Renewable and Sustainable Energy Reviews 47 (2015) 634–653

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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

Aquatic biomass (algae) as a future feed stock for bio-refineries: A review on cultivation, processing and products

B. Bharathiraja [a,*], M. Chakravarthy [a], R. Ranjith Kumar [a], D. Yogendran [a], D. Yuvaraj [a], J. Jayamuthunagai [b], R. Praveen Kumar [c], S. Palani [c]

[a] Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai 600062, Tamilnadu, India
[b] Centre for Biotechnology, Anna University, Chennai 600025, India
[c] Department of Biotechnology, Anna Bio Research Foundation, Arunai Engineering College, Tiruvannamalai, Tamilnadu, India

ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form
25 October 2014
Accepted 8 March 2015
Available online

Keywords:
Biofuels
Biodiesel
Downstream processing techniques
Algal farming
Macroalgae
Microalgae

ABSTRACT

Global outlook of biofuels turns out to be a full-fledged search focusing the viability and sustainability assets. The present day option for immediate and sustainable alternate fuels lies with algal biofuels. Algae are the most sustainable fuel resource in terms of food security and environmental issues. Inefficient and unsustainable biofuel derived from food crops twosome food security issues thus increasing interests towards algal energy. CO_2 mitigation, quick biomass accumulation accomplishing simultaneous bioremediation have gathered progressive attention. Cultivation of biomass, harvesting, processing and fuel production by chemical/biochemical reactions are the sequential stages in algal biofuel production. Currently, biofuels produced from algal biomass is not economical since biomass cultivation, processing and separation of fuel products appears costly although certain advancements in culturing techniques have been recently unearthed. Further improvements with the biomass processing strategies may step up the third-generation biofuel concept a profitable one in the near future. This article reviews various cultivation methods, processing techniques and stages in algal biofuel production thereby extensively investigating their potential application in biofuel refineries.

Contents

* Corresponding author. Tel.: +91 9843557513; fax: +91 44 26841061.
E-mail address: btrbio@gmail.com (B. Bharathiraja).

http://dx.doi.org/10.1016/j.rser.2015.03.047
1364-0321/

1. Introduction

Energy security is a major issue faced by countries all over the world. The increasing energy consumption has dropped the fuel resource by maximum. The total global petroleum consumption is still increasing due to intensified energy consumption. In 2007, there were 806 million cars and light trucks [1] which is expected to increase up to 1.3 billion by 2030 and 2 billion by 2050 [2]. Currently, one-fifth of the global CO_2 emission is due to transportation and trucking. It is critical to realize the negative impacts imparted on the global environment by fossil fuels that has drifted the exploitation of alternate fuels. The green replacement of fossil based petro fuel is the trending strategy that has gained much attention from scientists all over the world. Biofuels have the potential to replace existing conventional fuels, reinforce energy security and reduce the emission of both greenhouse gases (GHGs) and other air pollutants. Biofuels are considered sustainable, renewable and environment friendly fuels. Biofuels such as bio-ethanol, bio-butanol and bio-diesel are produced from sugar beet, sugar molasses, soybean or rapeseed. Biodiesel is alkyl esters obtained from transesterification of fatty acids obtained from renewable biomass. Biodiesel is a proven fuel and its production and processing techniques are known for more than 5 decades. Biomass fuels potential include wood, short-rotation woody crops, agricultural wastes, short-rotation herbaceous crops, animal wastes, and a host of other materials. Various sources of hydrocarbons used in commercial biodiesel production include canola oil, animal fat, palm oil, corn oil, jatropha oil and waste cooking oil. Using agricultural crops as biofuel feedstock denotes competing with food production as large quantity of fresh water and land supply is needed which raises serious issues on raw material sustainability [3,4]. However currently 95% of world's biodiesel is produced from edible oils [5]. This concern is discussed as food versus fuel dispute in long term vision where food scarcity will be the consequence if considering agro crop based biofuel production. Though there are several other non-edible oil sources, sustainable production of land based non-edible biomass is difficult to feed large scale biodiesel plants. Due to rolling food cost from the competition, global economies have become unstable since 2006 and the global food stocks of major grains such as rice, wheat, and corn were at their lowest for the past 2 decades. For countering these reasons, algae based biofuel production progressed recklessly in the midst of biofuel research.

Autotrophic micro-organisms such as algae seem to be a promising way out for unceasing energy appetite [7]. Algae can be directly converted to energy. The hydrocarbon content of algae distinctively the fatty acid (FAs) and acyl glycerides [8] have the potential to counter the diminishing fossil assets [9]. The oil extracted from algae can be used for biodiesel production. The residual biomass rich in sugar fraction can be used for production of bio-butanol and bio-ethanol by fermentation. The algal cells suspended in nutrient rich water acts as a reliable bio-mechanism that efficiently converts nutrients and CO_2 to hydrocarbons. Microalgae are photosynthetic micro-organisms that convert sunlight, water and carbon di-oxide to algal biomass [10]. Microalgae are being explored at a faster rate as potential oil source for biodiesel production. About 25,000 species are reported out of which only 15 are employed as commercial oil producers [11]. Active researches are promoted in the field of micro-algal biodiesel in scope of evaluating it as a potential source for sustainable biodiesel production. On the other hand, macroalgae commonly called as seaweeds remain as untapped marine mysteries for fuel production. Only few investigations have been reported for macro-algae based biodiesel. Marine macroalgae is a potential biomass for biofuel production because of their higher productivity rates than terrestrial biomass such as corn and switch grass [12]. Annual biomass production of macroalgae is too high [13] and the ease degree of maintenance in large scale cultivation of marine seaweed serves as a key factor for using marine macroalgae as biofuel feedstock. Although many researchers have been trying to utilize lignocellulosic biomass that is not used for food, this biomass can still incur the same environmental consequences associated with land use and water consumption [14]. Thus, terrestrial biomass-based bio-refinery seems not to be sustainable choice for scale up at present due to environmental as well as economic impacts. Microalgae and marine macroalgae have the high potentials to fully and partly displace terrestrial biomass and produce sustainable bioenergy and biomaterials. This review focuses the technical aspects of algae based biofuel production and its potential to replace fossil fuels.

2. Algae

Algae are diverse group of photosynthetic organisms ranging from unicellular (Microalga) to multicellular (Macroalgae) forms. They have chlorophyll as primary photosynthetic pigment and do not have a common ancestor. Commonly, algal population falls under two broad categories (1) Microalgae: Microscopic algae that grows in fresh water and marine environment and (2) Macroalgae: Comparatively large, multicellular organisms that grows in marine environment. There are two main populations of algae: filamentous and phytoplankton algae. These two species, in particular phytoplankton, intensifies rapidly to form algal blooms [15]. Though the main storage compound of these algae is starch, oil can also be produced or induced to accumulate within the biomass. The faster growth rate and greater lipid content of microalgae compared to oilseed crops urge researchers to develop technologies for algae utilization in biodiesel production instead of plant oils [16]. Algae based biofuel production has very less degree of intrusion in the food versus fuel dispute of tomorrow which is an added advantage [17].

2.1. Microalgae

Microalgae are microscopic, unicellular and phototrophic organisms that falls under the categories (1) Diatoms (*Bacillariophyceae*), (2) Green algae (*Chlorophyceae*) and (3) Golden algae (*Chrysophyceae*). Cyanobacteria (*Cyanophyceae*) such as *Arthrospira platensis* and *Arthrospira maxima* are also referred to as microalgae. Diatoms are the dominant life form in phytoplankton and probably represent the largest group of biomass producers on earth. Green algae are abundantly found in fresh water than in marine waters. The golden algae are similar to diatoms and produce oils and carbohydrates. Microalgae are efficient producers of lipids and other great metabolites that work by utilizing nutrients in the presence of solar energy. The algal species found suitable for biofuel research includes different species of *Chlorells* sp., *Dunaliella* sp., *Botryococcus braunii*, *Nannochloropsis* sp., and many others [3]. Autotrophic micro-algae are capable of using carbon dioxide and solar energy to synthesize organics such as protein and lipid for their growth. Most of the production of autotrophic microalgae for biodiesel production occurs in indoor photobioreactors that consumes heavy illumination for photosynthesis. In comparison, heterotrophic microalgae are more flexible for the cultivation condition (can grow under light free condition), and been found capable of accumulating higher lipid in the cells. The lipid content of heterotrophic *Chlorella protothecoides* was 3 times higher than that of the autotrophic ones [18–20]. Microalgae commonly double their biomass within 24 h and biomass doubling time during exponential growth can be as short as 3.5 h and under specific cultivation conditions, their oil content can exceed 50% by weight of dry biomass [7]. Microalgae require less land for cultivation than terrestrial crops, can grow in non-portable water, and do not displace food crops [8,21]. Strategies such as selection of carbon source and nitrogen source for enhancing lipid accumulation in microalgae have been reported extensively. Nanomaterials are found capable of enhancing the microbial activities and hence, it could be considered that the addition of nanomaterials to microalgae cultivation medium could impact on lipid accumulation [22,23]. Nano-materials act as carriers of chemicals to cell thereby performing the role of organic solvents. They also have a significant role in immobilization of lipase in enzymatic transesterification [24]. Productivities of microalga and plants are discussed in Table 1.

2.1.1. Microalgae diversity and collection centers

It is estimated that 50,000 species of microalgae exists, but only few were used commonly [26]. About 20,000+ species are yet to be isolated and analyzed. National Institute for Environmental Studies Collection (NIES), in Japan holds a collection of about 2150 strains and 700 species of different algae. Fresh water microalgae collection of Coimbra (Portugal) accommodates 4000 strains and 1000 species. The CSIRO Collection of Living Microalgae (CCLM) in Australia holds about 800 strains from Australian waters. Collection of Göttingen University has about 2213 strains and 1273 species. University of Texas Algal Culture Collection lodges about 2300 different species of microalgae [27]. Several other microalgae collection centers are being established to support algal process and products.

Table 1
Estimated productivity of microalgae compared with other plants [25].

Land plants/microalgae production systems	Annual productivities (Mg DM/ha)
C3 land plants	8–10
C4 land plants	10–30
Open raceway ponds (paddle wheels)	20
Tubular reactor (mixing via air and CO_2)	60
Tubular reactor (dilution of light)	80
Flat panels (intense mixing, short light–dark periods)	100

2.1.2. Cultivation methods

There are many types of cultivation methods available for microalgal farming that includes open ponds or raceways, closed ponds, photobioreactors (PBRs), plastic bag systems, well systems etc. As of now, raceways and PBRs are the well-established methods promoting photobiological reactions at affordable cost. Bioreactors favoring desired biochemical conversions are selected for efficient biomass production. Selection of culture systems greatly depends on the metabolic nature of algae. Autotrophic members need illumination facilities where illuminated raceways, tubular PBRs are great choice for cultivation. Heterotrophic cultures can be grown in conventional bioreactors and where photo-heterotrophs need PBR and open ponds for normal growth. Mixotrophic cultures can be cultured in raceways or PBR. Time period of exposure to light also has great effect on productivity of biomass and other co-products in autotrophic and mixotrophic cultures. Depending on the species, growing conditions and growth stage, microalgae have been shown to produce various types of lipids including triglycerides, phospholipids, glycolipids and betaine lipids [28]. Productivity is a measure of how much algal biomass is produced per area per unit of time. Production of up to 127,000 kg ha^{-1} yr^{-1} can be achieved in high-rate raceway ponds [7]. Productivity rates between 20 and 30 g m^{-2} day^{-1} (73–109,000 kg ha^{-1} yr^{-1}) are in the range of usual open raceway performance [29]. Productivity is estimated to be a function of photosynthetically active radiation coupled with data for insolation and radiation [30]. Algal cell concentrations can be determined using a particle sizer and enumerator (Multi-Sizer III, Beckman-Coulter). Cell-free samples of filtered cultures can be analyzed daily for the determination of residual nutrient concentrations. Few milliliter samples are filtered through a 0.22 μm filter and can be stored frozen for analysis and data evaluation in later stages of experimentation. Biomass productivity can be measured by weighing dry biomass and optical density measurements. The mass balance based on system input, output relating growth, oxygen evolution rate, nutrients and carbon dioxide consumption rate is given [31–33].

