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

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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 CO₂, 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

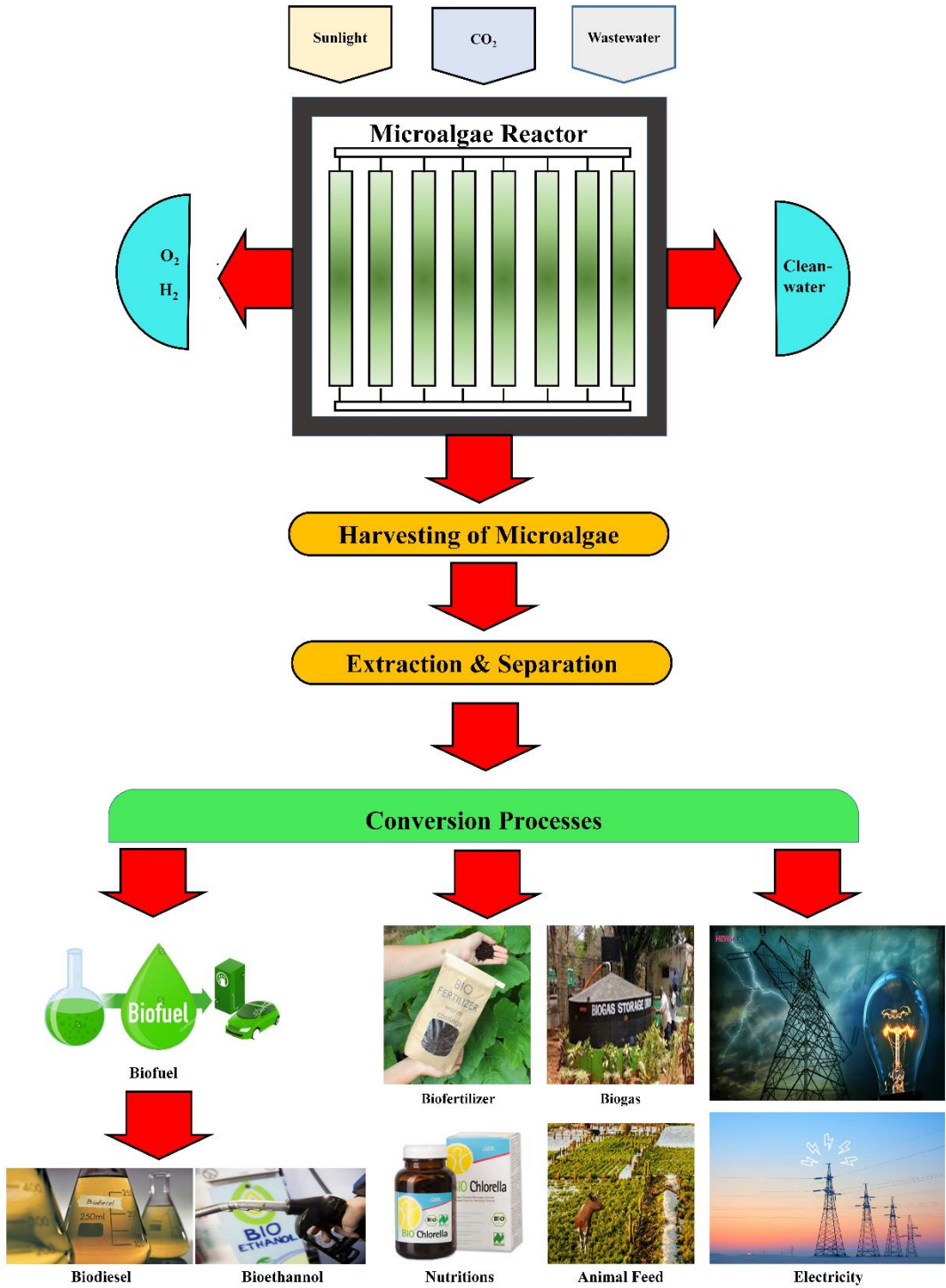
4 **1 Introduction**

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 CO₂ 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 CO₂ 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 Table 1.

