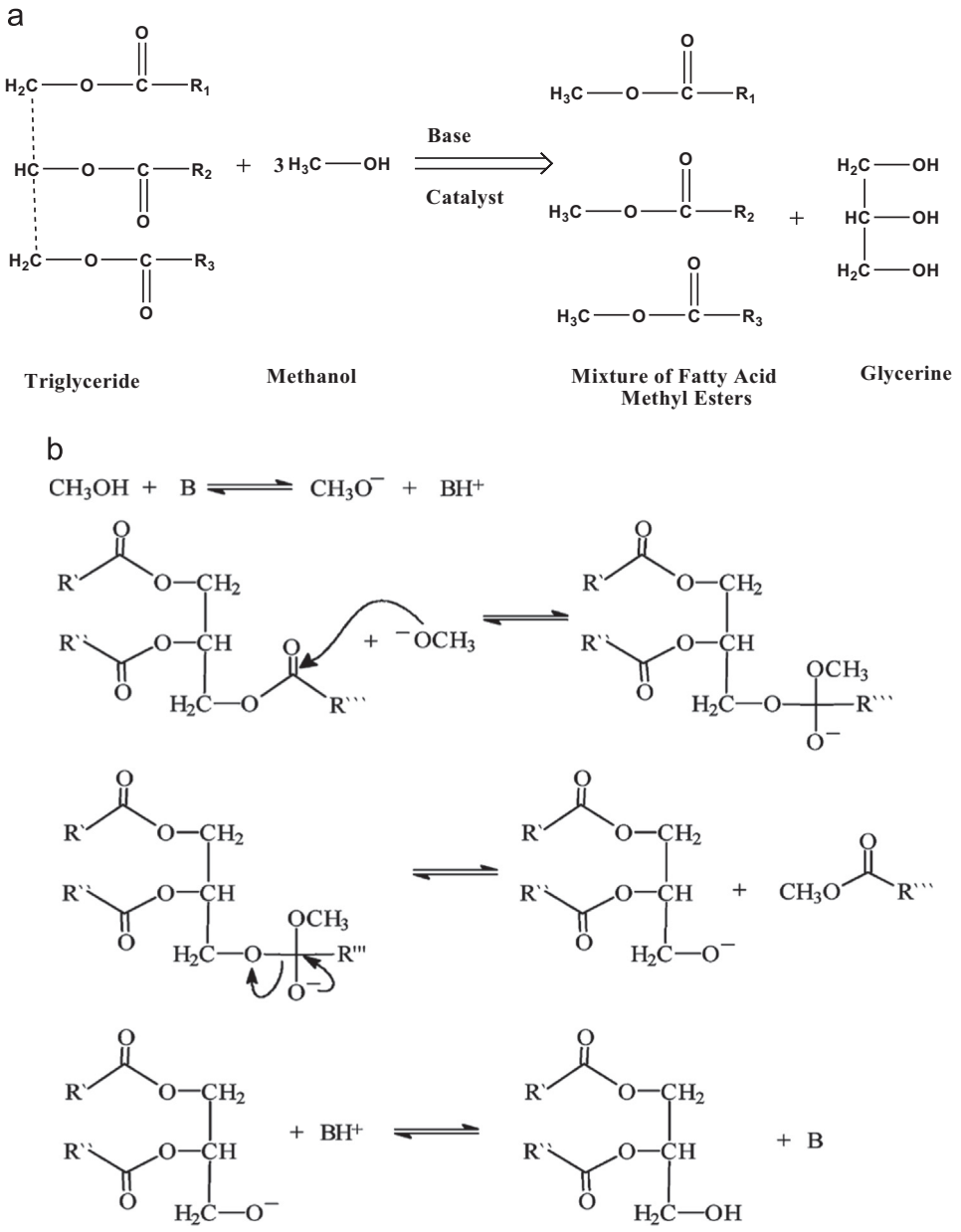

Fig. 9. Base catalyzed transesterification. (a) Reaction. (b) Mechanism [97,124,126].

Yield of biodiesel from extracted algal oil was calculated as *C. vulgaris* (95%), *Rhizoclonium hieroglyphicum* (91%) and mixed algae culture (92%) [127]. Macroalgae *Caulerpa peltata*, *Enteromorpha compressa* was subjected to transesterification reaction for the production of biodiesel. Acid–base catalyzed transesterification was applied to this macroalgal biomass to convert algal oil into biodiesel [128,129].

3.1.2.5. Direct methanolysis for biodiesel production. Conventional method for the production of biodiesel from algae involves various stages; oil extraction, purification (degumming, deacidification, dewaxing, dephosphorization, dehydration, etc.) and esterification/transesterification. The requirement of these multiple processing stages constitutes over 70% of the total production cost of biodiesel [130]. Therefore, development of direct methanolysis is also known as in-situ extraction, direct transesterification or simply as a reactive extraction that has the potential to cut down the processing cost. Reactive extraction differs from the conventional biodiesel production process in which the oil bearing material contacts with the alcohol directly instead of reacting with extracted oil. In other words, extraction and transesterification proceed in a single step, with alcohol acting as an extraction solvent and as a transesterification reagent [131]. Therefore, reactive extraction eliminates the requirement of two separate stages of biodiesel production processes, such as, costly oil extraction process and transesterification reaction process, thus reducing processing time, production cost, amount of solvent required and procedure of the production system [132].

Haas and Wagner [133] achieved 83% of maximum biodiesel yield from algal biomass through in-situ transesterification method at methanol/fatty acid molar ratio of 220:1, 65 °C reaction temperature for 2 h reaction time. Biodiesel was produced from *Chlorella pyrenoidosa* using in-situ transesterification process [134].

Suganya et al. [135] carried out an ultrasound-enhanced rapid in-situ transesterification of marine macroalgae *Enteromorpha compressa* for biodiesel production. The maximum biodiesel yield was calculated as 98.89% from this direct methanolysis process.

In the similar way, Amin [103] discussed the main conversion processes of microalgae into energy. Since microalgae have high water content, not all biomass energy conversion processes can be applied. The properties of the microalgae product are almost similar to those of offish and vegetable oils and therefore, it can be considered as a substitute of fossil oil.

4. Biofuel production and environmental pollution control using algae: integrated approaches

In last few decades, various researchers have worked on the use of seed oils, for the production of biofuels. Production of second generation fuels such as bioethanol and biodiesel from biomass grown on arable lands, specially the use of oil-seeds for biodiesel, have raised the food prices. Third generation biofuels from algal cells grown on non-arable land are the exact results of the food-fuel competition. Wastewater and flue gases are the best options for reducing the environmental burden from the cultivation of algal biomass [136].

4.1. Bio-mitigation of CO_2 emission in algal cultivation

One of the key advantages of using microalgae for biofuel production lies in the ability of microalgal species to tolerate high CO_2 content in feeding air streams [137]. It allows an efficient capturing of CO_2 from high-CO_2 streams such as flue gases and flaring gases (CO_2 content 5–15%) [138] in comparison with terrestrial plants, which typically absorb only 0.03–0.06% CO_2 from the atmosphere. The benefit of microalgae is evident in terms of CO_2 mitigation [46]. Microalgae can typically be used to capture CO_2 from three different sources: atmospheric CO_2, CO_2 emission from power plants and industrial processes and CO_2 from soluble carbonate. Capture of atmospheric CO_2 is probably the most basic mechanism, to sink carbon and relies on the mass transfer from the air to the microalgae in their aquatic growth environments during photosynthesis. Flue gas emission from an industrial process unit (e.g. from fuel-fired power plants) as a source of CO_2 for the microalgae growth is envisioned to have a great potential to diminish CO_2 and to provide a very promising alternative to current GHG emissions mitigation strategies. A number of microalgae species are able to assimilate CO_2 from soluble carbonates such as Na_2CO_3 and $NaHCO_3$ [44]. Zeiler et al. [139] demonstrated that, *Monoruphidium minutum* algae could efficiently utilize flue gas containing high levels of carbon dioxide, as well as sulfur and nitrogen oxides, as a feedstock to produce substantial biomass. The green algae Chlorophyta showed the ability to fix CO_2 while capturing solar energy with an efficiency of 10–50 times greater than that of terrestrial plants [44]. Chlorococcum littorale, a marine alga, showed remarkable tolerance to high CO_2 concentration of up to 40% [140]. *Chlorella* Strains from hot springs, also showed to be tolerant to high temperatures up to 42 °C, for CO_2 fixation from industrial flue gases containing up to 40% CO_2 [141].

Chang and Yang [137] found that certain species of *Chlorella* could grow in an atmosphere containing CO_2 up to 40% (v/v). Doucha et al. [142] recorded 10–50% reduction in CO_2 concentration in flue gases using *Chlorella* sp., with the efficacy, decreasing with an increasing rate of flue gas injection into microalgae culture. de Morais and Costa [143], using *Spirulina* sp. obtained a maximum daily CO_2 biofixation of 53.29% for 6% (v/v) CO_2 and 45.61% for 12% (v/v) CO_2 in the injected flue gas, with the highest mean fixation rate being 37.9% for 6% (v/v) CO_2. They reported that the microalgae species, *S. obliquus* and *C. kessleri* are capable to grow in media containing up to 18% (v/v) CO_2. They also achieved biofixation rates of 28.08% and 13.56% for 6% (v/v) and 12% (v/v) CO_2 respectively, using *S. obliquus*. Kadam [77] demonstrated the potential benefits of recycling CO_2 for microalgae biomass production through co-firing coal and microalgae to reduce the environmental impact of power generation. Their LCA results showed that co-firing reduced CO_2 and methane. Hence, GHG emissions through the recycling of microalgae biomass and the reduction in coal use, also registered lower net SO_x and NO_x particulates.

Comparison study was conducted by Yoo et al. [144] with three species of microalgae such as *B. braunii*, *C. vulgaris* and *Scenedesmus* sp. under flue gas conditions. From the study, it was found that *Scenedesmus* sp. were the most suitable for CO_2 mitigation due to high rates of biomass production (0.218 g l^{-1} per day). *B. braunii* and *Scenedesmus* sp. were found to grow better using flue gas as compared to air enhanced with CO_2.

