



Javed, F. et al. (2019) Microalgae-based biofuels, resource recovery and wastewater treatment: A pathway towards sustainable biorefinery. *Fuel*, 255, 115826.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/190526/>

Deposited on: 25 September 2019

Enlighten – Research publications by members of the University of Glasgow  
<http://eprints.gla.ac.uk>

# **Microalgae-based Biofuels, Resource Recovery and Wastewater Treatment: A Pathway Towards Sustainable Biorefinery**

3 Fahed Javed<sup>a,e</sup>, Zufishan Shamair<sup>a</sup>, Muhammad Aslam<sup>a\*</sup>, Naim Rashid<sup>a,c\*</sup>, Asim Laeeq Khan<sup>a</sup>, Muhammad Yasin<sup>a</sup>,  
4 Tahir Fazal<sup>a,e</sup>, Ainy Hafeez<sup>a,e</sup>, Fahad Rehman<sup>a,e</sup>, Muhammad Saif Ur Rehman<sup>b</sup>, Zakir Khan<sup>a, c</sup>, Javed Iqbal<sup>b</sup>, Aqeel  
5 Ahmed Bazmi<sup>a, d</sup>

<sup>6</sup> <sup>a</sup>Department of Chemical Engineering, COMSATS University Islamabad, Lahore Campus, Pakistan

<sup>b</sup> Department of Chemical Engineering, Khawaja Farid University of Engineering and Information Technology, Rahim Yar Khan, Pakistan

9 © Systems Power and Energy, School of Engineering, University of Glasgow, Glasgow, G12 8QQ, UK

<sup>d</sup> Process and Energy Systems Engineering Center-PRESTIGE, Department of Chemical Engineering, COMSATS Institute of Information Technology, Lahore, Pakistan

<sup>e</sup> Biorefinery Engineering and Microfluidics (BEAM) Lab, Department of Chemical Engineering, COMSATS Institute of Information Technology, Lahore, Pakistan

14 [www.ijerph.com](http://www.ijerph.com) International Journal of Environmental Research and Public Health

\* Corresponding Authors: [maslam@cuilahore.edu.pk](mailto:maslam@cuilahore.edu.pk) (M. Aslam)

[naimkanwar@yahoo.com](mailto:naimkanwar@yahoo.com) (N. Rashid)

17 Abstract

18 Intense utilization of natural fuel resources is threatening the global environment and societal  
19 sustainability. It triggers up the need for finding environmental-friendly and sustainable sources  
20 of energy. In this perspective, microalgae have emerged as a potential alternative. Microalgae are  
21 featured with distinct ability to provide ecological services and respond to the sustainability  
22 challenges simultaneously. Microalgae can fix atmospheric CO<sub>2</sub>, valorize waste resources, and can  
23 produce a wide variety of bio-products. The promising features of microalgae pitch the idea of  
24 establishing a sustainable bio-refinery to draw multifaceted benefits and reinforce the objectives  
25 of resource efficient bio-economy. Unfortunately, in the last few years, preferential studies have  
26 been carried out to assess the potential of microalgae-based integrated bio-refinery. This review  
27 critically discussed the recent developments, opportunities, and barriers in the microalgae bio-  
28 industry and wastewater treatment. Particularly, microalgae potentials for biofuels and resources  
29 recovery are addressed towards sustainable biorefinery. Moreover, techno-economic and

1 commercial viability of microalgae-led bio-refinery is reviewed to drive this technology towards  
2 practicality.

3 **Keywords:** Microalgae; biofuels, wastewater, biomass, biorefinery

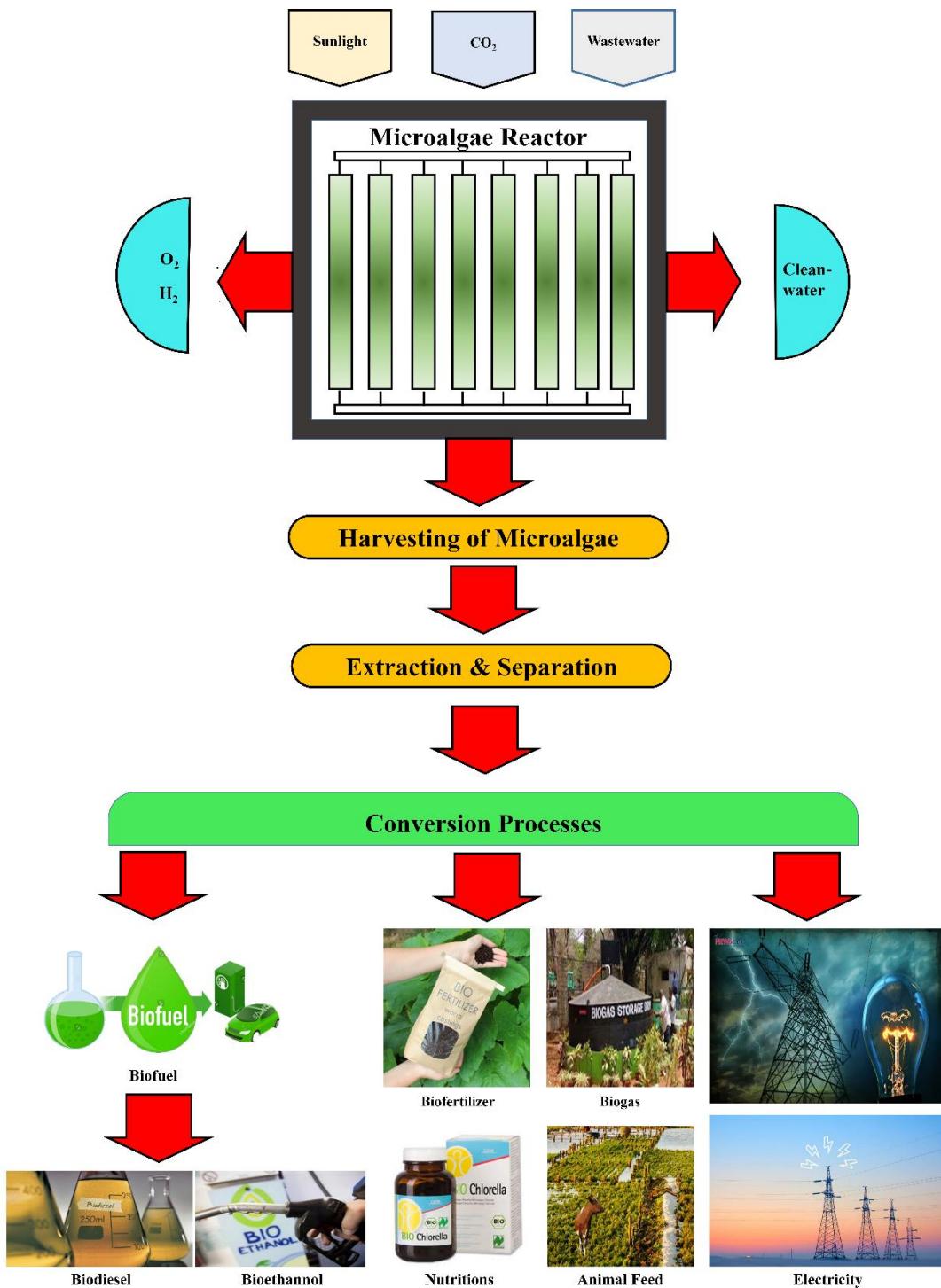
4 **1 Introduction**

5 Urbanization, industrialization, and overuse of fossil fuels cause an increase in greenhouse  
6 gas (GHG) emissions. Wastewater discharge without treatment threatens both human and  
7 ecological health. It triggers up the need of exploring clean, renewable and sustainable resources  
8 of energy [1-3]. Recently, research has been emphasized to use waste streams (including solid,  
9 liquid and gaseous) as resources to recover and produce biofuels such as biodiesel, bio-methane,  
10 bio-hydrogen, bio-oil, and other value-added products [4]. Microalgae is one of the most  
11 executable biomass for industrialization without any harmful effects on the environment.  
12 Microalgae can grow in fresh, waste, or sea water. Algae cultivation does not require freshwater,  
13 agricultural land, and yet it has high biomass yield with large starch and oil content. Techniques  
14 employing microalgae, such as anaerobic digestion to methane, microalgae oil to biodiesel and  
15 photobiological conversion to hydrogen production can produce several different renewable  
16 biofuels. The production of biofuel is not a new concept. However, currently it is investigated  
17 more earnestly due to growing demand and increasing price of fossil fuels. However, production  
18 of biofuel through microalgae is impeded by many commercialization challenges such as  
19 deficiency of energy and cost-intensive processes for algae growth and harvesting with a  
20 significant amounts of nutrients needed like nitrogen (N) and phosphorous (P), with conventional  
21 cultivation methods [5]. Moreover, membrane integrated systems are emerging as potential  
22 alternatives not only in microalgae cultivation and harvesting but also to produce and recover

1 biofuels such as biogas, bio-hydrogen, biodiesel, bioethanol, and other value-added products [6-  
2 11].

3 Wastewater treatment through microalgae growth and cultivation has gained promising  
4 attention as an economical and environmental-friendly route for microalgae-based biofuels  
5 production [12]. For instance, algae consume nutrients that can be “procured” from wastewater  
6 providing bioremediation while reducing treatment costs [12]. Integrating this with CO<sub>2</sub> emission  
7 plants would result in more efficient carbon capture and sequestration. Biomass generated through  
8 this process can be used to produced biofuels and other by-products as shown in Fig. 1. Microalgae  
9 cultivation in wastewater is a long-known technology. Microalgae generate oxygen through  
10 photosynthesis during cultivation. The produced oxygen can be utilized to degrade organic matter  
11 and bio-remediate inorganic compounds present in the wastewater. Thus, photosynthetic oxygen  
12 displaces the need for providing air through conventional aeration process reducing the cost  
13 significantly. Microalgae also have an ability to recover resources from the wastewaters which  
14 have promising applications in bio-refinery [13].

15 Microalgae cultivation in wastewater is a promising bio-refinery approach; however, it  
16 confronts with many challenges including contamination, low biomass yield, complex nutrients  
17 removal mechanism, and impurities in the biomass after downstream processing. For a sustainable  
18 and economical bio-refinery, future research should be dedicated to address these challenges. This  
19 review provides a perspective on recent trends and developments in microalgae-based wastewater  
20 bio-refinery. The possibility of integrating the wastewater industry with microalgae cultivation and  
21 biofuels production is critically reviewed. Life cycle and techno-economic analysis are also  
22 presented to assess the sustainability potential of microalgae industry.



1

2

**Fig. 1.** Schematic concept of converting microalgae to biofuels and value-added products

1      **1.2 Algal biofuels and wastewater treatment**

2            Wastewater treatment is a growing issue worldwide, wastewater treatment through algae  
3       was first studied in California, USA in 1950s. Algae were used as a tiny aerator to produce oxygen  
4       for bacteria to consume and simultaneous degradation of organic matter wastewater. Bacteria  
5       produce CO<sub>2</sub> and other nutrients ( such as N and P) which are highly needed for microalgae growth  
6       during photosynthesis [14, 15]. The symbiotic system efficiently removed nutrients from the  
7       system. Initially, algal ponds were designed to treat the secondary effluent before discharging the  
8       water to minimize the chances of eutrophication [16]. Algae could also remove the nutrients like  
9       K and N more efficiently in sewage than conventional treatment methods. Sewage process shows  
10      great potential for algal cultivation, wastewater treatment and biofuel production [17].

11          Aquatic Species Programs in 1978 demonstrated that the concept of algae growth in  
12       wastewater for biodiesel production is cost-effective as compared to petroleum-based diesel in the  
13       closed report supported by the United State Department of Energy [18]. This report clearly  
14       indicates that production of algal-biofuel is economically feasible when wastewater treatment is  
15       combined with cultivation. Not only photoautotrophic growth showed high biomass growth rate,  
16       but also heterotrophic microalgae utilize organic wastewater to increase the growth rate as well as  
17       it gives higher biomass and lipid productivity [19-24]. Wastewater contains both organic and  
18       inorganic sources of carbon which facilitate the conversion of microalgae through mixotrophic and  
19       heterotrophic mode. These techniques for growing microalgae have many advantages over  
20       photoautotrophic mode such as higher growth and productivity rate [24], low light [25] and  
21       contamination rate [23].

22          Mixotrophic growth systems are also considered to be efficient in uptake of ammonium  
23       and nitrogen enzymes. For example, in *Scenedesmus obliquus* (green algae) autotrophic medium

1 acetate is added and cultivation occurs under mixotrophic condition. As a result, ammonium  
2 uptake is increased four times than autotrophic conditions. In addition, harvesting cost is also  
3 decreased due to the presence of higher density biomass especially in organic-rich wastewater with  
4 the additional benefit of low downstream processing cost [22, 23]. Algal biomass production in  
5 organic-rich wastewater (municipal) is widely studied and reported in the literature [25-27].  
6 Additionally, many strategies have been developed for algal biomass conversion into value-added  
7 products. For example, a study proposed a photoautotrophic–mixotrophic two-phase culture  
8 (PMM) model in which organic materials (such as sucrose and glucose) are used as carbon-source  
9 for algal-based biodiesel production [28]. A similar model, photoautotrophic–heterotrophic  
10 (PHM) culture mode is designed to increase algal cell density production [29]. Recently, a new  
11 model hetero-photo-autotrophic (HPM) culture mode was developed which shows increase  
12 removal of nutrient and low production of biofuels in concentrated municipal wastewater, leading  
13 to next step in algae cultivation systems [30]. Different microalgae species and their productivity  
14 are shown in Table1.