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</i> sp. UMACC 112	Green	53.7	
<i>Chlorella vulgaris</i> CCAP 211/11b	Green	170	
<i>Ellipsoidion</i> sp.	Eustigmatophytes	47.3	
<i>Monodus subterraneus</i> UTEX 151	Eustigmatophytes	30.4	
<i>Pavlova salina</i>	Prymnesiophytes	49.4	[31]
<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	[37]
<i>Nannochloropsis oculata</i> .	Green	84-142	
<i>Phaeodactylum tricornutum</i>	Diatoms	44.8	
<i>Nannochloropsis oculata</i> NCTU-3	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 CO₂, 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 CO₂ uptake for microalgae growth- (i) CO₂ as
12 discharge gas from industry, (ii) from atmosphere and (iii) from carbonates [41]. Recent reports
13 revealed that air contains 400 ppm of CO₂ [42], but most microalgae utilize higher CO₂ levels [36].
14 Therefore, microalgae production uses some external sources of CO₂ such as industrial discharge
15 or soluble carbonates at commercial-scale [42]. Fig. 2 shown conventional routes of microalgae
16 cultivation.

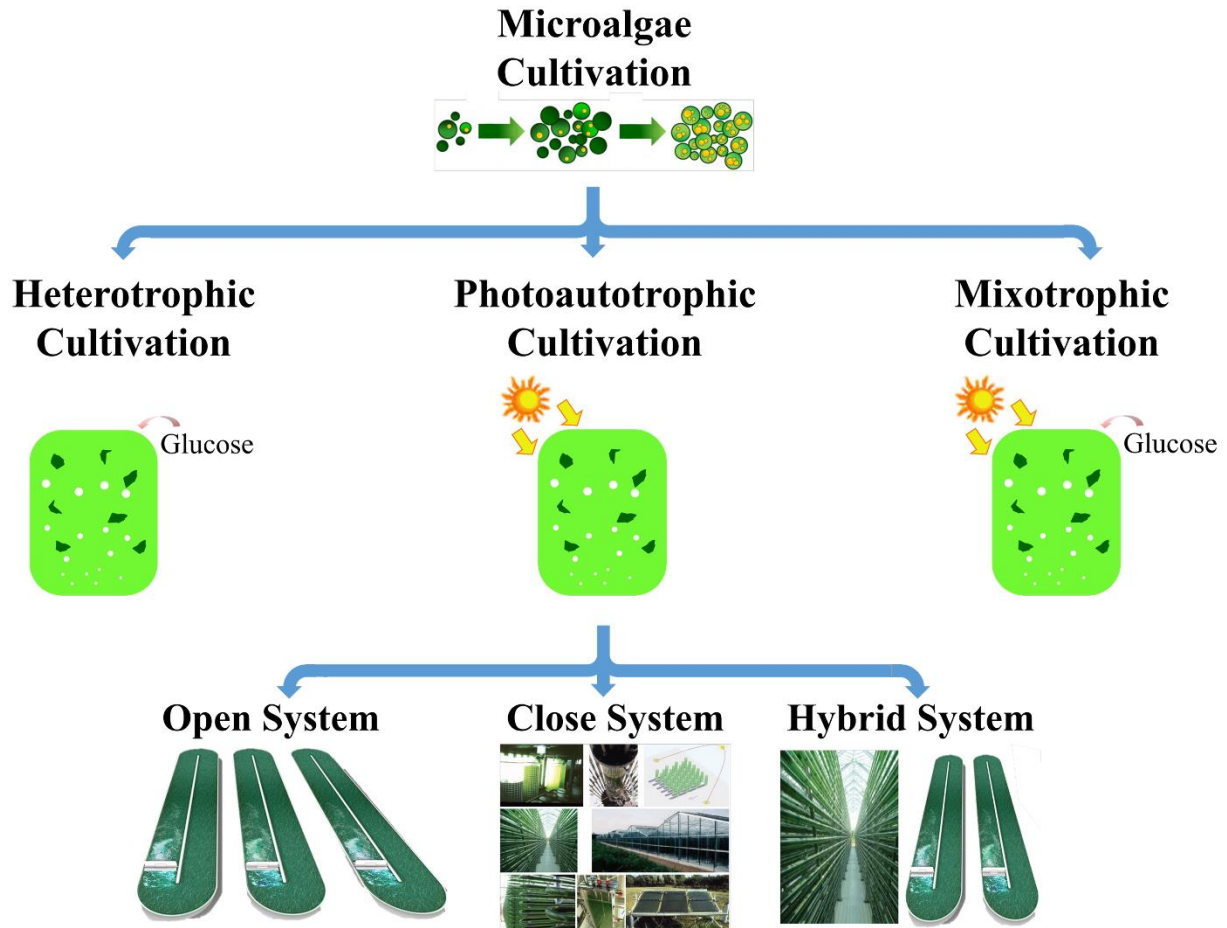


Fig 2. Microalgae cultivation system

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 CO₂ and O₂). Typically, closed systems consist of four phases: solid, liquid, gas, and light
16 phase. Microalgae as solid phase, growth media liquid phase, CO₂ and O₂ 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 25m³ at Mera Pharmaceuticals Hawaii [55], and even larger at Klotze, Germany having a volume
5 of 700 m³ [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].

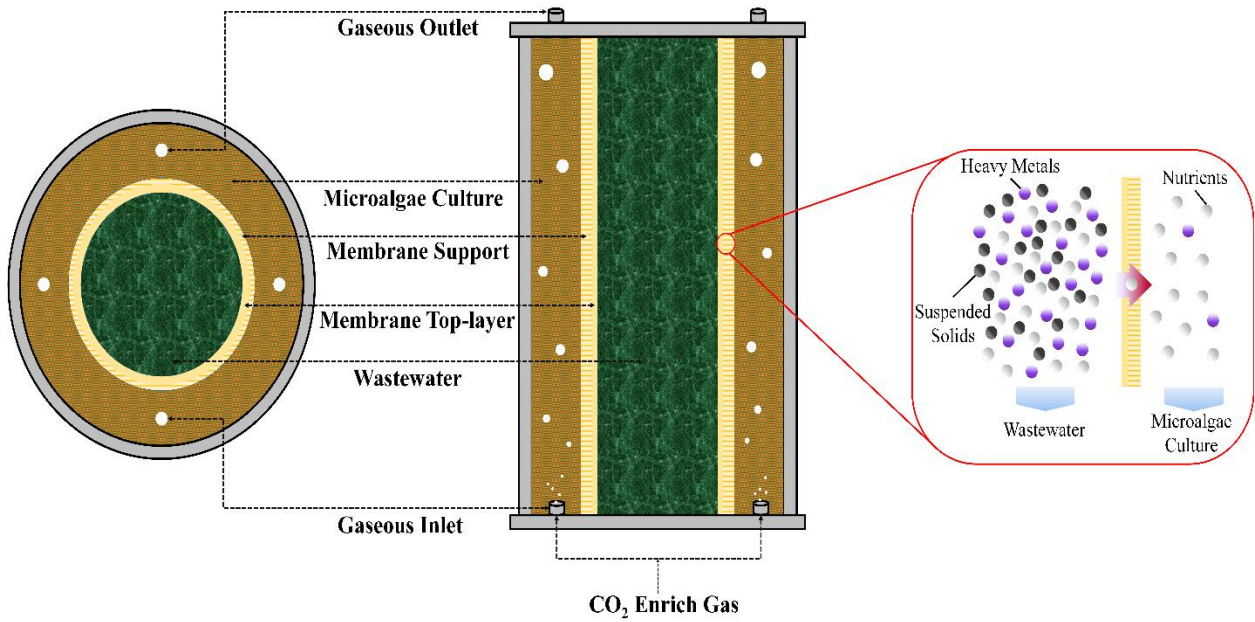
8 **2.1.2.1 Membranes photobioreactors**