At present, the high cost of biodiesel is the major obstacle for its commercialization [26]. Bio-mitigation of CO_2 emissions provides a complementary function that may be exploited to reduce cost and to enable sustained utilization of microalgae as a biofuel resource [56].

4.2. Co-processing of wastewater treatment and microalgae farming

Heterotrophic cultivation systems involving microalgae production and wastewater treatment (e.g. of amino acids, enzyme, or food industries wastewaters) seem to be quite promising for microalgae growth combined with environmental cleaning [43]. There is a unique opportunity to carry out co-processing of wastewater treatment and nutrients supplement to algae growth using nutrient-rich effluent streams (nitrogen and phosphorus).

This leads to find a pathway for the removal of chemical and organic contaminants, heavy metals and pathogens from wastewater while producing biomass for biofuel production [145]. Wastewater treatment using algae has many advantages. It offers the feasibility to recycle these nutrients into algae biomass as a fertilizer and thus can offset treatment cost. Oxygen rich effluent is released into water bodies after wastewater treatment using algae [146]. Wastewater rich with CO_2 provides a conducive growth medium for microalgae because the CO_2 balances the Redfield ratio (molecular ratio of carbon, nitrogen and phosphorus in marine organic matter, C:N:P=106:16:1) of the wastewater allowing for faster production rates, reduced nutrient levels in the treated wastewater, decreased harvesting costs and increased lipid production [56,147]. Additionally, microalgae can mitigate the effects of sewage effluent and industrial sources of nitrogenous waste and at the same time, contributing to biodiversity. Moreover, removing nitrogen and carbon from water, microalgae can help to reduce the eutrophication in the aquatic environment [43].

Most of the used water turns into wastewater polluting the environment and creating health hazards. If 50% (495 billion m^3) of this consumed water is available for algae production, it could generate about 247 million tons of algal biomass and about 37 million tons of oil. Yet the variations in the composition of wastewater limit such a notion, since only specific algae may perform to their potential [148]. Therefore, it is essential to select strains, capable of growing in variety of wastewaters and producing feedstock for biofuels that can compete in terms of land and water use, carbon sequestration and GHG emission savings, etc. Wastewater generated by carpet mills along with sewage from Dalton area in North Central Georgia (40–55 million m^3 $year^{-1}$) has the potential to generate up to 15,000 t of algal biomass which can produce about 2.5–4 million L of biodiesel and remove about 1500 t of nitrogen and 150 t of phosphorus from the wastewater in one year [148].

Several applications in wastewater treatment have been found in the literature. For example, Sawayama et al. [149] used *B. braunii* to remove nitrate and phosphate from sewage after primary treatment along with the production of hydrocarbon-rich biomass. Martinez et al. [150] achieved a significant removal of phosphorus and nitrogen from urban wastewater using the microalgal *S. obliquus*. They were able to achieve 98% elimination of phosphorus and a complete removal (100%) of ammonium in a stirred culture at 25 °C and 183 h retention time, respectively. Gomez-Villa et al. [151] experimented with outdoor cultivation of microalgal *S. obliquus* in artificial wastewater, achieved the final dissolved nitrogen concentrations which were 53% and 21% of initial values in winter and summer, respectively. Phosphorus, which was only removed during the day, achieved a total reduction of 45% in winter and 73% in summer [155]. Aslan and Kapdan [152] used *C. vulgaris* for nitrogen and phosphorus removal from wastewater with an average removal efficiency of 72% for nitrogen and 28% for phosphorus. Hodaifa et al. [153] recorded 67.4% reduction in BOD_5 with *S. obliquus* cultured in diluted (25%) industrial wastewater from olive-oil extraction.

Yun et al. [154] successfully grew *C. vulgaris* in wastewater discharge from a steel plant to achieve an ammonia bioremediation rate of 0.022 g NH_3 l^{-1} per day. For the biodegradation of hazardous or toxic compounds, it is possible to use microalgae to generate the oxygen required by bacteria to biodegrade pollutants such as polycyclic aromatic hydrocarbons

(PAHs), phenolics and organic solvents. Photosynthetic oxygen generated from the microalgae production reduces or eliminates the need for external mechanical aeration [145]. Chojnacka et al. [155] proved that *Spirulina* sp. acted as a bio-sorbent, thus was able to absorb heavy metal ions (Cr^{3+}, Cd^{2+} and Cu^{2+}).

Additionally, extensively used microalgae cultures for contaminants' removal from wastewater are *Chlorella* sp. [156,157], *Scenedesmus* sp.[150] and *Spirulina* sp. [158], *Nannochloris* [159], *B. brauinii* [160] and cyanobacterium *Phormidium bohneri* [161,162] and also their pollutants' removal have been reported.

5. Commercial application of algae

Algae were promising organisms for providing both novel biologically active substances and essential compounds for human nutrition [163]. Therefore, an increasing supply for algal extracts, fractions or pure compounds for the economical sector was needed [164]. In this regard, both secondary and primary metabolites production from algal cells are shown in Fig. 10.

5.1. Commercial applications of microalgae

Microalgae have three fundamental attributes that can be exploited to useful ends: They are (1) very diverse, (2) often phototrophic and (3) virtually unexplored. These attributes can provide significant technical and commercial advantages. Microalgae provide a large and untapped reservoir of potential new products and applications [58].

Table 9 indicates microalgae as a potential source, for high-value products in commercial scale level.

5.1.1. Microalgae in human and animal nutrition

5.1.1.1. Human nutritional products. Nutritional supplements produced from microalgae have been the primary focus of microalgal biotechnology. Microalgae have been used for food by humans for thousands of years. Microalgae are observed as having a protein quality value, greater than other vegetable sources, for example, wheat, rice and legumes, but poorer than animal sources, for example, milk and meat [43]. Microalgae for human nutrition are now-a-days marketed in different forms, such as tablets, capsules and liquids. They can also be incorporated into pastas, snack foods, candy bars or gums and beverages [165,166]. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants [62,167,168]. The commercial applications are dominated by four strains: Arthrospira, *Chlorella*, *D. salina* and *Aphanizomenon* flos-aquae.

Dried biomass or cell extracts produced from *Chlorella* [165,169], *Dunaliella* [170] and *Spirulina* [171] have dominated the commercial opportunities. These products are directed mainly at the nutraceutical or health food market and are collectively worth many hundreds of million dollars [62].

Chlorella is sold as a health food or dietary supplement [172]. Health benefits of this microalgae are efficacy on gastric ulcers, wounds and constipation together with preventive action against both atherosclerosis and hyper-cholesterol and antitumor activity [57]. The most important active substance is b-1,3-glucan which is believed to be an active immune-stimulator, free radical scavenger and a reducer of blood lipids [61]. As affirmed by Barrow and Shahidi [173] *Chlorella* sp. presents several health benefits, when their extracts are ingested. For example, it can increase hemoglobin concentrations, lower blood sugar levels and act as hypocholesterolemic and hepatoprotective agents during malnutrition and ethionine intoxication. Polysaccharide complexes from *C. pyrenoidosa* and *Chlorella ellipsoidea* contain glucose and any combination of galactose, rhamnose, mannose, arabinose, N-acetyl glucosamide and N-acetyl galactosamine [173]. These complexes have immuno-modulating properties, specifically immune stimulatory activity and can inhibit the proliferation of *Listeria monocytogenes* and *Candida albicans* [43]. In addition, *Chlorella* extracts may be administered to mammals, to increase the proliferation of

splenocytes and production of cytokines and can be used as a supplement to further stimulate their immune response [173].

Spirulina (*Arthrospira*) is used in human nutrition because of its high protein content and excellent nutrient value [49,59,174,175]. It is also a valuable source of the essential fatty acid (linolenic acid) that cannot be synthesized by humans [57]. In addition, this microalgae has various possible health-promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal Lactobacillus and suppression of elevated serum glucose level [176]. Many companies are producing "nutraceuticals" (food supplements with claimed nutritional and medicinal benefits) made from *Spirulina* [61]. *Spirulina* has been exploited by ancient people in both Chad and Mexico as a source of food [28,177]. The market value of dried *Spirulina* was estimated to be US$40 million in 2005 [60]. *Spirulina* sp. has been shown to increase the production of plaminogen-activating factor in vascular endothelial cells and thus facilitate cardiovascular disease prevention [173]. Additionally, several antioxidant compounds (e.g. dimethylsulfoniopropionate, mycosporines or mycosporinelike amino acids, b-carotene, astaxanthin and other carotenoids) have been isolated from microalgal sources, having the potential to protect against oxidative stress, cause a wide spectrum of diseases and ageing. A significant amount of *Arthrospira* production is realized in China and India.

There are health concerns over the ingestion of cyanobacteria (e.g. *Spirulina*). Cox et al. [178] studied over 50 strains of cyanobacteria and found that nearly all the strains produced the neurotoxin B-N-methylamino-L-alanine (BMAA). BMAA is linked to amyptrophic lateral sclerosis–Parkinsonism dementia complex, Lou Gehrig's disease (ALS) and Alzheimer's disease.

Dunaliella sp. (especially *Dunaliella salina*) has become popular as a food grade green microalgae. In particular, due to their lipids and protein contents, glycerol concentration, β-carotene content (up to 4% of dry weight) and they have an exceptional ability to grow under brackish conditions [173]. *D. salina* is exploited for its β-carotene content that can reach 14% of dry weight [179]. For human consumption, Cognis Nutrition and Health, the world's largest producer of this strain, offers *Dunaliella* powder as an ingredient of dietary supplements and functional foods [59].

At present this microalgae is cultivated by several companies, in both Israel and Australia, as sources of vitamins A and C and as dietary supplements and powders [180]. Furthermore, it has been postulated that the carotenoids found in *Spirulina* sp. and *Dunaliella* sp. may be more potent anticancer agents than β-carotene [51,181].

The last major important commercial application strain is *Aphanizomenon flos-aquae*. According to many research studies, used alone or in combination with other nutraceuticals and natural food products, *A. flos-aquae* promotes overall good health [182–184].