Biomass productivity can be measured using the below formula

$$\frac{dC_x}{dt} = (r_x) - \frac{C_x}{\tau} = (r_x) - DC_x$$

where C_x (g L^{-1}) is the biomass conc., r_x is the mean biomass volumetric growth rate, τ is the mean residence time and D (d^{-1}) is the dilution rate.

Dilution rate (D, d^{-1}) or specific growth rate (μ, d^{-1}) is given by

$$D = \mu = \frac{\text{Volumetric liters harvested (Sample)}}{\text{Total volume} \times T_n}$$

T_n denotes the no. of days taken to harvest the sample (if 1 l of the sample is harvested in 1 day, T_n = volume harvested = 1).

Volumetric productivity (P, g L^{-1} d^{-1}) is obtained by product of specific growth and biomass concentration (C_x, g L^{-1})

$$P = \mu \times C_x$$

Notable fall in the biomass concentration during night due to the absence of light can be observed. This can be calculated from the below formula

$$\text{Average Biomass loss during night time} = \frac{\ln(C_{x(0)}) - \ln(C_{x(t)})}{\Delta t_{night}}$$

Where $C_{x(0)}$ denotes the concentration of biomass at time 0 (initial), $C_{x(t)}$ denotes the concentration of biomass at time t (final),

and Δt_{night} denotes the time interval between sunset and sunrise in hours (h).

Dissolved ammonium and phosphate in the cultures can be determined using commercially available kits such as colorimetric assay kits with portable spectrophotometer [34]. Post-cultivation harvesting process involves biomass recovery from the culture medium that may contribute to 20–30% of the total biomass production cost [35]. Medium cost plays a significant role in cultivation of algal population for biofuels. In case of biodiesel production, the glycerol byproduct can be recycled as feed for mixotrophic and heterotrophic cultures [36]. However there are some effective methods and cost effective mediums established and being researched (Tables 2 and 3).

2.1.2.1. Open ponds and raceways. Currently, suspend-based open ponds and enclosed PBRs are commonly used for algal–biofuel production. Open ponds are a very efficient and cost-effective method of cultivating algae, but they become contaminated with unwanted species very quickly. About 98% of commercial algae biomass production is currently with open ponds, even for high value nutritional products, that are sold for prices over hundreds or thousands fold higher than allowable biofuels [39]. Open ponds constructed in excavated pits or raised above ground level is equipped with isolation material such as thick silpauline sheets to carry water and nutrients in tones. Paddle wheel is the commonly used mechanism for mixing the culture in raceways. Other mechanisms include water jet and air pump for mixing. Raceways can be closed by building greenhouse over it to ensure preliminary safety against species contamination and other contaminants. Raceways are advised to be launched in highly selective environments since there is high possibility of other algal species and protozoa to thrive in. Though the biomass productivity of raceways is fair, light utilization is poor. These ponds can be operated as batch or semi-continuous routine. Ponds use more energy to homogenize nutrients and the water level cannot be kept much lower than 15 cm (or 150 L/m^2) for the microalgae to receive enough light. Gas utilization by autotrophic cultures is good at the surface but poor at depth. Atmospheric air has 0.03–0.06% of CO_2 which is very less for good mass transfer [27]. Evolved gas cannot be collected and analyzed. Hence this method proves to be incorrect for bio-hydrogen collection by algae accomplishing co-photolysis. Biomass productivity is poor compared with photobioreactor systems. Area/volume ratio is too low as the system requires large surface area and the risk factor is directly proportional to the size of pond. Evaporation results in significant changes of ionic composition in the medium [41] and temperature maintenance due to seasonal variations are the main problematic outlooks. Low capital, operational costs, simple and easy maintenance of ponds adds up for scale up feasibility.

Table 2
Cost efficient medium for microalgae cultivation.

Substrate	Biomass (g/L)	Oil content (%)	Oil yield (g/L)	Conversion ratio of biomass/sugar	Conversion ratio of oil/sugar	Reference
Glucose	51.2	50.3	25.7	ND	ND	[37]
MHL	97.1	57.1	55.4	0.542	0.318	[37]
MHD	70.9	57.6	40.8	0.481	0.277	[37]
Sugarcane	121.3	45.0	54.8	0.409	0.185	[37]
Cassava	53.6	53.0	28.4	ND	ND	[37]
Sorghum	3.6	52.5	ND	ND	ND	[38]

MHL – Molasses hydrolysate N limited medium=molasses hydrolysate+base medium.
MHD – molasses hydrolysate is the sole component in the medium.

2.1.2.2. Photobioreactors. Photobioreactors can be tubular or flat, serpentine or manifold, helical, inclined, vertical or spiral, pyramids made of acrylic glass. PBRs are flexible for biomass production where operational conditions can be easily matched with optimal growth condition of cultures (Fig. 1). PBRs are exclusive for cultivation of phototrophs and mixotrophs. Tubular PBRs provide good biomass productivity in outdoor environment. Tubular and flat plate PBRs provide large surface area at relatively cheaper cost than column PBRs. Flat plate and column reactors provide low hydrodynamic stress on algal cell's nutrient penetration [42]. Volumetric productivity is found to be 8 times and cell concentration is 16 times higher than open pond production. Sophisticated construction, difficulty in maintenance of reactor, high capital investment, bio-fouling, illumination are drawbacks counterbalanced by automation availabilities, high productivity, less contamination, low oxygen buildup, good mass transfer etc. (Fig. 1). Cultivation of microalga in ponds and raceways for products other than biofuels are given in Table 4.

2.1.3. Proposed location for construction of microalgae farms

Though microalgal biofuel production is possible in laboratory scale, meaningful scale up is viable only if cost reducing technologies are adopted and integrated with the existing technologies. Co-location of algal plants with industries producing recommended resources will be the only intelligent way to reduce process economics. Power plants and other chemical industries can be chosen for co-location of algal plants since they produce large amount of heat and CO_2 which is very much required for algal production and processing. The proposed

Table 3
Requirements and risk factor in farm scale cultivation of microalgae.

Type of cultivation	Biomass production	Risk factor	Maintenance	Investment and operational cost	Agitation and flow	Particular requirements	Common requirements
Open ponds	++	++++	Easy	Low	Paddle wheel, water jet, air pump	Water proof sheets (preferably white), frame setups for raised wells or wells in excavated pits, green house,	Nutrients, mother culture, cell counter, circulation pump, heating/cooling setup, filter harvesters and ph meter, exit gas analyzer.
Closed ponds	+++	+++	Easy	Low (but higher than open ponds)			
Photobioreactors	++++	+	Difficult	High	Compressible circulators, air pumps and spargers	Acrylic tubes, glass constructions, feed mixing tank, automated controllers for medium, temp and pH.	
Plastic bags	+++	++	Simple	Low	Air pumps	Plastic bags and construction frames, hangers.	
Well systems	+++	+++	Trouble-free	Average	Agitators and air pumps with spargers	Excavated pits with metal tanks, water proof sheets (preferably white).	

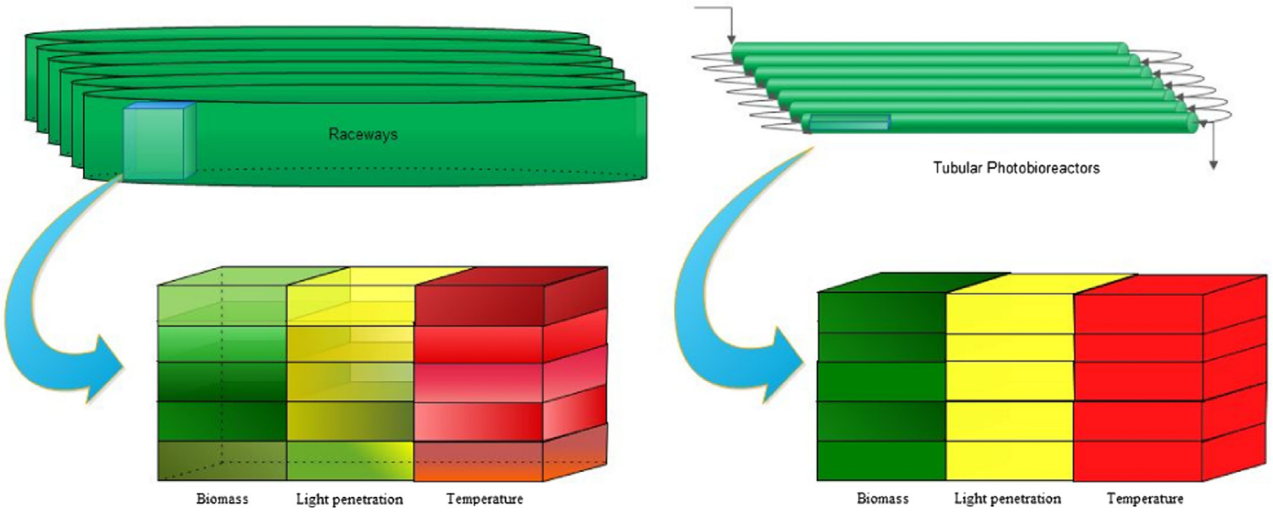

Fig. 1. Graphical illustration showing gradient concentration of biomass, light penetration and temperature across a unit volume of open algal pond in comparison with photobioreactor. Photobioreactor superiors open ponds by providing equal biomass concentration, light and temperature throughout the reactor by intense and equal mixing.

Table 4
Microalgae cultivation, mode of production and useful bio-products obtained [43–58].

Species	Production	Mode	Annual production (in dry wt)	Product	Area of application	Price (€/kg)
Spirulina	OP, PBRs	P	3000 tonnes	SCP, phycobiliproteins, γ-linolenic acid	Health care, cosmetics	11–35
Chlorella	OP, PBRs	P, H	2000 tonnes	Protein isolates, feed for aquatic organisms	Aquaculture, health care	36–50
Dunaliella salina	OP, PBRs	P, H	1200 tonnes	B-carotene	Health care and cosmetics	215–2150
Aphanizomenon flos-aquae	OP, PBRs	P	500 tonnes	Nutritional additive	Human nutrition	–
Haematococcus pluvialis	OP, PBRs	P, H	300 tonnes	Carotene, astaxanthin	Health care	501–7150
Crypthecodinium cohnii	PBRs	H	240 tonnes	Dihydroxy acetone oil	Health care and nutrition	43
Schizochytrium	PBRs	H	10 tonnes	Dihydroxy acetone oil	Health care and nutrition	43
Porphyridium	PBRs	P, H	–	Arachidonic acid	Human nutrition	–

locations are already being investigated for efficiency and may bring fortunate improvements towards cost-effective algal cultivation.