9 The biomass productivity of microalgae cultivation system largely depends on CO₂
10 delivery. Generally, CO₂ 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 CO₂ than conventional bioreactors.

13 CO₂ 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 CO₂
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/m³/h was reported for *Chlorella*
21 *vulgaris*. The analysis shows that a combination of wastewater treatment, CO₂ 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 microalgae 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 ₂ capture and removal of nutrients	Aeration: 0.156 L/min Pore size: 0.1 μm Flux: 2.2-4.4 L/m ² /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 ² /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 ² /h	[61]
Polyacrylonitrile ultra-filtration flat sheet	<i>Haslea ostrearia</i>	Production of exocellular metabolite (marennine pigment)	Flux: 3-10 L/m ² /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 ² /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 tricorutum</i>	1.2	[65]
Tubular system	<i>Phaeodactylum tricorutum</i>	1.9	[47]
Inclined tubular system	<i>Chlorella sorokiniana</i>	1.47	[66]
Undular row tubular system	<i>Arthrospira platensis</i>	2.7	[67]
helical tubular system	<i>Phaeodactylum tricorutum</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	[74]
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/M²/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>Cryptocodinium cohnii</i>	Docosahexaenoic acid	Batch	56	[80]
<i>Cryptocodinium cohnii</i>	Docosahexaenoic acid	Batch	42	
<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.