5.1.1.2. Animal nutrition

5.1.1.2.1. Aquaculture feeds. Aquaculture animals must obtain all their nutrients through the food chain. Algae are the basic producer in the food chain. The nutrient properties of the algae are critical for the growth and survival of larvae and adults. In a typical food chain, algae are consumed by zooplankton (rotifers, cladocerans, brine

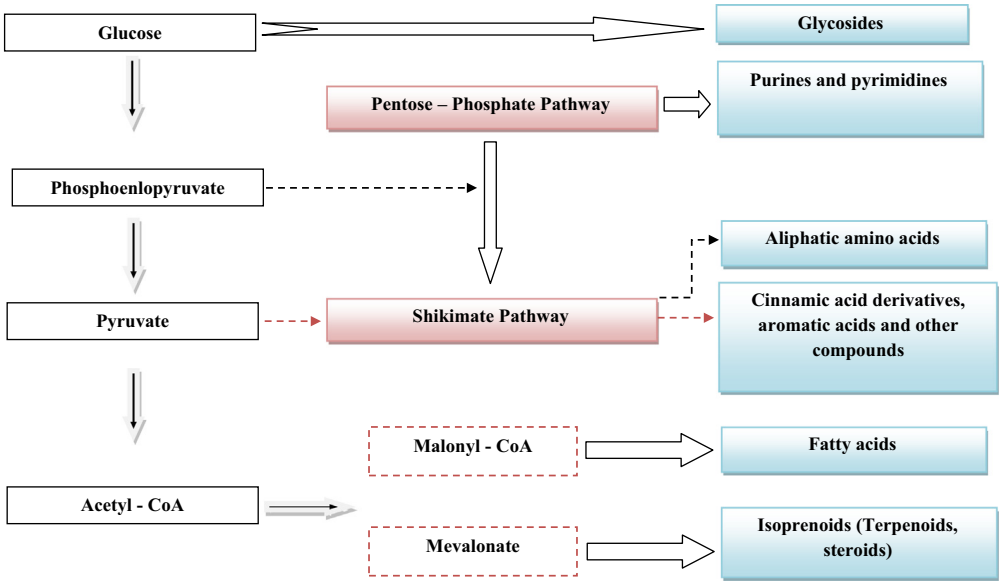

Fig. 10. Secondary and primary metabolites produced from algal cell.

Table 7
Potential of microalgae as primary PUFA resources [56,59].

PUFA	Structure	Potential application	Algal species
γ-Linolenic acid (GLA)	18:3 ω6, 9, 12	Infant formulas for full-term infants, Nutritional supplements.	*Arthrospira*
Arachidonic acid (AA)	20:4 ω6, 9, 12,15	Infant formulas for full-term/preterm infants, Nutritional supplements.	*Porphyridium*
Eicosapentaenoic acid (EPA)	20: 5 ω3, 6, 9, 12, 15	Nutritional supplements, Aquaculture.	Nannochloropsis, Phaeodactylum, *Nitzschia*
Docosahexaenoic acid (DHA)	22: 6 ω3, 6, 9, 12, 15, 18	Infant formulas for full-term/preterm infants, Nutritional supplements, Aquaculture.	Crypthecodinium, Schizochytrium

Table 8
Carotenoids from microalgae.

Molecule	Origin	Isomer	Price (US$)	Principal producer
β-Carotene	*Dunaliella*	All-*trans* and 9-cis	300–3000/kg	Cognis Nutrition and Health (Hutt Lagoon and Whyalla, Australia), Cyanotech (Kona, Hawaii, USA), Inner Mongolia Biological Eng. (Inner Mongolia, China), Nature Beta Technologies (Eilat, Israel), Tianjin Lantai Biotechnology (Tianjin, China)
Astaxanthin	*Haematococcus*	All-*trans* 3S, 3′S	2500/kg	Cyanotech (Kona, Hawaii, USA), Mera Pharmaceuticals (Kailua-Kona, Hawaii, USA), Bioreal (Kihei, Hawaii, USA), Parry's Pharmaceuticals (Chennai, India), Algatech (Kibbutz Ketura, Israel) Phaffia yeast 3 R, 3′R DSM (Heerlen, The Netherlands)

Table 9
High-value products from microalgae [59].

Product name	Price (US$)	Distributor
R-phycoerythrin	3.25–14/mg	Cyanotech
Allophycocyanin	6–17/mg	Cyanotech
Streptavidin: B-phycoerythrin	145/mg	Martek
Goat anti-mouse IgG: R-phycoerythrin	165/mg	Martek
Sensilight PBXL1: Anti-GST	1500/mg	Martek
Mixed fatty acids	60/g	Spectra stable isotopes
^{13}C-mixed free fatty acids	200/g	Spectra stable isotopes
^{13}C-DHA (> 95%)	38,000/g	Spectra stable isotopes
^{15}N-alanine	260/g	Spectra stable isotopes
$^{2}H_7$, ^{13}C, $^{15}N_4$-arginine	5900/g	Spectra stable isotopes
dATP-CN	26,000/g	Spectra stable isotopes

shrimp or copepods), which in turn are consumed by fish larvae [180,185,186]. The algal species are commonly cultured for aquaculture feed, which include *Skeletonema*, *Chaetoceros*, *Isochrysis*, *Dunaliella*, *Phaeodactylum*, *Navicula*, *Pavlova*, *Amphora*, *Nannochloropsis*, *Cyclotella*, *Tetraselmis*, *Nitzschia and Chlorella* [187–189].

Isochrysis galbana and *Tetraselmis suecica* are considered the best food for larval bivalves, growing much better in unfiltered seawater to which these algae have been added. Drum-fried *Scenedesmus* can be used as Artemia food and *Chlorella* (marine, freshwater, or dried) is well suited for the rotifer *Brachionus plicatilis* cultivation [185]. The cost of producing dry algal biomass feed, in Australia, varies from US$80/kg to US$800/kg [57,59]. Other cost estimates have given costs between US$50/kg to US $150/kg with a peak value of US$1000/kg [47]. In fact, 30% of the current world algal production is sold for animal feed applications [190] and over 50% of the current world production of *Arthrospira* is used as feed supplement [165]. In order to be used in aquaculture, a microalgal strain has to meet various criteria. It has to be easily cultured and nontoxic. It should also have the correct size and shape to be ingested and should have high nutritional qualities and a digestible cell wall to make nutrients available [191,192].

Although algae are an important part of any aquaculture facility, the reliability of the algal supply is a major problem in attaining a profitable operation [186]. If there is an interruption in the supply of algae, the entire food chain could be broken, resulting in loss of fish larvae and eventually decreased production of adult fish. This need for reliability to support zooplankton and larvae has led to a number of different designs for algal culturing systems, ranging from ponds to tanks and to sophisticated photo bioreactors [186,193–195]. Photo bioreactors are generally designed and constructed with input from engineers as well as biologists, so they can be very efficient in growing algae.

While microalgae provide food for zooplanktons, they also help to stabilize and improve the quality of the culture medium. Indeed, for numerous freshwater and seawater animal species, the introduction of phytoplanktons to rearing ponds (green-water technique) leads to much better results in terms of survival, growth and transformation index than that of the clear-water technique [196–198]. The reasons for this are not entirely known, but may include [186,199] water quality improvement and stabilization by algal oxygen production and pH stabilization, the action of some excreted biochemical compounds along with the induction of behavioral processes like initial prey catching and the regulation of bacterial population have probiotic effects [200] and the stimulation of immunity [59].

Another challenge for aquaculture that is being addressed by phycologists is improvement of larval nutrition to achieve higher larval survival rates [201]. Given the substantial cost of maintaining the food chain for larvae, any increase in larval survival can have a significant impact on the economics of an aquaculture facility. Improving the nutritional properties of the rotifers and *Artemia* by feeding them more nutritionally balanced algae is a simple way to improve larval nutrition [202]. Protein content is a major factor determining the nutritional value of microalgae. In addition, highly unsaturated fatty acid (e.g., eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA)) content is of major importance [203]. Most of the researchers have focused on the importance of polyunsaturated fatty acids in larval growth and development [189,204,205]. In particular, DHA, EPA and more recently AA have been recognized as important nutrients for larvae [206,207]. Schizochytrium, Crypthecodinium and other algae that contain high levels of DHA have been used as a source of DHA for the aquaculture food chain [205,208]. *Schizochytrium* has been shown to enrich and boost the fatty acid and DHA content in rotifers and *Artemia* and to improve larval growth [205]. In addition, EPA is recognized as an important fatty acid in larval nutrition and the ratio of DHA/EPA is critical for larval development [201]. Recently, AA is also receiving attention as a potentially important nutrient for larval nutrition and it is possible that the ratio of these three fatty acids might be more important than their absolute levels [62,207]. Indeed, some fatty acids are essential for many marine animals [209] and similar requirements exist for the growth and metamorphosis of many larvae [190,210]. Microalgal vitamin content also has to be taken into account as it may be equally important [165,191].

Alterations in pigmentation can also be an important criteria for organisms grown in culture because they can affect commercial acceptability. Artificial diets typically lack the natural sources of pigments, that give organisms, such as, salmon, and trout their characteristic coloration. As a result, the carotenoid astaxanthin is used as a supplement feed [211]. In the natural food chain, algae are the primary source of astaxanthin and other pigments. For artificial diets, synthetic sources are commonly used because of reduced costs. The algae *Hematococcus* has been found to be an abundant producer of astaxanthin [212] and several companies have successfully commercialized *Hematococcus* as a source of natural astaxanthin for animal feeds [213,214]. In fact, microalgal astaxanthin has been approved in Japan and Canada as a pigment in salmonid feeds [215]. Feeds including 5% to 20% Arthrospira (rich in carotene pigments), which enhance the red and the yellow patterns in carp, while leaving a brilliant white color. This clarity and color definition increases their value (Resource center for *Spirulina* and microalgae; official web page, 2005 [216]). Another example is the traditional French technique called the greening of oysters. It consists of creating a blue-green color on the gills and

labial palps of oysters using the diatom *Haslea ostrearia*. This increases the product's market value by 40% [199].

The main applications for algal biomass in aquaculture are: fish feed [180] including larval nutrition for molluscs or peneid shrimp [199]; coloring for farmed salmonids [199]; stabilization and improvement of quality of culture medium ('green-water' technique) [196]; inducement of essential biological activities in bred aquatic species [199] and enhancement of the immune systems of fish [217].