2.1.3.1. Co-location with sugar mills. The ability of cane mills to provide CO_2, water and heat are already studied and discussed [59]. Sugar is the main product of cane industry with bagasse and molasses as important co-products. Molasses is a thick dark colored liquid, strongly acidic in pH with high BOD and COD [60] is a rich source of glucose which can be fed to algae for higher biomass yield. Bagasse is a fiber residue that can be burnt to heat boiler for electricity generation. Heat produced by boiler is used to convert water to stem and rotate turbine for electricity generation. Since drying the algae up to 90%ds expenses 60% of process economy [61], a proportion of heat lost in boiler can be driven to heat the wet algal biomass and the electricity produced can be optionally used for illumination of algal reactors. Drying the wet algal biomass is the serious issue faced in post-harvest processing as the energy content of algal biomass has lower energy content than required for its drying. Lohrey and Kochergin in 2012 investigated sugar mill integrated microalgal biodiesel production and reported the requirements as 2.5 kg CO_2, 3.4 kWh energy and 1.9 L/kg water to produce per kg algae dry wt. For sustainable biodiesel production, algal meal can be utilized for co-energy generation in boilers [62] along with glycerol byproduct obtained in biodiesel production that has heating value of 5.8 kWh/kg [63]. Flue gas from boiler heating setup is a rich source of CO_2 recommendable as carbon source for biomass cultivation. Yan et al. [37] cultured heterotrophic *C. protothecoides* in molasses hydrolysate medium and the oil from algae is converted to biodiesel which had palmitic acid methyl esters, linolenic acid methyl esters and oleic acid methyl esters contributing to total of 80% comparable with that of glucose fed microalgal biodiesel [64]. Further advancements and researches targeting integration of these two technologies can bring out significant counts of viable microalgal biofuel industries (Fig. 2).

2.1.3.2. Cultivation in waste water. Municipal waste water is found to be rich in nitrogen, phosphorus and potassium and hence can be used as cultivation medium for microalgae [65,66]. Aquaculture of microalgal strains is a promising method for economical algal cultivation coupled with bioremediation advantage. Waste water with high organic content and inorganic salts left in environment can cause eutrophication thus leading to decrease in dissolved oxygen content of water bodies. Serious environmental issues are being faced with waste water disposal. Biological treatment of waste water can be incorporated in the concept of microalgae cultivation. Some of the members such as *Chlorella* sp. [67,68], *Arthospira* sp. [69], *Botryococcus* sp. [70] are already investigated extensively. At an average *Chlorella vulgaris* can remove about 72% of nitrogen and 28% of phosphorous from waste water [71]. 14 Potential strains have been investigated by Li et al. [72] where *C. kessleri* and *C. protothecoides* were found to be capable of mixotrophic growth when cultivated on centrate with high biomass yield of 2.01 g L^{-1} and 1.30 g L^{-1} respectively. Higher light intensity and exogenous CO_2 concentration with longer lighting period promotes good biomass growth and nutrient accumulation in waste water. Comparative efficiency of microalgae production in piggery waste water and recycled harvest water was evaluated for *Chlorella zofingiensis* [73]. *Neochloris oleoabundans* was investigated for its growth ability on anaerobically digested diary waste for biodiesel production [74]. Coastal microalgae culturing plants enjoys unlimited water supply that suits mass cultivation of marine microalgae. Use of sea/sewage water can reduce nitrogen usage by 94% and eliminates

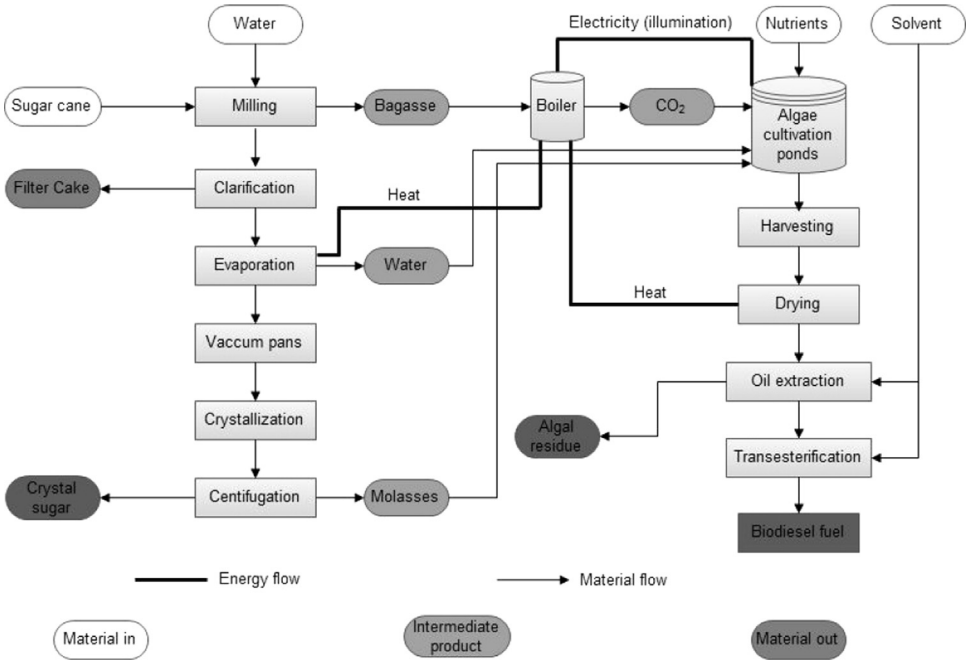

Fig. 2. Possible material and energy flow in an algal biofuel plant integrated with sugar mill. Data adopted [62] and modified in the present form.

Table 5
Cultivation of microalgae in industrial, synthetic and farm waste water.

Waste water	Microalgal species	Biomass productivity (mg/L/d)	Lipid productivity (% dry cell wt)	Lipid productivities (mg/L/d)	Reference
Carpet mill	*Botryococcus braunii*	–	13.2	4.5	[76]
	Chlorella saccharaphila	–	18.1	4.2	[76]
	Duniella tertiolecta	–	15.2	4.3	[76]
	Pleurochrysis carterae	–	12.0	4.0	[76]
Nitrate and ammonia	*Scenedesmus* sp.	–	12.8	16.2	[77]
Science industrial park	*Chlamydomonas* sp. *TAI-2*	–	20.9	31.0	[78]
Brewery industry	*Chlorella vulgaris*	–	18	26.8	[79]
	Chlorella sp.	–	20	40.0	[79]
Thiocyanate	*Algae–bacteria mixed*	–	15.2	40.1	[80]
Road waste water reclamation facility	*Nannochloropsis salina*		–	–	[81]
Digested dairy waste	*Neochloris oleoabundans*	88.3 ± 7.9	–	2.57 ± 0.9	[74]
		55 ± 5.5	–	16.5 ± 2.5	[74]
Piggery waste water	*Chlorella zofingiensis*	86.30 ± 6.61	–	–	[73]
		266.66 ± 18.82	–	–	[73]

addition of potassium, magnesium and sulfur [75]. Arid regions will be of good choice for culture systems requiring large surface area such as open ponds. Cultivation of algae in alternate mediums other than conventional nutrient medium is discussed in Table 5.

2.1.3.3. Co-location with power plants. The bulk amount of carbon dioxide gas released by power plants on coal burning results in strong environmental pollution. To direct the destructive effect of waste CO_2 on air, microalgae can be used to mitigate the exhaust gas. Co-location of microalgal farm with power plant can be of greater advantage since most of the carbon source needed by the algal biomass is supplied free of cost by exhaust CO_2. With CO_2 supply, the medium pH can also be controlled [82]. With this integration, per kg of algal biomass can be produced from 1.83 kg of carbon dioxide [7]. At an average, 60 wt% of lipids can be accumulated in unit weight of algal biomass. 60% of lipids can be harvested from biomass cultivated to enable biodiesel conversion [74]. Drying of algal biomass can be facilitated by driving the surplus heat from power plants. Algal oil will have same density as that of soybean oil, 0.92 kg/l [83] and based on conversion type the yield results. Supply of CO_2 to microalgal mass culture system is the only difficulty to be solved [84–86].

2.2. Macroalgae

Macro-algae or "seaweeds" are fast growing multicellular plants growing in salt or fresh water that can reach sizes up to 60 m in length [87]. Seaweeds are lower level plants with undifferentiated roots, leaves and stems. Seaweeds are classified into three broad groups based on their pigmentation: (1) brown seaweed (*Phaeophyceae*), (2) red seaweed (*Rhodophyceae*) and (3) green seaweed (*Chlorophyceae*) [88]. Macroalgae are photoauxotrophic and thus produce and store organic carbons (i.e., carbon sources for biorefinery) by utilizing either atmospheric CO_2 or HCO_3 [89]. Most macroalgae directly uptake HCO_3 rather than CO_2 for their growth because the diffusion rate of CO_2 is found to be extremely slow in seawater. Due to the high photosynthetic ability of macroalgae, they have the potential to generate and store sufficient carbon resources needed for biorefinery.

Evaluating macroalgal species as feedstock for biofuel is the emerging trend in biorefinery research that has grabbed attention world-wide since one can grow the same in enormous amount where the globe will share approximately 3/4th area for it. Advantages of macroalgae as biofuel feedstock includes atmospheric CO_2 mitigation, entrapment of HCO_3 in the water bodies there by reducing the acidic nature of water bodies and acid rain hazards, promoting green fuel for green earth etc. Analysis of macroalgal biomass as fuel feedstock is given in Table 6.

2.2.1. Macroalgae diversity and collection centers

90% of marine plants are algae contributing 40% of global photosynthesis [92]. Global diversity and count of seaweed species still remains mysterious (Fig. 3). Macroalgal Culture Collection of Kobe University, Japan had about 95 genera, 161 species, 338 strains of macroalgae of which most of them are native to Japan and some foreign. This includes 45 genus and 71 species of *Phaeophyceae*, 42 genus and 63 species of *Rhodophyceae*, 7 genus and 26 species of *Chlorophyceae* and 1 genus and 1 species of *Schizocladiophyceae* according to 2011 update. Currently, the center holds 600 species. Research Culture Collection of Institute of Biotechnology (VAST), Vietnam holds about 177 strains comprising 32 genera and 81 species of fresh and marine microalgae, cyanobacteria and macroalgae isolated from soil, fresh water and marine water of Vietnam. Culture Collection of Baltic Algae belonging to Institute of Oceanography, University of Gdańsk stores 300 species of which 200 species are abroad collections and 100 are novel collections. Only few established collection centers are available for macroalgae that preserves seeds by cryopreservation technique.

Table 6
Analysis of biomass composition of marine macroalgae [13,91].