15 **Table 1** Comparison of microalgae species and their lipids productivity

Microalgae species	Alga type	Productivity of lipids (mg/L/day)	Reference
<i>Chaetoceros muelleri</i>	Diatom	21.8	
<i>Chlorella sorokiniana</i>	Green	44.7	
<i>Chlorococcum sp. UMACC 112</i>	Green	53.7	
<i>Chlorella vulgaris CCAP 211/11b</i>	Green	170	
<i>Ellipsoidion</i> sp.	Eustigmatophytes	47.3	
<i>Monodus subterraneus UTEX 151</i>	Eustigmatophytes	30.4	[31]
<i>Pavlova salina</i>	Prymnesiophytes	49.4	
<i>Pavlova lutheri</i>	Prymnesiophytes	40.2	
<i>Scenedesmus quadricauda</i>	Green	35.1	
<i>Scenedesmus</i> sp. DM	Green	40.8-53.9	
<i>Skeletonema</i> sp.	Diatoms	27.3	
<i>Skeletonema costatum</i>	Diatoms	17.4	

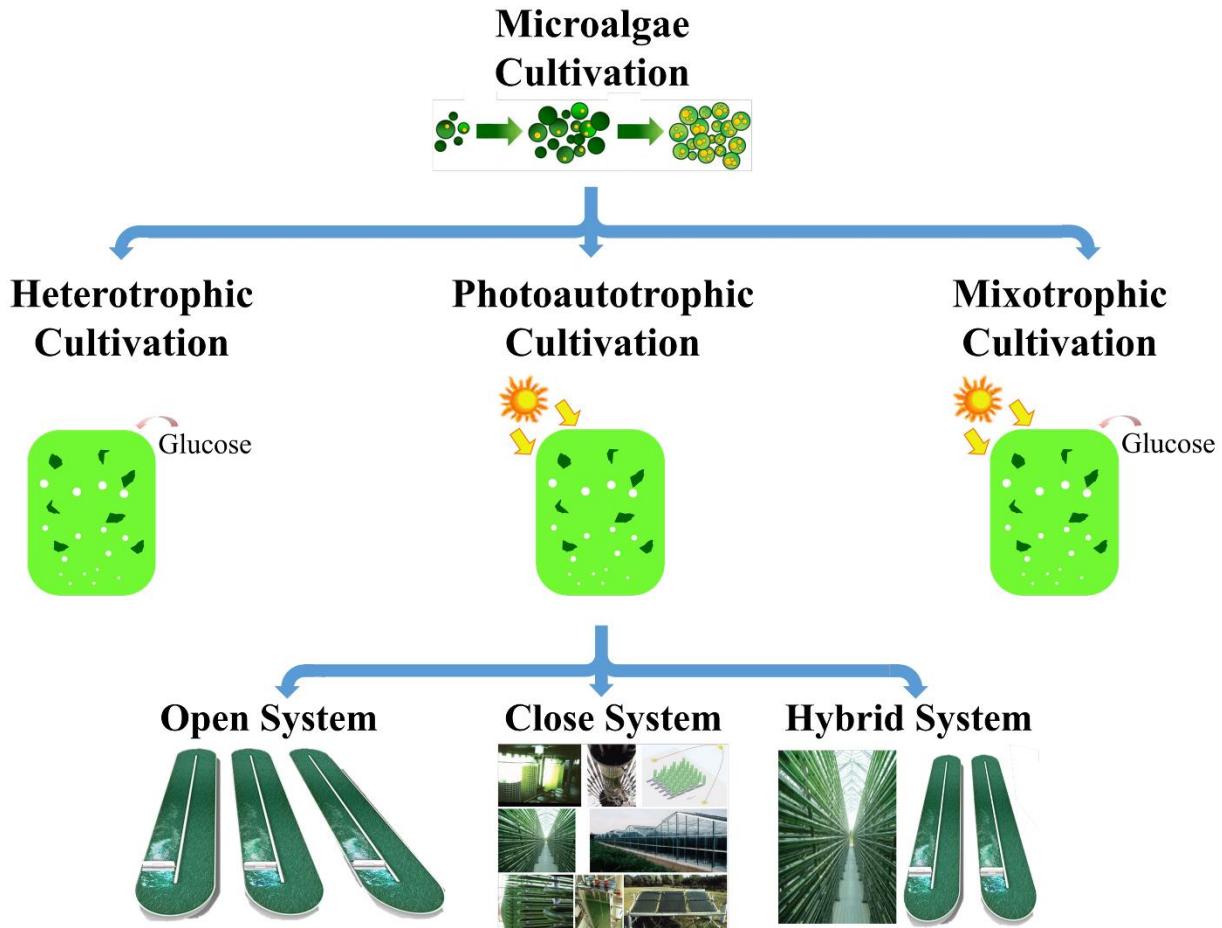
<i>Thalassiosira pseudonana</i>	Diatoms	17.4	
<i>Tetraselmis</i> sp.	Green	43.4	
<i>Chlorella</i> sp.	Green	18.7	[32]
<i>Dunaliella salina</i>	Green	46	[33]
<i>Isochrysis</i> sp.	Prymnesiophytes	37.8	
<i>Nannochloropsis</i> sp.	Eustigmatophytes	37.6-90	[34-37]
<i>Tetraselmis suecica</i>	Green	27-36.4	
<i>Nannochloris</i> sp.	Green	60.9-76.5	
<i>Nannochloropsis oculata</i>	Green	84-142	[37]
<i>Phaeodactylum tricornutum</i>	Diatoms	44.8	
<i>Nannochloropsis oculata NCTU-3</i>	Green	142	[36]
<i>Porphyridium cruentum</i>	Red	34.8	[38]

1

2   **2   Cultivation of microalgae**

3              Microalgae are prokaryotic photosynthetic microorganisms. In a natural environment, they  
 4       fix atmospheric CO<sub>2</sub>, use organic carbon from the wastewater, and light from the sun. To cultivate  
 5       microalgae in an artificial environment, the resource input should well match with the natural  
 6       environment [39]. The most important factor of hindering in commercialized production of algae  
 7       is the limited access of sunlight. To minimize this factor, an artificial source of light is implemented  
 8       for the cultivation of microalgae such as fluorescent light. But artificial light source derived from  
 9       petroleum energy diminishes major aim of producing a cost-effective method and it also increases  
 10      the carbon footprint [40].

11             There are three different sources for CO<sub>2</sub> uptake for microalgae growth- (i) CO<sub>2</sub> as  
 12      discharge gas from industry, (ii) from atmosphere and (iii) from carbonates [41]. Recent reports  
 13      revealed that air contains 400 ppm of CO<sub>2</sub> [42], but most microalgae utilize higher CO<sub>2</sub> levels [36].  
 14      Therefore, microalgae production uses some external sources of CO<sub>2</sub> such as industrial discharge  
 15      or soluble carbonates at commercial-scale [42]. Fig. 2 shown conventional routes of microalgae  
 16      cultivation.



**Fig 2.** Microalga cultivation system

### 2.1 Photoautotrophic cultivation

Phototrophic cultivation is the cheapest mode of microalgae cultivation. Phototrophic cultivation can be carried out in open ponds as well as closed bioreactors at lab scale [43]. Open pond systems are more beneficial because these are cheaper than photobioreactors, but the limited number of microalgae species are cultivated in open pond.

#### 2.1.1 Photoautotrophic open cultivation system

Open pond cultivation was first carried out in 1950s. Most of the open ponds are raceway type which was proposed by Oswald 1969. The most common design of raceway ponds often

1 consists of the rectangular channel with the flow from one end to another [44]. For raceway pond  
2 length, depth and diameter/width is an important parameter. Increase in pond width may results in  
3 current speed decrease leading to a lower mass transfer and mixing. Depth and length are  
4 dependent on the amount of culture volume used and light penetration. Open cultivation systems  
5 have been demonstrated cost-effective and sustainable mode of cultivation. They require less  
6 energy, easy to maintain and clean and, consequently, have the potential to return high net  
7 production of energy. However, water loss, lower light utilization, and large required space are the  
8 major limitations of open pond systems. Moreover, limited type of algae production, impure  
9 culture growth, insufficient mixing and low biomass productivity are the constraints of the  
10 technology [44].

### 11 **2.1.2 Photoautotrophic closed cultivation system**

12 Photobioreactors are mostly closed containers used for the production of phototrophic  
13 microalgae, where energy is provided through the artificial source of light [45]. They offer uniform  
14 distribution and efficient utilization of light distribution resulting in high mass transfer of gases  
15 (like CO<sub>2</sub> and O<sub>2</sub>). Typically, closed systems consist of four phases: solid, liquid, gas, and light  
16 phase. Microalgae as solid phase, growth media liquid phase, CO<sub>2</sub> and O<sub>2</sub> are a gaseous phase, and  
17 light phase [46]. Closed systems include tubular [47], flat plate [48, 49], and column  
18 photobioreactors [50]. An ideal closed system would have high transparent surface and high  
19 minimum illuminated parts. Closed systems are considered best for cultivation of a specific species  
20 in a controlled environment.

21 Flat-plate reactors were used as closed systems [51]. They have high surface area and high  
22 densities of microalgae cells, greater than 80 g/L [52]. They are made up from a transparent  
23 material and have a high capture rate of solar energy and these reactors have high photosynthesis

1 efficiency as compared to tubular bioreactor [53, 54]. Scaling up of tubular reactor is troublesome.  
2 Large tubular reactor can only be manufactured by joining smaller units presenting an operation  
3 and maintenance problems. However, significantly tubular reactor are working worldwide such as  
4 25m<sup>3</sup> at Mera Pharmaceuticals Hawaii [55], and even larger at Klotze, Germany having a volume  
5 of 700 m<sup>3</sup> [56]. To overcome these problems, Column bioreactors were proposed. They have a  
6 high volumetric mass transfer, controllable growth conditions, compact design and easy to operate  
7 [50].

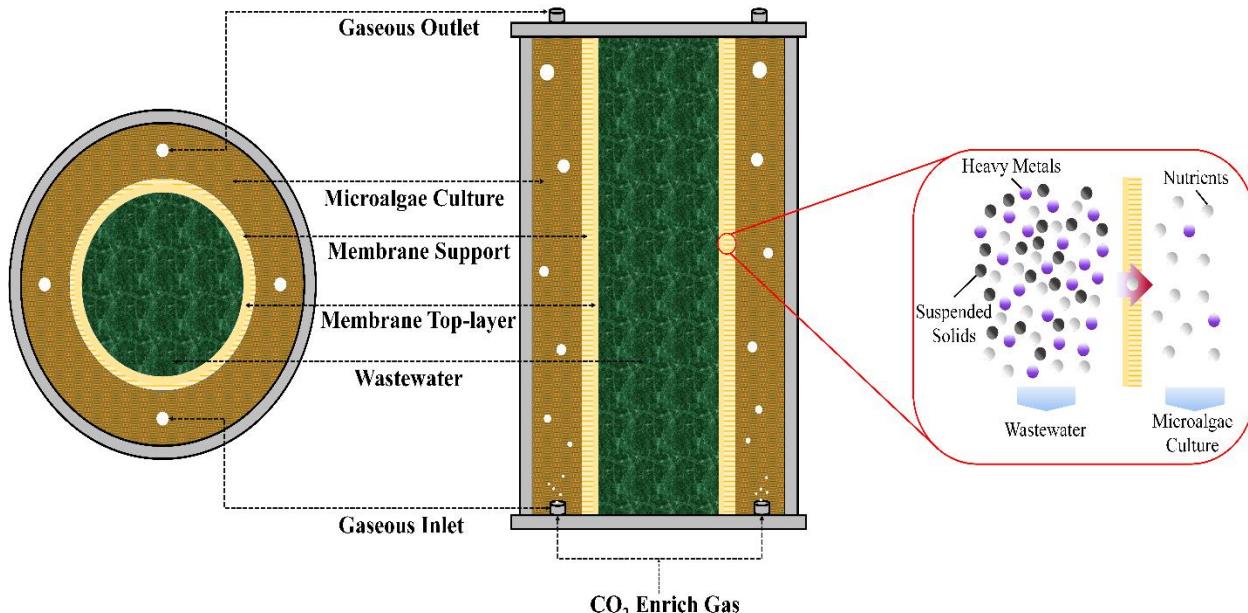
8 **2.1.2.1 Membranes photobioreactors**

9 The biomass productivity of microalgae cultivation system largely depends on CO<sub>2</sub>  
10 delivery. Generally, CO<sub>2</sub> delivery is carried out by simple air bubbling. Mixing is insufficient  
11 causing poor mass transfer and low efficiency. This problem can be addressed by using membrane  
12 aided bioreactors offering higher mass transfer of CO<sub>2</sub> than conventional bioreactors.

13 CO<sub>2</sub> sequestration using a membrane photobioreactors is as shown in Fig. 3 [57]. This  
14 process is carried out in two steps: (1) membrane is used to remove water continuously while  
15 biomass is retained on the membrane and (2) during cultivation, membrane is used to provide CO<sub>2</sub>  
16 either as contacted or as sparger. Over time many membrane systems have been studied for  
17 microalgae cultivation.

18 Hollow fiber membrane photobioreactors are adopted for microalgae cultivation due to  
19 their increased interfacial area and high algal biomass for biofuel conversion from wastewater with  
20 high nutrients strength. Highest biomass productivity of 4 g/m<sup>3</sup>/h was reported for *Chlorella*  
21 *vulgaris*. The analysis shows that a combination of wastewater treatment, CO<sub>2</sub> sequestration, and  
22 biofuels production is a promising route for microalgae cultivation [58]. Different applications for

1 membranes photobioreactors are shown in Table 2. Comparison of different photobioreactors and  
 2 their biomass productivity as shown in Table 3.



3  
 4 **Fig. 3.** Membrane photobioreactor for wastewater treatment and microalga cultivation. Reproduced from  
 5 Ref. [57]. Copyright 2016 Elsevier.

6 **Table 2** Summary of membrane photobioreactors for microalgae cultivation

Membrane	Microalgae	objective	Parameters	Reference
Polyvinylidene fluoride hollow fiber	<i>Chlorella vulgaris</i>	CO <sub>2</sub> capture and removal of nutrients	Aeration: 0.156 L/min Pore size: 0.1 µm Flux: 2.2-4.4 L/m <sup>2</sup> /h	[59]
Hydrophilic poly (ether sulfones) hollow fiber	<i>Chlorella vulgaris</i> <i>Scenedesmus</i> <i>Quadricauda</i> <i>Scenedesmus</i>	Removal of nutrients	Aeration rate: 4 L/min Pore size: 0.45 µm Flux: 12 L/m <sup>2</sup> /h	[60]

Hydrophilic polyvinyl chloride (PVC) / silica	<i>Chlorella vulgaris</i>	Coupled cultivation and harvesting of <i>Chlorella vulgaris</i>	Aeration rate: 2 L/min Pore size: 0.05-5 µm Flux: 1.5-8.63 L/m <sup>2</sup> /h	[61]
Polyacrylonitrile ultra-filtration flat sheet	<i>Haslea ostrearia</i>	Production of exocellular metabolite (marennine pigment)	Flux: 3-10 L/m <sup>2</sup> /h	[62]
Chlorinated polyethylene	<i>Chlorella vulgaris</i>	MBBR for an MBR effluent polishing	Aeration: 0.0014-0.0035 L/min Pore size: 0.2 µm Flux: 3 -16 L/m <sup>2</sup> /h	[63]

MBBR: membrane biomass retention photobioreactor; MBR: membrane bioreactor

1

2 **Table 3** Photobioreactors and their biomass productivity

Photobioreactors	Microalgae species	Productivity of biomass (g/L/day)	Reference
Tubular system	<i>Porphyridium cruentum</i>	1.5	[64]
Tubular system	<i>Phaeodactylum tricornutum</i>	1.2	[65]
Tubular system	<i>Phaeodactylum tricornutum</i>	1.9	[47]
Inclined tubular system	<i>Chlorella sorokiniana</i>	1.47	[66]
Undular row tubular system	<i>Arthrosphaera platensis</i>	2.7	[67]
helical tubular system	<i>Phaeodactylum tricornutum</i>	1.4	[68]
Parallel tubular system	<i>Haematococcus pluvialis</i>	0.05	[55]
Bubble column	<i>Haematococcus Pluvialis</i>	0.06	
Tubular system	<i>Haematococcus Pluvialis</i>	0.41	[69]
Tubular system	<i>Spirulina platensis</i>	0.42	[70]

---

Flat plate system	<i>Nannochloropsis sp.</i>	0.27	[71]
Flat plate system	<i>Chlorella</i>	3.8	[72]
Column system	<i>Tetraselmis</i>	0.42	[73]
Parabola system	<i>Chlorococcum</i>	0.09	[74]
Dome system	<i>Chlorococcum</i>	0.1	
Membrane system	<i>Chlorella vulgaris</i>	0.08	[58]

1

## 2 2.1.3 Photoautotrophic hybrid cultivation system

3            Hybrid cultivation consists of two-step microalgae growth in photobioreactor and in open  
 4    ponds. In the first step, photobioreactors are used in which controlled conditions are provided to  
 5    minimize the chances of contaminations and favored cell division. In second step, cells are exposed  
 6    to the nutrients, which help to increase lipid productivity [31]. The second step is preferably an  
 7    open pond, where environmental effects help in the production of microalgae. Huntley and Redalje  
 8    [75] studied hybrid system for oil and astaxanthin production by using *Haematococcus pluvialis*  
 9    and achieved oil production rate 10 tons of oil /ha. They also showed that oil production rate can  
 10   be achieved 76 tons of oil/ha/year by using high oil content species.

11           Rodolfi et al. [31] gave the concept of two-stage oil production, in which one part of plant  
 12   is dedicated for biomass under nitrogen sufficiency and rest of the plant is assigned for oil  
 13   production under nitrogen deprivation. Due to this process, 10 kg of lipid/ha/day produced in the  
 14   first section of the plant and 80 kg of lipid /ha/day produced in the second section and overall  
 15   production rate is 90 kg of lipid/ha/day. For tropical areas of the world where average solar  
 16   radiation is 20 MJ/M<sup>2</sup>/day can produce almost 30 tons of oil/year.

## 1    2.2    Heterotrophic cultivation

2              Algal biomass can successfully be produced by heterotrophic production as shown in Table  
3    4 [76, 77]. In this process algae grows on carbon substrate such as glucose in fermenters. In  
4              heterotrophic production, algae growth is independent of light energy making the process simpler  
5              and easy to scale-up [50, 78]. These processes have a higher growth rate and also lowering the  
6              harvesting costs due to high cell densities [79]. The installation cost is lower. However, this process  
7              consumes more energy than photosynthesis as it requires an organic source for initiation of the  
8              process [44]. Many other studies suggested a higher production rate in heterotrophic as compared  
9              with photoautotrophic [80-82]. Miao and Wu [77] studied that heterotrophic cell could be almost  
10          4 times greater than autotrophic cells in similar conditions. Hence, the cultivation through  
11          heterotrophic can result in higher lipid and biomass content.

12    **Table 4** Heterotrophic microalgae species and their lipid content

Species	Product	Culture	Total lipid content (%)	Reference
<i>Chlorella protothecoides</i>	Biodiesel	Batch	57.8	
<i>Chlorella protothecoides</i>	Biodiesel	Batch	55.2	[82]
<i>Chlorella protothecoides</i>	Biodiesel	Batch	50.3	
<i>Cryptothecodinim cohnii</i>	Docosahexaenoic acid	Batch	56	
<i>Cryptothecodinim cohnii</i>	Docosahexaenoic acid	Batch	42	[80]
<i>Chlorella protothecoides</i>	Biodiesel	Batch	46.1	
<i>Chlorella protothecoides</i>	Biodiesel	Batch	48.7	[81]
<i>Chlorella protothecoides</i>	Biodiesel	Batch	44.3	

13

1    **2.3 Mixotrophic cultivation**

2       Some microalgae species use metabolism for growth (heterotrophic or photoautotrophic),  
3       with the ability to grow on photosynthesis as well as ingest prey from organic material [83] as  
4       shown in Table 5. These species can rely on both photosynthesis and organic substrates such as  
5       *cyanobacteria spirulina platensis* [76]. The growth of microalgae is highly influenced by media  
6       during dark and light phase, due to which biomass losses were minimal during dark phase [84].  
7       To get maximum biomass formation from mixotrophic cultures, integration of both photo and  
8       heterotrophic processes during cultivation can be investigated. These factors indicate that  
9       mixotrophic production can play a significant role for microalgae to biofuel production.

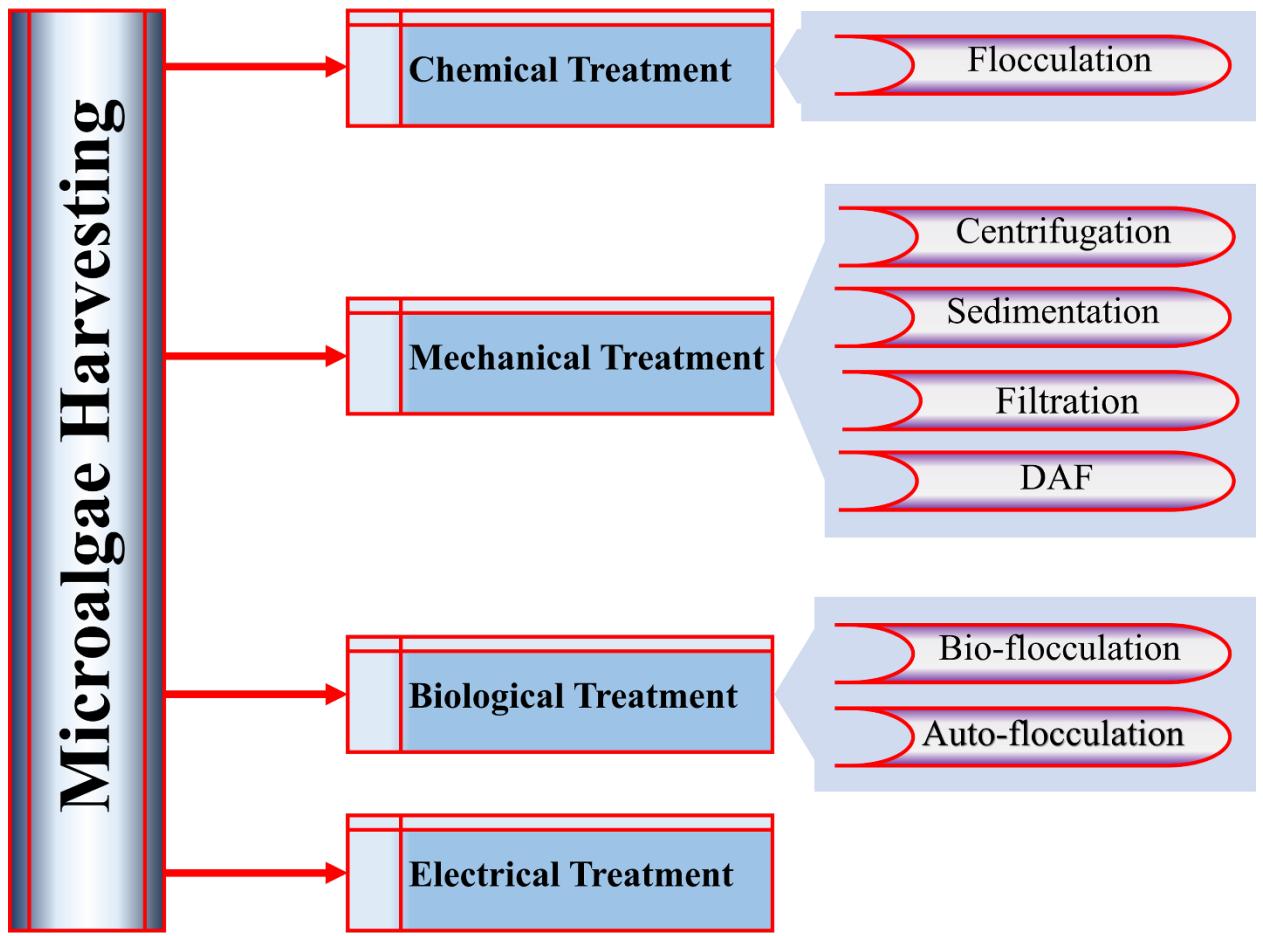
10      **Table 5** Mixotrophic species and biomass concentrations

Species	Organic carbon source	Biomass Concentration (g/L)	Reference
<i>Spirulina platensis</i>	Glucose	2.66	[76]
<i>Spirulina platensis</i>	Acetate	1.81	[76]
<i>Spirulina sp.</i>	Glucose	2.50	[85]
<i>Spirulina platensis</i>	Molasses	2.94	[84]

11

12      **3 Microalgae Harvesting**

13       Harvesting microalgae economically, due to its smaller size (3-30  $\mu\text{m}$ ), is a barrier yet to  
14       overcome [86]. Harvesting complicate downstream processing inflicting process economic s  
15       adversely. Currently, harvesting is carried out by chemical based, mechanical based, biological  
16       based and to some extent electrical methods as shown in Fig 4. All these methods have been  
17       investigated intensively to reduce the harvesting cost. However, no single method has provided an  
18       economical solution. [87].



**Fig 4** Techniques for microalgae harvesting

### 3.1 Chemical treatments

Owing to smaller cellular size, microalgae tend to remain suspended in aqueous solution and difficult to remove from the medium due to stable suspension. The addition of chemical flocculants can help to destabilize the cells and agglomerate them to settle down through gravitational forces. Synthetic polymers and electrolytes are used to neutralize the charge and flocculate the cells [88]. Ferric chloride and aluminum sulfate are used for neutralization of charge due to presences of +3 charges on their cations. The use of metal salts in flocculation and coagulation is not recommended considering the downstream processing for the bio-based

1 products formed by algae because aluminum and sulfate shows the methanogenic activity. For  
2 land applications, aluminum treated sludge have increased uptake and causes phosphorous  
3 deficiencies in plants. To minimize the concern of secondary pollution, natural polymers are used  
4 as flocculants. The use of natural polymer is not extensively reported. Divakaran and Pillai [89]  
5 worked on flocculation and settling of algae by adding chitosan sheds some light on the application  
6 of natural polymers. Starch is also used in freshwater as an efficient flocculating agent for the lab-  
7 scale experiment.

### 8 **3.2 Mechanical treatments**

9 Centrifugation is one of the most efficient method for microalgae recovery. In this process  
10 centrifugal force accelerates the separation of particles on the basis of their density difference [90].  
11 Most of the microalgae gave 80 to 90% recovery within 2 to 5 min for a pond effluent of 500 to  
12 1000xg. Centrifugation offers high recovery, but it's an energy-intensive process. This method is  
13 preferred for algal cell recovering, especially for concentrated aquaculture The factor affecting  
14 biomass recovery are: cell settling characteristics, settling depth and residence time [91].  
15 According to Shelef et al. [95], disc centrifuges can be used for all types of microalgae; also they  
16 can easily be sterilized and cleaned but high principal investment and operational costs limits the  
17 application of mechanical treatment to industrial use only.

18 Sedimentation process is mostly used for separating microalgae from water in which  
19 separation depends on microalgae particle size and density [90]. This process is recommended for  
20 microalgae size greater than 70  $\mu\text{m}$ . This process requires a low capital investment and recover  
21 1.5% concentration of biomass [92]. However, the reliability of this process is also very low due  
22 to the dilute densities of algal cells. Sedimentation process is slow, for example, 0.1 to 2.6 cm/h  
23 with biomass losses during settling time as well [93].

1 Dissolved air flotation (DAF) is used for sludge removal from wastewater treatment [94].  
2 This method is preferred over sedimentation in algae-rich water containing 3-6% algal biomass.  
3 The main advantage of DAF is that it can be employed at a large scale. However downstream  
4 processing becomes difficult due to addition of flocculants [93]. Recently, Hanotu et al. [95]  
5 reported microbubble harvesting known as microflotation using oscillatory flow through fluidic  
6 oscillator (FO). Using FO, the size of the bubbles generated were reported to be 10 times smaller  
7 than produced by DAF. Microflotation recovered 99% algae in 30 min. Oscillatory flow has been  
8 used for mass transfer enhancement for several other applications as well [95, 96] [97].

9 Harvesting of microalgae is still facing challenges due to its smaller size, similar densities  
10 to water, and recovery of biomass costing 20 to 30% of the total cost of biomass production.  
11 Membrane technology is known for low energy-intensive process compared to other technologies,  
12 due to their isothermal behavior and no phase change. Moreover, membranes provide complete  
13 retention of biomass and additional chemicals such as coagulants or flocculants are not required.  
14 It also helps removal of viruses and protozoa from liquid effluents [98-100]. For smaller algal  
15 cells, membrane filtration is more appropriate as compared to simple filtration. Membrane  
16 technology for harvesting purposes was first reported in 1995. Petrushevski et al. [101] studied the  
17 potential of a tangential flow filtration system. The result indicated that almost 70 to 80 % of  
18 biomass was recovered. It is an efficient method of microalgae harvesting. However, membrane  
19 fouling is a major issue in commercialization of this technology [102-104]. Overcoming membrane  
20 fouling is energy intensive and frequent chemical cleaning is required which results in reduced  
21 membrane lifetime and increased operating cost [105-112].

22 Different membrane materials have been tried for microalgae harvesting. However, a  
23 specific criterion for selection of membrane material for microalgae harvesting has not been

1 developed so far. Rossi et al. [101] have studied different materials for microalgae membrane  
 2 filtration. They studied 11 commercial polymers for harvesting microalgae by using cross-flow  
 3 microfiltration and ultrafiltration. Hydrophilic membrane showed more efficient results than other  
 4 membranes. These membranes are easy to clean, due to the negatively charged membrane fouling  
 5 and cake formations [10, 101, 113-116]. Different mechanical harvesting methods and their  
 6 comparison are shown in Table 6.

7 **Table 6** Different mechanical harvesting methods and their comparison [92, 93, 117, 118]

Method	Concentration after harvesting	Recovery	Energy Usage		Merits		Demerits	
	(%)	(%)						
Centrifugation	12-22	>90	-	Energy Intensive	-	Reliable Solid concentration is high	-	High cost
Membrane Filtration	5-22	70-90	-	0.4 kWh/m <sup>3</sup>	-	Reliable Solid concentration is high	-	Fouling High cost
Sedimentation	0.5-3	10-90	-	Very high 8 kWh/m <sup>3</sup>	-	Cheap	-	Time consuming process Unreliable
Dissolved air flotation	3-6	50-90	-	High dissolved air flotation 10–20 kWh/m <sup>3</sup>	-	Large scale process	-	Flocculants are required

8

### 9 **3.3 Electrical treatments**

10 For electrical treatment, electrophoresis method is used for harvesting algae cells. Due to  
 11 the negative charge of algae, they can be moved by applying electric field [87]. The only benefit

1 of this process is no chemicals are used for harvesting of microalgae. But high power consumption  
2 makes this process significantly cost intensive making it an unattractive option for harvesting at  
3 large-scale production [92].

#### 4 **3.4 Biological treatments**

5 The two basic terms used for biological harvesting of microalgae are auto-flocculation and  
6 bio-flocculation. Auto-flocculation occurs at high pH and dissolved CO<sub>2</sub> concentration. Due to  
7 high pH, supersaturation of calcium and phosphate ions occurs. Positive charge on calcium ions  
8 attract the negative charge on algae facilitating the settling of microalgae [119]. The optimum pH  
9 for this process is 8.5 to 9 where 3.1 to 6.2 mg/L of phosphate and 60 to 100 mg/L calcium is  
10 reported. This is very difficult to maintain, with the additional constraint of no possibility of auto-  
11 flocculation in all types of water. This limitation is rectified by Oswald in 1995 by adding lime in  
12 raceway pond. This method gives 90% removal of N, K, and algae. The bio-flocculation process  
13 is carried out by flocculation using bio-polymers. Passow [120] reported controlled diatoms bloom  
14 underwent mass flocculation increased by biopolymers. Extracellular polymeric substance (EPS)  
15 produced by biofilms increased solid flocculation in clarifier operation [120].

### 16 **4 Conversion of biomass into value-added products**

#### 17 **4.1 Biomass to biofuels**

18 Biofuels are sustainable energy sources that can replace fossil fuels. Biofuels are produced  
19 from biomass such as residues, spent coffee grounds, wastewater, algae, and other biological  
20 materials [121]. Biomass can be converted into different forms of energy such as heat, electricity,  
21 and biogas, etc. [122]. The production of biofuel is categorized into three generations according to  
22 their feedstock [123]. In the 1<sup>st</sup> generation, the feedstock is food crops such as sugarcane, soybeans,

1 and corns, etc. This feedstock raises the conflict between food versus fuel and also increases the  
2 price of feedstock which adversely affects the world food market [5, 124, 125]. Also, the biofuels  
3 produced from first generation feedstock such as soybean biodiesel are not compatible in term of  
4 energy yield per acre [5]. The 2<sup>nd</sup> generation biofuels tackled the first-generation problems as they  
5 are produced from non-edible feedstock such as Jatropha and Pongamia. However, cultivation of  
6 energy crops for biofuel production requires vast land. Consequently, energy required for  
7 harvesting and transport these corps is increased as well. Moreover, the biofuels generated from  
8 the second generation has half of the energy content as compared with conventional fuels such as  
9 coal [124, 126].

10 In 3<sup>rd</sup> generation, algae are used to produce biofuels. It is a viable alternate energy source  
11 as it tackles all the major issue with 1<sup>st</sup> and 2<sup>nd</sup> generation biofuels. Microalgae have high  
12 photosynthetic efficiency. Moreover, it has high oil yield productivity compared to other oil crops  
13 [127]. Microalgae use solar energy to produce oils and convert it into biofuel and can produce 200  
14 to 5000 gallons of biofuel per acre per year [5]. Comparison of all three generations is given in  
15 Table 7.

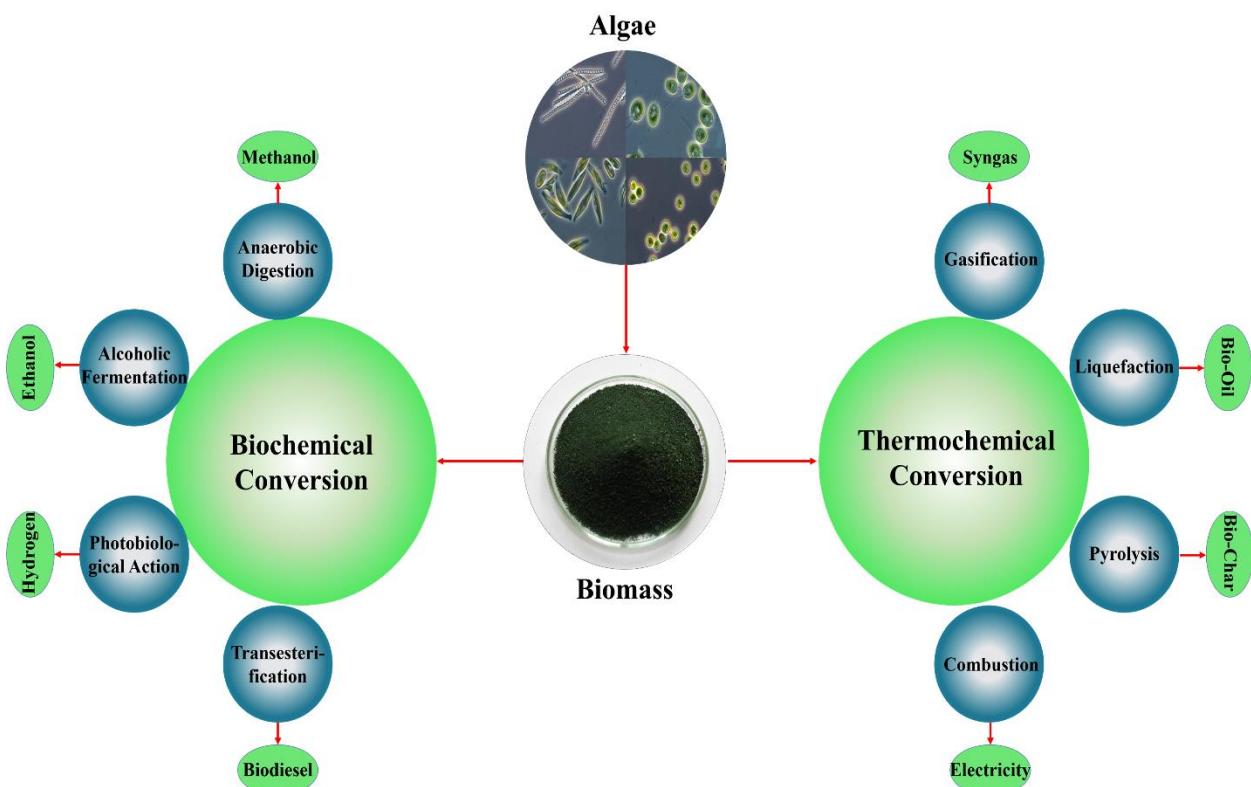
16

**Table 7** Comparison of all three generation biofuels

Generation	Technology	Products	Merits	Limitations
<b>First generation Biofuels</b>				
Corn				
				
Sugar-Beet	<ul style="list-style-type: none"> <li>– Alcoholic fermentation</li> <li>– Transesterification</li> </ul>	<ul style="list-style-type: none"> <li>– Bio-ethanol</li> <li>– Bio-diesel</li> </ul>	<ul style="list-style-type: none"> <li>– Environmentally pleasant</li> <li>– Financially viable</li> </ul>	<ul style="list-style-type: none"> <li>– Inadequate Feedstock</li> <li>– Food versus fuel competition</li> <li>– Blending with orthodox fuel</li> </ul>
Sugar Cane				
				
<b>Second generation</b>				
Wood Residue				
				
Straw	<ul style="list-style-type: none"> <li>– Pretreatment,</li> <li>– Hydrolysis</li> <li>– Fermentation,</li> <li>– Transesterification</li> </ul>	<ul style="list-style-type: none"> <li>– Hydro-treating oil</li> <li>– Bio-oil</li> <li>– Lignocellulose</li> <li>– Bio-alcohols</li> </ul>	<ul style="list-style-type: none"> <li>– Environmental friendly</li> <li>– Not competing with food</li> </ul>	<ul style="list-style-type: none"> <li>– Agricultural land depletion</li> <li>– Complex procedures</li> </ul>
Energy Crops				
				
<b>Third generation</b>				
Microalgae	<ul style="list-style-type: none"> <li>– Thermal and biochemical conversion of algal biomass</li> </ul>	<ul style="list-style-type: none"> <li>– Biodiesel</li> <li>– bioethanol</li> <li>– biohydrogen</li> <li>– biomethane</li> </ul>	<ul style="list-style-type: none"> <li>– Environmental friendly</li> <li>– Oil productivity is high</li> <li>– High reproduction rate</li> <li>– Biofuel contains nontoxic</li> <li>– Highly biodegradable</li> </ul>	<ul style="list-style-type: none"> <li>– Less yield</li> <li>– Reduced biomass production</li> </ul>

#### 1    4.1.1 Algal biomass conversion technologies

2        Algal biomass conversion into energy encompasses different processes, entirely dependent  
3        on types of biomass, conversion options, and its end use. Microalgal biomass conversion is  
4        categorized in two types-(i) thermo and (ii) biochemical conversion [128-130] as shown in Fig. 5.  
5        Influencing factors for conversion of biomass are quality of biomass, feedstock, and desire energy  
6        content [124].



8            **Fig. 5.** Microalgae conversion technologies for the production of biofuels

##### 9    4.1.1.1 Thermochemical conversion

10        In this process, biomass is thermally decomposed to produce fuel products. The technique  
11        includes combustion, gasification, liquefaction, and pyrolysis [131]. Comparison of all thermal  
12        technologies is shown in Table 8.

1      **4.1.1.1.1 Gasification**

2            In this process, hydrocarbons are converted under limited supply of oxygen to produce  
3            combustible gaseous mixture (syngas) [132]. It can burn directly or use as a fuel for gas engines.  
4            However, it has a low calorific value of 4-6 MJ/m<sup>3</sup>. There are two different routes for transporting  
5            fuels through syngas: (1) hydrogen production by water gas shift reaction; and (2) Fischer Tropsch  
6            conversion of hydrocarbons to produce liquid fuels/chemicals such as methanol [133, 134].  
7            Several studies have been reported for converting microalgae biomass into biofuel through  
8            gasification. Hirano et al. [135] studied Spirulina partially oxidized at high temperature at 850 to  
9            1000 °C to determine the gas composition required to generate methanol. Highest theoretical yield  
10          0.64 g of methanol for 1 g of biomass was obtained at 1000 °C. They estimated energy balance  
11          that showed marginal positive balance. [135].

12      **4.1.1.1.2 Liquefaction**

13            In this process, wet-algal biomass is used to produce liquid fuel. Microalgae cells derived  
14          from mechanical harvesting have high moisture and are used as raw material for liquefaction [129,  
15          136]. Generally, this process produces oils of high viscosity and requires biomass, solvents, and  
16          gases like H<sub>2</sub> or CO as well as catalysts. Liquefaction can consume wet biomass and converts it  
17          into the smaller molecular material with higher energy densities [132, 137-139]. Dote et al. [34]  
18          reported liquefaction of *B. braunii*, with 64% yield (dry weight) at 300 °C. *Dunaliella tertiolecta*  
19          gave oil yield of 42% dry weight. This clearly reveals that liquefaction is also a vital option for  
20          biomass conversion [34].

21      **4.1.1.1.3 Pyrolysis**

22            Algal-biomass can be used to produce various products such as biochar, bio-oil, and syngas  
23          in the absence of air at 350 to 700 °C [140]. Pyrolysis can convert biomass to liquid fuels and can

1 be up-scaled for large-scale production. Flash pyrolysis, occurring at 500 °C, is one of the most  
2 important techniques for future petroleum replacement with liquid biofuels. As it can achieve high  
3 biomass to liquid ratio of almost 95.5% [141]. However, commercialization of the microalgal  
4 pyrolysis still requires to address some major challenges. Oil generated in this process is acidic in  
5 nature, highly viscous, has solid and water in it. The process also requires further development in  
6 hydrogenation and catalytic cracking to remove alkali and lower the oxygen content [132].

7         The conventional process is divided into three stages. In first stage, decomposition of  
8 biomass at 550-950 K is known as pre-pyrolysis. In this step, water is removed following by bond  
9 breakage and then carbonyl and carboxyl groups are formed. In second stage, pyrolysis products  
10 are formed as a result of solid decomposition. In third stage, char decomposes to carbon-rich  
11 residues. Fast pyrolysis process occurs at 850 to 1250 K, as a result, biomass decomposes and  
12 pyrolysis products are formed. The product's composition is bio-oil (60 to 70%), biochar (15 to  
13 25%), and non-condensed gases (10 to 20 %). Microalgae biomass contain high moisture content,  
14 thus it needs to go through the drying process before subjecting to pyrolysis [142]. Miao et al.  
15 [143] achieved almost 18-24% bio-oil yield for *Chlorella prothotecoides* and *Microcystis*  
16 *aeruginosa* through fast pyrolysis [143].

17         **4.1.1.4 Combustion**

18         In all thermochemical process, combustion is one the easiest ways of producing energy.  
19 The fuel is burnt, typically, in excess air. in which fuel react in the presence of air known as  
20 burning. The major products are CO<sub>2</sub>, H<sub>2</sub>O, and release of heat. Combustion usually occurs at a  
21 high temperature above 800 °C. It usually occurs in the boiler, furnaces where steam is generated  
22 to produce electricity [144]. According to the life cycle assessment (LCA) combustion of algae,

1 biomass leads to the lower CO<sub>2</sub> emissions. However, further investigation and modified burner to  
2 deal algae are required for further scale-up of the technology [145].

3 **Table 8** Thermochemical technologies and lipid productivity

Microalgae species	Process	Temperature (°C)	Pressure (MPa)	Lipid Productivity (% dry wt.)	Reference
<i>Botryococcus braunii</i>	Liquefaction	300	3	64	[34]
<i>Dunaliella tertiolecta</i>	Liquefaction	300	3	42	[146]
<i>Chlorella protothecoides</i>	Pyrolysis	450	0.101	57.9	
<i>Chlorella protothecoides</i>	Pyrolysis	450	0.101	16.6	[143]
<i>Chlorella protothecoides</i>	Pyrolysis	500	0.101	18	
<i>Chlorella protothecoides</i>	Pyrolysis	500	0.101	24	
<i>Chlorella protothecoides</i>	Pyrolysis	502	0.101	55.3	[141]

4

5 **4.1.1.2 Biochemical conversion**

6 Biomass conversion through the biological process (e.g. anaerobic digestion, fermentation,  
7 and transesterification) is used to produce biofuels.

8 **4.1.1.2.1 Anaerobic digestion (AD)**

9 In this process, organic matter is converted into gas and energy content. AD process is one  
10 the most efficient method for higher moisture feed and is best suited for wet algal biomass. This  
11 process is divided into three stages: hydrolysis, fermentation, and methanogenesis [147]. Complex  
12 compounds are broken into soluble sugars in the first stage. It is followed by fermentation where  
13 soluble sugar converts into alcohols, acetic acid and gaseous products mainly hydrogen and carbon  
14 dioxide. These gases are metabolized into methane and carbon dioxide by methanogens during the  
15 third stage [148, 149]. Yen and Brune [150] experimentally showed that AD of combined waste

1 of paper and algal biomass significantly increased methane production. Methane production rate  
2 was doubled by using 50% waste paper in algal biomass instead of pure algal biomass. However,  
3 the products have about 20 to 40% of less heating value.

4 **4.1.1.2.2 Fermentation**

5 Starch or cellulose are converted into alcohols like bioethanol in fermentation [144].  
6 Mostly, yeast is used to convert sugars into ethanol. Distillation is required for separation of  
7 products and purification of alcohols [151]. The residue from fermentation can be reused in  
8 gasification. This reduces the process cost and makes this process economically more viable.  
9 [132]. Microalgae like *Chlorella Vulgaris* are the most common species to produce ethanol due to  
10 high starch content, and maximum ethanol conversion of 65% reported [152]. Ueno et al. [153]  
11 reported that conversion of algal biomass through dark fermentation process provided ethanol  
12 productivity of 450 µmol/g dry wt. at 30 °C.

13 **4.1.1.2.3 Photo-biological process**

14 Hydrogen is a clean and efficient energy carrier. Microalgae have necessary enzymatic  
15 characteristics to produce hydrogen gas [154]. During photosynthesis of microalgae, molecules of  
16 water are converted into oxygen and hydrogen ions under anaerobic conditions. Eukaryotic  
17 microalgae are used for this process than hydrogenase enzymes, which convert hydrogen ions into  
18 hydrogen gas [147]. Fundamentally, photosynthesis of hydrogen production occurs by two  
19 processes. In the first process, hydrogen synthesis process divided into two parts, (1) algae is grown  
20 at normal condition and (2) anaerobic conditions are introduced in microalgae which stimulate  
21 continuous hydrogen production. The advantage of this process is that it does not generate harmful  
22 or toxic chemicals. Moreover, it provides useful byproducts as a result of biomass[155].

1 Photo-biological process can also produce hydrogen and oxygen through photosynthesis  
2 simultaneously. In this method, photosynthetic H<sub>2</sub>O oxidation electron is released. The electron is  
3 directly fed into the hydrogenase-mediated H<sub>2</sub>-evolution process [154]. This process has greater  
4 efficiency than two-step process because of simultaneous production. Melis and Happe [156]  
5 studied that theoretical yield through two-step process for hydrogen gas production was 198  
6 kg/h/day [156].

7 **4.1.1.2.4 Transesterification**

8 In transesterification process [157], an alkoxy group of an ester is substituted by alcohol.  
9 The reaction is reversible in nature in which triglycerides and methanol react in the presence of a  
10 catalyst to produce biodiesel [158]. Miao and Wu [77] studied *Chlorella protothecoides* for  
11 biodiesel production through conventional process. The reaction occurs at 30 °C and at a molar  
12 ratio of oil to methanol is 56:1. The efficiency of transesterification process for biodiesel  
13 production was determined for *Chlorella vulgaris*, *Rhizoclonium hieroglyphicum*, and mixed algae  
14 culture. The results indicated higher conversion for *Chlorella vulgaris* with 95% and than mixed  
15 algae culture, *Rhizoclonium hieroglyphicum* with 92% and 91% respectively [159].

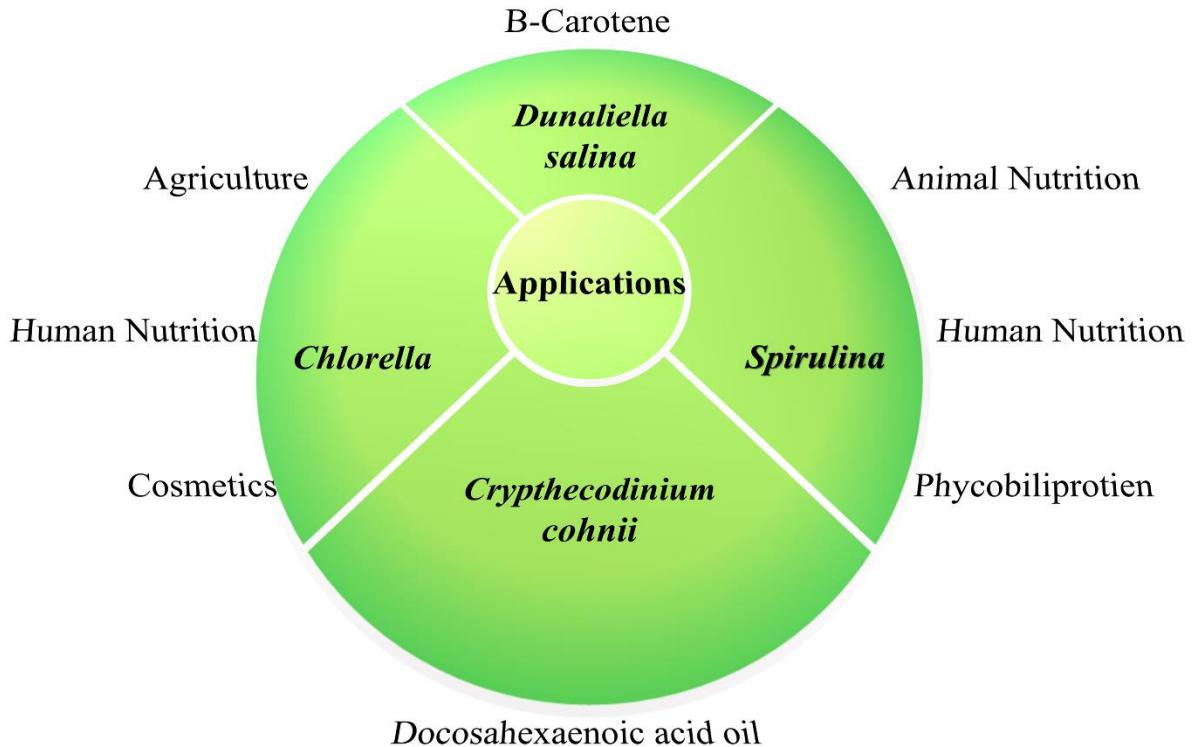
16 **4.2 Commercial applications of algal biomass**

17 Microalgae have several commercial applications as shown in Fig. 6. Mostly these are  
18 phototrophic in nature, which gives significant technical and commercial advantages such as food  
19 food nutrition. Wastewater grown microalgae have numerous applications in fuel as discussed  
20 above. But it also has numerous non-fuel applications such as fertilizer, cosmetics, and animal  
21 feed. The production of biochar from microalgae as a result of many technologies such as pyrolysis  
22 has great potential for fertilizer and carbon sequestration as an agricultural application [160]. It

1 can also be used in bioenergy conversion as a process fuel. For carbon sequestration purposes, it  
2 reduced carbon emission up to 84% [161].

3 A limited number of microalgae biomass is allowed for human consumption. Few species  
4 such as *Spirulina*, *Dunaliella* and *Chlorella* are available in the market. These species dominate  
5 the microalgae world market and they are available in the form of powder and tablets as food  
6 additives. It is also used in medical application such as curing growth promotion of intestinal  
7 Lactobacillus and renal failure [162]. Use of microalgae as food nutrition have some adverse  
8 effects like Alzheimer's and Lou Gehrig's disease (ALS) due to ingestion of cyanobacteria mostly  
9 present in *Spirulina* [163].

10 *Chlorella* and *Arthrospira* have commonly used microalgae species in cosmetics  
11 industries, mostly in skin care products like anti-irritants in peelers and anti-aging creams.  
12 Microalgae extensively are employed in sun and hair care products. A commercial example of  
13 microalgae products and their properties is *C. vulgaris*, which stimulates collagen synthesis in the  
14 skin by providing support to tissues regeneration and also helps in wrinkle reduction. *Arthrospira*  
15 helps to minimize the early aging signs and also helps to lighten the skin [164].



**Fig 6.** Applications of commercially used microalgae

Microalgae can be used for the synthesis of animal feed supplements. Microalgae present in animal feed have multiple benefits such as improved fertility, healthier skin, increased immunity, and better weight control. However, prolonged feeding of this supplement can also have an adverse effect due to *cyanobacteria*. Mostly, *Scenedesmus*, *Spirulina*, and *Chlorella* have been used in animal supplement. Algae are also used as a natural food source for many aquatic species like fish, shrimps, and molluscs. The major applications of algae in aquaculture are fish feed. It increase the immunity of fish and stabilize the quality of culture medium [165]. Comparison of different commercial produced microalgae and their costs are summaries in Table 9.

11

12

1 **Table 9** Microalgae annual production and their prices [124]

Microalgae Species	Production	Price (€/kg)
<i>Spirulina</i>	3000 ton/year	- 36
<i>Chlorella</i>	2000 ton/year	- 36
<i>Dunaliella salina</i>	1200 ton/year	- 215-2150
<i>Cryptocodinium cohnii</i>	240 ton/year	- 43000

2

3 Polyunsaturated fatty acid (PUFAs) is one of the vital roles of human development and  
 4 physiology [166]. The major advantage of PUFAs is to minimize the risk of cardiovascular disease.  
 5 At present, fish oils are used as the main source of PUFA, but its implementations are limited in  
 6 food additives due to fish odor, unpleasant taste, the presence of mixed fatty acid and poor  
 7 oxidative stability. Microalgae PUFA is used in many other commercial applications such as an  
 8 additive in infant milk and used in chicken feed to produce eggs enriched with omega-3 [167].

## 9 **5 Review of Techno-economic and life cycle analysis**

10 There are many challenges for the commercialization of algal biomass for bioenergy  
 11 production such as high operating and capital cost. To understand the current scenario, many  
 12 studies have been concluded for both open pond and photobioreactors. To find the way for  
 13 reducing costs, a brief economic analysis was performed by Davis et al. [168]. \$8.52/gal for 25  
 14 g/m<sup>2</sup>/day were achieved for open system and cost for photobioreactor achieved \$18.10/gal for 1.25  
 15 kg/m<sup>3</sup>/day, which provided biodiesel cost of \$9.84/gal, \$20.53/gal for open bond and  
 16 photobioreactor, respectively [168]. Therefore, current microalgae biodiesel price is not  
 17 compatible as compared to fossil fuels for large-scale biofuel production.

1       The major reason of cost intensiveness of microalgae biofuels, is nutrients (N and K) and  
2   freshwater microalgae cultivation. This is almost 20 to 30 % of total costs of biodiesel production  
3   for microalgae [12, 169, 170]. Use of wastewater for microalgae growth has been proposed to be  
4   the most economical and sustainable option for biofuel production on a commercial scale [12, 18,  
5   19, 22]. For example, all required nutrients can be supplied by wastewater due to which not only  
6   cultivation cost is reduced but also wastewater can be treated simultaneously. Moreover,  
7   significantly higher biomass and liquid contents can be achieved if inorganic wastewater is used  
8   for algae cultivation through mixotrophic cultivation. Therefore, wastewater-based biofuel can  
9   reduce the cost upto 50% making it comparable to petroleum diesel.

10       Besides the economic advantages of wastewater-based algae cultivation, it also reduces the  
11   ecological footprint. Many studies have been published for LCA, using wastewater for the  
12   production of biofuel. Use of wastewater was found to be more sustainable and environmentally  
13   friendly. For example, Clarens et al [12] performed LCA for microalgae, corn, grass, and canola.  
14   It suggested that wastewater based microalgae cultivation offset the environmental burdens related  
15   to microalgae [12]. Another study was conducted by Yang et al [171], which indicated that by  
16   using wastewater for microalgae, 90% of freshwater can be saved. In addition, nitrogen  
17   consumption reduced to 94% and other nutrients such as sulfur, potassium, and magnesium,  
18   reduced 100%.

19       The life cycle of microalgae cultivation is shown in Fig.7. The use of wastewater for  
20   microalgal cultivation is also difficult to process due to a variety of wastewater streams.  
21   Wastewater location, pretreatment methods, and nutrients inculcates uncertainties for the  
22   production of algal-biomass. Such as nutrients content in wastewater are unsuitable for microalgae,  
23   incompatible for carbon to nitrogen ratio, presence of inhibitors, resulting in reduced process

1 efficiency and low biomass production [172]. Due to which, downstream processing might  
2 increase and as a result cost of the process can increase.



**Fig 7.** The life cycle of biofuel from microalgae

5 Recently, Mu et al. [173] studied multiple ways for wastewater based algal biofuels  
6 including (1) cultivation methods for algae in open pond or bioreactors; (2) different conversion  
7 technologies for biomass; (3) nutrient sources [173]. The results indicated that microalgal biofuel  
8 production through wastewater was better than freshwater microalgal biofuels. However, the  
9 effectiveness of this process was greatly dependent on nutrient profile and downstream processing.  
10 The availability of suitable wastewater also restricted the implementation of these processes on a  
11 large scale. Due to which further improvements are needed in current production technologies  
12 before their commercial implementations.

1    **6 Conclusions and future perspectives**

2         This review presents critical prospects of adopting an integrated approach to advocate the  
3         sustainability of microalgae bio-refinery. It is argued that microalgae have meritorious attributes  
4         to treat wastewater, produce energy, and recover value-added bio-products at the same time. These  
5         have the potential to replace conventional fuel resources; however, the entire chain of microalgae  
6         bioprocessing need improvements to prove their sustainability. The techno-economic analysis  
7         reveals that the major cost of microalgae cultivation is rendered on nutrients supply. Wastewater  
8         can source the nutrients to feed microalgae. A wide variety of wastewaters can be employed for  
9         microalgae cultivation. Microalgae require a specific nutrients composition and concentration to  
10      grow. The major challenge in using wastewater is the *dilution*, which would demand an additional  
11      cost for water supply. Therefore, the wastewater satisfying the microalgae nutrients demand should  
12      be identified, so that the dilution cost could be eliminated. The cultivation cost can be reduced by  
13      increasing the biomass productivity. To this end, mixotrophic cultivation seems a promising  
14      choice. Hybrid cultivation which involves cultivation in the close pond, as well as open pond  
15      system, can receive attention in future since it offers high biomass productivity by controlling  
16      contamination. Cascade cultivation should be attempted as it reduces resource input supplied in  
17      the form of light, nutrients, and water.

18         In harvesting, bio-flocculation and auto-flocculation should be exploited, as  
19         auto-flocculation is induced by the polysaccharides, which are abundantly available in  
20         wastewaters. The scope of auto-flocculation should be extended to an attached-cultivation system  
21         in which the microalgae are grown on a solid surface avoiding the complexity of an aqueous  
22         medium. Microalgae cultivation in membrane photobioreactors can also displace the need for  
23         dewatering. This study also points out the pyrolysis and liquefaction can be effective routes to

1 extract bio-products from the microalgae biomass. It is concluded that the focus of microalgae bio-  
2 refinery should be re-directed towards resource recovery and value-added products instead of  
3 relying on traditional bio-processing of microalgae. Forward-looking steps should be taken to  
4 project the scalability and sustainability of the proposed integrated system.

5 **Acknowledgment**

6 This work was funded by Higher Education Commission (HEC) under NRPU program project  
7 number 20-3982/14 and 20-4547/14.

8

9

10

11

12

13

14

15

16

17

18

## References

- [1] Ramachandra T, Mahapatra DM, Gordon R. Milking diatoms for sustainable energy: biochemical engineering versus gasoline-secreting diatom solar panels. *Industrial & Engineering Chemistry Research* 2009;48(19):8769-88.
- [2] Demirbas A. Progress and recent trends in biodiesel fuels. *Energy conversion and management* 2009;50(1):14-34.
- [3] Wijffels RH, Barbosa MJ. An outlook on microalgal biofuels. *Science* 2010;329(5993):796-9.
- [4] Atabani A, Shobana S, Mohammed M, Uğuz G, Kumar G, Arvindnarayan S, et al. Integrated valorization of waste cooking oil and spent coffee grounds for biodiesel production: Blending with higher alcohols, FT-IR, TGA, DSC and NMR characterizations. *Fuel* 2019;244:419-30.
- [5] Elrayes GM. Microalgae: prospects for greener future buildings. *Renewable and Sustainable Energy Reviews* 2018;81:1175-91.
- [6] Maaz M, Yasin M, Aslam M, Kumar G, Atabani AE, Idrees M, et al. Anaerobic membrane bioreactors for wastewater treatment: Novel configurations, fouling control and energy considerations. *Bioresource technology* 2019;283:358-72.
- [7] Khalid A, Aslam M, Qyyum MA, Faisal A, Khan AL, Ahmed F, et al. Membrane separation processes for dehydration of bioethanol from fermentation broths: Recent developments, challenges, and prospects. *Renewable and Sustainable Energy Reviews* 2019;105:427-43.
- [8] Saqib S, Rafiq S, Chawla M, Saeed M, Muhammad N, Khurram S, et al. Facile CO<sub>2</sub> Separation in Composite Membranes. *Chemical Engineering & Technology* 2019;42(1):30-44.
- [9] Charfi A, Park E, Aslam M, Kim J. Particle-sparged anaerobic membrane bioreactor with fluidized polyethylene terephthalate beads for domestic wastewater treatment: Modelling approach and fouling control. *Bioresource technology* 2018;258:263-9.
- [10] Charfi A, Thongmak N, Benyahia B, Aslam M, Harmand J, Amar NB, et al. A modelling approach to study the fouling of an anaerobic membrane bioreactor for industrial wastewater treatment. *Bioresource technology* 2017;245:207-15.
- [11] Aslam M, Yang P, Lee P-H, Kim J. Novel staged anaerobic fluidized bed ceramic membrane bioreactor: Energy reduction, fouling control and microbial characterization. *Journal of Membrane Science* 2018;553:200-8.
- [12] Clarens AF, Resurreccion EP, White MA, Colosi LM. Environmental life cycle comparison of algae to other bioenergy feedstocks. *Environmental science & technology* 2010;44(5):1813-9.
- [13] Green FB, Lundquist T, Oswald W. Energetics of advanced integrated wastewater pond systems. *Water Science and Technology* 1995;31(12):9-20.
- [14] Oswald W. J. Gotaas, H. B., Golueke, CG. Kellen, W R-Algae in Waste Treatment, Sewage and Industrial Wastes 1957;29(4):437.
- [15] Park J, Craggs R, Shilton A. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource technology* 2011;102(1):35-42.
- [16] Oswald WJ, Golueke CG. Biological transformation of solar energy. *Advances in applied microbiology*. Elsevier; 1960;2:223-62.

- [17] Lau P, Tam N, Wong Y. Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution* 1995;89(1):59-66.
- [18] Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the US Department of Energy's aquatic species program: biodiesel from algae. *National Renewable Energy Laboratory* 1998;328.
- [19] Zhou W, Li Y, Min M, Hu B, Chen P, Ruan R. Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal wastewater for biofuel production. *Bioresource Technology* 2011;102(13):6909-19.
- [20] Li Y, Chen Y-F, Chen P, Min M, Zhou W, Martinez B, et al. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource technology* 2011;102(8):5138-44.
- [21] Zhou W, Cheng Y, Li Y, Wan Y, Liu Y, Lin X, et al. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. *Applied biochemistry and biotechnology* 2012;167(2):214-28.
- [22] Zhou W, Li Y, Min M, Hu B, Zhang H, Ma X, et al. Growing wastewater-born microalga Auxenochlorella protothecoides UMN280 on concentrated municipal wastewater for simultaneous nutrient removal and energy feedstock production. *Applied Energy* 2012;98:433-40.
- [23] Zhou W, Min M, Li Y, Hu B, Ma X, Cheng Y, et al. A hetero-photoautotrophic two-stage cultivation process to improve wastewater nutrient removal and enhance algal lipid accumulation. *Bioresource Technology* 2012;110:448-55.
- [24] Li Y, Zhou W, Hu B, Min M, Chen P, Ruan RR. Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains Chlorella kessleri and Chlorella protothecoides cultivated in highly concentrated municipal wastewater. *Biotechnology and bioengineering* 2012;109(9):2222-9.
- [25] Min M, Wang L, Li Y, Mohr MJ, Hu B, Zhou W, et al. Cultivating Chlorella sp. in a pilot-scale photobioreactor using centrate wastewater for microalgae biomass production and wastewater nutrient removal. *Applied biochemistry and biotechnology* 2011;165(1):123-37.
- [26] Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, et al. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. *Bioresource technology* 2010;101(8):2623-8.
- [27] Min M, Hu B, Mohr MJ, Shi A, Ding J, Sun Y, et al. Swine manure-based pilot-scale algal biomass production system for fuel production and wastewater treatment—a case study. *Applied biochemistry and biotechnology* 2014;172(3):1390-406.
- [28] Zhou W, Chen P, Min M, Ma X, Wang J, Griffith R, et al. Environment-enhancing algal biofuel production using wastewaters. *Renewable and Sustainable Energy Reviews* 2014;36:256-69.
- [29] Xiong W, Gao C, Yan D, Wu C, Wu Q. Double CO<sub>2</sub> fixation in photosynthesis–fermentation model enhances algal lipid synthesis for biodiesel production. *Bioresource technology* 2010;101(7):2287-93.
- [30] Hu B, Min M, Zhou W, Du Z, Mohr M, Chen P, et al. Enhanced mixotrophic growth of microalga Chlorella sp. on pretreated swine manure for simultaneous biofuel feedstock production and nutrient removal. *Bioresource technology* 2012;126:71-9.

- [31] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and bioengineering* 2009;102(1):100-12.
- [32] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. *Journal of industrial microbiology & biotechnology* 2009;36(2):269-74.
- [33] Weldy CS, Huesemann M. Lipid production by *Dunaliella salina* in batch culture: effects of nitrogen limitation and light intensity. US Department of Energy Journal of Undergraduate Research 2007;7(1):115-22.
- [34] Dote Y, Sawayama S, Inoue S, Minowa T, Yokoyama S-y. Recovery of liquid fuel from hydrocarbon-rich microalgae by thermochemical liquefaction. *Fuel* 1994;73(12):1855-7.
- [35] Sawayama S, Minowa T, Yokoyama S-Y. Possibility of renewable energy production and CO<sub>2</sub> mitigation by thermochemical liquefaction of microalgae. *Biomass and Bioenergy* 1999;17(1):33-9.
- [36] Chiu S-Y, Kao C-Y, Tsai M-T, Ong S-C, Chen C-H, Lin C-S. Lipid accumulation and CO<sub>2</sub> utilization of *Nannochloropsis oculata* in response to CO<sub>2</sub> aeration. *Bioresource technology* 2009;100(2):833-8.
- [37] Kim S-K. *Handbook of marine microalgae: biotechnology advances*. Academic Press; 2015.
- [38] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renewable and sustainable energy reviews* 2010;14(1):217-32.
- [39] Janssen M, Tramper J, Mur LR, Wijffels RH. Enclosed outdoor photobioreactors: Light regime, photosynthetic efficiency, scale-up, and future prospects. *Biotechnology and bioengineering* 2003;81(2):193-210.
- [40] Pulz O, Scheibenbogen K. Photobioreactors: design and performance with respect to light energy input. *Bioprocess and algae reactor technology, apoptosis*. Springer; 1998;59: 123-52.
- [41] Wang B, Li Y, Wu N, Lan CQ. CO<sub>2</sub> bio-mitigation using microalgae. *Applied microbiology and biotechnology* 2008;79(5):707-18.
- [42] Fazal T, Mushtaq A, Rehman F, Khan AU, Rashid N, Farooq W, et al. Bioremediation of textile wastewater and successive biodiesel production using microalgae. *Renewable and Sustainable Energy Reviews* 2018;82:3107-26.
- [43] Borowitzka MA. Microalgae for aquaculture: opportunities and constraints. *Journal of Applied Phycology* 1997;9(5):393.
- [44] Chisti Y. Biodiesel from microalgae. *Biotechnology Advances* 2007;25:294–306.
- [45] Andersen R. Recipes for freshwater and seawater media. *Algal culturing techniques* 2005.
- [46] Posten C. Design principles of photo-bioreactors for cultivation of microalgae. *Engineering in Life Sciences* 2009;9(3):165-77.
- [47] Molina E, Fernández J, Acién F, Chisti Y. Tubular photobioreactor design for algal cultures. *Journal of biotechnology* 2001;92(2):113-31.
- [48] Sierra E, Acién F, Fernández J, García J, González C, Molina E. Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal* 2008;138(1-3):136-47.
- [49] Slegers P, Wijffels R, Van Straten G, Van Boxtel A. Design scenarios for flat panel photobioreactors. *Applied energy* 2011;88(10):3342-53.
- [50] Eriksen NT. The technology of microalgal culturing. *Biotechnology letters* 2008;30(9):1525-36.

- [51] Samson R, Leduy A. Multistage continuous cultivation of blue-green alga spirulina maxima in the flat tank photobioreactors with recycle. *The Canadian Journal of Chemical Engineering* 1985;63(1):105-12.
- [52] Hu Q, Kurano N, Kawachi M, Iwasaki I, Miyachi S. Ultrahigh-cell-density culture of a marine green alga Chlorococcum littorale in a flat-plate photobioreactor. *Applied Microbiology and Biotechnology* 1998;49(6):655-62.
- [53] Richmond A. Microalgal biotechnology at the turn of the millennium: a personal view. *Journal of Applied Phycology* 2000;12(3-5):441-51.
- [54] Richmond A, Cheng-Wu Z, Zarmi Y. Efficient use of strong light for high photosynthetic productivity: interrelationships between the optical path, the optimal population density and cell-growth inhibition. *Biomolecular Engineering* 2003;20(4-6):229-36.
- [55] Olaizola M. Commercial production of astaxanthin from Haematococcus pluvialis using 25,000-liter outdoor photobioreactors. *Journal of Applied Phycology* 2000;12(3-5):499-506.
- [56] Pulz O. Photobioreactors: production systems for phototrophic microorganisms. *Applied microbiology and biotechnology* 2001;57(3):287-93.
- [57] Chang H-X, Fu Q, Huang Y, Xia A, Liao Q, Zhu X, et al. An annular photobioreactor with ion-exchange-membrane for non-touch microalgae cultivation with wastewater. *Bioresource technology* 2016;219:668-76.
- [58] Kumar A, Yuan X, Sahu AK, Dewulf J, Ergas SJ, Van Langenhove H. A hollow fiber membrane photo-bioreactor for CO<sub>2</sub> sequestration from combustion gas coupled with wastewater treatment: a process engineering approach. *Journal of Chemical Technology & Biotechnology* 2010;85(3):387-94.
- [59] Honda R, Boonnarat J, Chiemchaisri C, Chiemchaisri W, Yamamoto K. Carbon dioxide capture and nutrients removal utilizing treated sewage by concentrated microalgae cultivation in a membrane photobioreactor. *Bioresource technology* 2012;125:59-64.
- [60] Singh G, Thomas PB. Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. *Bioresource technology* 2012;117:80-5.
- [61] Bilad M, Discart V, Vandamme D, Foubert I, Muylaert K, Vankelecom IF. Coupled cultivation and pre-harvesting of microalgae in a membrane photobioreactor (MPBR). *Bioresource technology* 2014;155:410-7.
- [62] Rossignol N, Lebeau T, Jaouen P, Robert J. Comparison of two membrane-photobioreactors, with free or immobilized cells, for the production of pigments by a marine diatom. *Bioprocess Engineering* 2000;23(5):495-501.
- [63] Marbelia L, Bilad MR, Passaris I, Discart V, Vandamme D, Beuckels A, et al. Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent. *Bioresource technology* 2014;163:228-35.
- [64] Rubio FC, Fernández FA, Pérez JS, Camacho FG, Grima EM. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. *Biotechnology and Bioengineering* 1999;62(1):71-86.
- [65] Fernández FA, Sevilla JF, Pérez JS, Grima EM, Chisti Y. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. *Chemical Engineering Science* 2001;56(8):2721-32.

- [66] Ugwu C, Ogbonna J, Tanaka H. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. *Applied microbiology and biotechnology* 2002;58(5):600-7.
- [67] Carlozzi P. Dilution of solar radiation through “culture” lamination in photobioreactor rows facing south–north: a way to improve the efficiency of light utilization by cyanobacteria (*Arthrospira platensis*). *Biotechnology and bioengineering* 2003;81(3):305-15.
- [68] Hall DO, Acién Fernández F, Guerrero EC, Rao KK, Grima EM. Outdoor helical tubular photobioreactors for microalgal production: modeling of fluid-dynamics and mass transfer and assessment of biomass productivity. *Biotechnology and bioengineering* 2003;82(1):62-73.
- [69] López MG-M, Sanchez EDR, López JC, Fernández FA, Sevilla JF, Rivas J, et al. Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors. *Journal of biotechnology* 2006;123(3):329-42.
- [70] Converti A, Lodi A, Del Borghi A, Solisio C. Cultivation of *Spirulina platensis* in a combined airlift-tubular reactor system. *Biochemical Engineering Journal* 2006;32(1):13-8.
- [71] Cheng-Wu Z, Zmora O, Kopel R, Richmond A. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp.(Eustigmatophyceae). *Aquaculture* 2001;195(1-2):35-49.
- [72] Secchi S, Kurkalova L, Gassman PW, Hart C. Land use change in a biofuels hotspot: the case of Iowa, USA. *Biomass and Bioenergy* 2011;35(6):2391-400.
- [73] Zittelli GC, Rodolfi L, Biondi N, Tredici MR. Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture* 2006;261(3):932-43.
- [74] Sato T, Usui S, Tsuchiya Y, Kondo Y. Invention of outdoor closed type photobioreactor for microalgae. *Energy conversion and management* 2006;47(6):791-9.
- [75] Huntley ME, Redalje DG. CO<sub>2</sub> mitigation and renewable oil from photosynthetic microbes: a new appraisal. *Mitigation and adaptation strategies for global change* 2007;12(4):573-608.
- [76] Chen F, Zhang Y, Guo S. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnology letters* 1996;18(5):603-8.
- [77] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. *Bioresource technology* 2006;97(6):841-6.
- [78] Eriksen NT. Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. *Applied microbiology and biotechnology* 2008;80(1):1-14.
- [79] Chen G-Q, Chen F. Growing phototrophic cells without light. *Biotechnology letters* 2006;28(9):607-16.
- [80] De Swaaf ME, Sijtsma L, Pronk JT. High-cell-density fed-batch cultivation of the docosahexaenoic acid producing marine alga *Cryptocodinium cohnii*. *Biotechnology and bioengineering* 2003;81(6):666-72.
- [81] Li X, Xu H, Wu Q. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnology and bioengineering* 2007;98(4):764-71.

- [82] Xiong W, Li X, Xiang J, Wu Q. High-density fermentation of microalga Chlorella protothecoides in bioreactor for microbio-diesel production. *Applied microbiology and biotechnology* 2008;78(1):29-36.
- [83] Zhang X-W, Zhang Y-M, Chen F. Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. *Process Biochemistry* 1999;34(5):477-81.
- [84] Andrade MR, Costa JA. Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. *Aquaculture* 2007;264(1-4):130-4.
- [85] Chojnacka K, Noworyta A. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. *Enzyme and Microbial Technology* 2004;34(5):461-5.
- [86] Grima EM, Belarbi E-H, Fernández FA, Medina AR, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology advances* 2003;20(7-8):491-515.
- [87] Kumar H, Yadava P, Gaur J. Electrical flocculation of the unicellular green alga *Chlorella vulgaris* Beijerinck. *Aquatic Botany* 1981;11:187-95.
- [88] Bernhardt H. Flocculation of micro-organisms. *Aqua* 1991;40(2):76-87.
- [89] Divakaran R, Pillai VS. Flocculation of algae using chitosan. *Journal of Applied Phycology* 2002;14(5):419-22.
- [90] Shah JH, Deokar A, Patel K, Panchal K, Mehta AV. A comprehensive overview on various method of harvesting microalgae according to indian perspective. *International Conference on Multidisciplinary Research & Practice. International Conference on Multidisciplinary Research & Practice*. 1. 2014:313-17.
- [91] Chen P, Min M, Chen Y, Wang L, Li Y, Chen Q, et al. Review of biological and engineering aspects of algae to fuels approach. *International Journal of Agricultural and Biological Engineering* 2010;2(4):1-30.
- [92] Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of renewable and sustainable energy* 2010;2(1):012701.
- [93] Greenwell H, Laurens L, Shields R, Lovitt R, Flynn K. Placing microalgae on the biofuels priority list: a review of the technological challenges. *Journal of the royal society interface* 2010;7(46):703-26.
- [94] Friedman A, Peaks D, Nichols R. Algae separation from oxidation pond effluents [Water pollution]. *Proceedings-Industrial Wastes Conference, Purdue University (USA)*. 1977.
- [95] Hanotu J, Bandulasena HH, Zimmerman WB. Microflootation performance for algal separation. *Biotechnology and bioengineering* 2012;109(7):1663-73.
- [96] Javed F, Shamair Z, Ali S, Ahmad N, Hafeez A, Fazal T, et al. “Pushing and pulling” the equilibrium through bubble mediated reactive separation for ethyl acetate production. *Reaction Chemistry & Engineering* 2019;4:705-14.
- [97] Rehman F, Medley GJ, Bandulasena H, Zimmerman WB. Fluidic oscillator-mediated microbubble generation to provide cost effective mass transfer and mixing efficiency to the wastewater treatment plants. *Environmental research* 2015;137:32-9.
- [98] Mouchet P, Bonnelye V. Solving algae problems: French expertise and world-wide applications. *Journal of water supply: research and technology-AQUA* 1998;47(3):125-41.
- [99] Judd S. The MBR book: principles and applications of membrane bioreactors for water and wastewater treatment. Elsevier; 2010.

- [100] Vandamme D, Pontes SCV, Goiris K, Foubert I, Pinoy LJJ, Muylaert K. Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater microalgae. *Biotechnology and bioengineering* 2011;108(10):2320-9.
- [101] Rossi N, Jaouen P, Legentilhomme P, Petit I. Harvesting of cyanobacterium *Arthrospira platensis* using organic filtration membranes. *Food and bioproducts processing* 2004;82(3):244-50.
- [102] Petrushevski B, Bolier G, Van Breemen A, Alaerts G. Tangential flow filtration: a method to concentrate freshwater algae. *Water Research* 1995;29(5):1419-24.
- [103] Aslam M, Ahmad R, Kim J. Recent developments in biofouling control in membrane bioreactors for domestic wastewater treatment. *Separation and Purification Technology* 2018;206:297-315.
- [104] Aslam M, Kim J. Investigating membrane fouling associated with GAC fluidization on membrane with effluent from anaerobic fluidized bed bioreactor in domestic wastewater treatment. *Environmental science and pollution research international* 2019;26(2):1170-80.
- [105] Eliseus A, Bilad MR, Nordin NAHM, Khan AL, Putra ZA, Wirzal MDH, et al. Two-way switch: Maximizing productivity of tilted panel in membrane bioreactor. *Journal of Environmental Management* 2018;228:529-37.
- [106] Ahmad R, Aslam M, Park E, Chang S, Kwon D, Kim J. Submerged low-cost pyrophyllite ceramic membrane filtration combined with GAC as fluidized particles for industrial wastewater treatment. *Chemosphere* 2018;206:784-92.
- [107] Charfi A, Aslam M, Kim J. Modelling approach to better control biofouling in fluidized bed membrane bioreactor for wastewater treatment. *Chemosphere* 2018;191:136-44.
- [108] Aslam M, Charfi A, Kim J. Membrane scouring to control fouling under fluidization of non-adsorbing media for wastewater treatment. *Environmental Science and Pollution Research* 2019;26(2):1061-71.
- [109] Aslam M, Lee P-H, Kim J. Analysis of membrane fouling with porous membrane filters by microbial suspensions for autotrophic nitrogen transformations. *Separation and Purification Technology* 2015;146:284-93.
- [110] Aslam M, McCarty PL, Bae J, Kim J. The effect of fluidized media characteristics on membrane fouling and energy consumption in anaerobic fluidized membrane bioreactors. *Separation and Purification Technology* 2014;132:10-5.
- [111] Aslam M, Charfi A, Lesage G, Heran M, Kim J. Membrane bioreactors for wastewater treatment: a review of mechanical cleaning by scouring agents to control membrane fouling. *Chemical Engineering Journal* 2017;307:897-913.
- [112] Ahmad R, Ahmad Z, Khan AU, Mastoi NR, Aslam M, Kim J. Photocatalytic systems as an advanced environmental remediation: Recent developments, limitations and new avenues for applications. *Journal of Environmental Chemical Engineering* 2016;4(4):4143-64.
- [113] Rossignol N, Vandajon L, Jaouen P, Quemeneur F. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. *Aquacultural Engineering* 1999;20(3):191-208.
- [114] Rossi N, Petit I, Jaouen P, Legentilhomme P, Derouinot M. Harvesting of cyanobacterium *Arthrospira platensis* using inorganic filtration membranes. *Separation science and technology* 2005;40(15):3033-50.

- [115] Aslam M, Ahmad R, Yasin M, Khan AL, Shahid MK, Hossain S, et al. Anaerobic membrane bioreactors for biohydrogen production: Recent developments, challenges and perspectives. *Bioresource technology* 2018;269:452-64.
- [116] Aslam M, McCarty PL, Shin C, Bae J, Kim J. Low energy single-staged anaerobic fluidized bed ceramic membrane bioreactor (AFCMBR) for wastewater treatment. *Bioresource technology* 2017;240:33-41.
- [117] Shelef G, Sukenik A, Green M. Microalgae harvesting and processing: a literature review. Technion Research and Development Foundation Ltd., Haifa (Israel); 1984.
- [118] Shen Y, Yuan W, Pei Z, Wu Q, Mao E. Microalgae mass production methods. *Transactions of the ASABE* 2009;52(4):1275-87.
- [119] Lavoie A, De la Noüe J. Harvesting of *Scenedesmus obliquus* in wastewaters: Auto-or bioflocculation? *Biotechnology and bioengineering* 1987;30(7):852-9.
- [120] Passow U, Alldredge AL. Aggregation of a diatom bloom in a mesocosm: The role of transparent exopolymer particles (TEP). *Deep Sea Research Part II: Topical Studies in Oceanography* 1995;42(1):99-109.
- [121] Chawla M, Rafiq S, Jamil F, Usman MR, Khurram S, Ghauri M, et al. Hydrocarbons fuel upgradation in the presence of modified bi-functional catalyst. *Journal of Cleaner Production* 2018;198:683-92.
- [122] Ghauri M, Bokhari A, Aslam M, Tufail M. Biogas reactor design for dry process and generation of electricity on sustainable basis. *International Journal of Chemical and Environmental Engineering* 2011;2(6):414-7.
- [123] Gharieb Y, Ibrahim Z. Alternative Track of Energy in Egypt. International Institute of Social and Economic Sciences; 2014.
- [124] Brennan L, Owende P. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and sustainable energy reviews* 2010;14(2):557-77.
- [125] Naik SN, Goud VV, Rout PK, Dalai AK. Production of first and second generation biofuels: a comprehensive review. *Renewable and sustainable energy reviews* 2010;14(2):578-97.
- [126] Singh KK. Economic competitiveness of Paulownia as a feedstock for ethanol and electricity production. Tennessee State University; 2015.
- [127] Mohammady NG, El-Sayed HS, Taha HM, Fakhry EM, Mahmoud NH, Mohamed JH, et al. Chlorella sp. as a Source of Biodiesel and By-Products: An Integral Study of Med-Algae Project; Part A. *Int J TechnoChem Res* 2015;1(3):144-51.
- [128] Bravo IN, Velásquez-Orta S, Cuevas-García R, Monje-Ramirez I, Harvey A, Ledesma MO. Bio-crude oil production using catalytic hydrothermal liquefaction (HTL) from native microalgae harvested by ozone-flotation. *Fuel* 2019;241:255-63.
- [129] Hu Y, Qi L, Feng S, Bassi A, Xu CC. Comparative studies on liquefaction of low-lipid microalgae into bio-crude oil using varying reaction media. *Fuel* 2019;238:240-7.
- [130] Sekoai PT, Ouma CNM, Du Preez SP, Modisha P, Engelbrecht N, Bessarabov DG, et al. Application of nanoparticles in biofuels: An overview. *Fuel* 2019;237:380-97.
- [131] Benemann JR, Weissman JC, Koopman BL, Oswald WJ. Energy production by microbial photosynthesis. *Nature* 1977;268(5615):19.
- [132] Demirbaş A. Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy conversion and Management* 2001;42(11):1357-78.

- [133] López-González D, Fernandez-Lopez M, Valverde J, Sanchez-Silva L. Comparison of the steam gasification performance of three species of microalgae by thermogravimetric–mass spectrometric analysis. *Fuel* 2014;134:1-10.
- [134] Adnan MA, Xiong Q, Hidayat A, Hossain MM. Gasification performance of Spirulina microalgae—A thermodynamic study with tar formation. *Fuel* 2019;241:372-81.
- [135] Hirano A, Hon-Nami K, Kunito S, Hada M, Ogushi Y. Temperature effect on continuous gasification of microalgal biomass: theoretical yield of methanol production and its energy balance. *Catalysis Today* 1998;45(1-4):399-404.
- [136] Hu Y, Gong M, Xu CC, Bassi A. Investigation of an alternative cell disruption approach for improving hydrothermal liquefaction of microalgae. *Fuel* 2017;197:138-44.
- [137] Minowa T, Sawayama S. A novel microalgal system for energy production with nitrogen cycling. *Fuel* 1999;78(10):1213-5.
- [138] McKendry P. Energy production from biomass (part 3): gasification technologies. *Bioresource technology* 2002;83(1):55-63.
- [139] Patil V, Tran K-Q, Giselrød HR. Towards sustainable production of biofuels from microalgae. *International journal of molecular sciences* 2008;9(7):1188-95.
- [140] Goyal H, Seal D, Saxena R. Bio-fuels from thermochemical conversion of renewable resources: a review. *Renewable and sustainable energy reviews* 2008;12(2):504-17.
- [141] Demirbaş A. Oily products from mosses and algae via pyrolysis. *Energy Sources, Part A* 2006;28(10):933-40.
- [142] Amin S. Review on biofuel oil and gas production processes from microalgae. *Energy conversion and management* 2009;50(7):1834-40.
- [143] Miao X, Wu Q. High yield bio-oil production from fast pyrolysis by metabolic controlling of Chlorella protothecoides. *Journal of biotechnology* 2004;110(1):85-93.
- [144] McKendry P. Energy production from biomass (part 2): conversion technologies. *Bioresource technology* 2002;83(1):47-54.
- [145] Kadam KL. Environmental implications of power generation via coal-microalgae cofiring. *Energy* 2002;27(10):905-22.
- [146] Minowa T, Yokoyama S-y, Kishimoto M, Okakura T. Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. *Fuel* 1995;74(12):1735-8.
- [147] Cantrell KB, Ducey T, Ro KS, Hunt PG. Livestock waste-to-bioenergy generation opportunities. *Bioresource technology* 2008;99(17):7941-53.
- [148] Olguín E. The cleaner production strategy applied to animal production. *Environmental biotechnology and cleaner bioprocesses* 2000;227-43.
- [149] Yang P, Tan G-YA, Aslam M, Kim J, Lee P-H. Metatranscriptomic evidence for classical and RuBisCO-mediated CO<sub>2</sub> reduction to methane facilitated by direct interspecies electron transfer in a methanogenic system. *Scientific reports* 2019;9(1):4116.
- [150] Yen H-W, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource technology* 2007;98(1):130-4.
- [151] Shokrkar H, Ebrahimi S, Zamani M. Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture. *Fuel* 2017;200:380-6.
- [152] Hirano A, Ueda R, Hirayama S, Ogushi Y. CO<sub>2</sub> fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. *Energy* 1997;22(2-3):137-42.

- [153] Ueno Y, Kurano N, Miyachi S. Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. *Journal of Fermentation and Bioengineering* 1998;86(1):38-43.
- [154] Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, et al. Microalgae: a green source of renewable H<sub>2</sub>. *Trends in biotechnology* 2000;18(12):506-11.
- [155] Melis A. Green alga hydrogen production: progress, challenges and prospects. *International Journal of Hydrogen Energy* 2002;27(11):1217-28.
- [156] Melis A, Happe T. Hydrogen production. Green algae as a source of energy. *Plant physiology* 2001;127(3):740-8.
- [157] Ahmad N, Javed F, Awan JA, Ali S, Fazal T, Hafeez A, et al. Biodiesel production intensification through microbubble mediated esterification. *Fuel* 2019;253:25-31.
- [158] Nwokoagbara E, Olaleye AK, Wang M. Biodiesel from microalgae: The use of multi-criteria decision analysis for strain selection. *Fuel* 2015;159:241-9.
- [159] Ahmad F, Khan AU, Yasar A. Transesterification of oil extracted from different species of algae for biodiesel production. *African Journal of Environmental Science and Technology* 2013;7(6):358-64.
- [160] Marrs E. Putting the carbon back: Black is the new green. *Nature Publishing Group*; 2006;442:624-26.
- [161] Lehmann J. A handful of carbon. *Nature* 2007;447:143-44.
- [162] Yamaguchi K. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *Journal of applied phycology* 1996;8(6):487-502.
- [163] Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, et al. Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences* 2005;102(14):5074-8.
- [164] Stolz P. Manufacturing microalgae for skin care. *Cosmetics Toiletries* 2005;120:99-106.
- [165] Chuntapa B, Powtongsook S, Menasveta P. Water quality control using *Spirulina platensis* in shrimp culture tanks. *Aquaculture* 2003;220(1-4):355-66.
- [166] Hu C, Li M, Li J, Zhu Q, Liu Z. Variation of lipid and fatty acid compositions of the marine microalga *Pavlova viridis* (Prymnesiophyceae) under laboratory and outdoor culture conditions. *World Journal of Microbiology and Biotechnology* 2008;24(7):1209-14.
- [167] Pulz O, Gross W. Valuable products from biotechnology of microalgae. *Applied microbiology and biotechnology* 2004;65(6):635-48.
- [168] Davis R, Aden A, Pienkos PT. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 2011;88(10):3524-31.
- [169] Benemann JR. Open ponds and closed photobioreactors—comparative economics. *5th Annual World Congress on Industrial Biotechnology and Bioprocessing*. Chicago. 30. 2008.
- [170] Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource technology* 2011;102(1):71-81.
- [171] Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y. Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresource technology* 2011;102(1):159-65.
- [172] Cheung K, Chu L, Wong M. Toxic effect of landfill leachate on microalgae. *Water, Air, and Soil Pollution* 1993;69(3-4):337-49.

- [173] Mu D, Min M, Krohn B, Mullins KA, Ruan R, Hill J. Life cycle environmental impacts of wastewater-based algal biofuels. *Environmental science & technology* 2014;48(19):11696-704.