5.1.1.2.2. Animal feed (pets and farming). Many nutritional and toxicological evaluations have proved the suitability of algal biomass as feed supplements [146]. *Arthrospira* is largely used in this domain and concerns many types of animals: cats, dogs, aquarium fish, ornamental birds, horses, cows and breeding bulls. Algae positively affect the physiology (by providing a large profile of natural vitamins, minerals and essential fatty acids, improved immune response and fertility, better weight control) and their external appearance (resulting in healthy skin and a lustrous coat) of animals [218].

In poultry rations, algae up to a level of 5–10% can be used safely as a partial replacement for conventional proteins. The yellow color of broiler skin and shanks as well as the egg yolk, is the most important characteristic that can be influenced by feeding algae [146].

5.1.2. Bioactive compounds

5.1.2.1. Fatty acids. Microalgae, especially marine microalgae, are also excellent sources of polyunsaturated fatty acids such as linolenic acid, AA, EPA and DHA [219,220]. These essential fatty acids are important for the treatment and prevention of a range of diseases and also important in human nutrition [221,222]. Algal species can be selected for the preponderance of a particular fatty acid and the content of these fatty acids can be manipulated by changing the culture conditions [223,224].

Certain microalgae produce large quantities of oils and fats containing long-chain omega-3 and omega-6 fatty acids (LC-PUFA). LC-PUFA are long-chain polyunsaturated fatty acids ('LC-PUFA), such as DHA and EPA [225]. LC-PUFA are essential to human nutrition and health and recent studies have indicated that certain of these LC-PUFA may be associated with physical, mental and visual development in infants [226]. In addition, omega-3 fatty acids are a part of a healthy diet that helps to lower the risks of diseases and include cardiovascular disease, various cancers, arthritis and dementia [227].

A number of algal groups have been identified that produce high levels of LC-PUFA, including diatoms, chrysophytes, cryptophytes, dinoflagellates and others [228,229]. Table 7 represents the predominant PUFA from different microalgal species [59].

DHA omega-3 LC-PUFA has 22 carbon atoms and 6 methylene-interrupted *cis*-double bonds (22:6). It is a dominant fatty acid in neurological tissue, constituting 20–25% of the total fatty acids in the gray matter of the human brain and 50–60% in retina rod outer segments. It is also abundant in heart muscle tissue and sperm cells [230–232]. Humans are not capable of synthesizing DHA *de novo* and their capacity to synthesize DHA from its precursor, a linolenic acid, is relatively poor. Thus, adequate supplies of DHA must be obtained from dietary sources [233].

DHA is the predominant structural fatty acid in the gray matter of the brain and retina and must be supplied, preformed, in the diet; particularly it is important for correct brain and eye development in infants. It is essential for the proper functioning of brains and has been shown to support cardiovascular health in adults [234,235]. Microalgae such as *Crypthecodinium cohnii* (40–50% DHA but negligible EPA), *Schizochytrium* (40% DHA, 17% docosapentaenoic acid) and *Ulkenia* sp. are well suited for the production of DHA [59,236].

The dino-flagellate *Crypthecodinium cohnii* can produce most of its fatty acid as DHA [229] with no other detectable LCPUFA, such as EPA or ARA. From 1990 onwards, a number of health and nutrition organizations specifically recommended the inclusion of DHA in infant formula for pre term and full term infants. The world wholesale market for infant formula is now estimated to be about US$10 billion per annum [235]. Martek's DHA oil for this application (DHASCO; Martek, Columbia, MD, USA) comes from *C. cohnii* and contains 40–50% DHA but no EPA or any other long-chain PUFAs [235,237,238]. DHA oil produced from *C. cohnii* is currently available worldwide (including Europe, Australia, Asia and the Middle East) [239].

DHA are also produced commercially from *Schizochytrium* and these are mainly used for adult dietary supplements (including cheese, yogurt, spreads, dressings, cereals) and foods for pregnant and nursing women [59]. Moreover, OmegaTech (USA), also owned by Martek, exploits *Schizochytrium* to produce a low-cost oil formerly known as DHA Gold [238]. The oil is currently used as an adult dietary supplement in food and beverages, health foods, animal feeds and maricultural products. Finally, the Nutrinova process (Frankfurt, Germany) uses *Ulkenia* sp. which grows in 80-m^3 fermenters. The oil is sold under the name of DHActive [238].

A number of algae have been proposed for the production of EPA, including *Nitzschia* sp. [240], *Nannochloropsis* [223], *Navicula* sp. [241], *Phaeodactylum* [242] and *Porphyridium* [243]. In addition, EPA is a LCPUFA, but with 20 carbons and 5 double bonds (20:5). Changes in EPA levels can significantly change an individual's coronary vascular status because the products of EPA metabolism are eicosanoids with antithrombotic and antiaggregatory effects [231]. A process for producing high-purity EPA, another omega-3 fatty acid (20:5), from *Phaeodactylum tricornutum* has been developed by the University of Almeria in Spain. An economic analysis, on a potential facility producing 430 kg 96% pure EPA per year, estimated the total cost of production at US$4602/kg, with 60% of the cost arising from the recovery process and 40% from the biomass production. It is believed that the cost needs to

be reduced by 80% to be economically viable. The residual biomass following the extraction of the EPA contains high amount of residual solvent to be sold for animal feed and therefore must be incinerated [244]. The annual worldwide demand of EPA is 300 t [60].

Algae can also serve as a source of genes involved in PUFA synthesis. Once the genes are isolated and characterized, they could be evaluated for suitability for transfer into other organisms, such as higher plants [245].

In addition, new algal sources for the very long chain n-3 polyunsaturated fatty acids (VLCPUFA) ($>$ 18 C) are being examined. These include the production of EPA in *Glossomastix chrysoplasta* [246] and screening of different strains of *Thraustochytrium* sp. for optimization of n-3 VLCPUFA production [247].

Harwood and Guschina [248] introduced the major cellular lipids and their fatty acids and also described how the PUFAs are synthesized. The discovery of different elongases and desaturases important for PUFA production and their application for biotechnology was detailed. Finally, the potential for algae in commercial applications was discussed, particularly in relation to the production of very long chain PUFAs and biofuel [248].

5.1.2.2. Carotenoids. Algae contain carotenoids, (yellow, orange or red pigments) that include the nutritional and therapeutic values, which are due to their ability to act as provitamin A, that can inturn be converted into vitamin A [249–251]. Carotenoids such as β-carotene and fucoxanthin also have anti-tumor and cancer preventive activity [252,253]. Moreover, carotenoids have intrinsic anti-inflammatory properties owing to their quenching action on relative oxygen species and a therapeutic chemo preventive

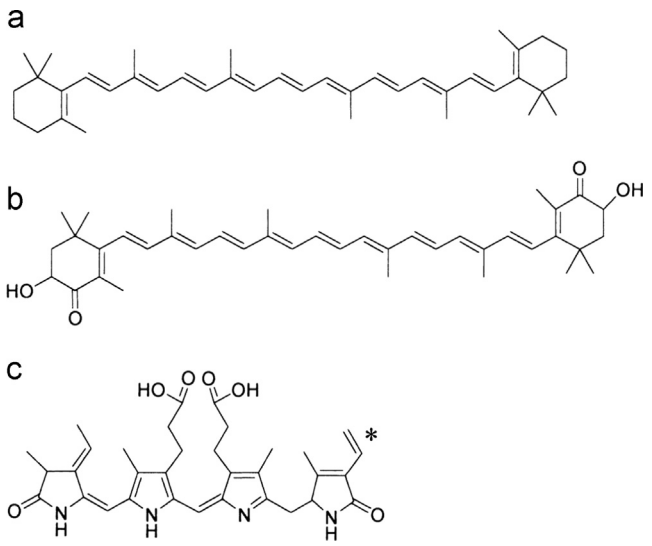

Fig. 11. Chemical structures of microalgal pigments. (a) β-Carotene, (b) Astaxanthin, (c) Phycoerythrin (in phycocyanin, the $CH=CH_2$ group noted an asterisk is replaced by $CH_3–CH_2$) [59].

anticancer effect is sometimes attributed to these molecules [250,254,255]. The carotenoids are widely used as food colorants and supplements for human and animal feeds (poultry, fish) [57,59]. Carotenoids also have applications in cosmetics [255]. Among 400 known carotenoids, only a very few are used commercially (β-carotene, astaxanthin) (Table 8) and, others are of lesser importance (lutein, zeaxanthin, lycopene and bixin) [255,176]. The structures of β-carotene, astaxanthin are presented in Fig. 11a and b [59].

5.1.2.2.1. β-Carotene. The average concentration of carotenoids in most algae is only 0.1–2%, but *Dunaliella* when grown under the right conditions of high salinity and light intensity will produce up to 14% β-carotene [29,57,59,60,172]. *Dunaliella* is, therefore, well suited for the commercial production of β-carotene and several industrial production plants are in operation around the world including Australia, Israel, USA and China [59]. It can be cultivated outdoors in open ponds owing to the extreme conditions under which it grows (hypersaline, low availability of nitrogen and high levels of solar radiation) [249,256].

The major producer of β-carotene is Cognis Nutrition and Health, whose farms cover 800 ha in Western Australia, Whyalla and South Australia [257]. Three categories of products which are derived from *D. salina* are: β-carotene extracts, *Dunaliella* powder for human use and dried *Dunaliella* for feed use. The prices of these products in 2004 varied from US$300 to US$3000/kg [59,258,259]. It has been reported that β-carotene from *Dunaliella* is a substantial growing industry and its commercial utilization is economically viable [60,28].

5.1.2.2.2. Astaxanthin. Astaxanthin is another carotenoid that can be derived from algae and is principally used in pharmaceuticals, cosmetics, nutraceuticals, agriculture and animal nutrition [260–263]. It is a potent antioxidant [264] and has possible roles in human health such as UV-light protection, immune enhancement, hormone precursor, pro-vitamin A source and for anti-inflammation [215]. It is also a strong coloring agent, with uses for coloring muscles in fish [217]. The annual worldwide aquaculture market for this pigment in 2004 was estimated to be US $200 million with an average price of US$2500/kg [265]. Astaxanthin can be produced by *Haematococcus*, a freshwater alga that normally grows in puddles, birdbaths and other shallow fresh water depressions [212,266]. *Haematococcus* contains up to 3% astaxanthin, but it requires a two stage culture process, which is not suited to open pond cultivation. The first stage of the process is designed to optimize algal biomass (green-thin walled flagellated stage with optimum growth at a temperature of 22–25 °C) and the second stage (thick walled resting stage) under intense light and nutrient poor conditions during which astaxanthin is produced [59,172].

