Clean Energy Production Technologies

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Microbial Strategies for Techno-economic Biofuel Production

Springer

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Series Editors

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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

Replacement of fossil fuels with renewable energy is important to save the environment from its hazardous effect as well as fulfilling the needs of society. Renewable energy production is a unique sustainable replacement option of fossil fuels in the form of biofuels production, which is cheap, renewable, and environment friendly. The cost of technology involved in this green fuel production process is the main limitation of its practical and commercial implementation. Production process such as enzymes used in biofuels production, processing of waste biomass for enzymatic hydrolysis, imbalance, the cost economy of this process, and so required attention for commercial utility and environmental protection. Various approaches like microbial and bioprocess improvement may be able to resolve this issue to some extent.

The publication of the book entitled *Microbial Strategies for Techno-economic Biofuel Production* is a commendable step in the proposed area. I am writing this message with satisfaction as a researcher in the same area. This book essentially contains ten chapters addressing various issues on practical ground level to improving the economy of the system. The book presents details of various microbial and microbes-related parameters used in different biofuels production technologies including biogas, biobutanol, bioethanol, biohydrogen, and biodiesel. In my view, the book will prove itself as an asset to those working and interested in this field, which includes scientists, researchers, teachers, students, and industries.

I appreciate the efforts of Dr. Neha Srivastava [IIT (BHU), Varanasi], Dr. Manish Srivastava [IIT (BHU), Varanasi], Prof. (Dr.) P.K. Mishra [IIT (BHU), Varanasi], and Dr. Vijai Kumar Gupta [TTU, Estonia] for bringing out the book entitled *Microbial Strategies for Techno-economic Biofuel Production*. The effort taken to complete this book will surely cover the whole and demand of industries, scientists, teachers, researchers, and students. I congratulate the editors for their hard work and bringing a final shape to this book.

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Anthonia O'Donovan

Acknowledgments

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 into the text inadvertently and for these we owe undiluted responsibility. We are grateful to all the authors for their contribution to this book. We are also thankful to Springer Nature for giving this opportunity to editors and the Department of Chemical Engineering & Technology, IIT (BHU) Varanasi, U.P., India, for all technical support. We thank them from the bottom of our heart.

Contents

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Neha Srivastava is an expert in the field of biofuel production, microbial bioprocessing and enzyme technologies. She is currently working as a post-doctorate fellow at the Department of Chemical Engineering and Technology, IIT (BHU) Varanasi, India. She has published 25 research articles in peer-reviewed journals and holds 3 patents and 1 technology transfer. She completed her PhD in Bioenergy at the Department of Molecular and Cellular Engineering, SHIATS, India. She has received 6 Young Scientist Awards. Her current research focuses on biofuel production (cellulase enzymes; production and enhancement, biohydrogen production from waste biomass, bioethanol production).

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Chapter 1
An Introduction to Algal Biofuels

Manisha Verma and Vishal Mishra

Abstract Desires of living a higher standard of life bring regular consumption of non-renewable energy resources. This conventional fuel consumption causes emission of CO_2, particulate matter, and greenhouse gases to the atmosphere; in such conditions, energy crisis brings ignited focus on algaculture for producing biodiesel and other liquid biofuels. Algal oil or algal biofuels are third-generation biofuels, emerged as a renewable alternative to conventional liquid fuels. These algal oils are also a replacement for conventional biofuels, which are obtained from agricultural sources like corn, sugarcane, oilseed plants, and some animal fats. Unlike oilseed crops, they do not need vast farmland and hence keep agrarian lands available for food crops. Algaculture is possible in freshwater, saline water, and wastewater from various sources with minimal impact. Algaculture has a significant effect on environmental pollution as it assimilates nitrate and phosphate present in wastewater while continuously contributing to CO_2 sequestration. Algal oils are biodegradable and comparatively less harmful to the surrounding if spilled. Open outdoor cultures are used for algae cultivation for their low cost, but generally, they are profoundly affected by environmental disturbances like light availability and temperature swings. Ongoing research in algal biofuels is focused on the rapid growth of biomass, high lipid production, and thermal tolerance.

Keywords Renewable energy · Algal biofuel · Algaculture · Wastewater · CO_2 sequestration · Third-generation biofuel

Abbreviations

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ASTM	American Society for Testing and Materials

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ATP	Adenosine triphosphate
C	Carbon
Ca	Calcium
CNRL	Canadian Natural Resources Limited
CO_2	Carbon dioxide
Cu	Copper
DNA	Deoxyribonucleic acid
Fe	Iron
GHG	Greenhouse gas
HAMGM	Highly assimilable minimal growth medium
HDRD	Hydrogen-derived renewable diesel
K	Potassium
K_2CO_3	Potassium carbonate
Mg	Magnesium
Mn	Manganese
Mtoe	Million tons of oil equivalent
N	Nitrogen
NO_X	Nitrogen oxides
P	Phosphorus
PBR	Photobioreactor
S	Sulfur
TAGs	Triacylglycerides
TPES	Total primary energy supply
Zn	Zinc

1.1 Introduction

Petroleum reservoirs are depleted due to unlimited consumption of petroleum as an energy source and cause an increased demand for fuel in developed and developing countries worldwide. Fossil fuel combustion causes serious climate issues such as global warming by emitting greenhouse gases (GHG) (Deng et al. 2020). Biofuels are emerged as a budding hope to reduce the reliance on crude oils with some limitations. Lipid-based fuels from algal sources are well-known renewable sources for many years. Algae are easy to grow and harvested at outdoor cultivations in freshwater, saline, or wastewater. Extracted algal oils contain various forms of lipids or triacylglycerides (TAGs) that can be processed further for the production of fatty acids, methyl ester (biodiesel), synthetic jet fuel, and hydrogen-derived renewable diesel (HDRD) which can be employed as fossil fuel alternatives (Lam et al. 2019). Photosynthetic microorganisms like microalgae can fix atmospheric carbon dioxide more rapidly than terrestrial plants or trees (Paul et al. 2020). Microalgae could be either prokaryotic or eukaryotic and have a reasonable growth rate in harsh conditions (Li et al. 2008a; Richmond 2008); it could be served as a basic raw material for