S. no.	Analysis and results					
1.	*Proximate analysis (different basis)*					
	Volatile matter	Fixed carbon	Moisture	Ash		Sum
	45.1	23.1	10.7	21.1		100.0
2.	*Proximate analysis (dry basis)*					
	Volatile matter	Fixed carbon	Moisture	Ash		Sum
	50.5	25.9	00.0	23.6		100.0
3.	*Ultimate analysis (dry ash-free basis)*					
	Carbon	Oxygen	Hydrogen	Nitrogen	Sulfur	Sum
	43.2	45.8	6.2	2.2	2.60	100.00

2.2.2. Culture methods

Few cultivation methods have been established and commercialized for mass cultivation of macroalgal biomass. Of all the macroalgae species reported so far, only few have been cultivated for commercial uses [96]. Approximately 200 species are known worldwide of which around 10 are cultivated massively. Some of the intensively cultured species include *Laminaria japonica*, *Undaria pinnatifida*, *Monostroma* sp., *Enteromorpha* sp., *Porphyra* sp., *Eucheuma* sp., *Kappaphycus*, *Gracilaria* etc. [97]. Small seaweeds such as *Enteromorpha*, *Ulva*, *Sargassum*, *Gracilaria* etc. can be cultured in artificial systems by pumping seawater in the pond like aquatic farms. Potential cultivation of seaweeds coupled with offshore wind farm has already been studied to economize the process. Large kelps are difficult to cultivate in artificial environment because of their monstrous growth. Cultivation of seaweeds, growth and biomass intensification depends on various factors such as nutrient composition, pH, temperature, climate, salinity etc. Seaweeds are mainly produced for these end uses in Asian countries such as China, Philippines, North and South Korea, Japan and Indonesia. The USA, Canada and European countries such as

Fig. 3. Macroalgae diversity [92–95].
1. *Caulerpa racemosa*, 2. *Gracilaria fergusonii*, 3. *Gracilaria pygmaea*, 4. *Hypnea musciformis*, 5. *Padina pavonica*, 6. *Callophyllis variegate*, 7. *Delisea pulchra*, 8. *Himantothallus grandifolius*, 9. *Palmaria decipens*, 10. *Sargassum duplicatum*, 11. *Kappaphycus alvarezii*, 12. *Durvillaea antartica*.

France, Germany and the Netherlands are attempting to establish large-scale seaweed cultivation [100]. China cultivates *Laminaria japonica* in large amounts. *Kappaphycus alvarezii* are being cultivated by fisher community in Rameswaram and other southern parts of India. Galician sea accommodates about 17,500 species of macroalgae some of which are used in agriculture traditionally [12]. For offshore culture, sandy clay bottom is required for less organic load. Water should be 0.3–0.5 m deep with specific gravity around 1.010. Optimal growth occurs at alkaline pH (7.0–8.0) and temperature between 15 and 30 °C. Pools, crab ponds, bays and straights are chosen as algae cultivation sites. Spores are naturally released from the existing cultures and on favorable conditions, they germinate. Shells of oysters and other molluscs can be used to act as cheap substrates for attachment. Culture techniques should be designed as per nature of the seaweed growth. For suspension culture, rope hangs can be chosen whereas seaweeds requiring bottom supports off-bottom setups, long lines etc. can be chosen. Kelp cultivation in china is done on artificial floating rafts. These species have low wet to dry biomass weight ratio. Temperature control has greater effect of growth. Spore release does not occur if temperature goes below 5 °C. Control of culture conditions and barriers against natural calamities are uncontrollable in macroalgal farming. Artificial pond culture of seaweeds in off-marine areas requires artificial seawater. Composition of artificial seawater has direct effect on algal biomass growth. Kaladharan achieved significant increase in the yields of *Gracilaria cortica and Ulva lactuca* of about 15.5% and 18% respectively [102]. Composition of seawater is given in Table 7. Cultivation techniques focusing enhanced biomass productivity towards biofuel production has not investigated in enough numbers. Economics of macroalgae cultivation for bioenergy remains untested but evidenced as efficient by majority of production in UK and Ireland. Macroalgae biomass harvest may be possible by wild harvest or artificial cultivation in ponds. Possibly, the environmental impacts of mass cultivation of macroalgae may be reduced by coupling it with compatible aquaculture systems [105]. Marine macroalgae farming suffers few drawbacks such as introduction of invasive species, grazing of weeds by fishes, fishing difficulties, changes in nutrient composition at the cost of prolonged cultivation in a same location, etc.

3. Harvest technologies

Harvesting refers to segregation of solid content. Ratio of mass of liquid to solid should be low at the time of harvest to ensure peak biomass produced. Macroalgae can be hand harvested manually. Microalgae harvesting and dewatering involves use of technical procedures such as filtration, vacuum flow filtration, tangential flow filtration etc. [106]. Centrifugation is the mostly preferred technique of harvesting [107,108]. Centrifugation uses centripetal acceleration against gravity to settle down the algal cells depending on their densities. Continuous feed fixed bowl centrifuge can be used to obtain 15–20% dry matter followed by vacuum dry around 70 °C, 500 Torr for dry powder [109]. Higher energy consumption is a limiting factor for centrifugal separation though it gives $>95\%$ separation efficiency [110]. Algal paste can be obtained by flocculation or coagulation. The algal paste thickening can be done by ammonium sulfate assisted coagulation for which algae concentration should be around 0.206 g L^{-1} [55]. Coagulated sludge can be allowed to settle in settling tanks and later separated. Oil rich microalgae naturally float on water surface and can be harvested easily using flat filter harvesters. Magnetophoretic harvesting technique involves attachment of magnetic nanoparticles coated with cationic substances by electrostatic interaction. Biomass harvesting is completed in few hours by application of external electric field in harvesting electrodes [111]. Lee et al. adopted this technique using biocompatible chitosan-Fe_3O_4 for recovering 90% of oleaginous *chlorella* sp. [112]. Starch and chitosan are also investigated as bioflocculants [113,114]. Chitosan is considered as widely used bioflocculant and effective in biomass recovery when used in culture medium at pH 10 [115]. Ammonium sulfate, ferric sulfate, ferric chloride etc. are chemical flocculants advised for large scale harvesting but known to have toxic effects on microalgae [116]. Ultra sonic aggregation is a non-hazardous, non-toxic efficient method for continuous biomass harvesting [117]. Flocculation is followed by filtration, flocculation or gravimetric separation [35]. Microalgae can also be immobilized in alginate beads and cultivated to reduce difficulties in harvesting but growth hinders to a notable extent. Wet biomass is generally sun dried, low pressure shelf dried or drum dried [118]. For valuable product extraction, spray drying [119], fluidized bed drying [120] and freeze drying [121] is recommended. Drying dehydrates the biomass and avoids spoilage. Microalgal drying seems to consume more of the total energy utilized in the process [35,107]. Renewable energy driving devices such as solar panels, heat reflecting windows can be used for drying. High capital investment is the set up hindering constraint.

Table 7
Seawater composition for alga cultivation (natural and artificially prepared).

Ion	Natural seawater[a]	Artifical seawater[b]
Cl^-	19.353	19.353
Na^+	10.76	10.764
SO_4^{2-}	2.712	2.701
Mg^{2+}	1.294	1.297
Ca^{2+}	0.413	0.406
K^+	0.387	0.387
HCO^{3-}	0.142	0.142
Br^-	0.067	0.066
Sr^{2+}	0.008	0.014
H_3BO_3	0.026	0.026
F^-	0.001	0.001

[a] Salinity 35% [103].
[b] Salinity 35% [104].

4. Post-harvest processing technologies

The biomass after collected from cultivation site is processed prior to fuel conversion steps. This includes (1) drying for dewatering the biomass, (2) extraction of products & co-products and (3) grinding for homogenization of biomass components with biological/chemical reactants during conversion.

4.1. Cell disruption techniques

Number of techniques has been practiced for cell lysis which broadly falls under (i) mechanical disruption, (ii) chemical disruption and (ii) enzymatic disruption. Mechanical press is the conventional method similar to grinding that involves disruption of cell by mechanical shear stress. Average of 65% oil can be obtained by this method when operated manually or engine powered. Filtration and degumming are necessary post-extraction treatments [122]. Cell debris often intrudes with the targeted product and mechanical press suits only for macroalgae. Dried cells can be disrupted and powdered using ball milling method. Electroporation done by electric charge variation can vary the size of pores on cell wall and possibly break if the charge variation exceeds than advised. High pressure homogenization seems energy expensive method. Osmotic shock can also be employed to outflow the cellular contents to aqueous medium. Polymixin, lysine, strong mineral acids such as HCL, H_2SO_4, organic acids such as acetone, methanol, Dimethyl Sulfoxide (DMSO) and all cationic agents can be used for chemical degradation of cell wall [123]. Addition of chemicals makes downstream costly and can impart toxic nature to the product. Possibility of quality degradation is high in this case and enzymatic treatment is considered comparatively safer method. Treatment using

papain, pectinase, neutrase, cellulase etc. [124] can effectively break cell wall in a product friendly manner but remains costly. Product extraction can also be made possible without cell death known by a process called milking. This method suits microalgae product extraction using suitable solvents [49]. However upcoming technologies like ultrasound, microwave assisted cell lysis suits appropriate for algal disruption.

4.2. Oil and biomass recovery

Bligh dryer method is one among the simplest and earliest discussed methods for lipid extraction [125]. For soxhlet extraction, wet/dry biomass can be loaded directly after proper chopping and pulverization. Dry biomass can be ball milled to crack cell wall and improve extraction efficiency. Chloroform: methanol in the ratio 2:1 is the mostly preferred solvent composition for lipid extraction [126]. Hexane is used to extract neutral lipids [109]. Selection of solvent is based on polarity difference existing between the solvent and compound to be extracted. General span of lipid extraction is 6–12 h where some unusual biomass loading may require 12–24 h or even more. In traditional solvent based lipid extraction, chlorophyll and magnesium associated compounds are usual contaminants spoiling the oil and biodiesel quality [127]. The extracted solvent along with lipid and coloring pigments are treated with charcoal and evaporation to recycle solvent and complete oil extraction. Wet lipid extraction combines the use of centrifugation and solvent extraction after acid/alkaline treatment of biomass [128]. Quality oil can be obtained but tedious steps in extraction supports only analytical purposes. Solvents are costly, volatile and hazardous. Use of n-hexane in extraction is environmentally hazardous as it releases toxic water as waste. Eco-friendly extraction is achieved by employing enzymes as molecular machines. But prolonged mining of products from biomass, enzyme cost has limited the establishment of this method [129].

Super critical carbon dioxide method is an alternate green technology that eliminates solvent assistance for oil extraction [130]. Super critical CO_2 facilitates a safe extraction due to its low toxicity, low flammability, and lack of reactivity [131]. Super critical methanol for direct conversion of oil from wet algae to alkyl esters was also investigated [132]. Optimal operation conditions would be as high as 50 °C and 200–250 bar pressure for which energy allocation is difficult in algae processing. Ultrasound Assisted Extraction (UAE) is the emerging and promising method for extraction of cell lysate [129]. Microwave Assisted Extraction (MAE) of lipids was identified as the simplest and efficient methods of lipid extraction [133]. UAE and MAE are considered for efficient and quality extraction in short processing time [134,135] UAE and MAE can be performed by taking biomass: solvent in w/v ratio of 1:100 at temperature ranging 30–50 °C for about 5–30 min [126]. These methods are non-toxic, energy efficient, environmental friendly and hence the choice of many. To eliminate the oil extraction step in the case of biodiesel production, direct conversion also called as in-situ transesterification of oil in biomass has been proposed [136,137]. Direct conversion of biomass showed much reduced phospholipid content as result of efficient extraction followed transesterification [138]. Various oil extraction techniques and corresponding yields are listed in Table 8.