Natural astaxanthin is preferred, for example in carp, chicken and red sea bream diets, due to enhanced natural pigment deposition, regulatory requirements and consumer demand for natural products [59,267].

Commercial production is being carried out in Hawaii, India and Israel, where Algatech sell a crushed *Haematococcus* biomass on the pharmaceutical market [59,172,268]. Cyanotech, in Hawaii claimed a market share of over 95% of the animal nutrition market for algae-based astaxanthin products [269]. Moreover, since the 1990's, human nutraceuticals have appeared as a new market possibility [254,264] and Algatech (Kibbutz Ketura, Israel) sells its product (crushed *Haematococcus* biomass rich in astaxanthin) in the pharmaceutical market [59].

5.1.2.2.3. Lutein. Lutein is a xanthophyll and one of the naturally available carotenoids. *Muriellopsis* sp. a microalgae, that is able to accumulate high levels of carotenoids, such as lutein, that is used for the prevention and treatment of degenerative diseases [51]. The lutein market is segmented into pharmaceutical, nutraceutical, food, pet foods and animal and fish feed. The pharmaceutical market is estimated to be around US$190 million, nutraceutical and food is estimated to be around US$110 million. Pet foods and other applications are estimated to be US$175 million annually. Apart from the customary age-related macular degeneration applications, newer applications are emerging in cosmetics, skins and as an antioxidant. It is one of the fastest growing areas in the US$2 billion carotenoid market.

5.1.2.3. Other bioactive compounds. Microalgae contain several different types of sterols, including clionasterol, isolated from *Spirulina* sp., which are shown to increase the production of plaminogen-activating factor in vascular endothelial cells and thus facilitate cardiovascular disease prevention [173]. Additionally, several antioxidants' compounds (e.g. dimethylsulfoniopropionate, mycosporines or mycosporinelike amino acids) have been isolated from microalgal sources, having the potential to protect against oxidative stress, a wide spectrum of diseases and ageing [43].

5.1.3. Fluorescent pigment

Pigments present in algal photosynthetic systems are being utilized for commercial applications [270]. The most widely used are the phycobiliproteins. Phycobiliproteins are a family of light-harvesting macromolecules that function as components of the photosynthetic apparatus in cyanobacteria and several groups of eukaryotic algae, including the red algae, cryptomonads and glaucophytes [271,272]. They are deeply colored (red or blue), water soluble, complex, proteinaceous compounds. These algae pigments have the potential as natural colorants for food, cosmetics and pharmaceuticals. Their main function is to trap light energy in the 495–650-nm wavelength range and transfer it to chl *a* of the photosynthetic reaction centers.

Phycobiliproteins can be divided into three major groups based on their spectral properties [62]:

i. phycoerythrin (PE) Amax = 560 nm, emission = 580;
ii. phycocyanin (PC) Amax = 620 nm, emission = 650;
iii. allophycocyanin (APC) Amax = 650 nm, emission = 660 nm.

Each of the different phycobiliproteins assemble into high-molecular-mass complexes composed of two non-identical polypeptide subunits (α and β) (Fig. 11c) [59]. The number of

chromophores present in these complexes range from 6 to 34 and these complexes have extremely high absorbance coefficients. When excited with light energy at the maximal absorbance, greater than 90% of the absorbed energy can be emitted as fluorescence [272].

Many characteristics make phycobiliproteins well suited for commercial applications: (1) they have large numbers of chromophores and high quantum yields, (2) they are capable of large Stokes shifts (displacement of absorption and emission wavelengths) with the fluorescence emission at wavelengths with minimal auto fluorescence from biological materials, (3) they form very stable conjugates with many materials, (4) they are fully water soluble and (5) they can be efficiently excited by argon or helium–neon lasers [62]. The ability to form stable conjugates with antibodies, strepavidin, biotin and so on are especially important for developing valuable applications for the phycobiliproteins. This allows the phycobiliproteins to function as fluorescent tags for labeling highly specific probes to identify cell types or proteins [273]. Some of the more significant applications are in flow cytometry and fluorescence-activated cell sorting. In these applications, PE is 20 times brighter on a molar ratio than on a fluorescein isothiocyanate and provides an important additional color for multicolor detection systems in conjunction with other fluorescent pigments. In addition, APC is a significant pigment for flow cytometry applications. Biliproteins have also been widely used in immune-histochemistry [61].

Phycobiliprotein complexes assemble into extremely large macromolecular complexes called phycobilisomes [272]. Phycobilisomes are unstable in low salt buffer and at dilute protein concentrations. Recently, methods have been developed to stabilize the phycobilisomes, by chemical crosslinking [274]. Stabilized phycobilisomes have the same advantages as individual biliproteins do but contain up to 1400 chromophores, making them the most powerful fluorescent pigments, currently available on a per-binding-event basis. They have broad wavelength adsorption characteristics, with the prominent absorption peaks corresponding to each of the phycobiliproteins present. This is well suited for excitation by both argon and helium-neon lasers. They can also have extraordinary Stokes shift of up to 178 nm. The emission wavelength of approximately 670 nm provides minimal overlap with mammalian cell auto-fluorescence. They are easily conjugated to the same materials as the individual biliproteins, which include antibodies, peptides, streptavidin, biotin and DNA. Stabilized phycobilisomes are commercially available as secondary labels for a variety of uses.

Phycobilisomes are well suited for direct fluorescent detection in immunoblots, in which they are capable of detecting sub-picogram levels of protein [275]. In microplate immunoassays, phycobilisomes are capable of detecting 40-femtomolar levels of antigenic protein, with a linear assay range of four orders of magnitude [276]. For use in flow cytometry, they are five-fold brighter than PE and thus well suited for detection of low density cell surface markers, which were previously undetectable through conventional fluors.

Phycoiliproteins from cryptomonads, which provide unique absorption and emission characteristics [277] along with relatively low molecular mass (< 50 kDa), are also commercially available. These have possible applications for use as intracellular markers or in cases, in which specialized absorption and emission requirements are desired.

Dino flagellates also produce a pigment, that has found limited application as an additional color in flow cytometry [278]. The peridinin chlorophyll proteins are water-soluble pigments containing carotenoids and chl *a*.

Dainippon Ink and Chemicals produce a blue food colorant from *Spirulina*, called Lina blue, that is used in chewing gum, ice slush, sweets, soft drinks, dairy products and wasabi [47,59,279]. Phycobiliproteins can be commercially produced from *Spirulina* and the red microalgae Porphyridium and Rhodella [57,59,60,172]. In 1997, the global market for Phycobiliproteins colorants was estimated at US$50 million and prices vary from US$3 to US$25/mg [59].

5.1.4. Stable-isotope biochemicals

Microalgae are ideally suited as sources of stable isotopically labeled compounds. They are easily handled and cultured and their ability to perform photosynthesis allows them to incorporate ^{13}C, ^{15}N and ^{2}H from relatively inexpensive inorganic compounds (i.e. $^{13}CO_2$, $^{15}NO_3$ and $^{2}H_2O$) into more highly valued organic compounds [62]. For unicellular microalgae, each cell is exposed to the isotope, resulting in uniform labeling of compounds. Closed photobioreactor systems make it possible to have a very high conversion of $^{13}CO_2$ into biomass, thus minimizing the cost associated with producing ^{13}C labeled substrates. Microalgae are metabolically very flexible and can be made to overproduce a variety of different products through simple manipulations of the culture environment [229,280,281].

One application for algal-produced stable isotopically labeled complex organic compounds is forming the basis of culture media of bacteria, yeast and mammalian cells. Stable isotopes provided in the media are incorporated into cellular components and, in particular, proteins. Proteins of interest can be produced in large quantity using molecular technology. It is coupled with recent developments in multidimensional NMR technology and stable-isotope-editing techniques [282]. These techniques are also used to determine the primary, secondary and tertiary structures of small and medium-sized proteins [283,284]. Structural information can be used to predict the interactions of substrates with the active sites of proteins and to detect the specific site which is used to alter biological activity of the protein [285,286].

Two commonly used stable isotopically labeled compounds for cell culture are glucose and glycerol. Many algae (especially chlorophytes) are known to accumulate high levels of glucose in the form of starch [280]. When these organisms are grown in the presence of $^{13}CO_2$, they will produce labeled starch that can be easily hydrolyzed and purified as crystalline ^{13}C-glucose. Similarly, *Dunaliella* produces high levels of glycerol and has been used for ^{13}C-glycerol production. In addition to the use of glucose and glycerol as cell culture nutrients, other stable isotopically labeled compounds derived from algae are being used to study macromolecular interactions and the elucidation of metabolic pathways [62]. For example, ^{13}C glucose has been included in growth media, enabling the algae to produce ^{13}C-DHA-containing triglyceride, which is used to study the metabolism and turnover of DHA [287].

Algal-derived stable isotopically labeled compounds have also been used as metabolic tracers to elucidate various metabolic pathways [288,289]; ^{13}C-palmitic acid has been used to measure palmitic acid flux in the blood [290] and labeled galactose has been used to follow carbohydrate metabolism in the liver [289]. A variety of labeled fatty acids has been used to monitor fatty acid metabolism. For example, ^{13}C-labeled linoleic acid and linolenic acid have been useful in studying the synthesis of polyunsaturated fatty acids in infants [291].

Breath tests for the diagnosis of medical disease and dysfunction represent another application for the use of microalgal-derived stable isotopically labeled products. A breath test is simply the determination and quantitation of the compounds in human breath. The principle of these tests is that a substrate labeled with ^{13}C is ingested, absorbed from the small intestine and ultimately metabolized to carbon dioxide. The magnitude and the rate of the appearance of $^{13}CO_2$ in the exhaled breath is used to diagnose the subject's physiological state. Several different

approaches to measure $^{13}CO_2$ have been developed. These include the use of isotope ratio-mass spectrometry, infrared spectroscopy and laser-based systems [292].