the production of biodiesel, ethyl alcohol, methane, and bio-hydrogen (Tsukahara and Sawayama 2005). Microalgae cultivation for biodiesel serves several other purposes, such as elimination of ammonia, nitrogen oxides, and phosphates from domestic or industrial effluents (Wang et al. 2008) and CO_2 sequestration during photosynthesis (Wang et al. 2008). Very less greenhouse gases are emitted during the production process of biodiesel, and the residual biomass left after oil extraction can be utilized as bio-fertilizer for agricultural lands (Directive 2003). It was also noticed that high lipid content was obtained when microalgae was cultivated under nutrient-limited condition (Rehman and Anal 2019). Algae generate more lipid content as compared to conventional oilseed crops (Moradi-kheibari et al. 2019). Recent trends of hike in the price of crude oil along with GHG and pollutant emission drive a new focus on microalgae biodiesel production. Several companies started a suitable market by selling either the complete process unit or some parts of it (photobioreactors and cell disruption units) for algaculture and biodiesel production (Barclay and Martek Biosciences Corp 2005; Behrens et al. 2007; Kanel et al. 1999). Algae cost more per unit biomass production as compared to other lignocellulosic non-food crops because of their expansive processing and operation (Carriquiry et al. 2011). To ensure availability and reducing the production cost of algal biofuels commercially, several industries and governments provide funds (Omidvarborna and Kim 2019).

1.2 Algal Species Involved in Biofuel Production

Table 1.1 represents different freshwater and marine algal species and the range of dry weight lipid content in percent (Chisti 2007; Meng et al. 2009; Li et al. 2008b; Terme et al. 2017).

Table 1.1 List of various algal species and their dry weight lipid content%

Algae	Type of algae	Lipid content% dry-weight	References
Botryococcus braunii	Colonial-green microalgae	25–75	Chisti (2007), Meng et al. (2009)
Chlorella sp.	Single-celled green algae	22–63	Li et al. (2008b)
Dunaliella primolecta	Single-celled, photosynthetic marine-green alga	23	Chisti (2007)
Crypthecodinium cohnii	Dinoflagellate microalgae	20	Meng et al. (2009)
Isochrysis sp.	Haptophytes	25–33	Chisti (2007)
Nannochloris sp.	Green algae	20–35	Chisti (2007)
Nitzschia sp.	Marine diatom	45	Chisti (2007), Meng et al. (2009)
Sargassum	Brown (class Phaeophyceae) macroalgae (seaweed)	0.13–2.96	Terme et al. (2017)

Different microalgae species accumulate different amounts of lipid. In order to enhance the yield of lipid from microalgae cultivation, algal cells can be cultivated under some favorable environmental conditions (Sheehan et al. 1998). In certain controlling circumstances, lipid content could arise up to 90% of the dry weight of microalgae, but in general, the average content of lipid ranges between 1 and 70% of dry weight biomass (Li et al. 2008a, c; Chisti 2007; Spolaore et al. 2006). Screening of seaweed, diatoms, and other algae species for biofuel production relies on growing efficiency, lipid content, oil yield, and ability of microalgae to assimilate available nutrients in certain environmental conditions. As represented in Table 1.1, widely used algal species are *Botryococcus braunii, Chlorella* sp., *Cylindrotheca, Crypthecodinium* sp., *Dunaliella* sp., *Isochrysis* sp., *Nitzschia* sp., and *Nannochloris* sp. These species contain up to 20–60% dry weight lipid content. Different algal species accumulate different compositions of fatty acids in their lipid content, which have an enormous impact on biodiesel characteristics. Usually, saturated and unsaturated fatty acids containing 12–22 carbon atoms are found in freshwater microalgae species (Thomas et al. 1984). Fatty acid composition could be affected by the growth phase of cells, and under certain nutritional limitations, environmental and cultivation conditions such as salt stress and nitrogen depletion in culture medium induce C18:1 accumulation (Thomas et al. 1984).

1.3 Types of Biofuels Produced from Microalgae

In the field of renewable energy fuels, microalgae part a huge contribution by being a producer of multiple biomass-generated energy sources. Efforts of producing biofuels for replacing fossil fuels are not novel; instead, now it is taken seriously by seeing the scarcity and escalating hike in the price of fossil fuels. Figure 1.1 represents renewable fuels generated from different processes by microalgae biomass. Anaerobic digestion of microalgae biomass produces methane (Serna-García et al. 2020). Algal lipids are used for biodiesel production by transesterification (Duran 2020). Direct or indirect photolysis process of algae yields bio-hydrogen (Jiménez-Llanos et al. 2020). Microalgae have also been reported as a promising feedstock for the alcohol fermentation because of their sufficient starch accumulation inside the cell and cell wall (Harun et al. 2010), which conclude that microalgae could have great potential to generate biodiesel as well as ethanol-based biofuels.

1.3.1 Biodiesel

Nowadays, biodiesel is a proven energy fuel produced from plants, animals, and microalgae oils. Biodiesel from soybeans is available in the United States. Jatropha, corn, palm, and canola have been used for agro-based diesel generation (Blackshaw et al. 2011). Under some specific growth conditions, microalgae produce a large

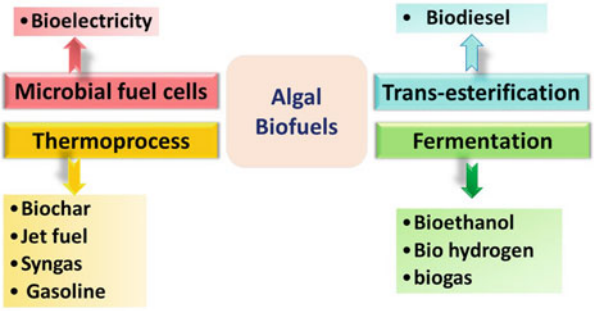

Fig. 1.1 Representation of different fuel alternatives produced from microalgae

amount of lipid in their biomass (Sharma et al. 2012a). Microalgae have more potential to deliver higher biodiesel content than cotton and palm oil (Singh et al. 2011). Algal oils are quite abundant in double bonds of polyunsaturated fatty acids as compared to other vegetable/seed oils (Belarbi et al. 2000). Biodiesel of microalgae oil origin is required to match with some standards. According to the European Union, two different grades are applied for biodiesel: (1) standard EN 14214 in automobile engines and (2) standard EN 14213 as heating or burning fuel, while in the United States, ASTM standard D 6751 biodiesel is used (Knothe 2006). Figure 1.2 represents the chemical reaction of lipid transesterification into methyl esters.

Algal oil consists of triacylglycerides, which have three moles of fatty acids esterified by a glyceride molecule. For obtaining biodiesel, triacylglycerides have to undergo a reaction known as alcoholysis or transesterification (acids, alkali, and lipase as catalysts) with 3 moles of methanol, which yield 3 moles of methyl esters of fatty acid (which is biodiesel) with 1 mole of glycerol (Fukuda et al. 2001). The transesterification process could be optimized for reducing the production cost of biodiesel, such as direct transesterification. Direct transesterification includes chemical extraction of complex algae cell walls and transesterification of these extracted algal oils in the same step (Li et al. 2011; Johnson and Wen 2009). Figure 1.3 represents microalgae processing steps for biodiesel production. Harvesting microalgae cells is the primary procedure performed by gravity sedimentation, dewatering, filtration, centrifugation, and flocculation. The selection of suitable harvesting techniques depends on the quality of the desired product (Richmond 2008). Processing is a significant aspect to optimize for the low-cost production of any commodity since it should be highly specific for the desired product. In microalgae processing, drying of algal biomass is the next step to extend the storage

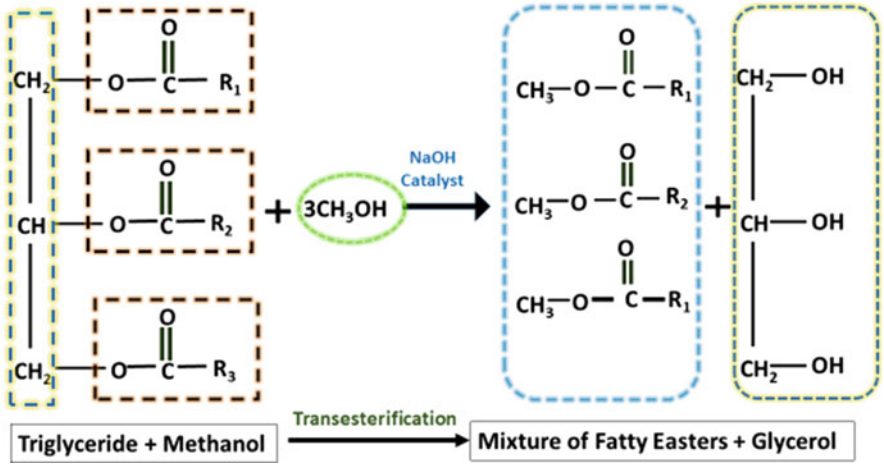

Fig. 1.2 Transesterification of lipids into methyl esters

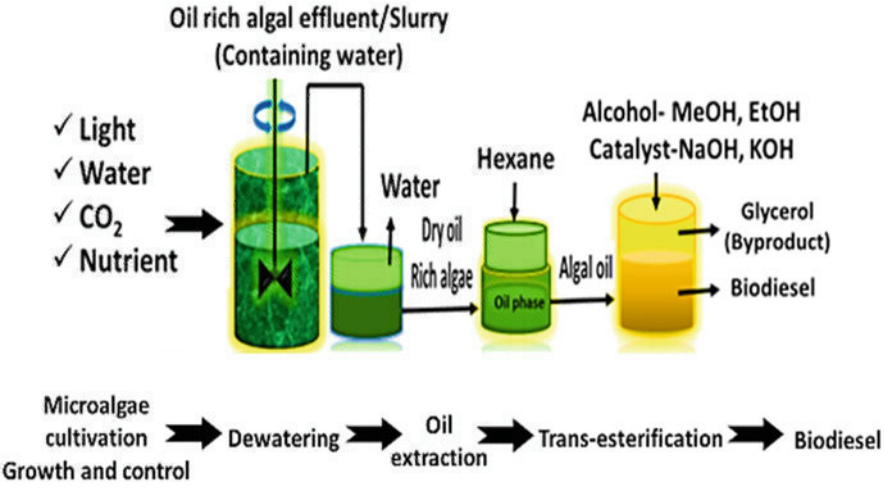

Fig. 1.3 Microalgae processing steps for biodiesel production

life of raw algal biomass, and spray dryer, freeze dryer, drum dryer, and sun drying are the main processes involved in this step (Richmond 2008). Cell disruption is the next step to obtain algal oil. Cell disruption has been done by mechanical (homogenization, ultrasonication) or any other chemical (acid–alkali treatment) or enzymatic methods. For lipid production, extraction of algal oils is the next process involving solvent extraction by using solvents such as ethyl alcohol, hexane, or hexane–ethyl alcohol mixture (Richmond 2008; Grima et al. 2003; Cravotto et al. 2008).

1.3.2 Biobutanol

Escalating price and scarcity of petroleum and other fossil fuels demand sustainable fuel at an economical cost. Up to 85% of biobutanol can be smoothly blended with gasoline; it is used as a substitute for energy in Japan, USA, and Europe, which include 8%, 33%, and 19% of their total fuel energy supplement, respectively. Up to 15% of biobutanol blend is allowed in Europe while a 16% blend is permitted in the United States. Butanol correctly fixes into the standards of replacing ethanol as a gasoline additive as it has very high viscosity with lower heat of vaporization and lowers volatility as compared to ethanol (Qureshi et al. 2001). Microalgae biomass contain carbohydrates, lipid, protein, and many biomolecules, so microalgae that consist of the majority of storage as glucose, starch, and cellulose serve as feedstock for fermentation media (Chen et al. 2013). Storage polysaccharides of microalgal cells are released by some biological (enzymatic), chemical, mechanical, and thermal pretreatment. Cellulose and lignin need to be hydrolyzed by some mild treatments. However, polysaccharide accumulating strains could be improved by genetic engineering for efficient biobutanol production (Passos et al. 2014). After algal lipid extraction, the leftover green cellular waste could be used as polysaccharide feedstock for butanol production. Fermentation could be carried out by some bacteria of genus *Clostridia* (Potts et al. 2012).

1.3.3 Biogasoline

Gasoline consists of C4–C12 n-alkanes and iso-alkanes with a mixture of various arenes, cycloalkanes, and oxygenates. The antiknock index is the main characteristic of gasoline and is measured by octane rating (Gibbs et al. 2009). Higher the octane number tends to raise the compression ratio, which generates more energy and enhances the functioning of the motor engine. After the three-step conversion of biomass-derived levulinic acid into angelica lactone dimer, gasoline-like branched C7–C10 hydrocarbon is obtained. Figure 1.4 shows the chemical conversion reactions of levulinic acid into alpha-angelica lactone dimer.

This levulinic acid derived from algal biomass undergoes intramolecular dehydration by using K10, i.e., montmorillonite clay and yields >90% angelica lactone. The next step is the dimerization of angelica lactone in the presence of anhydrous K_2CO_3, which yields >94% alpha angelica dimer; this dimer is used as feedstock in hydro-deoxygenation reaction, in the presence of some noble catalysts, and yields C7–C10 hydrocarbons of gasoline volatility range (Mascal et al. 2014).

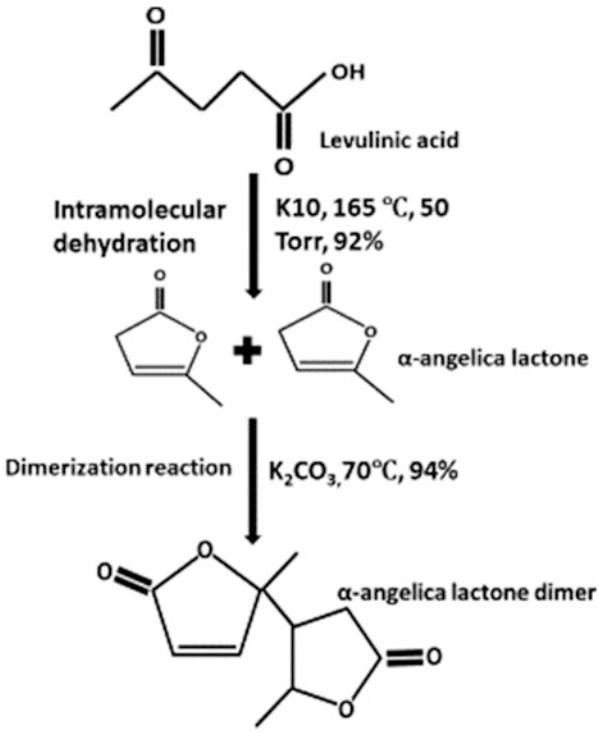

Fig. 1.4 Formation of alpha-angelica lactone dimer for biogasoline production

1.3.4 Methane

Microalgae have substantial nutritional value. They contain proteins, carbohydrates, and lipids in abundant quantities. For anaerobic digestion and methane production, *Chlamydomonas reinhardtii, Dunaliella salina, Arthrospira platensis, Euglena gracilis*, and *Scenedesmus obliquus* are some microalgae species that have been utilized (Mussgnug et al. 2010). Processed algae leftovers (after oil extraction) are the finest raw material for methane generation by anaerobic digestion as compared to unprocessed microalgae (microalgae that do not undergo the process of lipid extraction). Unprocessed microalgae have lipid content which generates volatile fatty acids that inhibit anaerobic digestion (Zhao et al. 2014).

1.3.5 Ethanol

Microalgae and algal species have cell walls derived from various monosaccharides and complex polysaccharides, the majority of which are cellulosic contents; also algae have starch accumulation as a storage substrate (Goh and Lee 2010). Up to 70% of polysaccharide content is found in some marine microalgae are cellulose,

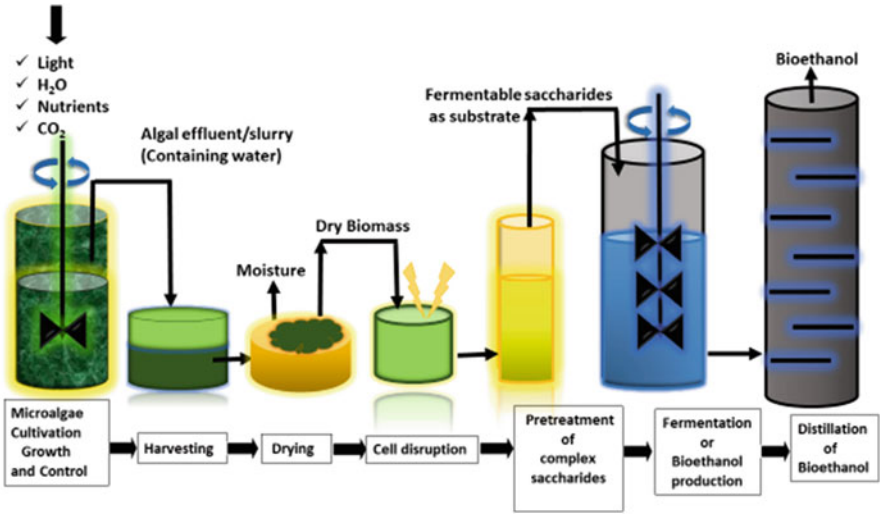

Fig. 1.5 Schematic representation of bioethanol production from microalgae feedstocks

hemicellulose, mannon, and xylan present as cell wall components. Agar, algin, and carrageenan present as intracellular saccharides, while floridean starch, laminarin starch, and amylopectin are present as food storage of cells (Okuda et al. 2008). All these polysaccharides are the finest feedstock and need some pretreatments to convert complex polysaccharides into fermentable saccharides for ethyl alcohol fermentation. Algal feedstock has various advantages over other crops to obtain saccharide or starch as fermentable sugar, which include utilization of different domestic/industrial effluents or saline/blackish wastewater, carbon dioxide recycling, high productivity in terms of area, and there is no need of traditional agricultural land; hence, algae are the most valuable nonedible feedstock for biofuel production (Wijffels and Barbosa 2010). *Laminaria hyperborean* is a brown seaweed used to extract mannitol, which is utilized as a raw substrate for bioethanol fermentation by the bacteria *Zymobacter palmae* (Horn et al. 2000). Up to 15% of bioethanol blends are used in transportation fuel and hence make 99% of overall biofuel utilization in the USA. However, ethanol has less energy efficiency as compared to gasoline, but it has many advantages, such as fewer pollutant emissions, and is produced from waste biomass (Rao and Bantilan 2007). Algae are a nonfood feedstock, and having high photosynthetic efficiency could be considered as the most promising feedstock for ethanol fermentation (Hossain et al. 2008; Wijffels et al. 2010). Figure 1.5 represents microalgae pretreatment and processing for the bioethanol fermentation.

Microalgae cell is the natural source to obtain fermentable saccharides. Utilizing microalgae for bioethanol fermentation is based on three consecutive processes: (1) preprocessing of microalgae biomass, (2) saccharification, and (3) bacterial or yeast fermentation of saccharides. Microalgae pretreatment is a necessary process for improving the production efficiency, which focuses on accessing all intracellular

saccharides as well as saccharides present in cell walls. Fermentable sugars can be extracted from algal biomass by utilizing some biological, chemical, mechanical and thermal methods of cell diruption and extraction. Cell disruption usually involved mechanical methods (high-pressure homogenization, ultrasonication, ultrasound, pulsed electric field) (Miranda et al. 2012; Zhao et al. 2013a) or non-mechanical methods like enzymatic hydrolysis of cell wall components by amylases, cellulases, and amyloglucosidase (Günerken et al. 2015). Obtained saccharides are further processed under the fermenter in the presence of some alcohol-producing yeast such as *Saccharomyces cerevisiae* or other *Saccharomyces* sp. (Eshaq et al. 2011*;* Abate et al. 1996) or bacteria like *Zymobacter palmae* (Horn et al. 2000) and *Zymomonas mobilis* (Abate et al. 1996).

1.4 Nutrients and Growth Inputs for Algal Growth

Microalgae are found either independently or in symbiosis with other living beings on both terrestrial and aquatic ecosystems; microalgae could be able to get efficient biomass and high growth rate by utilizing light energy, CO_2, and water via photosynthesis. Microalgae cultivation gains massive concerns for some therapeutic proteins and the sustainable biofuel generation (Abdeshahian et al. 2010). Large-scale cultivation of microalgae is affected by various aspects such as nutrient, light availability/intensity, color, and temperature conditions (Xenopoulos et al. 2002). Studies show that chemical elements like N, K, Ca, Cu, Fe, Mg, Mn, P, S, and Zn are crucial for microalgae growth in the form of salts; however, the amount of these micro- and macronutrients varies for every algal growth medium (Kaplan et al. 1986; Oh-Hama and Miyachi 1988). The selection of growth medium is a significant aspect, and it depends on the chemical composition of media that affects the biomass growth (Borowitzka 2005). Bold's basal (BB) medium, acidified Bold's basal medium, Chu10 medium, BG (Blue-green) 11 medium, and modified Hoagland's medium are mostly used for culturing microalgae (Ilavarasi et al. 2011).

1.4.1 Bold's Basal Medium (BBM)

One liter of Bold's basal medium is prepared by adding 10 mL of each stock solution from Table 1.2, items 1–6, and 1 mL of items 7–10 in a volumetric flask with 1 L distilled water.

Table 1.2 Composition of Bold's basal medium (Ilavarasi et al. 2011)

S No.	Stocks of chemicals	g/L
1.	$NaNO_3$	25.00
2.	KH_2PO_4	17.50
3.	$MgSO_4 \cdot 7H_2O$	7.50
4.	K_2HPO_4	7.50
5.	NaCl	2.50
6.	$CaCl_2 \cdot 2H_2O$	2.50
7.	**Trace elements**:	
	• $ZnSO_4 \cdot 7H_2O$	4.42
	• $CuSO_4 \cdot 5H_2O$	1.57
	• $MnCl_2 \cdot 4H_2O$	1.44
	• Co $(NO_3)_2 \cdot 6H_2O$	0.49
	• MoO_3	0.71
8.	H_3BO_3	11.40
9.	**EDTA and KOH solution**:	
	• EDTA Na_2	50.00
	• KOH	31.00
10.	$FeSO_4 \cdot 7\ H_2O$ with 1.0 mL concentrated H_2SO_4	4.98

1.4.2 Acidified Bold's Basal Medium

Add 250 mg of $(NH_4)_2SO_4$ in 800 mL of deionized water, dissolve it completely, and add each of the stock solutions from Table 1.3, items 1–6, and then 6.0 mL from trace element solution. Add vitamin B_1 and B_{12}. Make up to 1 L with deionized water and bring the pH to 3.0 with HCl.

1.4.3 BG11 (Blue-Green Medium)

Make up to 1 L with deionized water and then adjust it to pH 7.1 with 1 M NaOH.

1.4.4 Chu10 Medium

Take 2.5 mL from each stock solution, and prepare the media by completing the volume to 1 L by adding deionized water.

Microalgae growth highly depends on the composition of media, and nutrient-rich water promotes more algal blooms (Castellanos 2013; Schenk et al. 2008). The presence of all essential micro- and macronutrients is necessary for media to produce adequate algal biomass (Dauta et al. 1990). Carbon is a significant ingredient for algal growth, obtained from various inorganic and organic substrates such as carbon

Table 1.3 Composition of acidified Bold's basal medium (Ilavarasi et al. 2011)

S. No.	Chemical formula of each salt	Distilled water, g/1000 mL	Stock solutions in 1 L final medium
	Stock solution		
1.	$NaNO_3$	25.0 g	30.0 mL
2.	KH_2PO_4	17.5 g	10.0 mL
3.	$K_2HPO_4 \cdot 3H_2O$	7.5 g	10.0 mL
4.	$MgSO_4 \cdot 7H_2O$	7.5 g	10.0 mL
5.	$CaCl_2 \cdot 2H_2O$	2.5 g	10.0 mL
6.	NaCl	2.5 g	10.0 mL
7.	**Trace element composition**	**mg/1000 mL**	**6.0 mL**
	• $MnCl_2 \cdot 4H_2O$.	41.0 mg	
	• $FeCl_3 \cdot 6H_2O$.	97.0 mg	
	• $ZnCl_2 \cdot 6H_2O$	5.0 mg	
	• $Na_2MoO_4 \cdot 2H_2O$	4.0 mg	
	• $CoCl_2 \cdot 6H_2O$	2.0 mg	
	• Na_2 EDTA	0.75 g	
8.	**Vitamin B_1**	**0.12 g/100 mL**	1.0 mL
9.	**Vitamin B_{12}**	**0.1 g/100 mL**	1.0 mL

dioxide, acetic acid, and peptone (Cysewski and Lorenz 2004). Nitrogen is another macronutrient that builds more than 10% of the total biomass content in the form of amino acids or proteins. Nitrogen is added to the culture medium in the form of nitrogen oxides such as nitrate (Cysewski and Lorenz 2004). It has been reported that nitrogen-deprived media in culture lead to decreased chlorophyll content and increased amounts of carotenoids and lipids (triacylglycerides) (Cysewski and Lorenz 2004). Phosphorus is the third essential element mostly available in the form of orthophosphate in the medium, and phosphorus is involved in cellular processes in the form of ATP/ADP/AMP for energy transmission and also a constituent of DNA (Cysewski and Lorenz 2004). Calcium, iron, magnesium, potassium, selenium, and sodium are other micronutrients. Boron, copper, manganese, molybdenum, and zinc are trace elements that are usually involved in the catalytic activity of enzyme reactions (Cysewski and Lorenz 2004) (Tables 1.4 and 1.5).

1.4.5 Wastewater as a Source of Nitrogen and Phosphate

Microalgae need inorganic nutrients, sunlight, and CO_2 for their growth. Utilizing wastewater for algaculture reduces the cultivation cost of adding culture media and minimizes the consumption of fresh water. Three significant origins for wastewater are agricultural, domestic, and industrial effluents. These effluents are rich in various organic and inorganic ingredients and nitrogen and phosphorus components.

Table 1.4 Composition of blue-green media BG11 (Ilavarasi et al. 2011)

S. No.	Chemical composition		Stock solutions (in mL) per 1 L final medium
	Stock solution	**Per 500 mL**	
1.	$NaNO_3$	75.0 g	10.0
2.	$MgSO_4 \cdot 7H_2O$	3.75 g	10.0
3.	K_2HPO_4	2.0 g	10.0
4.	$CaCl_2 \cdot 2H_2O$	1.80 g	10.0
5.	Ammonium ferric citrate	0.30 g	10.0
6.	Citric acid	0.30 g	10.0
7.	Na_2CO_3	1.00 g	10.0
8.	EDTA Na_2	0.05 g	10.0
9.	**Trace metal solution**	**Per 1000 mL**	**1.0**
	• $ZnSO_4 \cdot 7H_2O$	0.22 g	
	• $MnCl_2 \cdot 4H_2O$	1.81 g	
	• H_3BO_3	2.86 g	
	• $Na_2MoO_4 \cdot 2H_2O$	0.39 g	
	• $Co(NO_3)_2 \cdot 6H_2O$	0.05 g	
	• $CuSO_4 \cdot 5H_2O$	0.08 g	

Table 1.5 Composition of Chu10 medium (Ilavarasi et al. 2011)

S. No	Chemical composition	Concentration in g/L	Stock solutions (in mL) per 1 L final medium
1.	K_2HPO_4	4.0	2.5
2.	$MgSO_4$	10	2.5
3.	$CaCl_2$	16	2.5
4.	$NaNO_3$	8.0	2.5
5.	$FeCl_3$	0.32	2.5
6.	Na_2CO_3	8.0	2.5
7.	NaCl	30	2.5
8.	EDTA Na	4.0	2.5
9.	**Trace elements**		
	• $MnCl_2 \cdot 4H_2O$	0.02	2.5
	• $(NH_4)\ 6Mo_7O_{24} \cdot 4H_2O$	0.028	2.5
	• H_3BO_3	0.288	2.5
	• $CuSO_4 \cdot 5H_2O$	0.08	2.5
	• $ZnSO_4 \cdot 7H_2O$	0.224	2.5
	• $COCl_2 \cdot 6H_2O$	0.004	2.5
10.	Na_2SiO_3	5.7	2.5

Ammonium, nitrates, and nitrites are sources of nitrogen, and phosphates are used as the source of phosphorous available in various wastewater effluents which could be utilized by microalgae cultivation. Some studies reveal assimilation of heavy metals, nitrogen, and phosphorus by microalgal cells, and hence, these are used for bioremediation of wastewater (Cho et al. 2013; Cabanelas et al. 2013). Microalgae can bio-mitigate the effects of industrial and municipal effluents by consuming carbon, nitrogen, and phosphorus compounds and simultaneously overcome the problem of eutrophication for maintaining the aquatic ecosystem (Cai et al. 2013). By utilizing microalgae for tertiary treatment of wastewater and combining biofuel production along with that, a zero-waste concept is implemented, so wastewater algaculture is considered as sustainable cultivation for biodiesel industries (Rawat et al. 2013).

1.4.6 Impact of Growth Conditions on Microalgal Biomass

Table 1.6 represents optimization of various growth conditions and their outcomes in terms of biomass, lipid, and protein content.

Optimization of several growth aspects such as the chemical composition of nutrient media, light availability, temperature, and some other factors has a significant impact on biomass, lipid, and protein accumulation. It has been reported that the pattern of lipid accumulation in microalgae is reflected from its usual pattern of lipid accumulation under several diverse conditions (Sato et al. 2000; Thompson Jr 1996; Guschina and Harwood 2006). Nitrogen starvation approach is the most studied technique to increase triacylglyceride production (Widjaja et al. 2009). Light availability could be optimized at different growth levels, and light–dark photoperiod cycles have a significant impact on microalgal lipid accumulation. Microalgae culture in stationary phase under 12:12 h continuous intense light conditions gained greater storage of triacylglycerides with monounsaturated and saturated fatty acids compared to a culture grown under the lower intensity of light (Juneja et al. 2013).

1.5 Different Microalgae Cultivation Methods

For ideal large volume growth of microalgae, two major approaches have been taken: the indoor or outdoor photobioreactor cultivation method with electric lights for photosynthesis and the outdoor pond cultivation method with natural sunlight. In artificial photobioreactors, species-specific optimization of growth conditions is convenient (Liu et al. 2015; Xu et al. 2009). The light source, reactor setup, the materials used for the vessel, nutrient medium, temperature, circulation system, and CO_2 supply are the basic condition to be optimized in photobioreactor (Liu et al. 2015; Xu et al. 2009). It is divided into closed or open systems and open cultivation directly exposed to sunlight and air, while the closed system is indoor away from

Table 1.6 Representation of various experimental growth conditions and their effects on biomass/lipid production

Growth condition/ composition	Experimental methods	Effects observed	References
By limiting nitrogen source (NH_4 and NO_3)	Optimization of $NaNO_3$, KH_2PO_4, NH_4HCO_3, $MgSO_4 \cdot 7H_2O$, K_2HPO_4, $(NH_4)_2HPO_4$ based on HAMGM and BBM	• Raised concentration of algal biomass: 40% (0.73 g/L) by BBM • Elevated lipid concentration: 85% (281 mg/L) by HAMGM	Widjaja et al. (2009)
Nitrogen sources: $CO(NH_2)_2$, KNO_3, $NaNO_3$, and NH_4NO_3	*Chlorella sorokiniana* Optimization the concentration of $CO(NH_2)_2$: 0–10 g/L	1.50 g/L $CO(NH_2)_2$ gives • More significant biomass generation (0.220 g/L) • Lipid content: 61.50%	Ramírez-López et al. (2016)
Optimization of nitrogen and phosphorus concentration	Nitrogen concentration optimized: 0–56 mg/L Phosphorus optimization: 0–19 mg/L	• Nitrogen/phosphorus ratio: 10.0 • Biomass concentration: 1.58 g/L	Sharma et al. (2015)
N and other trace elements	Optimize Ca, Mn, N	• Approximately threefold lipid accumulation	Alketife et al. (2017)
NO_3	Optimizing nitrate utilization and accumulation of protein	• Protein accumulation raised to 44.30%	Morschett et al. (2017)
Photoperiod and light intensity	By optimizing light condition	• Appropriate light intensity: 2000 lux • Optimum light–dark period: 12:12 h	Xie et al. (2017)
Elevation of light intensity	400 µmol photon/ms	• Yellow color develops in microalgae due to high xanthophyll production (molecular sunglasses) • Xanthophyll antioxidants protect algal cells from radiation	Lu et al. (2013)
Variation in illumination methods	• Continuous light • Periodic light–dark durations • No light • Continuous dark with flashing light	Flash lighting elevates the total fatty acid accumulation and the growth rate significantly in *Chlorella vulgaris*	Grudzinski et al. (2016)
Optimization in red light intensity	Intensities at 800–2000 µmol/m^2/s	• Optimal light for best biomass growth: Red wavelength • The optimal light concentration: 1200–1600 µmol/m^2/s	Choi et al. (2017)
Nitrogen starvation and light limitation	Outer shading and dimming the lights	• Reduction in algal growth	Zhao et al. (2013b)

(continued)

Table 1.6 (continued)

Growth condition/ composition	Experimental methods	Effects observed	References
Optimization of carbon sources: Glucose, acetate, and glycerol	Autotrophic, heterotrophic, and mixotrophic modes	• The maximum biomass produced at heterotrophic mode: 8.90 g/L • Lipid content is highest in a heterotrophic way: 36.19% in culture medium	Schreiber et al. (2017)
Optimization for CO_2 concentration light intensity N deficiency P uptake	• CO_2: 0.03–12% • Intensity of light: 40–200 µmol photons/m/s • Nitrogen starvation • Phosphorus (optimized)	Best results obtained at • CO_2 concentration: 4% • Light intensity: 200 µmol photons/m/s • Polyphosphate maximum uptake rate: 2.08 mg/L/day • Observe better performance in N-deficient condition than N-rich condition	Morowvat and Ghasemi (2016)
Temperature	• Ranged between 20 and 30 °C	• Optimum temperature for lipid productivity: 27 °C • Optimum N concentration:1.50 g/L • Optimum cell density: 50%	Chu et al. (2014)

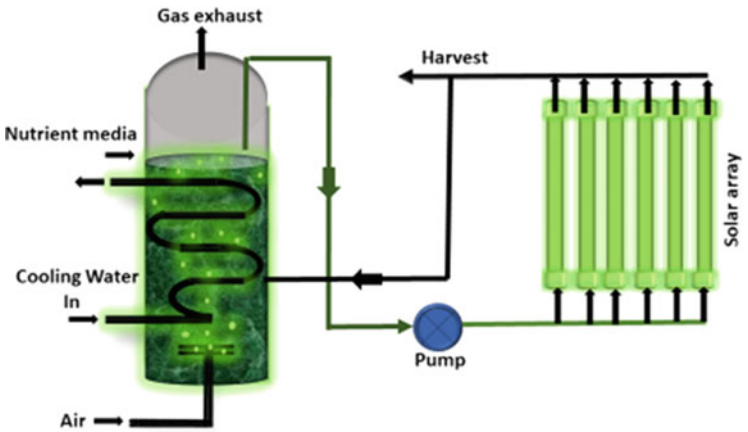

Fig. 1.6 Schematic representation of a photobioreactor assembly for microalgae cultivation

sunlight (Juneja et al. 2013). Figure 1.6 represents the schematic assembly of a photobioreactor for microalgae cultivation.

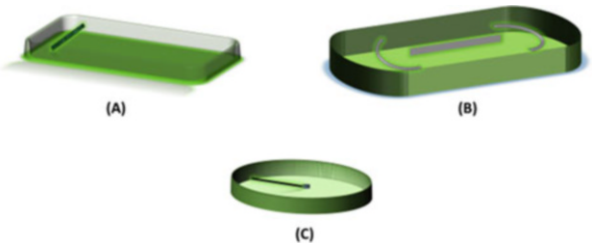

Fig. 1.7 A diagrammatic representation of open cultivation by using ponds. (**a**) Unstirred pond. (**b**) Raceway pond. (**c**) Circular pond

1.5.1 Open System

In open systems, pond's depth control light availability, stirring optimity, temperature, and evaporation. A shallow pond is better for light availability to the algae cells while a minimum depth is required to control the evaporation rate and proper mixing; shallow lakes and ponds provide extensive surface evaporation, which results in an ionic imbalance in the growth medium (Tredici 2004). Open cultivation systems are easy to build in the form of ponds and lakes. Pond designs include variety such as open, covered, raceway, circular, inclined, and big shallow ponds (Borowitzka 1999; Mata et al. 2010). However, the risk of bacterial, fungal, microalgal, and protozoal contamination is high in an open pond system. Figure 1.7 represents open systems (unstirred pond, raceway pond, and circular pond) for the cultivation of microalgae.

1.5.2 Closed Systems or Indoor Photobioreactors (PBRs)

An indoor photobioreactor is made up of any transparent material which provides optimization for several growth conditions such as aeration rate, CO_2 supply, cell density, pH, temperature, light, and water. For PBR designing, the surface to volume ratio is the main parameter regarding light availability for photosynthetic efficiency (Singh and Gu 2010). The most widely used PBR architecture is tubular and flat plate photobioreactor in continuous mode (Singh and Gu 2010), which provides a high surface to volume ratio (Ilavarasi et al. 2011). In the flat plate reactor, narrow panels are arranged in a vertical or horizontal manner and provide adequate exposure to light and air, generally used to get high biomass (Singh and Gu 2010), whereas the tubular photobioreactors consist of transparent tubes of approximately 1 dm in diameter arranged in helical, horizontal, or vertical parallel loops. These closed tubes have an advantage over external contaminants (Carvalho et al. 2006; Morweiser et al. 2010). Figure 1.8 represents various configurations of photobioreactors designs which have a general surface to volume ratio of 80–100 m^2/m^3 (Posten 2009).

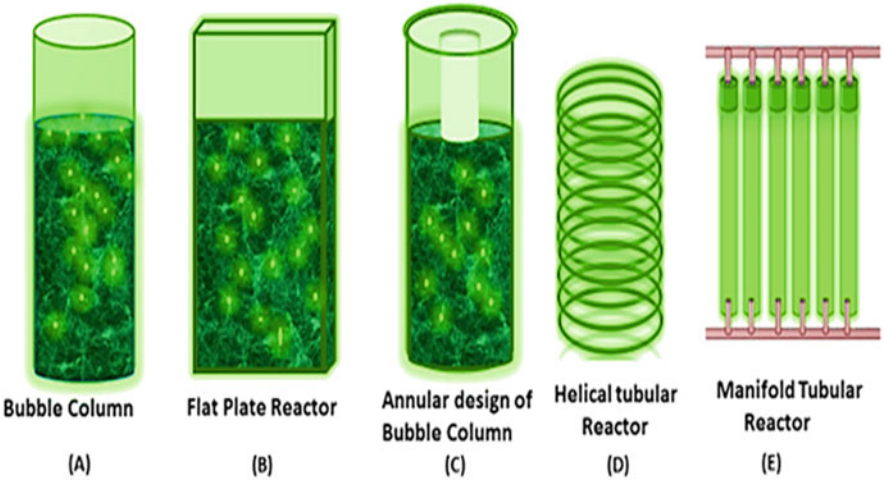

Fig. 1.8 Common geometries of closed photobioreactors. (**a**) Bubble column. (**b**) Flat plate reactor. (**c**) Annular design of bubble column. (**d**) Helical tubular reactor. (**e**) Manifold tubular reactor

Table 1.7 provides a comparative study on the open and closed system of microalgae cultivation (Carvalho et al. 2006; Chen 1996; Del Campo et al. 2007; Canela et al. 2002; Piccolo 2010).

1.6 Concept of Biorefineries

Microalgae is a rich source of carbohydrate, lipid, and protein; they uptake carbon dioxide and light from the atmosphere, just like plants, so it could be considered as a bio-based crop. Figure 1.9 demonstrates how phototropic microalgae biomass is useful for various commodities such as biofuel, animal feed application, protein supplements, and carbohydrate feedstocks (Williams and Laurens 2010). Microalgal biomass needs a complete fractionation and valorization similar to any petrochemical or oil refineries, which bring the concept of biorefineries for microalgae. Biorefinery facilities include harvesting of microalgae cells, cell disruption, and then product extraction and fractionation (Wijffels et al. 2010; Vanthoor-Koopmans et al. 2013).

1.6.1 Evaluation of the Biorefinery Processes

Nowadays, techno-economic advancements in large-scale cultivation of microalgae and its downstream processing are the basis on which a biorefinery should be evaluated. Figure 1.10 demonstrates a simplified view of biorefinery processes.

Table 1.7 Comparison of microalgae growth conditions in the closed and open system

Culture conditions	Photobioreactor	Ponds
Space requirement	Depends on productivity	Same as PBR
Light efficiency	Highly efficient utilization of light	Less-efficient utilization
Sterility	Sterilizable	Could not be sterilize
Contamination control	Convenient	Difficult to prevent contaminants
Contamination risk	Less	High risk of contamination
Area/volume	High	Low
Cell density	High	Low
Operation mode	Batch and semicontinuous	Batch and semicontinuous
Mixing and aeration	Uniform throughout PBR	Nonuniform
Evaporation rate (culture media)	Less evaporation	A high rate of evaporation
Temperature control	Uniform temperature throughout the system	Difficult to maintain the same temperature at various depths
Operating cost	High	3–10 times lesser than photobioreactors
Biomass yield	High, 3–5 times more in PBR	Less yield than PBR
Processing and control mechanism	Convenient	Difficult
Investment	High investment	Needs less investment than PBRs
Monitoring and control of gas transfer	Highly efficient	Less efficient
Scale-up of system	Demanding/complex	Demanding/complex

A complete biorefinery should have total revenues greater than total reproduction and economically feasible (Ruiz et al. 2016; Grima et al. 2003). Table 1.8 summarizes all the practices involved in a biorefinery approach to get multiple commodities along with biofuel production from microalgae biomass.

Biomass recovery is a significant criterion which contributes up to 25–30% of net algal biomass generation costs (Grima et al. 2003). Before applying harvesting techniques, an additional step of flocculation, flotation, or a combining approach of both is used on algal cells for their aggregation. Microalgal cell aggregation causes ease in biomass harvesting due to large effective particle size (Grima et al. 2003), then various recovery practices such as centrifugation, filtration, sedimentation, and ultra-filtration are applied to recover microalgal biomass (Grima et al. 2003). Recovered biomass is further processed by dewatering or dehydration step to increase the storage life of raw feedstock (biomass) and most commonly include drum dryer, freeze dryer, conventional sun drying technique, and spray dryer (Richmond 2008). Cell disruption is the next process to release of desired metabolites (carbohydrate, lipid, protein, pigments, and some other high-value compounds) from dry algal biomass. The selection of cell disruption techniques for microalgae usually relies on the composition of the cell wall/cell membrane and the nature of the