4.3. Fuel conversion processes

Fuel conversion strategies are generally thermochemical or biochemical in nature. Thermochemical conversion includes gasification, liquefaction, pyrolysis and direct combustion. Biochemical method covers biological production of biogas, fermentable production of alcoholic solvents and biophotolysis for hydrogen gas.

4.3.1. Oil transesterification

Oil having triacylglycerides (TAG) and Free fatty acids (FFA) is transesterified with alcoholic solvents in the presence of suitable catalyst (Fig. 4). Prior to transesterification, oil is washed with water to remove impurities. 0.88% KOH can be used to wash oil to reduce lipid loss during washing [146]. Washing of oil is followed by pretreatment to reduce FFA content. Methanol is the best acyl acceptor known to give peak conversion and quality biodiesel [147]. Other acyl acceptors include ethanol, isopropanol, n-butanol, n-propanol, isobutanol, isoamyl alcohol etc. Poor solubility of short chain alcohols may have inhibitory effect on the active site of enzyme for which solvents like tert-butanol, petroleum ether, n-hexane can be used [148]. Solvents enhance mutual solubility of hydrophobic TAG with hydrophilic alcohols. Batch wise addition of acyl acceptor is advised for solvent

Table 8
Various oil extraction methods and lipid yield [130,139–145].

Extraction method	Technique adopted	Conditions	Lipid yield (%)
Chemical method	n-Hexane in soxhlet	–	95–99
	Chloroform, ethanol, deionized water	8 h	49 ± 2.4
	Aqueous oil extraction	2 h	38
	Ultrasound assisted aqueous oil extraction	50 °C, pH 9, 6 h	67
	1st acetone, n-hexane	48 h	–
	Subcritical ethanol extraction	Ethanol:alga 20:1 (v/w), 105 °C, 100 min	72.82
Enzymatic method	Aqueous enzymatic oil extraction with cellulase/hemicellulose	60 °C, pH 4.5, 2 h	73
	Aqueous enzymatic oil extraction with alkaline protease	60 °C, pH 7, 2 h	86
		50 °C, pH 9, 6 h	64
	Ultrasound assisted alkaline protease treatment	50 °C, pH 9, 6 h	74
Mechanical methods	Engine driven	–	68
	Screw press	–	80
		–	79
	Ram press	–	62.5
Microwave method	B20 as co-solvent	80 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	13.1 ± 0.8
		100 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	16.5 ± 1.6
		120 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	11.8 ± 2
	B40 as co-solvent	80 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	32.2 ± 5.9
		100 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	38.2 ± 7.7
		120 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	56.6 ± 7.9
	Chloroform + ethanol	80 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	15.5 ± 0.7
		100 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	45.5 ± 2.2
		120 °C, 1.2 kW, 2.45 GHz, 15 min hold, 30 min, cool-down	53.1 ± 2.6
Super critical method	Super critical CO_2	80 °C, 250 bar	14.1

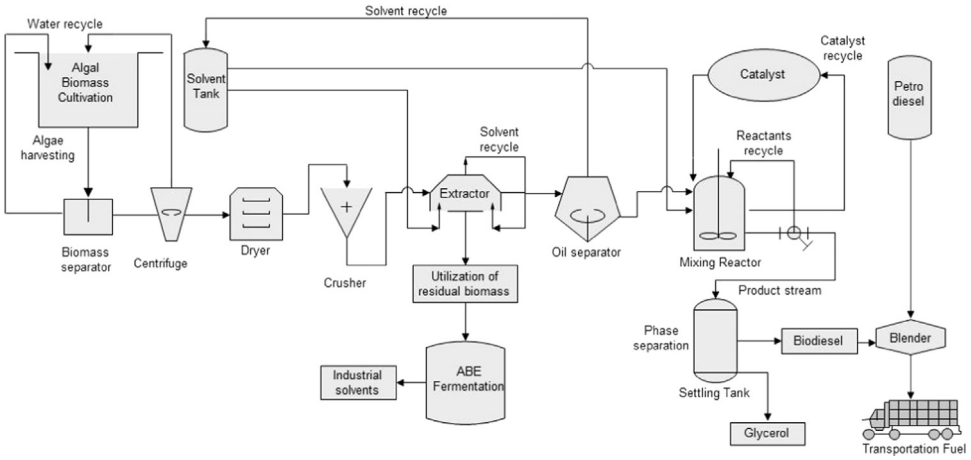

Fig. 4. Algal Biodiesel production and processing.

Table 9
Comparison of different technologies for biodiesel production [159].

Variable	Base	Enzyme	Supercritical	Monolithic	Resin	Acid
Temp. [°C]	60–70	30–50	200–350	50–180	60–180	50–80
Products from FFA	Soaps & esters	Esters	Esters	Esters	Esters	Water & esters
Effect of water[a]	↓	↓			↓	
Yield to ester	Normal	High	High	Normal	Good	Normal
Purification of glycerol	Difficult	Simple	Simple	Simple	Simple	Difficult
Reaction time[b]	1–2 h	8–70 h	4–10 min	6 h	Variable	4–70 h
Ester purification	Difficult	Simple	Simple	Simple	Simple	Difficult
Cost	Cheapest	Expensive	Expensive	Medium	Medium	Cheaper
Amount of equipment	High	Low	Low	Low	Low	High

[a] In this case the down arrow means that water is a draw back while the line means that is not effected and the system will be able to treat a raw material with some amounts of water.Base – down arrow – saponifies the fatty acid.Enzyme – down arrow – water in small quantity activates enzyme, but large quantity water deactivates the catalyst.Resin – down arrow as well as a line – water has different effects over different solid catalysts. Monolithics – line – leaching is not caused by water but for a non-stability of the catalyst.

[b] The reaction time set in this table is what it is most likely, however, it is important to point out that other times for the same technology could be found in the open literature.

free system [149]. Use of novel solvents such as dimethyl carbonate eliminates complex glycerol separation [150,151], ethyl acetate [152] and methyl acetate [153] are other novel acyl acceptors eliminating glycerol production and micro-emulsion formation during biodiesel synthesis. Unfortunately, solvent cost remained inhibitory factor for the process commercialization. Effect of both homogeneous and heterogeneous acid/base catalysts has been exclusively reviewed [154]. Homogeneous (H_2SO_4, trifluoro acetic acid, HCl etc.) and heterogeneous (SO_4/SnO_2, ZrO_2/SO_4^{2-}, S-ZrO_2, $Al_2O_3/ZrO_2/WO_3$, etc.) acid catalysts are used for conversion of oil with high Free Fatty Acid (FFA) content [155]. Alkali catalysts (NaOH, KOH, KF/Al_2O_3, CaO/Al_2O_3, Lithium-doped ZnO, $Ca(OCH_2CH_3)_2$, etc.) are conventionally used for biodiesel production from low FFA oil. Enzymes are good possible biological catalysts to produce biodiesel. Enzymes can easily treat fatty acid as well as triglycerides to produce biodiesel from non-edible or waste oil, reaching high conversion [156,157]. Use of immobilized enzyme and whole cell catalyst gives clear glycerol and biodiesel product [158]. Application of nanomaterials in biodiesel transesterification is the current trend under experimentation. *Rhizopus* species is the most widely used whole cell catalyst in biodiesel transesterification to achieve good conversion of oil. For aquatic microalgal biomass, when the solid content reaches 90%, transesterification similar to soybean oil can be performed [29]. It is also estimated that 2–10 l of water/l of biodiesel is needed [16]. Esterification of oil from various sources and resulting ester production is extensively reviewed [158]. Main parameters affecting biodiesel production includes FFA content, moisture, acyl acceptor, nature and concentration of catalyst, organic co-solvents, reaction temperature, pH, reaction time, agitation, mode of reaction system etc. Biodiesel transesterification methods are featured in depth by Marchetti [159] provided in Table 9.

Biodiesel quantification can be done by the following formula:

For, direct single step conversion (direct SCM, UAE, MAE assisted esterification)

$$\text{FAAE (\% by wt of algae loaded)} = \frac{\text{wt of FAAE obtained}}{\text{wt of algae taken}} \times 100$$

For, two step biodiesel production (oil extraction followed by separate transesterification)

$$\text{FAAE (\% by wt of oil taken)} = \frac{\text{wt of FAAE obtained}}{\text{wt of oil extracted}} \times 100$$

Integrated microalgae photobioreactor equipped with biogas and biodiesel production facility was developed and economy analysis was also done [106]. Unreacted Free fatty acids in biodiesel can be removed by EFAR process [160]. Glycerol byproduct from biodiesel production can be valorized for economic benefit [106]. Direct Supercritical methanol and In-situ transesterification reduces the need for lipid extraction step but bounces drop in yield %. Blending of biodiesel with petro-diesel is recommended for use in engines. B6-B20 blends are covered by American Standards for Testing of Materials (ASTM D7467) [161].

4.3.2. Alcoholic fermentation

ABE fermentation is a high valued biochemical process known to produce Acetone, butanol and ethanol in the ratio 3:6:1. Two significant phases in ABE fermentation are (1) Acidogenesis: Production of organic acids in exponential phase, (2) Solventogenesis: Production of solvents from acids. Aldehyde dehydrogenase, butanol dehydrogenase and alcohol dehydrogenase are the enzymes involved in production of butanol. Marine macroalgae and microalgae has been utilized as a good source of saccharides for ethanol fermentation [162,163]. For this purpose, conventional batch reactors remain simple and consistent [164] but suffers severe disadvantage like prolonged lag phase, product inhibition etc. [165]. To overcome substrate inhibition and increase biomass concentration, fed batch fermentation is advised [166]. Further increase in biomass productivity and product yield can be achieved by using cell recycle or immobilized bioreactors where the reactant and product stream flows continuously. Immobilized reactor allows the flow of broth alone arresting the movement of microbial strain preventing cell damage from shear forces of the flowing broth enabling cell reuse. Recommended strains for ABE fermentation are *Clostridium acetobutylicum*, *Clostridium beijerinckii*, *Clostridium saccharoperbutyllacetonicum* and *Clostridium Saccharobutylicum* [167]. Hydrolysate of green *Ulva lactuca* was used for the production of acetone, butanol and ethanol (ABE) by *C. acetobutylicum* and *C. beijerinckii*. Hydrolysate-based media were fermentable without nutrient supplementation. *C. beijerinckii* utilized all sugars in the hydrolysate and produced ABE at high yields (0.35 g ABE/g sugar consumed), while *C. acetobutylicum* produced mostly organic acids (acetic and butyric acids) [168]. Prior to fermentation, pretreatment of biomass is done to hydrolyze the complex cellulosic biomass. Ammonia fiber expansion (AFEX) process decrystallizes cellulose, depolymerizes hemicelluloses, and depolymerizes and removes lignin from cellulose/hemicelluloses. This method is found to be effective pre-treatment for promoting enzymatic hydrolysis of a wide variety of biomass sources. *Aspergillus niger*, *Aspergillus oryzae*, *Fusarium solani*, *Penicillium occitanis*, *Trichoderma reesi*, *Clostridium acetobutylicum*, *Bacillus subtilis*, *Pseudomonas cellulose*, *Streptomyces levedans* are highly recommended for biological pretreatment. NaOH is the strongest alkaline used in pretreatment [169]. Hot water treatment also called autolysis is economic and degradation of xylose is less. Acid treatment involves the use of sulfuric acid for desaccharification from cellulose. Release of glucose from cellulosic substrate increases with removal of lignocellulosic fraction. Assortment of algae as ethanol feedstock is done by evaluation of sugar fractions, lignin and relative sugar content in the biomass. Lignin content can be evaluated by method discussed by Hill et al. [170]. Carbohydrate content can be determined by sulfuric acid–phenol assay. Yield of ethanol from *Ulva reticulata* is estimated to be about 60 l/tonne after hydrogen peroxide pretreatment after acid hydrolysis of cellulosic biomass [171]. More of the macroalgae species has to be investigated for promising ethanol outcome from algal biorefinery.