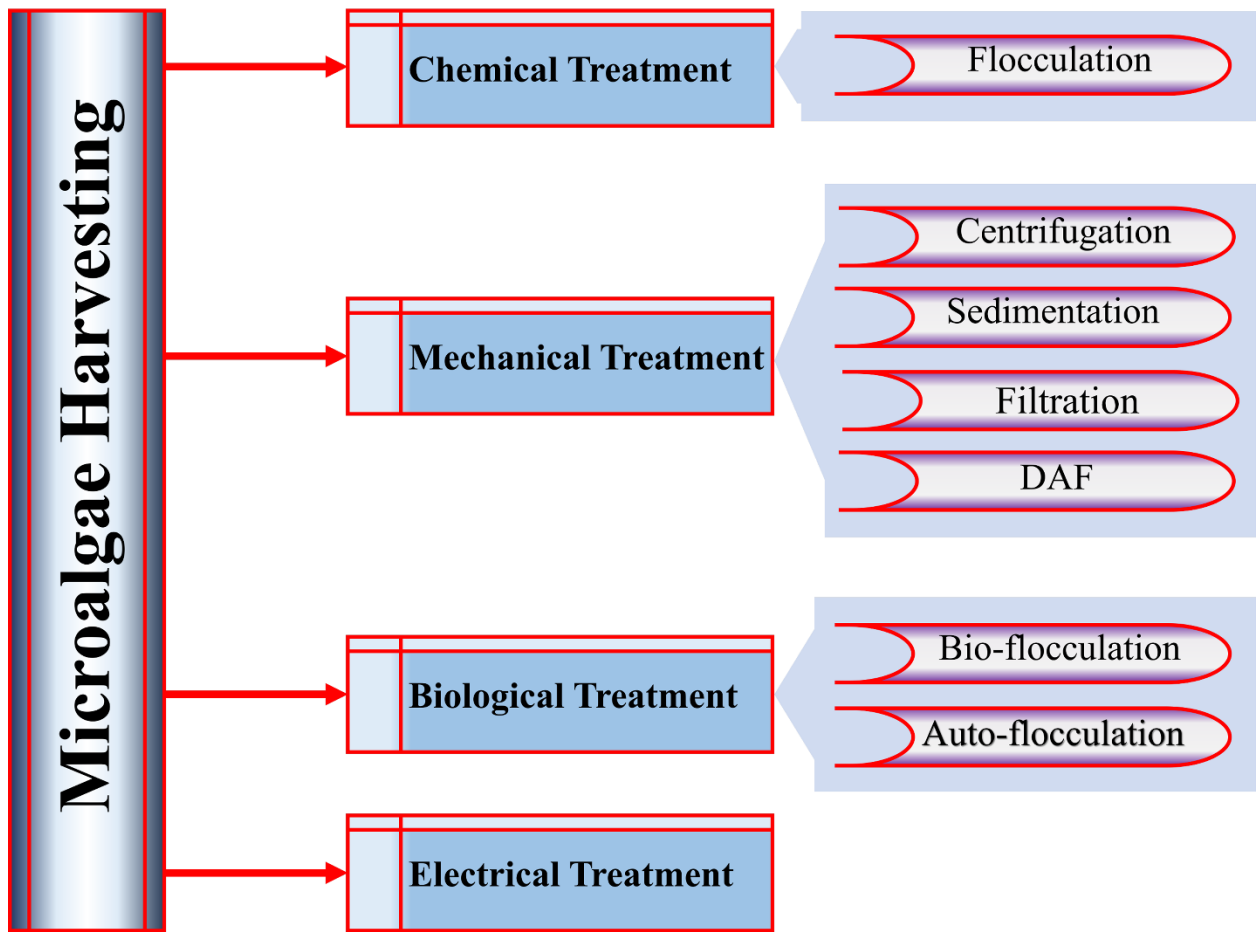
10 **Table 5** