Clean Energy Production Technologies

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Substrate Analysis for Effective Biofuels Production

Springer

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The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

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Foreword

Never before has the need for clean renewable fuels been a greater focus, due to both the pollution caused by and the limited lifespan of fossil fuels. It is well-known that sourcing a viable cost-efficient and commercial alternative to fossil fuels is still challenging. In the series of renewable energy production, biofuels are the most promising. Production of biofuels from organic and cellulosic biomass waste grabs global attention due to its renewable, cost-effective and eco-friendly nature. Cellulosic feedstocks provide the raw material for biohydrogen, biogas, biomethane and biobutanol production, whereas wastes such as sewage, sludge and algal biomass provide the feedstocks for biodiesel and bio-oil production.

The major bottleneck in turning to mainstream commercialized biofuels has been the seemingly insurmountable costs involved. The costs of effective substrate, pretreatment, enzymes and associated bioconversion and fermentation technologies have retarded the route of biofuels to mainstream markets. In the past few years, modifications and improvements in biofuel technologies have been noted, and developments in substrate-based bioprocess technologies associated with biomass to biofuels contribute towards making this process more economically viable. Developing this green economically sustainable process demands focused attention on each individual parameter and subsequently considering the parameters collectively at a global scale.

Publication of the book entitled *Substrate Analysis for Effective Biofuels Production* is a notable effort in collating relevant information in the field of biofuels. I am writing this message with great joy and satisfaction as a researcher fascinated in the area of green fuel production. This book essentially holds ten chapters focusing on various biofuel production technologies with defined strategies to improve their production at an economic level. The book is focused on the effect of substrates and related parameters on different biofuel production technologies including biogas, biobutanol, bioethanol, biohydrogen and biodiesel. While addressing existing rollbacks in different biofuel production technologies, the book also discusses way-out technologies related with the production of different biofuels. It is my opinion that this book won't disappoint in providing a unique collection of practical information for scientists, researchers, teachers, students and industries that are interested in biofuel production technologies.

I appreciate the meticulous work of Dr. Neha Srivastava [IIT (BHU), Varanasi], Dr. Manish Srivastava [DU, Delhi], Prof. (Dr.) P. K. Mishra [IIT (BHU), Varanasi], and Dr. Vijai Kumar Gupta [TTU, Estonia] in effectively compiling this book. The effort made by the editors will certainly satisfy the knowledge gap in the field and fulfil the demands of scientists, researchers, teachers, students and industries. This book is an excellent resource for further research and advancement in the area of effective substrate selection in biofuel production processes. I am sure the readers will find this book as an informative source of relevant information in the field of biofuels, which also includes a reliable resource of supporting reference material.

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We, the editors, are thankful to all the academicians and scientists whose contributions have enriched this volume. We also express our deep sense of gratitude to our parents whose blessings have always prompted us to pursue academic activities deeply. It is quite possible that in a work of this nature, some mistakes might have crept in text inadvertently, and for these, we owe undiluted responsibility. We are grateful to all authors for their contribution to present book. We are also thankful to Springer Nature for giving us this opportunity and the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, UP, India, for all technical support. We thank them from the core of our heart. Editor Manish Srivastava acknowledges the DST, Govt of India, for awarding the DST-INSPIRE Faculty Award [IFA13-MS-02] 2014 and also Science & Engineering Research Board for SERB-Research Scientist award 2019.

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About the Editors

Neha Srivastava is currently working as Postdoctorate Fellow in the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, India. She has published 25 research articles in peer-reviewed journals with 3 patents and 1 technology transfer. She completed her PhD from the Department of Molecular and Cellular Engineering, SHIATS, India, in 2016 in the area of bioenergy. Furthermore, she has received 6 Young Scientist Awards. Presently, she is working on biofuel production (cellulase enzymes, production and enhancement, biohydrogen production from waste biomass, bioethanol production).

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Vijai Kumar Gupta, ERA Chair of Green Chemistry, Department of Chemistry and Biotechnology, School of Science, Tallinn University of Technology, Tallinn, Estonia, is one of the leading experts in the area of microbial biology and biotechnology. He is a Member of the International Subcommission on Trichoderma and Hypocrea, Austria, and of the International Society for Fungal Conservation, UK, and Secretary of the European Mycological Association. He is also the Fellow of the prestigious Linnean Society of London, UK; of the Indian Mycological Association; and of the Mycological Society of India. He has been honoured with several awards in his career, including Indian Young Scientist Award for his advanced research achievements in the field of fungal biology and biotechnology. He is the Editor of a few leading scientific journals of high repute and has many publications in his hands with h-index 21. He has edited many books for publishers of international renown such as CRC Press, Taylor and Francis, USA; Springer, USA; Elsevier Press, The Netherlands; Nova Science Publishers, USA; DE Gruyter, Germany; and CABI, UK.

1 Algal Biomass: Potential Renewable Feedstock for Biofuel Production

Archana Tiwari and Thomas Kiran Marella

Abstract

Algae possess immense potential to yield a myriad of valuable products which find wide application in waste water remediation, nutraceuticals, and aquaculture. The natural products from algae have attained significant attention owing to their extraordinary efficiency which makes them suitable for a plethora of application as biofuels, waste water remediation agents, therapeutics, food and feed, nutraceuticals, biocontrol agents, and aquaculture. Despite the huge potential of algae, studies are limited owing to the fact the difficulties in isolation and cultivation, the significant impact of nutrients, contaminants, and sessional variations. Waste waters tend to act as a source of nutrients, thereby facilitating the growth of algae, which in turn quench the excessive nutrients and heavy metals leading to the phycoremediation of waste waters. Algae can produce a plethora of biofuels including biodiesel, biogas, and bioethanol to name a few. This chapter elaborates the potential of algae biomass as a renewable feedstock for biofuel production and their further utilisation as a promising source of nutraceuticals and high-value products, which can be a sustainable solution making the best out of waste for a better global environment and economy.

Keywords

Algae · Biofuel · Nutraceuticals · Phycoremediation · Wastewater treatment

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1.1 Introduction

The problem of global environmental pollution and rapid depletion of nonrenewable energy sources call for exploring alternate energy sources. Worldwide, researchers are exploring options for eco-friendly, renewable fuels. Algae can serve as a promising option as the most appropriate energy generating organisms as they are efficient in consuming the atmospheric carbon dioxide, which would limit the carbon dioxide emissions. They have been widely explored as the source of a plethora of biofuels and bioproducts (Laurens et al. 2017; Show et al. 2017; Halim et al. 2012; Dong et al. 2016; Li et al. 2017a, b). The acumen of algae in biofuel production coupled with long-chain polyunsaturated fatty acids (PUFAs) was reported to be encouraging (Harwood and Guschina 2009) and can grow promptly doubling algal biomass by 24 h, while in the exponential growth phase, the doubling time is nearly 3.5 h. Algae are abundant in oil content, which can exceed 80% of dry biomass weight (Spolaore et al. 2006).

There exists a variation in the presence of oil, lipids, hydrocarbons, etc. in different algal species, for example, *Botryococcus braunii* yields copious hydrocarbons, particularly oils in triterpene form, which are nearly 30–40% of its dry weight. The main hydrocarbons include *Botryococcus* oils: botryococcenes, alkadienes, and alkatrienes (Banerjee et al. 2002). The major product of *Botryococcus braunii* is quite similar to the fossil fuel compounds and these valuable bio-oils from photosynthetic algae can yield a wide range of fuels like diesel, gasoline, and jet fuel which is in fact the extreme demand of the present time (Xu et al. 2006).

Biofuels are the need of today. Algae are wonderful tiny factories that can convert biomass to bioenergy resolving the problems related to the exhaustion of fossil fuels and global increase in pollution. In addition to biofuels, algae are also exclusive reservoirs of a plethora of extraordinary products ranging from nutraceuticals to antimicrobial products, UV protectants, high-value products, and aquaculture (Fig. 1.1). Focusing on better growing and harvesting methods will help in early commercialisation. Emphasis on screening new algal strains, techniques to improve production of algal biofuels, and improvisation in downstream processing will open new doors in the area of algal biofuels. Biofuels can attribute towards conservation of our nonrenewable sources of energy and environment, a way towards better future.

Algae-derived biofuel generation needs sufficient innovative tool considerations for the extensive exploitation of biotechnological techniques, greater culture condition optimisation, new strains and consortium, and above all understanding fundamental mechanisms (Rodionova et al. 2017). The production of hydrogen from algae has been well documented, yet the commercial implementation requires further investigations. Amongst the major biofuels envisaged till date, the algae biodiesel has established the most focus and perhaps is the only algal biofuel venture which has been upscale to the pilot-scale and full-scale utilisation, though algae-based bioethanol production requires proper attention and adequate consideration.

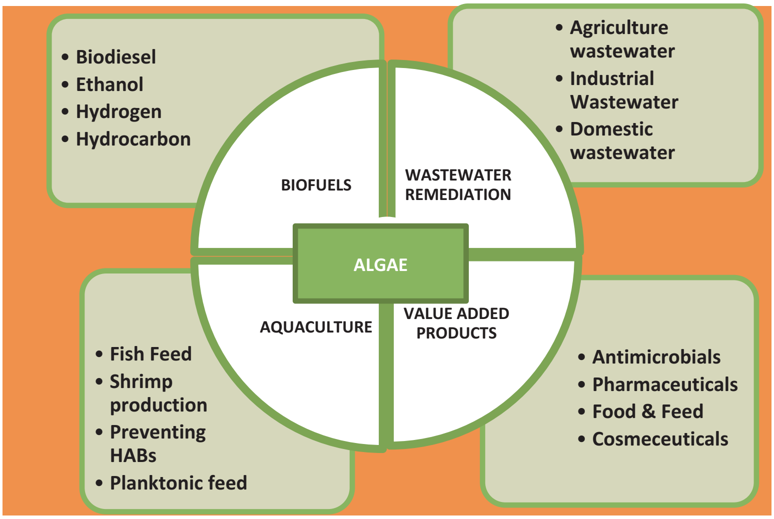

Fig. 1.1 Application of algae

1.2 Algal Biomass: A Unique Reservoir

Algae possess a special photosynthetic system which makes them ideal organisms for biofuel production with distinctive properties, and scientific reports have indicated the biofuel production efficiency in many algal species ranging from cyanobacteria, green algae, diatoms, to macroalgae (Archana and Anjana 2012; Marella et al. 2018). Algae are photosynthetic organisms inhabiting varied habitats and performing a plethora of ecological roles which are irreplaceable and commendable for the environment. They are efficient photosynthetic autotrophic systems that can convert atmospheric carbon dioxide utilising the sunlight into energy so proficiently that they can exponentially increase their algal biomass by twofold. The process of oxygenic photosynthesis takes place in almost all algae, and actually most of the knowledge about the photosynthetic process was for the first time elucidated in *Chlorella*, which is green alga. Similar to the higher plants in algae, photosynthesis also involves two reactions – light reactions and dark reactions (or Calvin cycle). Carbon dioxide is fixed by the oceanic primary producers during photosynthesis. The photosynthetic pigment systems which are the pioneers of the solar energy entrapment are also more of similar in nature compared to the higher plants. The algal pigments include chlorophyll, phycocyanin, phycoerythrin, carotenoids, fucoxanthin, etc. depending upon the algae class. The efficiency of the algal photosynthetic system is imparted by the pigments present in the diverse classes of algae and helps the algae to inhabit the range of habitants (Archana 2014).

The algal biomass is considered as the fastest-growing biomass on the planet and omits oxygen which is one of the survival sources for the living beings on the earth (Chakrabarti et al. 2018; Cheng et al. 2006). They are the potential source of oils and feedstocks (Chu 2017). Algae produce a number of chemicals such as chemicals used for the synthesis of resins and plastics (Mekonnen et al. 2013; Wang et al. 2016). Algae produce much higher concentrated lipid substances, and they have antibacterial properties (Satoru et al. 2007; Minhas et al. 2016) under stress conditions. During silicon deficiency, they can change activities of those enzymes which are responsible for the accumulation of lipids (Roessler 1988). They release secondary metabolites and other substances which have cytotoxic effects on other species of microalgae, invertebrates, and cell lines of mammalians (Ruocco et al. 2018; Dhanker et al. 2015). Red algae produce carotenoids which help to cure retinal damage in animals (Sathasivam and Ki 2018). Algae became even the priority research source for space missions for the sustainable production of feedstock, recycling of carbon dioxide, and oxygen replenishment on future long-distance interplanetary missions (https://www.nasa.gov/mission_pages/station/research/experiments_category). Diatoms are key elements of aquaculture (Wang 2015; Clifford and Kevan 2014) and are commercially used as feeds to secondary consumers (Mann and Droop 1996; Chen et al. 2018).

Several investigations are being conducting for the optimum utilisation of microalgae for the production of desired compounds (Gerardo et al. 2015; Chew et al. 2017) and make them as hot candidates for biofuel production (Saranya et al. 2018), as a food supplement for human and animals (Chew et al. 2017), to cure the problem of waste water treatment (Marella et al. 2018, 2019), their aquaculture importance (Li et al. 2017a, b), natural insecticidal and industrial uses of their fossilised form (Korunić et al. 2016), and many more.

When we compare the capability of oil production per acre in algae, it is nearly 15 times more compared to other biofuel-producing plants. The ability of algae to process sugar from sources rich in cellulose like wood chip or grass is incredible and definitely more proficient compared to other microorganisms. The processing of cellulosic biomass is challenging for microorganisms as the presence of lignin in such biomass inhibits the microorganism and the biomass has to be processed prior to removal of lignin. But the forbearance of the algae to lignin makes it possible to avoid the process of lignin removal from cellulosic biomass, thereby making the process less cumbersome, convenient, and cost-effective. The advantages of algae as source of different biofuels are highlighted below:

- Algae consume atmospheric carbon dioxide, thereby leading to the mitigation of greenhouse gas.
- The growth requirements of algae are minimal in nature, and they can be readily cultivated in the areas which are not fit for the plant growth making the land arable for agriculture. They can grow in salty water, freshwater, or waste waters.
- The algal photosynthetic machinery produces the bio-oils utilising solar power, carbon dioxide, and water. The biofuels originating from the algal oil have similar molecular structures to the existing fossil-based fuels like petroleum and diesel making them more suitable for use in the current automobile engines without many changes.

- The biofuel production per acre of algae is more compared to the other sources of biofuel generation. The productivity of algae-based biofuels is more as they can be rapidly cultured requiring less time.

1.3 Algae-Derived Biofuels

Biofuels in the present context have seen the first two initial generations. The first-generation biofuels originate from the crop-based plants, thereby committing with the agriculture and the food demands of the population, while the second-generation biofuels are derived from the residues of the forest and agriculture and feedstocks which are not food based in nature. There were several limitations associated with these two generations of biofuels, and the third-generation biofuels aptly addressed the lacuna with the previous generations of biofuels. The biofuels originating from the algae are quite diverse in their nature as algae produce copious amounts of biomolecules like carbohydrates, lipids, and proteins, and these can be readily sort out into biofuels like biodiesel, ethanol, hydrocarbons, lipids, hydrogen, and high-value substances with wide applications (Fig. 1.2).

1.3.1 Algal Biomass

The algal biomass in dry form can be converted directly to yield electricity and heat. The technique of high temperature can be used to generate the fuel gas from dry algal biomass; also high pressure can generate fuel oil from the dry algal biomass since these processes require the dry algal biomass; there are many apprehensions associated with their use to generate the biofuels. The algal biomass drying demands

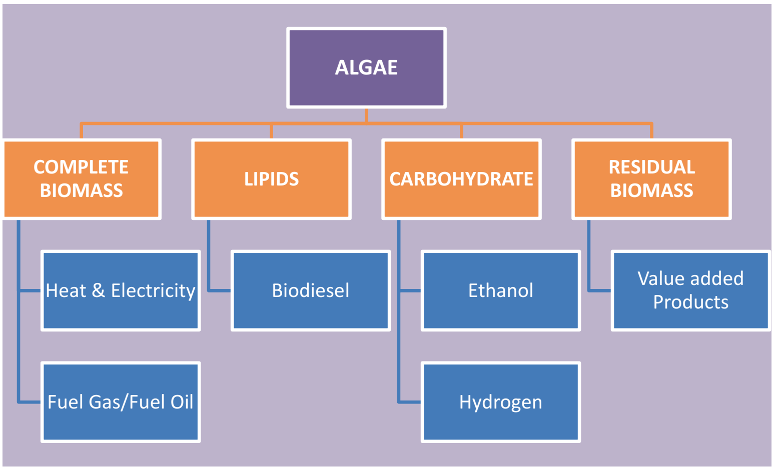

Fig. 1.2 Diverse fuels derived from microalgae

a good amount of energy, which is quite negative in part of the energy balance and investment costs of essential equipment (Wijffels 2007) though the sustainable approaches advocate the application of solar energy in order to dry algal biomass.

Another option to dry algal biomass is through the process of liquefaction which can take place through the application of both heat and chemicals and involves treatment at a very high pressure and temperature (Banerjee et al. 2002; Tsukahara and Sawayama 2005), but this technique is quite naïve and in the incipient phase (Meuleman 2007). The process of anaerobic digestion can be used as an alternate method to process algal biomass, and through this method biogas can be produced from the wet stream wherein the energy requirements are relatively less compared to the thermal process. The biogas contains nearly 55–75% methane which can be converted to generate electricity and heat (de Mes et al. 2003).

1.3.2 Lipids

The lipids comprise the key constituents of algae, and these lipids find application as a liquid fuel as straight vegetable oil (SVO) in the adapted engines, while the free fatty acids and triglycerides can be transformed into biodiesel by the action of the catalysts which can be homogeneous or heterogeneous in nature. A number of algal strains have been extensively documented to produce lipids (Li et al. 2017a, b). A comparative analysis between the straight vegetable oil (SVO) and algal oil indicates that the algal oil is basically unsaturated, thus less suitable for automobile engines which are sensitive. Intricate investigation is much essential for finding more novel algal species capable of yielding good amount of lipids, and improving the lipid production through innovative methods is certainly essential for prompt commercialisation so that the algal lipids can see the light of the day.

1.3.3 Biodiesel

Biodiesel is a non-polluting eco-friendly substitute fuel resulting from renewable sources (Hossain et al. 2008) which is at par in fuel performance to the existing fossil fuels like petrol and diesel with lesser amount of particulate matter and sulphur release (Miao and Wu 2006; Scragg et al. 2002). The green algae *Chlorella protothecoides* has been reported as a suitable genus for the production of biodiesel because it has the potential to utilise diverse sources of carbon, namely, acetate, glucose, and glycerol, and can produce good amount of biomass and lipids (Xu et al. 2006). In algae, the biodiesel and glycerol are produced by the transesterification of triacylglycerols (TAGs) with the aid of an acid or alkali catalyst (Johnson and Wen 2009). The transesterification of algal oil and processing of fatty acid methyl esters (FAME) lead to the biodiesel production from algae, and this process requires a catalyst of either an acid or alkali. The key benefits of the biodiesel from algae oil are included in Fig. 1.3.

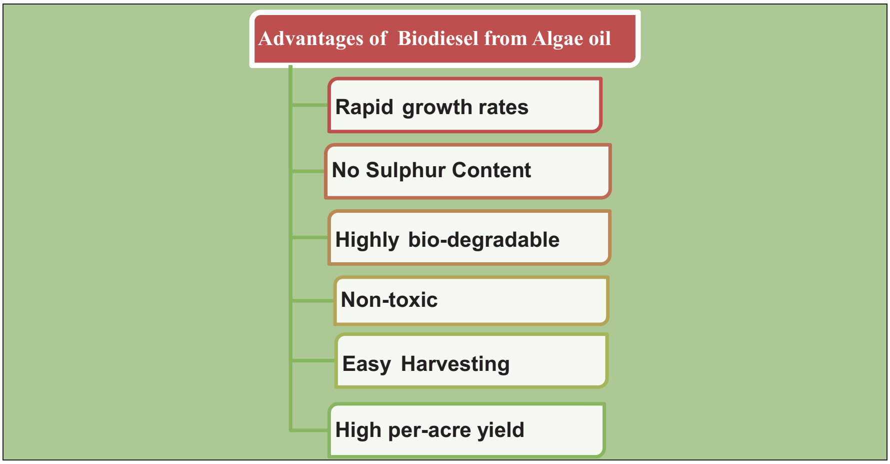

Fig. 1.3 Advantages of biodiesel

Biodiesel production can be mediated through two routes:

1. Direct transesterification of biomass from algae (Lewis et al. 2000)
2. Two-step process wherein the lipids are extracted, collected, and transesterified (Johnson and Wen 2009)

Both these biodiesel production techniques need the extraction of lipids utilising the amalgamations of different alcohols and solvents like chloroform/methanol, hexane/isopropanol, or petroleum ethers and methanol (Johnson and Wen 2009; Mulbry et al. 2009). Amongst the two production methods, the direct method is profitable, since it conglomerates the lipid extraction and transesterification into one process, thus saving more time compared to the two-step extraction-transesterification process (Fig. 1.4).

Though the biodiesel is a suitable alternative to the nonrenewable petroleum diesel, there are several bottlenecks associated with the biomass-based production, which need to be efficiently addressed through technological interventions in order to eradicate the current fossil-based diesel crunch. More exploratory studies are indeed required which investigate new strains or novel consortium with a much higher rate of growth and oil yield. In this direction, Ali et al. (2017) have advocated a new chemical-based method for increasing the algal biomass and production of lipid and the fatty acid composition alteration in the green algae *Chlorella protothecoides* employing exogenous bioactive molecules – phytohormones and antioxidants.

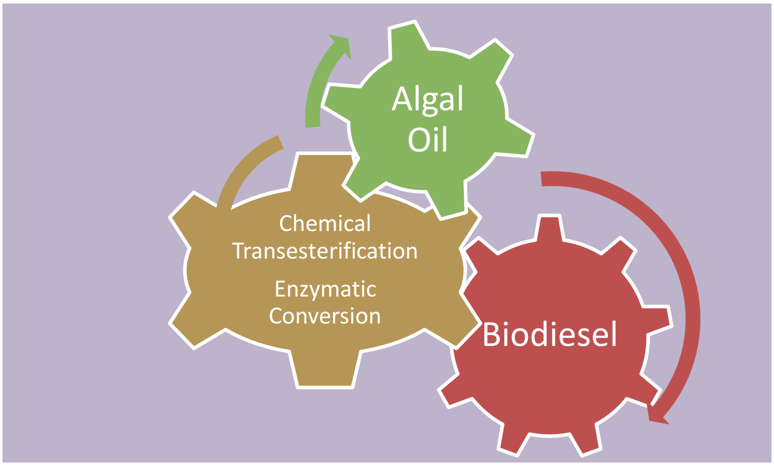

Fig. 1.4 Origin of biodiesel from algal oil

1.3.4 Hydrocarbons

Algae can produce hydrocarbons, and similar to petroleum, algal hydrocarbons can be converted to fuels like diesel, gasoline, and kerosene. A renowned alga, *Botryococcus braunii*, is extremely popular owing to its potential to yield hydrocarbons (Hillen et al. 1982). In comparison to other algal species which generally contain nearly 1% of hydrocarbons, *B. braunii* possesses nearly 20–60% dry weight which can reach up to 80% (Wijffels 2006). On the basis of the algal strains, the hydrocarbons are either C30 to C37 alkenes or C23 to C33 odd-numbered alkenes (RangaRao and Ravishankar 2007). The algal hydrocarbons are majorly gathered on the outer surface of the cell, which makes the process of their extraction relatively simpler and convenient (Wijffels 2006).

1.3.5 Hydrogen

Algae produce hydrogen, which has great potential as a future fuel owing to numerous advantages (Archana and Anjana 2012). Hydrogen as fuel has immense prospects as it liberates only water and no toxic emissions are there, which makes it environment savvy but he major associated lacuna is the non-availability of a sustainable system of hydrogen production. At present, the hydrogen gas is formed by the fossil fuel steam reformation process and the water electrolysis, but these processes are electricity consuming, making the hydrogen yield not at all economic. Biologically, hydrogen can be produced by a range of resources like the bio-oil steam reformation (Wang et al. 2007), dark and photofermentation of organic materials (Kapdan and Kargi 2006) and algae-mediated photolysis of water (Ran et al. 2006). However, algae can produce hydrogen straight from sunlight and water, but only under the condition wherein oxygen is not present in the vicinity.

In a nutshell it can be stated that the conditions required for hydrogen production are either expensive or difficult, making the production very challenging, so making the production commercially possible, exhaustive investigations are needed. Exploring new algal species producing high yield of hydrogen needs to be envisaged for an advanced understanding of the enhanced hydrogen production mechanism, and use of modern biological tools like genetic engineering are indeed needed (Ashutosh et al. 2007; Archana et al. 2019).

1.3.6 Ethanol

Bioethanol is unique as it can be an excellent substitute to the existing fossil fuel – petrol. Though the ethanol production from algae is quite promising, at present it is in the incipient research stage and a lot of attention is required for the functionalisation of the large-scale system of its production. In algae the fermentation of the polysaccharides like starch, cellular, and cellulose leads to the production of ethanol. Algae are rich in carbohydrate content, for example, in some species the carbohydrate content can rise up to 70% under optimum conditions, which makes them suitable organism for the bioethanol production. Algal strains like *Chlorella, Scenedesmus, Chlamydomonas,* and *Dunaliella* produce a good amount of starch, and hence they are very suitable candidates for the production of bioethanol. The cell wall of algae comprises of outer and inner cell wall layer, wherein the outer cell wall is trilaminar and is rich in polysaccharides such as agar, alginate, and pectin and the inner part of the cell wall comprises of mainly hemicellulose and cellulose. Though the abundance of cellulose and starch in algae, which can be further fermented to ethanol, makes them ideal for bioethanol production, elaborative exploratory research is needed to transform the preliminary research into concrete large-scale commercially profitable process. Table 1.1 highlights the biofuels from algae and their yield (Cuellar-Bermudez et al. 2014).

1.3.7 High-Value Products

The sustainable approaches towards algal biofuels necessitate the demand of valuable co-products to make the whole process economically pragmatic. Rapid commercial implementation of third-generation biofuels from algae requires innovative initiatives on algal energy generation. Alga culture can turn out to be a promising option as algae are excellent feed and generate yields with a greater commercial value compared to energy.

Algae have magnificent metabolism, and they produce an array of secondary metabolites which find vivid application as food, food supplements, food colourants and nutraceuticals, fish feed, shrimp and shellfish, and omega-3-fatty acids like DHA, EPA etc. (Grima Molina et al. 2003; Reith 2004; Li et al. 2017a, b).

Table 1.1 Microalgal biofuels

Species	Product	Yield
Chlamydomonas reinhardtii (CC124)	**Biohydrogen**	102 mL/1.2 L
		0.58 mL/hL
		0.30 mol/m^2
		0.6 mL/L h
Chlamydomonas reinhardtii (Dang 137C mt+)		175 mL/L
		4.5 mmol/L
		71 mL/L
Chlorella vulgaris MSU 01		26 ml/0.5 L
Scenedesmus obliquus		3.6 ml/μgChl a
Platymonas subcordiformis		11,720 nL/h
		7.20 mL /h
		0.339 mL/hL
Dunaliella tertiolecta	**Bio-oil**	43.8%, 34 MJ/Kg
		42.6%, 37.8 MJ/Kg
		25.8%, 30.74 MJ/Kg
Chlorella protothecoides		52%
		57.9%
Chlorella sp.		28.6%
Chlorella vulgaris		35.83%
Nannochloropsis sp.		31.1%
Chlorella vulgaris	**Biogas**	0.63–0.79 LCH_4/gVS
Dunaliella salina		0.68 LCH_4 /gVS
Euglena graciis		0.53 LCH_4 /gVS
Scenedesmus		140 LCH_4 /KgVS
Scenedesmus (biogas from lipid-free biomass)		212 LCH_4 /KgVS
Scenedesmus (biogas from amino acid-free biomass)		272 LCH_4 /KgVS
Scenedesmus obliquus		0.59–0.69 LCH_4/gVS
Botryococcus braunii	**Lipid content for biodiesel**	25–75%
Chlorella sp.		28–32%
Chlorella vulgaris		56%
Crypthecodinium cohnii		20%
Monallan thussalina		20–70%
Nannochlorisis sp.		20–35%
Nannochloropsis sp.		31–68%
Neochloris oleoabundans		35–54%
Nitzschia sp.		45–47%
Scenedesmus dimorphus		6–40%
Scenedesmus obliquus		11–55%
Schizochytrium sp.		77%

(continued)

Table 1.1 (continued)

Species	Product	Yield
Chlorella pyrenoidosa	**Carbohydrate content for bioethanol**	26%
Chlorella vulgaris		12–17%
Dunaliellasalina		32%
Scenedesmus obliquus		10–17
Porphyridium cruentum		40–57%
Euglena gracilis		14–18%

Cuellar-Bermudez et al. (2014)

1.4 Cultivation of Algae for Biofuels

Algae can produce a diverse range of biofuels and till date many algal species are reported to yield biofuels. The process of cultivation of algae has substantial benefits as algae have autotrophic ability to utilise sunlight to yield sugar, via photosynthesis, so it is relatively convenient to cultivate them in open ponds, closed system like photobioreactors, and hybrid system. Table 1.2 highlights the advantages and disadvantages of the various cultivation systems.

The selection criterion for algal-based biofuels includes:

1. Easy cultivation and rapid growth on minimal requirements
2. High content of oil and good productivity
3. Good ability of harvesting with easy purification
4. Contamination resistance and endurance
5. Tolerance towards high concentration of oxygen and temperature extremes
6. Adaptation to the altered culture conditions encountered in the growth ponds

1.4.1 The Open System

The open system is perhaps the oldest and the simplest method meant for algal cultivation. In the open system the natural resources are utilised for the algal growth. The energy is obtained via sunlight, which converts the atmospheric carbon dioxide to sugars. There are a couple of options available with open ponds with algae cultivation, which include raceway pond, circular pond, and unstirred pond. There are several advantages and disadvantages associated with the open system. The open systems are cost-effective and low maintenance demanding systems, but they are directly under the influence of environmental factors, and in countries where weather is not very suitable for algal cultivation, it is a difficult system.

1.4.1.1 Raceway Ponds

The raceway ponds can be built as an individual as well as after joining more than one raceway ponds together having depth of 15 and 30 cm. Channels may be made in concrete, plastics, and compacted earth. Paddlewheel, pumps, and airlifts are

Table 1.2 Different types of cultivation methods with advantages and disadvantages

Cultivation system	Advantages	Disadvantages
Open system	Low construction and maintenance cost	More prone to different kinds of contamination such as bacterial, virus, fungal, and other microalgal species including invasive ones
	Easy to scale up	
	Possibility to integrate with waste water	Low productivity – limited photosynthetic activity of algal species due to poor mixing and water and gas transfer
		Difficult to control cultivation parameters such as seasonal temperature, light, temperature, pH, and nutrient
		High harvesting cost: due to mostly small-sized algae and poor algal cell
		High evaporation of water especially in tropical or desert areas
		High volume to surface ratio
Closed system	Higher cell biomass production and cell density due to better mixing and maximised photosynthetic capabilities which directly reduced algae harvesting and drying costs	Prohibitively high construction cost at large scales
	Less contamination risk due to less exposure to the environment	Lowest volume to surface ratio
	Possible contamination from bacteria and fungi, even from other algae species also	
	Better control of cultivation parameters such as light, temperature, pH, and nutrient adjustment	
Immobilised system	Easy harvesting of algal biomass because algal cells are enclosed in small spaces (e.g. beads) or attached to solid carriers	Unavailability of appropriate cost-effective and durable supportive matrix for higher algal biomass growth
		Can be utilised for selected species only
		Having low lipid content
	Improved productivity – can improve nutrient/gas transfer and prevent light shielding	Difficult to scale up outdoor immobilised systems
	Main purpose is to remove nutrients not to produce biomass and oil production	

used to drive water continuously. However, paddlewheel is mostly popular for generating water velocity in comparison to others. The raceway ponds are more safe and cheaper in comparison to other open pond systems for commercial algal production. Because mixing is done in an open pond, the system is more efficient in capturing carbon dioxide and sunlight. Additionally, the water-derived movement continuously prevents the settling down and deposition of algae and helps make the operation more successful. The construction cost maintenance cost of these ponds are very low. Many companies around the world are using this type of system for large-scale production of food supplements or β–carotene. The algal productivity is varying with environmental factors such as light duration and intensity, temperature, and salinity.

1.4.1.2 Circular Ponds

Circular ponds have depth of 30–70 cm and, normally, having a diameter of 45 m. It has a central pivoted agitator. These ponds are being used at large scale for b carotene production and higher annual algal biomass production in Taiwan and Japan. These ponds are basically used commercially for β-carotene production. The most commonly used algae in this production is *Chlorella* sp. The integrated use of algal biomass production (such as *Chlorella* sp. and *Oscillatoria* sp.) and waste water treatment is conducted in circular ponds for a long time (Sheehan et al. 1998).

1.4.1.3 Unstirred Ponds

Unstirred ponds are the natural uncovered body. These ponds can be as large as the lake. These ponds are economically more efficient and need less technical support in comparison to other commercial production ponds. Generally, the depth of these ponds is not more than 50 cm deep. The unstirred ponds have been used for carotene production in few parts of Australia by some companies. However, these kinds of ponds are more prone to contaminations such as growth of invasive microalgal growth and bacterial, protozoan, and fungal infections. Similarly, in another study done by Lee in 1997, they noted approximately 32 tons of microalgal biomass production per year from natural lakes in Southeast Asia.

1.4.2 The Closed System

The closed systems have controlled set of conditions suited for the maximum growth of algae for enhanced biofuel production. The dependence on the climatic conditions is not a constraint in closed system. The chances of contamination are reduced in the closed systems compared to the open systems (Posten and Schaub 2009; Yeang 2008).

There are multiple options of photobioreactors employed in algal cultivation ranging from flat-panel to horizontal systems. The photobioreactor (PBR) systems are assembled to culture algae in tubes, bags, and transparent materials wherein they are not secluded from the direct influence of the environment (Lehr and Posten 2009; Shen et al. 2009). For nutrient supply either waste water is used or supplements are to be provided for algal growth. The Biomass productivity is much higher because the algae are exposed to abundant sunlight (Chisti 2008a).

The various culture systems have been highlighted in Fig. 1.5.

The tubular photobioreactors (TBPR) are specialised as they possess more ratios in terms of surface-to-volume (Shen et al. 2009). The following are the different types of TBRs:

- The helical tubular PBRs
- The fence-like tubular PBRs
- The horizontal tubular PBRs

All these types of TPBRs establish and standardise flow rate to avert the algal biomass process of sedimentation (Chisti 2008a). In these systems there is production of oxygen in high amounts due to dense algal growth, and this high oxygenic conditions lead to photosynthesis and algal cell damage owing to photo-oxidation (Chisti 2008a). Since the removal of dissolved oxygen is not possible, it is suggested to degas the reactor vessel (Chisti 2007). The optimum temperature can be maintained by the cooling or heating of the vessel coupled with use of agitators. Tubular PBRs are highly productive systems due to their large surface-area-to-volume area. They have been used to produce a significant amount of biomass algal productivities. The world's largest PBRs are vertical PBRs which is found in Germany which is arrayed in a greenhouse. The algal biomass production is about 35 to 41 gm2 per day. In another study, the highest productivity of 47 gm2 per day was noted in tubular PBRs with south–north orientation. A horizontal airlift tubular PBR with a size of 0.2 m2 was recorded with the average biomass production of 25.2 gm^{-2} d^{-1} in the case of *Phaeodactylum tricornutum*.

Flat-panel photobioreactors (FPBR) are made up of transparent substances and are thin rectangular vessels with unique angles for utmost solar power utilisation. Though they have much solar light exposure, the algal productivity is reported to be similar to that of the tubular photobioreactors (Shen et al. (2009). The advantage of flat-panel photobioreactor is the complete direct light illumination available throughout the reactor (Hu et al. 1996). The inclined and vertical alignment are the

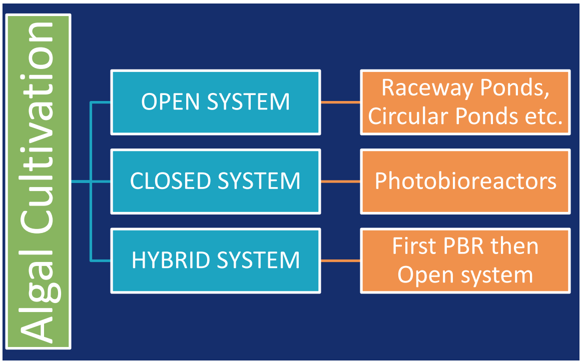

Fig. 1.5 Systems for algal cultivation

two most commonly used plate PBRs. These are flat panels having a transparent rectangular container whose light path generally lies between 1 and 30 cm (Hu et al. 1996). A seasonal variation in the production of *Synechocystis aquatilis* is recorded by Zhang et al. They successfully cultivated this algal species under 11.2 ± 2.1 MJ m^{-2} d^{-1}, an average irradiation. They have calculated an average algal biomass production of 30gm^{-2} d^{-1} with a cell concentration of 1–2 gL^{-1}. In another study, Hu and Sommerfeld have used the vertical plate PBRs, and the biomass productivity and have achieved 12.5 gm^{-2} d^{-1} cell density of about 7 g L^{-1} in Arizona.

Novel photobioreactor systems are innovative algal cultivation systems called the Offshore Membrane Enclosure for Growing Algae (OMEGA) which has been designed by the National Aeronautics and Space Administration (NASA) and consists of bags that are semipermeable in nature and float in waste water (Trent 2009).

1.4.3 Immobilised Culture Systems

It is the least studied algal culture system amongst all algal culture methods. In these systems, the unialgal cultures are immobilised in a polymeric matrix, or attached algal communities grow in shallow, artificial streams or on surfaces of rotating biological contactors. These systems can be classified as enclosure and non-enclosure methods.

1.4.3.1 Enclosure Methods

These methods are mostly used by the industries. These methods use the polymeric matrix for the algal culture, which helps the algae to attach to the substrates. However, these methods have been explored at a very limited scale for the algal production. However, it is extensively utilised for bacterial, yeast, and enzyme technology for industrial purposes for algal cultivation. The algal production is mostly utilised for waste water treatment through this method, because this system is good for species control and has high efficiency of waste removal, etc. (Robinson 1986). Some researchers have indicated this method as more efficient compared to free cell production systems. For instance, the algae *Chlamydomonas reinhardtii* cells are immobilised in Ca alginate. Due to highly costly polymeric fibre, it has limited use in large-scale cultivation of algae.

1.4.3.2 Non-enclosure Methods

Non-enclosure methods are similar to the enclosure method because this method is also applied to waste water treatment mostly. However, the difference between enclosure and non-enclosure is to grow algae on solid support without enclosure. Algal Turf Scrubber is one of the examples of the non-enclosure methods (ATS). The main component of this system is the solid support for growth and algal harvesting as well as wave surge for agitation. According to Kebede-Westhead et al., the algae removed nitrogen and phosphate from waste water efficiently and have very higher biomass productivity with mean biomass productivities between 7.1 and 9.4 g m^{-2} d^{-1}.The algal cells allow to grow on polymer matrix and on gel beads.

1.4.4 The Hybrid System

These systems amalgamate the features of both open and closed systems. Such systems are well articulated to eliminate the limitations of both open and closed systems of algal cultivation. In the hybrid cultivation system, the algal cultivation is done in controlled closed systems with optimum growth factors and later on the algal cultures are shifted to the open system of cultivation thereby facilitating enhanced algal growth and biofuel production. This system holds substantial features and is an outstanding cultivation technique for promoting biofuel production via algae (Archana and Kiran 2018).

1.5 Algal Harvesting Techniques

The water content is removed from algal cells through a process known as harvest. The algal harvesting is a downstream processing. There are so many downstream techniques available which are utilised for algal harvesting. However, due to higher cost, no harvesting technique is fully efficient for commercial large-scale harvesting till date. The basic steps which are involved in algal harvesting are screening, coagulation, flocculation, flotation, sedimentation, filtration, and centrifugation (Brennan and Owende 2010). However, other techniques are electroflotation, electrophoresis, and ultrasound which are less important compared to the above-mentioned general steps (Heasman et al. 2003). Generally, a particular type of harvesting technique should be applied on the basis of algal cell size and cell density with lower operating cost to achieve desirable yield of biomass. Further, the applied technique must have little influence on the further processes (Kim et al. 2013). Basically, harvesting technique can be divided into two types: first, based on the bulk separation of algal biomass using sedimentation process and, second, filtering of the biomass and preparing slurry through the process of filtration and centrifugation.

1.5.1 Centrifugation

The mixture of different-sized particles is separated on the basis of density through spinning in the centrifugation process. The centrifugation works on the principle of centripetal force. Although it is the most widely accepted and efficient technique for harvesting, it is costly when applied at large-scale production (Sim et al. 1988). In another study, Heasman et al. (2003) based on the harvesting efficiency trial, 90–100% harvesting efficiency was recorded via centrifugation. However, high gravitational force and shear stresses which are applied during the centrifuge process can damage the algal cell structure.

1.5.2 Gravity Sedimentation

In sedimentation procedure, the particles settled down in the bottom out of the fluid in which they are mixed. Gravity is one of the forces which are responsible for the sedimentation. In gravity sedimentation harvesting procedure, the heavier algal particles are settled down to the bottom against the barrier. A sample having higher cell density and large-sized algae are efficiently harvested through this procedure (Edzwald 1993) in comparison to lower cell density, but this procedure is effected by the density and radius of the microalgal cells, other factors are algal particle settling time, specific gravity suspension, and gravity of particles.

1.5.3 Filtration

This procedure is applied to separate solid matter from fluids or gases through different kinds of mechanical, physical, and biological processes by proving a medium through which only liquid can pass (Brennan and Owende 2010). For this procedure the filters with specific pore size are used (Chen et al. 2011; Mohn 1980). The filtration process can be of several types such as microfiltration, dead-end filtration, vacuum filtration, ultrafiltration, and tangential flow filtration (TFF). Out of all filtration process, the least energy is consumed in the pressure filtration and TFF that is why they are better for harvesting than others. However, overall, the filtration is an expensive process because in it we need to change filters and membrane frequently.

1.5.4 Microstrainers

It is a closed cylindrical structure having a cylindrical screen within. It is commonly used in sewage water for separating the microalgae and other plankton species from water. This system is rotated by applying the centrifugal force which pushes the larger particles away from the screen in turn preventing the clogging and maintaining the continuality of the procedure (Chen et al. 2011). It is made of a rotating drum in which a stainless steel micromesh is fabricated and partially dipped in the water. A range of mesh size such as 15–64 μm is used so that maximum species of algae and plankton can be trapped. The microstrainers have a simple function and manufacturing capacity. The filtration ratios are high under low investment and low energy consumption. The disadvantages with microstrainers are partial removal of solid particles. Another thing is that if the alga is in higher concentration than moderate, it can block the screen; due to this the small-sized algae can be driven away from the screen very easily.