4.3.3. Anaerobic digestion

Anaerobic digestion promotes energy recovery from sunlight via photosynthetic system in the form of Biogas (CO_2 and CH_4). Methanogenic digestion of oil extracted algal biomass was proposed to reduce external energy demands [29]. Concept of combining anaerobic digestion to economize the microalgal cultivation was first proposed in 1959 [172]. Anaerobic digestion is appropriate for biomass with high moisture content (80–90%) and thus can be used for microalgae [173]. Anaerobic digestion can convert the carbon and nitrogen content of delipidized algal biomass to methane worth energy obtained from extracted lipids [174] in few steps (1) Biopolymers to saccharides (hydrolyzation), (2) saccharides to carboxylic acids and alcohol (fermentation), (3) acids and alcohol to acetate, hydrogen and carbon dioxide (acetogenesis), and (4) acetogenic products to methane and carbon dioxide (methanogenesis).

$$\text{Biomass } (C_\alpha H_\beta O_\gamma N_\omega) \rightarrow \text{Methane } (CH_4) + \text{Ammonia } (NH_3/NH_4^+) + \text{Carbon dioxide } (CO_2) + \text{Residue}$$

Biomethane production can be calculated from the formula

$$M = M_{BG} \times (\%CH_4) \times (M_{BM} + M_S + M_C + M_G)$$

where M is the mass of biomethane produced, M_{BG} is the mass of biogas produced by anaerobic digestion of biomass, M_{BM}, M_S, M_C and M_G is mass of biomass, sludge, waste paper and glycerin.

Good carbon to nitrogen (C/N) ratio is expected in the biomass to operate the fermentation process with positive impressions [173]. Rate of degradation of organic matter (volatile solids) is of major importance in determining the process performance which is 45% for algae and 60% for waste water sludge [175]. Delipidization leaves the algal cells with good C/N ratio thus making it more suitable for anaerobic fermentation. More optimal C/N ratio can be obtained by mixing sewage sludge, waste paper pulp, glycerin with biomass [176].

C/N ratio can be calculated from formula

$$CN_{min} \le (a \times CN_{BM} + b \times CN_S + d \times CN_C + k \times CN_G) \le CN_{max}$$
$$(a; b; d; k;) = 1 \text{ and } 0 \le a; b; d; k \le 1.$$

Subscripts BM, S, C and G stand for biomass, sludge, waste paper and glycerin, respectively. a, b, d, and k are the different percentages of the respective feedstock.

Efficient and economic biomethane production depends on algal concentration after cultivation. Unfermented residue can be used as fertilizer or animal feed. The fermented remains of biomass are proposed to recycle in algal cultivation to conserve the outflow of nutrients from the system [172]. The pH of fermented residue lies between 6.6 and 7.6 implying nearer to normal pH thus ready for nutrient recycle after solid–liquid extraction. Resultant biogas is mainly composed of methane and carbon dioxide, but also traces of hydrogen sulfide, dinitrogen, dihydrogen and other volatile compounds [177]. Methane has low solubility in water than H_2S and CO_2. Hence water is passed through a packed bed pressurized with biogas to dissolve out gases other than methane [178]. Methane yield from various biomass are given in Table 10.

4.3.4. Gasification

Biomass oxidation at high temperature between 800 and 1000 °C results in syngas formation. Syngas contains CO, CO_2, H_2, N and CH_4 in varying compositions [185]. Biomass reacts with oxygen and steam (H_2O) to produce syngas. Nitrogen content evolves out after mineralization as ammonia. Gasification can be done to any biomass for

Table 10
Yield of methane produced from different feedstocks.

Biomass	Methane yield ($m^3\ kg^{-1}$)	Reference
Laminaria sp.	0.26–0.28	[180]
Gracilaria sp.	0.28–0.4	[181]
Sargassum sp.	0.12–0.19	[181]
Macrocystis	0.39–0.41	[180]
L. digitata	0.5	[182]
Ulva sp.	0.20	[183]
Water hyacinth	0.13–0.21	[180]
Sorghum	0.26–0.39	[180]
Poplar	0.23–0.32	[180]
Food waste	0.54	[180]
Tetraselmis *suecica*	0.20–0.41	[106]
Microalgae – ACAD model	0.54	[179]

Biomethane yields depend highly on the fermentation conditions, therefore the data derived in the same studies are comparable, but not necessarily the data between individual studies.

syngas generation. Gasification of *Spirulina* at 1000 °C yielded 0.64 g of methane per gram of biomass [186]. *Chlorella vulgaris* is gasified and by adopting novel nitrogen recycle mechanism, ammonia rich fertilizer grade biomass is produced [187]. Energy spent for gasification and drying seems not to be significantly beneficial. However, if biomass to syngas conversion is efficient, gasification may turn out advantageous and much favored for biogas outlet.

4.3.5. Pyrolysis

Pyrolysis also known as thermal cracking is thermal decomposition of biomass in the absence of oxygen. It is a promising method of biomass conversion technique but complex and influenced by various factors. Pyrolysis is a conventional technique for biomass–liquid fuel conversion potential for large scale biofuel production. This process is simple, effective and complete, yielding products with good physical and chemical properties. Any lignocellulosic biomass will have three components: Hemicellulose, cellulose and lignin in 20–40, 40–60 and 10–25 wt% respectively. Thermal cracking involves exposure of biomass to high temperature (up to 700 °C) in the presence of suitable catalyst. Conversion is a four step process: (i) moisture evolution, (ii) hemicellulose decomposition, (iii) cellulose decomposition, and (iv) lignin decomposition. Variety of organic compounds such as alkenes, alkanes, carboxylic acids and other aromatics are derived in this process [122]. Biomass conversion efficiency peaks 95.5% and quality products are obtained by adopting hydrogenation and catalytic cracking treatments. Pyrolytic treatment of cellulose, D-glucose, chlorogenic acid and xylan biomass can be analyzed using FTIR for gas compounds evolved from the process. Pyrolytic yields of sugar fraction rich biomass is given in Table 11. Pyrolytic experiments with *Chlorella prothothecoides* are much extensive and microalgal pyrolysis data has reached enough entries ready for commercialization. Bio-oil yield of *Chlorella prothothecoides* is found as 51.8% (39.7 MJ kg^{-1}) and 57.9% (41 MJ kg^{-1}) [18]. Ash and carbon residue disposal are major issues involved with the process.

4.3.6. Biophotolysis

Algae are capable of producing biohydrogen by photolytic reaction of water against photonic energy from sun light. Hydrogen is a naturally occurring molecule carrying good energy and so categorized as efficient fuel. There are various methods to produce biohydrogen from algal biomass (Tables 12 and 13). Methods include pyrolysis, gasification, microbial conversion, thermolysis, electrolysis etc., but, bio-photolysis and photofermentation occurs with live condition of algae. Algae produce hydrogen by direct photolysis of water while some species of bacteria photofermentate organic acids to give hydrogen. Microalgal metabolism of organic compounds can also release H_2 along with CO_2 by indirect photolysis.

(a) Direct photolysis: $2H_2O \rightarrow light \rightarrow 2H_2 + O_2$
(b) Indirect photolysis: (1) $12H_2O + 6CO_2 \rightarrow light \rightarrow C_6H_{12}O_6 + 6O_2$
(2) $C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6CO_2$

During photosynthesis, water is broken to give hydrogen ions (H^+) and oxygen (O_2). Hydrogenase enzyme converts hydrogen ions to hydrogen. Photosynthetic oxygen production inhibits the activity of hydrogenase enzyme resulting in decreased hydrogen production. In simultaneous production of photosynthetic oxygen and hydrogen, electrons released upon photosynthetic break down of water is directly converted to hydrogen by hydrogenase. Generally hydrogen production by photolysis is a two-step reaction where algae is cultivated photosynthetically [204] and subjected to anaerobic conditions for stable hydrogen production. By this two stage technique, green alga is assumed to produce 198 kg H_2 h^{-1}/day theoretically [205]. Biohydrogen yield and conversion efficiency can be determined by using the formula below [206]

$$\text{Biohydrogen Yield } (\%) = \frac{\text{Amount of hydrogen produced (mol)}}{\text{Amount of substrate consumed (mol)}}$$

$$\text{Conversion efficency } (\%) = \frac{\text{Amount of substrate utilized}}{\text{Total subsrate supplied}} \times 100$$

Inhibitors can also be employed to increase H_2 generation but considered inferior to the above process [206]. Some species of *Platymonas* and *Chlamydomonas* are insensitive against above described sulfur deprivation where inhibitors and nitrogen deficiency can bring out enhanced hydrogen production [207]. Photoproduction of seaweed *Platymonas helgolandica* has also been investigated for hydrogen producing potential. This method of biological production of hydrogen is environmentally safe, efficient enough and non-hazardous [208]. Co-cultivation of algae with bacteria can effective intensive hydrogen production [209] but the system will be difficult to understand as it involves complex biological reaction. Though cultivation under stressed condition gives good results, genetic engineering application in biohydrogen production will be a novel attempt and expected to bring notable fortunes [207].

Table 11
Gas components evolved by pyrolysis of hemicellulose, cellulose and lignin [188].

Sample	Gas product yield (mmol/g biomass)					
	H_2	CO	CH_4	CO_2	C_2H_4	C_2H_6
Hemicellulose	8.75	5.37	1.57	9.72	0.05	0.37
Cellulose	5.48	9.91	1.84	6.58	0.08	0.17
Lignin	20.84	8.46	3.98	7.81	0.03	0.42

5. Algal biofuels and characteristics

Currently, algal biofuels are non-viable due to cost factors, but expected to be practically viable in the near future. Various fuel products are obtained from algae of which methane, ethanol, butanol and biodiesel are most significant. Other fuel products include bio-oil, hydrogen and other hydrocarbon derivatives (Fig. 5).

5.1. Algal oil and biodiesel

Biodiesel is a high valued monoalkylester product considered as emerging alternate energy resource. Biodiesel obtained by various methods such as pyrolysis, dilution, transesterification, microemulsion

Table 12
Biohydrogen production systems.

Production method	Advantage	Disadvantage
Direct photobiolysis	Simple and average yield	Deactivated by excess oxygen, separation of oxygen and hydrogen from out stream is difficult
Substrate catabolism	Sustainable production, suits indoor and does not need illumination or light	Expensive, scale up difficulties, complexities in process control and maintenance
Two stage biophotolysis	Sustainable process for intensive H_2 production	Process parameters are difficult to evaluate and imply in the reaction environment

Table 13
Various species of microalgae employed in photobiological hydrogen production.

Species	Mode of cultivation	Type of culture	Reactor	Time	H_2 production	Reference
Chlamydomonas reinhardtii (CC124)	Batch	Photomixotrophic	Tubular	48 h	0.6 ml L^{-1} h^{-1}	[189]
Chlamydomonas reinhardtii (CC124)	Batch	Photomixotrophic	Stirred tank	192 h	1.5370.05 mL L^{-1} h^{-1}	[190]
Chlamydomonas reinhardtii (CC124)	Batch	Photomixotrophic	Stirred glass bottles	140 h	102 ml	[191]
Chlamydomonas reinhardtii (CC124)	Continuous	Photomixotrophic	Stirred glass bottles	4000 h	0.58 ml L^{-1} h^{-1} PBR	[192]
Chlamydomonas reinhardtii (Dang 137Cmt+)	Batch	Photomixotrophic	Stirred glass bottles		175 ml L^{-1}	[193]
Chlamydomonas reinhardtii (Dang 137Cmt+)	Batch	Photoautotrophic	Flat glass bottles	100 h	7173 ml L^{-1}	[194]
Platymonas subcordiformis	Batch	Photoautotrophic	Bottle flask	50 h	11,720 nL h^{-1}	[195]
Platymonas subcordiformis	Batch	Photoautrophic	Bottle flask	8 h	0.339 mL L^{-1} h^{-1}	[197]
Chlorellapy renoidosa C-101	Batch	Photoautotrophic	Bubble column	60–65 h	6.9×10^{-2} m^3 kg^{-1} cell	[196]
Chlorella vulgaris MSU 01	Batch	Photoautotrophic	Stirred tank	6–7 days	26 ml	[198]
Spirulina platensis (NIES-46)	Batch	Photoautotrophic	Erlenmeyer flask	20 h	2 mmol mg DW^{-1}	[199]
Tetraspora sp. CU2551	Batch	Photomixotrophic	Vials	>24 h	17.3–61.7 mmol mg^{-1} Chl a h^{-1}	[200]
Anabaena variabilis (ATCC29413)	Continuous	Photoautotrophic	Vials	18–100 h	5 nmol h^{-1} mg Chl a^{-1}	[201]
Anabaena variabilis (CCAP 1403/4B)	Continuous	Photoautotrophic	Stirred container	35 days	12–14 mL g^{-1} DW h^{-1}	[202]
Anabaena variabilis (ATCC29413)	Batch	Photoautotrophic	Panel	50 h	40–50 ml	[203]

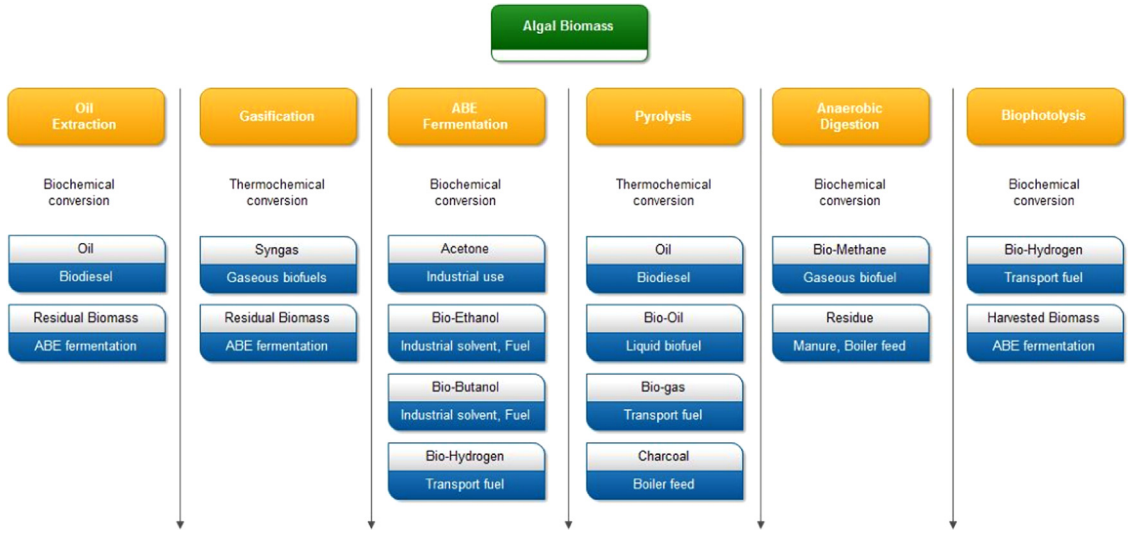

Fig. 5. Available conversion processes and corresponding fuel products obtained from algal biomass.

and supercritical processes have different qualities and properties. Properties of biodiesel and petro-diesel are given in Table 14. Quality of biodiesel product greatly depends on the feedstock quality and extent of reaction. Cetane number of biodiesel depends on fatty acid composition of oil directly affecting the fuel combustion. Moderate cetane number of 50 is preferred for proper operation of ignition engines with good cold start properties. Low cost short chain methanol and ethanol yields quality biodiesel [122]. Long and branched chain alcohol can also be employed but costs higher. Biodiesel has good combustion characteristics, reduces smoke and carbon dioxide emissions by 78% compared to petro-diesel [210]. Biodiesel production and utilization can promote us forward for a safer living, lights rural development by generating employment opportunities.

5.2. Biobutanol and bioethanol

Biobutanol and bioethanol are clean burning fuel and considered environmentally safe as greenhouse gas emission is comparatively lesser than fossil fuels. On combustion, they emit carbon di oxide and water.

$C_2H_60 + 3O_2 \rightarrow 2CO_2 + 3H_2O$ (ethanol combustion)

$C_4H_{10}O + 6O_2 \rightarrow 4CO_2 + 5H_2O$ (butanol combustion)

Micro- and macroalgae are fast growing carbon accumulating biomass venturing alcoholic fuel production. Quantity of alcohols obtained from algae is much dependent on the reaction parameters [211]. Bioethanol from microalgae can be produced by fermentation of biomass or direct cellular reactions [212]. Extracted algal biomass can be pretreated to convert starch to glucose after which biological hydrolysis is done to produce ethanol. Biobutanol production is generally done by biochemical reactions facilitated by clostridium species. Genetic engineering of fermentating organisms enabling utilization of fixed carbon to directly excrete biosolvents in fermentation medium is also considered. *Zymomonas mobilis* was introduced into *Streptococcus* microalgal culture assuming that bacterium will efficiently utilize the CO_2 for ethanol conversion in many attempts. This method of ethanol production reduces downstream processing of algal biomass and novelty lies with product excretion in the culture medium [213]. Fuel properties of butanol and ethanol are given in Table 15.

5.3. Biomethane

Biomethane from biogas is a fuel source that can be converted to heat and electrical energy on combustion [172,214]. Combustion of methane in Internal Combustion (IC) engines happens by oxidation of methane. Combined heat and power gas (CHG) engine burns methane to CO_2 completely [178].

$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O + Heat$

Table 14
Reported properties of algal biodiesel compared with 1st generation biofuels and ASTM biodiesel standards [109,122].

Test	1st generation biodiesel	Microalgae biodiesel	Macroalgae biodiesel	Biodiesel ASTM D6751 standards	Petro-diesel ASTM D975 standards	ASTM methods
Flash point (°C)	100–170	> 160	149	Min. 100–170	60–80	D93
Distillation, 90% recovery	~339	–	371	360 °C Max.	–	D1160
Carbon residue (wt%)	59.5–81	–	0.018	77	84–87	D4530
Total glycerin	0.2–0.3	0.102	0.169	Max. 0.24% (m/m)	–	D6584
Free glycerin	Min. 0.001 to Max 0.009	0.014	0.006	0.02% (m/m)	–	D6584
Water and sediment	< 0.005	< 0.005	< 0.005	Max. 0.05 (vol%)	0.05 (vol%)	D2709
Sulfur, total	0.0–0.0015	0.6	8.43	Max. 0.05% (m/m)	0.05% (m/m)	D5453
Cetane number	45–65	–	71.67	Min. 47	40–55	D613
Cloud point (°C)	−2 to −12	3.9	−16	−3 to −12	−20	D2500
Sulfated ash	< 0.005	< 0.005	0.008	Max. 0.02% (m/m)	–	D874
Copper strip corrosion	1	1	1	Max. 3	1	D130
Acid number	< 0.6	0.003	0.01	Max.0.50	0.062	D664
Kinematic viscosity at 40 °C (cSt)	3.6–9.48	–	9.8	1.9–6.0	2.6	D445
Cold soak filtration	–	–	126	–	–	D6217
Phosphorus	< 0.1	< 0.1	< 1	Max. 0.001	–	D4951

Table 15
Properties of bioethanol and biobutanol as vehicular fuels.

Properties	Butanol	Ethanol
Melting point (°C)	−89.3	−114
Specific gravity	0.810–0.812	0.79
Ignition temperature(°C)	35–37	276–456
Auto-ignition temperature (°C)	343–345	422
Flash point (°C)	25–29	12.77
Relative density (water: 1.0)	0.81	0.805–0.812
Critical temperature (°C)	287	239.85
Explosive limits (vol% in air)	1.4–11.3	3.3–19
Vapor pressure (kPa at 20 °C)	0.5	5.95
Boiling point (°C)	117–118	78
Density at 20 °C (g/ml)	0.8098	0.7851
Energy density (MJ/l^{-1})	27–29.2	19.6
Energy content/value (BTU/gal)	110,000	84,000
Liquid heat capacity (Cp) at STP (kJ/kmol °K)	178	112.3
Research octane number	96	129
Motor octane number	78	102
Viscosity (10^{-3} Pa s)	2.593	1.078

Carbon dioxide from biogas and flue gas from combustion of biomethane can be recycled back to the culture system as nutrients [215]. Recycling flue gas and CO_2 from biogas as feed for carbon mitigating autotrophs can take the process in feasible way (Fig. 6). Recycling the culture water can reduce nutrient usage by 55% [75]. Syngas obtained by gasification method is a typical source of methane. Syngas has low calorific value of 4–6 MJ m^{-3} that still can be used to run engines for heat, power and drive turbine for electricity.

5.4. Bio-oil

Bio-oil, the liquid content obtained by thermal treatment of biomass such as pyrolysis, thermal liquefaction etc. shares much similar properties with petroleum oil. Bio-oil possesses various fuel compounds but as whole has a good heating value ranging from 29 to 46.5 MJ kg^{-1}. Density of oil is nearly equal to petroleum oil. Viscosity factor greatly varies according to composition and purity of bio-oil. Though many useful products are recovered from bio-oil, the reactor for thermal liquefaction of biomass and fuel feed are complex and expensive. Initial capital cost limits the process scale up, but operational costs can be overcome by promising energy outcomes. Energy outcome to input ratio such as 6.67:1 [216] and 2.94:1 [217] denotes conversion of biomass-bio-oil is a viable option for fuel conversion. Characteristics and composition of bio-oil samples are given in Table 16.

5.5. Biohydrogen

Biohydrogen is a zero emission fuel considered to be much safer than all other fuels. Hydrogen carries energy that can be used to replace gasoline in the vehicles. On combustion, it reacts with oxygen to give water.

$$H_2 + O_2 \rightarrow H_2O \text{ (water)}$$

Water produced is radiant and energy obtained is lesser than spent in production. Combustion in internal engines can drive out power and electricity useful for beneficial activities. Hydrogen as fuel is used in space craft propulsion as it has the highest heating value. Efficient utilization of hydrogen fuel for running vehicles is been considered as the hot topic in hydrogen combustion science. Electric power of 5HW is generated by utilizing hydrogen at volumetric flow rate of 119.7 mol/h using proton exchange membrane and fuel cell [220]. Biohydrogen production is coupled with fuel cells to harvest energy proficiently [221,222]. Possibly, hydrogen can be used in Fuel Cell Vehicles (FCV) (Fig. 7) and Hydrogen Internal Combustion Engines (H-ICE). Hydrogen with wide range of flammability draws much attention towards safer storage and handling. High flame speed, lower density, high diffusivity and low ignition energy provides increased fuel-power ratio improving fuel economics. Biohydrogen production strategies are still on the verge of exploitation and offers improvement opportunities. Commercialization of biohydrogen fuel may take few years if supported by novel research activities. Technical approaches addressing scale up and operational issues can fortunately improve production economics for viable biohydrogen fuel in the future.

6. Algal genomics and genetic engineering

Genetic engineering of algae for beneficial and profitable fuel production is the current trend in algal biofuel production. Algal genomics concerns the alteration of metabolic pathways, enzyme production, identification of genetic elements and assisting proteins for improving the genetic manipulation options. A number of fresh water algal species are genetically engineered to improve biomass productivity and bioproducts accumulation. Algal genetic manipulation addresses the industrial problems of biomass sustainability, high valued by product coproduction to hold up process economics in profiting side, construction of artificial photoautotrophs for a crucial systemic manufacturing technology [223–225]. Various transformation methods are used to engineer algal genome that includes trans-conjugation, electroporation, microinjection and use of *Agrobacterium* sp. and other DNA

viruses as transformation vectors. *Agrobacterium* mediated vir inducers are more potent than the acetosyringone like synthetic vir inducers. Still vanillin, coumarin and cinnamic acid have greater potency than natural vir inducers. First report of stable genetic transformation was done in marine seaweed *Porphyra yezoensis* [226]. Silicon carbon whishkers method is also efficient in transforming algal cells of 10^{-5} per cell. Cell wall lacking species such as *Duniella* sp. and *Porphyra* sp. are transformed with glass bead method which is simpler and independent working. Use of artificial transposons is strongly recommended for avoiding unintentional genetic changes and high frequency recombination [227]. Many softwares and applications are available to optimize and reduce codon usage [228]. Though synthetic biological techniques have greater potential in biotechnology applications, the available design tools limits the functional complexity of synthetic devices [223]. The ability to build novel biomolecules compatible with algal cells and the metabolic networks to reprogram them functionally is intricate and exciting. Construction of effective strategies, complex devices and simple methodologies utilizing innovative approach can contribute to broader range of genetic engineering application of algae for biofuel farming and human health.

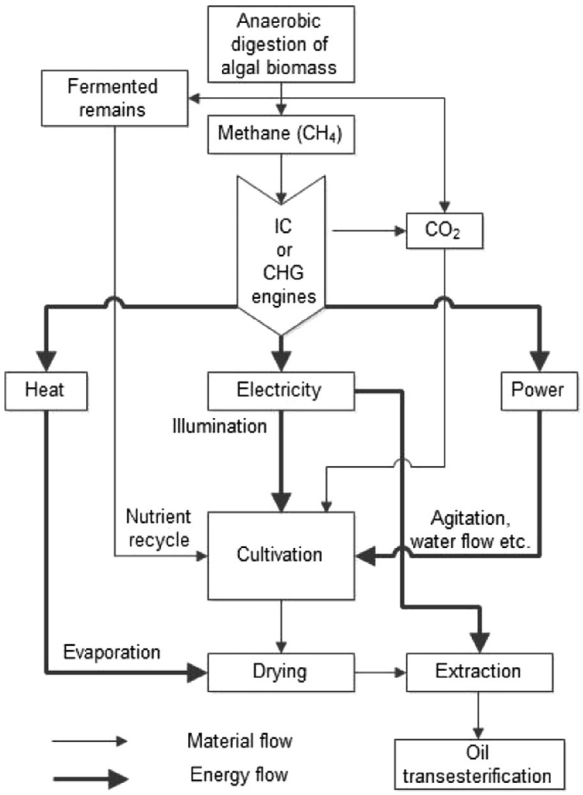

Fig. 6. Reutilization of resources in integrated algal biodiesel–biogas plant describing material and energy flow possibilities.

7. Significance of algal biomass for biofuels

Aquatic biomass could also be used as raw material for co-firing to produce electricity, for liquid fuel (bio-oil) production via pyrolysis, or for bio-methane generation through fermentation [88]. Cell walls of diatoms have been composed of polymerized silica and accumulate oil and chrysolaminarin. The fresh water green algae *Haematococcus pluvialis* is commercially important as a source for astaxanthin, *Chlorella vulgaris* as a supplementary food product, and the halophilic algae *Dunaliella* species as a source of β-carotene. Extracted biomass proposed to be used as fertilizer or animal feed is significant in adding economic value to the process. Co products such as pigments, agar, carragen and other bioactive compounds are value added products that can be removed before fuel conversion of biomass [48]. Methane, butanol and ethanol can be effectively produced by fementative digestion of residual algal biomass by selective microorganisms [229]. Microalga utilizes 1.83 kg of carbon/kg of dry weight of biomass [7]. Though algae are aquatic, they use less amount of water than terrestrial plants [230]. No herbicide or pesticide is recommended in algal cultivation [21]. Growth of algal population in waste water promotes them as dual purpose choice for biofuel production and organic load degradation [231]. Biochemical composition of algal biomass can be changed by varying the growth parameters thus inducing richness of targeted biomolecular fractions in resultant biomass steering to the purpose of cultivation [232].

8. The Indian scenario

India, a tropical South Asian country has a stretch of about 7500 km coastline, excluding its island territories with 2 million km^2 Exclusive Economic Zone (EEZ) and 9 maritime states. The algal flora of India is highly diverse and comprises mostly of tropical species,

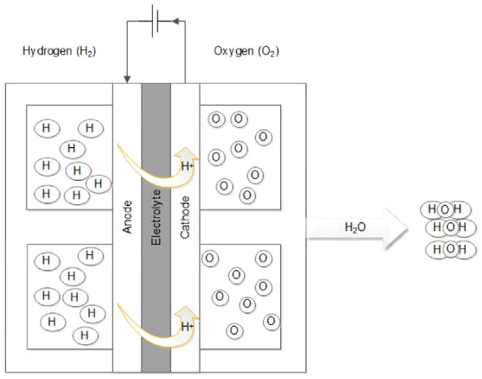

Fig. 7. Fuel cell consuming hydrogen and oxygen to produce water and electrical energy.

Table 16
Composition and properties of bio-oils obtained by fast pyrolysis [18,158,218,219].

Properties	Typical values			ASTM testing methods
	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	ASTM D-6751
Viscosity (Pa s)	0.04–0.20 (at 40 °)	0.10 (at 40 °C)	2–1000	ASTM D-445
Higher heating value (MJ kg^{-1})	21	29–45.9	42	ASTM D-6751

but members of subtropical and temperate zones are also reported. Highly established species of microalgae farming is practiced in a moderate scale to supply nutraceuticals and pharmaceuticals. However 271 genera and 1153 species of marine algae are reported and many are yet to be explored [233]. Due to geographical, climatic and physiographic change, the coastal habitats predominantly the subtidal algal population has been reduced in a great number. Many of the rocky beaches available with, mudflats, coral reefs and lagoons along the coast provides ideal habitat for seaweed growth. Most of the agarophytes occur throughout the year where significant number of alginophytes and carrageenophtes occur only in particular months. Approximately 7.5–8 million tons of seaweeds are harvested globally per year [87]. Currently India has 46 seaweed based industries out of which 21 agar and 25 alginate based industries that does not perform up to the mark due to the shortage of raw materials. There is a drastic decrease in seaweed production in the past few decades [234]. Sustainable and mass seaweed cultivation is still in experimental stage. Over-utilization coupled with short supply of algal biomass due to natural calamities and human intervention in marine environment and continuous depletion of standing crop are the current challenges needs to be addressed. Encouraging regular cultivation practices and adopting sustainable utilization practices such as planned cultivation and harvest might result in better conservation of marine algal biomass resulting in boon of sustainable algal biorefineries in India.

9. Compromises and future outlook of algal biofuels

Future of algal fuels is based on developing cost effective strategies by adopting suitable technologies such as sugar mill integration, biogas-biodiesel production, co-cultivation of algae with other living aquatic entities, promoting co-product recovery and marketing etc. Working on technical aspects of cultivation systems thus making it more efficient for intensified biomass production, reduced material and energy loss by technically advanced system fabrication are expected as next level improvements for profitable fuel production. Exploiting algal species with higher CO_2 mitigation rate can be effective enough in reducing greenhouse gas thus bringing mother earth back to naturalistic manner. Fuel studies are made within the limited species of interest considering them research friendly. Heterotrophic algae though superiors autotrophic ones in accumulating mass lipids, it consumes high carbon, nitrogen and phosphorous nutrients for which sewage water is proposed as alternate cradle. Selecting outdoor culture systems for mixotrophic members can eliminate illumination requirements and promotes energy saving. Contamination risk of outdoor ponds and capital investment of closed photobioreactors limits the construction of commercial plants. Strain selection should be done by considering climatic conditions prevailing around the production facility. Appropriate reactor design and processing strategies should be designated pondering the biology of algal biomass. Progress in biochemical and genetic engineering can stimulate higher productivity of targeted product and co-products. Algal biomass based biofuel production will emerge as multi-purpose process if suitable strategies are developed and placed properly in process sequence for sustainable outcome of value added co-products along with main fuel products.

10. Conclusion

This review highlights the technical advancements in upstream/downstream processes of algal fuel production. Co-extraction of valuable products can take the process in a feasible way thus promoting industrialization and commercialization of the process. Versatility of algae is a promising parameter for them being prompted to be used as a biofuel resource. Easy adaptability nature of algae for surviving in wide range of climatic conditions make them most wanted in the scientific market spread around the globe for the purpose of biofuel research and implementation. Distinguished hybrid reactors are fortunate outcomes of ground breaking strategies and technology advancements for promoting aquatic biofuels. Sustainable supply of oil can be made possible by combining microalgal oil with macroalgal oil in case of biodiesel transesterification. Algal oil can be possibly combined with other plant oils even attempting absolute use of all oil feedstocks. Transesterification standards are to be evaluated and established for commercialization of mixed oil conversion. Though technologies for efficient use of residual biomass are already developed, more of its other side is to be made effective for efficient down-streaming of products. Cost effective algal biofuel is possible only when the energy content of the same remains higher than consumed in production and processing strategies. Sooner in the future, advancements in technology boosted by convention of knowledge from chemical, biotechnology and marine science can turn out algae energy a sustainable, renewable, economical and green fuel for safer world. Novel approaches utilizing alga and other fuel feedstock effectively has guaranteed market in the scientific community, possibly quicker progress to emerge as first-rate gear in race for budgeting biofuels.

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