Several *Chlamydomonas* sp. are known to produce high levels of a galactose containing polysaccharides [229], which can be hydrolyzed to produce monosaccharides; ^{13}C-galactose has been used to measure liver function [229,293,294] and its non-invasive nature gives it an advantage over liver biopsy. In addition, ^{13}C-xylose has been produced from Chlamydomonas, which can produce nearly 25% of its biomass as xylose; ^{13}C-xylose has been used to diagnose bacterial overgrowth of the small intestine [295] because xylose is poorly absorbed from the small intestine and is metabolized largely by colonic microflora.

Finally, ^{13}C-labeled mixed triglycerides (known as Hiolein) have been produced from *Neochloris* and used to diagnose fat malabsorption [296]. Hiolein is a triglyceride oil, that contains over 50% oleic acid and it is functionally equivalent to other triglycerides that have been used as breath test substrates [297,298]. Market value of stable-isotope compounds from algae is probably higher than US$13 million/year [59]. Spectra Stable Isotopes (Columbia, MD, USA), a division of Spectra gases (part of Cambridge Isotope Laboratories) sells its marked amino acids at prices in the range from US$260/g to US$5900/g and its marked nucleic acids at about US$28/mg [59,299]. Moreover, it has recently developed a process for the autotrophic production of labeled PUFAs from microalgae using $^{13}CO_2$, in which $^{13}CO_2$ is directly sparged into the culture as required. Thus, the carbon loss is high and there is a low efficiency of labeled carbon use. In spite of these considerations, this company is manufacturing more than 400 g per year of labeled fatty acids at US$38,000/g [62,300] (Table 9).

5.1.5. Drug screening

Algae are a very diverse group of organisms, that occupy a wide variety of ecological niches. As such, they have the potential to be a rich source of bioactive compounds.

A large number of bioactivities have been reported in algae, including anticancer, antimicrobial, anti-HIV, antiviral and various neurological activities [301–305]. Some species of blue green algae and dinoflagellates can produce highly potent toxins [303]. For example, the microcystins are a group of circular peptides produced by blue green algae and some of the more potent derivatives have an LD50 of 50 μg/kg [306]. Saxitoxin and the brevetoxins are produced by dinoflagellates and each has significant bioactive effects on humans and fish [307]. Table 9 presents the high value bioproducts from microalgae.

In addition to toxins, many other bioactive compounds have been found in algae [301,302,304]. The National Cancer Institute (NCI) demonstrated that algal-sulfolipids had in vitro activity against the HIV virus [308]. More recently, NCI discovered cyanovirin from the blue-green alga *Nostoc ellipsosporum* [309]. This compound is a low-molecular-weight protein that can be produced as a recombinant molecule in *E. coli*. Cyanovirin irreversibly inactivates HIV, without adversely affecting the host cells [62].

5.1.5.1. Antimicrobial agents from microalgae. A large number of microalgal extracts and/or extracellular products have been found to have antimicrobial (antibacterial, antifungal, antialgal, antiprotozooal) activity [310]. Table 10 represents the antibacterial and antifungal substances identified from microalgae [311–329].

Microalgae antimicrobial compounds serve as useful leads to new synthetic antibiotics or may find application in agriculture. For example, the tjipanazoles, isolated from the cyanobacterium, Tolypothrix tjipanensis, are indolo (2,3-a) carbazoles, similar to those found in actinomycetes and slime molds, but without a pyrrolo (3,4-c) ring [319]. They show little cytotoxicity and no *in vivo* activity against Candida albicans, however tjipanazole Al and A2 show appreciable fungicidal activity against rice blast and leaf rust wheat infections.

Table 10
Antibacterial and antifungal substances identified from micro algae [311].

S. no.	Antibacterial and antifungal substances	References
1.	Fatty acids	[312–313]
2.	Glycolipids	[314]
3.	Acrylic acid	[315]
4.	Phenolics	[316]
5.	Bromophenols	[317]
6.	Terpenoids, carbohydrates	[314,318]
7.	N-glycosides	[319]
8.	Peptides	[320]
9.	Polysaccharides	[321–322]
10.	Acrolyl-choline	[323]
11.	Acrolyl-diketone	[324]
12.	Isonitrile (indole alkaloids such as haploindole)	[325]
13.	Nodularin, goniautoxin, saxitoxin, okadaic acid and ciguatoxin	[326–329]

Other algal toxins may also be of interest in environmental management. For example, the algacides produced by some cyanobacteria, such as the y-lactone, cyanobacterin, produced by *Scytonema hofmanni* [330], fischerellin from *Fischerella muscicola* [331] and an unidentified extracellular product of an *Oscillatoria* sp. [332–333] may find use in the control of algal blooms. Cyanobacteria have also been patented as a herbicide [330].

5.1.5.2. Antiviral activity of microalgae. A number of cyanobacteria and very few other microalgae, have been screened for antiviral activity [334]. For example, Rinehart et al. [335] have found that over 5% of the extracts of cultured cyanobacteria screened by them showed antiviral activity against *Herpes simplex* virus type II and $>5\%$ had activity against respiratory syncytial virus. Lau et al. [336] have also screened extracts of over 900 strains of cyanobacteria for inhibition of reverse transcriptases of avian myeloblastosis virus and human immunodeficiency virus type 1 and they found that over 2% of these algae showed promising activities. The active compounds have, however, not been identified, yet with the exception of an anti-AIDS sulfolipid [337].

5.1.5.3. Anticancer activity of microalgae. Amongst the cyanobacteria, numerous cytotoxic compounds, some of which have potential as anticancer drugs have been characterised [334]. These compounds include tubericidin and toyocamycin [338,339] and new unique macrolides such as scytophycin B isolated from *Scytonema pseudohofmanni*. The scytophycins show cytotoxicity against the KB (a human nasopharyngeal carcinoma) cell line, as well as moderate activity against murine, intraperitoneally implanted P388 lymphocytic leukemia and Lewis lung carcinoma [340,341]. Similar activities have been reported for the scytophycin, tolytoxin, from *Tolypothrix conglutinate* var. *colorata* and *S. mirabile* [342,343] and for indocarbazoles isolated from *Nostoc* [344]. The cytostatic effect of tolytoxin apparently results from an inhibition action of polymerization, thus disrupting microfilament organization in eukaryotic cells [345,346]. The acutiphycins from *Oscillatoria acutissima*, are another group of macrolides with cytotoxicity against KB, as well as activity against murine, intraperitoneally implanted, Lewis lung carcinoma [347]. Macrolides with antitumour action have also been isolated from dinoflagellates; i.e. amphidinolide-A from an *Amphidinium* sp. [348]. An alternative screen for potential anti-cancer activity, including protein kinase C, protein tyrosine kinase and inosine monophosphate dehydrogenase has also resulted in a range of compounds from cyanobacteria, cryptophytes and chrysophytes [349].

Concordance with the current discussion, Borowitzka [311] stated that the great biochemical diversity of microalgae makes them a valuable potential renewable source of new drugs, growth regulators and other useful chemicals. The author reviewed the status of microalgae as sources of pharmaceuticals and other biologically active molecules.

5.1.6. Microalgal recombinant proteins

Recently, tremendous advances have been made in the tools available to study a variety of algae. Highly sophisticated molecular systems are being used to dissect biological processes in many cyanobacteria [350]. The cyanobacteria can be readily transformed with autonomously replicating plasmids and endogenous genes can be disrupted by homologous recombination. Although a number of commercial possibilities have been proposed for recombinant cyanobacteria [351,352], the potential is yet to be realized.

A novel application of recombinant techniques was to transfer the *cryIVC* gene for producing Bt toxin to *Synechococcus* [353–354]. Cyanobacteria are a food source for mosquito larvae and the Bt toxin is capable of inhibiting larval development. In principle, recombinant cyanobacteria could be dispersed in areas of high mosquito infestation and as the larvae consume the cyanobacteria, larval development would be inhibited. An attempt is being made to commercialize cyanobacteria containing Bt toxin [355], but this venture faces obvious difficulties because of the potential for widespread dispersal of recombinant organisms that might rapidly lose their effectiveness because of the development of resistant larvae.

Advances in eukaryotic algal recombinant techniques have recently been extensively reviewed [356]. *Chlamydomonas* has developed into a sophisticated molecular system that has made important contributions to the understanding of photosynthetic processes. Although recombinant *Chlamydomonas* does not have direct commercial applications, the technology developed for *Chlamydomonas* has provided the direction for the development of transformation techniques in other algae.

Recently developed transformation techniques for *Chlorella* [357] and diatoms [358,359] have potential use in direct commercial applications. At the very least, recombinant techniques in economically valuable algae provide an important tool for elucidating and understanding the biochemical pathways responsible for the synthesis of products of interest (e.g. biosynthesis of PUFAs). At this point, recombinant techniques have not contributed directly to a commercial product. However, with public and government acceptance of recombinant and continued progress in developing methodologies for algal systems, significant contributions could be realized in the near future.

5.1.7. Microalgae in cosmetics

Some microalgal species are used in cosmetics industries, especially in the skin care market, the main microalgae species are *Arthrospira* and *Chlorella* [360]. Microalgae extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers). Microalgae are also utilized in sun protection and hair care products.

Examples of commercially available products and their properties claimed by their companies; 1. A protein-rich extract from *Arthrospira* repairs the signs of early skin aging, exerts a tightening effect and prevents stria formation (Protulines, Exsymol S.A.M., Monaco); 2. An extract from *C. vulgaris* stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction (Dermochlorella, Codif, St. Malo, France).

Recently, two new products have been launched by Pentapharm (Basel, Switzerland): 1. An ingredient from *Nannochloropsis oculata* with excellent skin-tightening properties (short and long-term effects) (Pepha-Tight); 2. An ingredient from *D. salina*, which shows the ability to markedly stimulate cell proliferation and turnover and to positively influence the energy metabolism of skin (Pepha-Ctive) [360].

5.1.8. Microalgae role as biofertilizer

Pyrolysis is the conversion of biomass to bio-oil, syngas and charcoal at medium to high temperatures (350–700 °C) in the absence of air [74]. This conversion process leads to the formation of the solid charcoal residue called "biochar" from algae, that has potential agricultural applications as a biofertilizer and for carbon sequestration [361]. Biochar can also be utilized as process fuel in bioenergy conversion. It is considered a long-term sink in carbon sequestration process, which could be used to reduce carbon dioxide emissions by up to 84%. This biochar sequestration offers the potential to produce a carbon-negative biofuel [362].

5.2. Commercial applications of macroalgae

The use of macroalgae as a potential source of high value chemicals and in therapeutic purpose has engrossed its commercial interest on macroalgae. Recently, macroalgae have been used as a novel food with potential nutritional benefits and in industry and medicine for various purposes. Furthermore, macroalgae have shown to provide a rich source of natural bioactive compounds with antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypercholesterolemia and hypolipidemic and anti-neoplasteic properties. Thus, there is a growing interest in the area of research on the positive effect of macroalgae on human health and other benefits [363].

Macroalgae have mainly been used as a raw material to extract alginates (from brown algae), agar and carragenates (from red algae). Moreover, algae also contain multitude of bioactive compounds (phenolic compounds, alkaloids, plant acids, terpenoids and glycosides) that have antioxidant, antibacterial, antiviral, anticarcinogenic, etc. properties [364]. Primarily brown algae, the largest and most conspicuous of the macroalgae and red algae, has a diverse algal group.

5.2.1. Foods from macroalgae

The major foods derived from macroalgae are illustrated in Table 11 [44,365]. The algal biomass for these products is derived from wild, managed, or cultivated stands of macroalgae that undergo a minor processing after harvest. In these cases, the product is the biomass itself, rather than chemicals extracted from the algae. The post-harvest processing serves only to clean and preserve the intrinsic character of the algae.

5.2.1.1. Nori. The major algal product in the world today is nori, the algal blade (called a thallus) of certain species of the red macroalgae *Porphyra*. Nori is a primary constituent of "SUSHI", a Japanese food item that is becoming increasingly popular in the West. The methods employed in the harvesting and drying of the seaweed are often small scale, traditional and primitive [366]. In the case of nori, however, modern techniques introduced in the 1960s have provided the means to rapidly increase the production yields [367]. Nori cultivation is a type of farming, in which seed like propagules, called conchospores, are seeded onto nori nets, which are hung in sheltered ocean areas. Before the mid 1960s, nori cultivation was limited to shallow, sandy bays, where the nori nets could be hung between poles stuck in the bottom. After this time, the nets were often attached to surface buoys, so that deeper water could be used for cultivation. Around 1970, a system was developed to raise the nets out of the water, which allowed the controlled drying and temporary storage of the porphyra thalli.

These sophisticated growth systems and procedures, coupled with fast growing cultivation forms and mechanized methods for processing, have provided ample production capacity and nori supply has recently exceeded demand [367]. Many nori products are available; of these, toasted nori sheets are the largest product segment. With a market value of approximately $2 billion and a product volume of 40,000 t per year [365], nori represents the most successful algal product group now. Although nori is primarily consumed in Japan, Korea and China, sales in other countries are rapidly increasing US sales in 1991, were estimated to be $20- $25 million [368].

5.2.1.2. Wakame. Another major algal food product is wakame, a group of related foods derived from a brown alga known as *Undaria pinnatifida*. This macroalgal product has been cultivated commercially since the middle of 1950s [369]. Similar to nori, wakame is produced mainly in Japan, Korea and China. Wakame is more extensively processed after harvest than most other macroalgal biomass products. The most popular wakame product is boiled and salted, which results in the green product most preferred by consumers. As with nori, the primary market for wakame products is Japan, where it is available in many forms (e.g., salted or dried cut) and is used as an ingredient in soups, salads and noodles. As of 1990, in the region of 20,000 t, with a market value of $600 million, were sold annually [49].

5.2.1.3. Kombu. The third major algal food product group is kombu, which is derived from *Laminaria japonica* and related species of brown macroalgae. These algae are collected during the summer, dried naturally and then boiled. A variety of kombu products are produced, which can be served with meat, fish, or soups or as a vegetable [370]. The annual market for these products is around $600 million.

5.2.1.4. Other macroalgal foods. Many other macroalgae are also used for human consumption as nutritious food items. For example, the red macroalga *Palmaria palmata*, known as “Dulse”, has been consumed by shoreline populations of northwest Europe since approximately the tenth century [371]. Another dulse, *Rhodymenia* sp., is harvested and consumed in parts of North America, particularly the Maritime Provinces of Canada [366], where it is promoted as a sea vegetable.

5.2.2. Industrial products from macroalgae

5.2.2.1. Hydrocolloids. Table 11 summarizes the uses and market values of the major polysaccharide products derived from macroalgae. These products, also referred to as “hydrocolloids”, make up the major industrial products derived from algae at the present time; their combined market value is well over $500 million. These products are prepared from wild or cultivated bed type marine macroalgal species (certain species of red and brown algae).

Carrageenans and agars are obtained from different species of red algae. Alginates are extracted from brown algae species. Unlike the food products described above, the macroalgal biomass for these products undergoes extensive extraction and processing to yield the final product [372]. The extreme case is agarose, which is derived from agar (already a processed product) by extensive separation and purification [373].

5.2.2.1.1. Alginates. The alginates are salts of alginic acid; these salts and the sodium salts in particular, are also known as algin. They are polymers composed of D-mannuronic acid and L-guluronic acid monomers. The sequences and proportions of these constituents vary with the source of the algin. The major commercial sources of alginates are brown macroalgae, particularly from *Laminaria* sp., *Macrocystis* sp. and *Ascophyllum* sp.

Table 11
Commercial Products from macroalgae [49].

Product	Use	Market value (million SUS)
Nori	Food	1800
Wakame	Food	600
Kombu	Food	600
Alginates	i. Food products ii. Paper products iii. Biomedical applications	230
Carrageenans	i. Food products ii. Cosmetics iii. Pharmaceutical products	100
Agars	Food products	160
Agarose	i. Biomedical applications ii. Biotechnology applications	> 50
Sea weed meal	Animal feed	5
Manure (“Maerl”)	Agriculture	10
Liquid fertilizer	Agriculture	5
Phycobili proteins	i. Biomedical applications ii. Biotechnology applications	2

Alginates are typically recovered from the macroalgal biomass by extracting the insoluble alginic acid salts with hot alkali reagent (sodium carbonate). The sodium alginate is then separated from the insoluble seaweed residue by filtration and purified [374]. The primary characteristic of alginates is, their ability to form viscous solutions when dissolved in cold water. Alginates provide thickening, gel-forming, water retaining and suspending properties to solutions containing them. These features trigger their importance in food, industrial and biotechnological applications. Approximately 27,000 t of alginates, with a value of $230 million, were sold annually in the year of 1990 [365].

5.2.2.1.2. Carrageenans. The carrageenans are a complex group of polysaccharides derived from red macroalgae. They are made up of galactose related monomers (a-1,3-D-galactose and P-1,4-3,6-anhydro-D-galactose) to which sulfate groups are attached. Three major types of carrageenans, designated kappa, lambda and iota. They are primarily derived from *Eucheuma cottonii*, *Chondrus crispus* and *Eucheuma spinosum*. The carrageenans are recovered from the macroalgal biomass by extraction with hot water. Subsequent processing depends on the characteristics of the product desired [375]. The carrageenans are commercially used to make gel, thicken, suspend and stabilize foods and other products. Approximately 15,500 t of carrageenans, with a value of $100 million, were sold annually at the beginning of this decade [365].

5.2.2.1.3. Agars. The agars are mixtures of polysaccharides extracted from certain red macroalgae. Their unifying characteristic is that, they are all composed of galactose related monomers (D-galactose and 3,6-anhydro-L-galactose). The agars contain varying amounts of sulfate, pyruvate and methoxy groups, the content of which vary with the source of the macroalgal biomass and the subsequent processing procedures. They are derived primarily from species of *Gracilaria*, *Gelidium*, *Pterocladia*, *Acanthopeltis* and *Ahnfeltia*. The agars are usually extracted with hot water. Subsequent processing steps serve to generate a concentrated filtrate, which is allowed to form a gel; this gel is then treated, dehydrated and milled [376]. The ability of agars to form stable gels that retain their characteristics under a range of conditions (e.g., temperature, humidity and chemical milieu) underlies their value in many applications. In addition to their use in foods, agars serve as media to grow microorganisms such as bacteria and yeast. Annual sales of these products are approximately $160 million, on a volume of 11,000 t, circa 1990 [365].

5.2.2.1.4. Agaroses. The agaroses are highly refined, specialized macroalgal products, that have played a pivotal role in the biotechnological revolution [377]. These products are manufactured

by isolating the less ionic fractions of agar, under highly controlled conditions, designed to minimize a lot-to-lot variation. The individual products are targeted to a variety of unique applications, each requiring specific quality assurance protocols. The main applications for the agaroses are in the biotechnology area and these products are key elements in powerful techniques such as gene mapping [377]. The value of more than $50 million per annum is a crude estimate. The unit value of some of these products can be ranging beyond $25,000/kg [49].

5.2.3. Phenolic compounds

Phenolic compounds are secondary metabolites and thus not directly involved in algal primary processes such as photosynthesis, cell division and reproduction. Phenolic compounds are characterized as stress compounds, it involves chemical protective mechanisms against biotic factors such as grazing [378,379], settlement of bacteria or other fouling organisms [380,381] and against abiotic stressors such as UV-radiation [382] and metal contamination [383,384]. However, some phenolic compounds, such as phlorotannins in brown seaweeds, also exhibit primary functions e.g. in growth and the development of the cell wall in *Fucales* [385].

Phenolic compounds contain one or more phenolic rings, which may be halogenated conferring different and often stronger biological activities [386,387]. Phenolic compounds are present in most algal groups; bromophenols are common to all major algal groups (Table 12). To our knowledge, contrary to terrestrial plants, no flavonoid phenolic compounds such as anthocyanins and flavones have been found in algae.

Bromophenols have been detected in red seaweeds mainly in Ceramiales (*Laurencia* sp.) [388,389], and also in Gelidiales [390] and Corallinales [391]. Bromophenols are also identified in brown [392] and green [390] seaweeds.

Mycosporine-like amino acids (MAA), water-soluble molecules, are most commonly produced by Cyanobacteria and Rhodophyta, but they have also been detected in several groups of microalgae and in *Prasiola* sp. [393,394]. Additionally, although these observations are still under debate, there are reports of the presence of MAA in macroalgae, belonging to other Chlorophyta and Phaeophyceae [394,395].

Phenolic terpenoids have been characterised in brown and red macroalgae; the former contain meroditerpenoids (plastoquinones, chromanols, chromenes) found almost exclusively in the Sargassaceae [396]. Red algae contain mainly diterpenes, with sesquiterpenes present in Rhodomelaceae particularly in *Laurencia* sp. [397] and the occurrence of a secondary cyclization forming a macrolide, which belongs to bromophycolides has been reported for *Callophycus serratus* [398]. In Phaeophyceae, the majority of described phenolic compounds are phlorotannins: polymers of phloroglucinol such as fucols, fuhalols, phlorethols [399], eckols and carmalols [400]. They are present only in brown seaweeds but are widespread amongst them, occurring in greatest abundance in the *Fucales* (up to 20% dry weight) [399].

Other non-typical phenolic compounds have been characterised such as colpol in the brown seaweed *Colpomenia sinuosa* [402]; tichocarpols (phenylpropanoid derivatives) in the red macroalga *Tichocarpus crinitus* [403]; coumarins in green seaweeds such as *Dasycladus vermicularis* [404] and some vanillic acid derivatives in another green macroalga, *Cladophora socialis* [405].

A polyphenolic pigment "marennine" in the diatom *Haslea ostrearia* responsible for the oyster "greening" [406]; finally another polyphenolic pigment, scytonemin and phenolic toxins such as microcystin are produced by some Cyanobacteria [395,407]. Lignin (polymerized hydroxycinnamyl alcohols) which commonly occurs in and was previously thought to be restricted to, vascular land plants has also been discovered in the calcified intertidal red seaweed *Calliarthron cheilosporioides* [408].

Table 12
Distribution of phenolic compounds in algal groups [425].

Algal group	Main class of phenolic compounds	Examples
Cyanobacteria	MAA	Asterina-330,euhalothece-362,mycosporine-alanine,mycosporine-glutaminol,mycosporine-glutaminol-glucoside,mycosporine-glycine, palythene, palythinol, porphyra-334, shinorine [393,394]
	Phenolic pigment	Scytonemin [395]
	Toxins	Microcystin [407]
Rhodophyta	Terpenoids	Diterpenes, sesquiterpenes; elatol;bromophycolides [397,398]
	Bromophenols	2-Bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol,2,4,6-tribromophenol [388–391]
	Phenylpropanoid derivatives	Tichocarpols [403]
	Polymerized hydroxycinnamyl alcohols	Lignin [408]
	MAA	Asterina-330, mycosporine-glycine, palythene, palythine, palythinol, porphyra-334,shinorine,usurijene [393,394]
Prymnesiophyceae	MAA	Mycosporine-glycine, mycosporine-glycine-valine, palythine, porphyra-334,shinorine [393,394]
Bacillariophyta	MAA	Mycosporine-glycine, mycosporine-taurine, palythene, palythine,porphyra-334,shinorine [393,394]
	Phenolic pigment	Marennine [406]
Dinophyceae	MAA	Mycosporine-glycine, palythene, palythenic acid, palythine, porphyra-334, shinorine, shinorine methyl ester,usujirene [393,394]
Phaeophyceae	Phlorotannins	Phloroglucinol,phloroglucinolwithaC_{20}acylsidechain;fucols,fucophlorethols,fuhalols,phlorethols; eckols,eckstolonol,phloroeckol, phlorofucofuroeckol-A, triphlorethol-A, dioxinodehydroeckol;carmalol, diphlorethohydroxycarmalol [399–401]
	C_6-C_4-C_6 metabolite	Colpol,8,9-dihydrocolpol [402]
	Meroditerpenoids	Plastoquinones,sargaquinoicacid,sargachromanols,chromenederivatives [396]
	Bromophenols	2-bromophenol, 4-bromophenol, 2,4-dibromophenol,2,6-dibromophenol,2,4,6-tribromophenol [392]
Raphidophyceae	MAA	Asterina-330, mycosporine-glycine, mycosporine-glycine-valine,shinorine [393,394]
Chlorophyta:	Bromophenols	Avrainvilleol,2-bromophenol,4-bromophenol,2,4-dibromophenol,2,6-dibromophenol,2,4,6-tribromophenol [390]
Chlorophyceae	Coumarin	3,6,7-trihydroxycoumarin [404]
	Vanillic acid derivative	Vanillic acid derivative andsulphateadduct [405]
Chlorophyta: Trebouxiophyceae	MAA	Mycosporine-324 [394]

5.2.3.1. Activity of phenolic compounds. Purified phenolic compounds exhibit many activities such as antioxidant [382,404,409], anti-radical [406,410], UV-protection [382,393,411], metal-chelation e.g. copper [383] and anti-fouling [381,412].

Activity of Phenolic compounds e.g phlorotannins varies according to concentration and molecular-size profile [413]. For example, eckols exhibit anti-viral (anti-HIV) [414] and anti-allergic [415] activities. They have anti-adipogenic [416] and neuroprotective [401] effects and have potential application in the treatment of Alzheimer's disease [417,418] and are inhibitors of melanin formation [419]. Bromophenols are used as “marine” flavor agents in farmed fish and prawns [390].

Some meroditerpenoids from *Sargassum fallax* exhibit anti-tumor activities [396] and elatol, a sesquiterpene found in *Laurencia obtusa* var. *dendroidea*, is an anti-parasitic agent [420].

Some specific terpenoids of *C. serratus*, bromophycolides, have anti-malaria properties due to their macrolide structure [398]. Some of these activities have been patented, for example as anti-allergic [421], anti-viral [422] and antioxidant [423] agents and natural UV-screen [424] (‘Helioguard® 365’ using MAA from the red macroalga *Porphyra umbilicalis*) [425].

5.2.4. Macroalgae as a meal and biofertilizer

In addition to the major products described, macroalgae provide several other products with significant commercial impact. For example, three agricultural products such as seaweed meal, manure and liquid fertilizer, each have annual sales of several millions of dollars annually (Table 11). Naylor [366] describes some of these products and their dissemination and use. An interesting group of high value macroalgal products, is the phycobiliproteins. These protein-containing pigments, which are distinctive to certain algae, serve as valuable fluorescent tags with many applications in high technology areas, such as flow cytometry, fluorescence activated cell sorting and histochemistry [273]. The major product in this area is R-phycoerythrin, which is currently derived from species of *Porphyra*, either cultured or harvested from the wild. The high cost of this process is balanced by the high value of the product as a biomedical reagent. The raw material (purified phycobiliprotein) currently sells for approximately $5000/g [49].

6. Conclusions

The growth in world population has resulted in a surge in energy demand and therefore, there is a need for secure energy sources. All the countries are grappling, with the problem of meeting, the ever increasing demand of transport fuels within the constraints of international commitments, legal requirements, environmental concerns and limited resources. Biofuels are an excellent substitute for conventional diesel fuel, because of being renewable, nontoxic and biodegradable. Algae are a potential alternative source for the conventional feedstocks. Current efforts and business investment are driving attention and marketing efforts on the promises of producing algal biodiesel and superior production systems. Algal biofuel production is potentially sustainable but economic feasibility is the major hindrance for its commercialization.

Apart from potential feedstock for biofuel production, Algae plays an important role in environmental pollution control, human health, animal and aqua nutrition, cosmetic industry, pharmaceutical filed and as a source for bioactive compounds, biomedical components and high value pigments.

Algae farming can be coupled with flue gas CO_2 mitigation and wastewater treatment. It can also be carried out with seawater as the medium, given that marine algal species are adopted, providing a feasible alternative for biofuel production to populous and dry coastal regions.

The key message arising from this study is cost-effective technologies and the processes to convert biomass into useful biofuels and bioproducts, with particular focus on algal biorefinery concepts. This biorefinery approach helps to improve the economic viability of the algal biofuels.

The paper has discussed the possible conversion technologies for biofuel production from both macroalgae and microalgae. The production technologies for different biofuels including biodiesel, bio-oil, bio-syngas, bio-hydrogen and methane were discussed briefly. By using thermochemical processes, bio-oil and gas (syngass and methane) can be produced and by using biochemical processes, bio-ethanol, diesel and bio hydrogen can be produced. Therefore, based on current knowledge and technology projections, third generation biofuels specifically derived from microalgae are considered to be a technically viable alternative energy resource that is devoid of the major drawbacks associated with the first and second generation biofuels.

The following conclusions can be drawn regarding commercial value assessment of algae:

i. Algae can be used to enhance the nutritional value of food (Nori, Wakame, Kombu, Arthrospira, Chlorella**,** Dunaliella) and animal feed owing to their chemical composition;
ii. Algae play a crucial role in aquaculture (Isochrysis galbana, Tetraselmis suecica); and
iii. Algae can be incorporated into cosmetics (Pepha-Tight, Pepha-Ctive).

Algae serve as a source for the production of following compounds:

i. Bioactive compounds such as Alginates Carrageenans, Agars, Agarose, Fatty acids, carotenoids (β-carotene and Astaxanthin), sterols and several antioxidants;
ii. Biofertilizer (Maerl);
iii. Fluorescent pigments (phycobili proteins);
iv. Algal recombinant proteins;
v. Algal Drugs (anticancer, antimicrobial, anti-HIV, antiviral); and
vi. Stable-isotope (^{13}C-palmitic acid and ^{13}C-labeled linoleic).

Evidence in this review suggests that, the concurrent production of valuable co-products that have wide applications in medicine, food and cosmetic industries with biofuel production, has significant potential.

Overall, Combining algal farming, novel bioproducts synthesis and the production of biofuels using biorefinery strategy is expected to significantly enhance the overall cost-effectiveness of the biofuels using algae technology. As a result, utilization of algae for biofuel production, provides dual benefits, it serves as a biomass for the production of biofuels and also save our environment from detrimental effects.

Acknowledgments

The authors would like to acknowledge University of Malaya for the financial support through Project no. UM.C/HIR/MOHE/ENG/60 and RP016-2012A and FP007-2014.

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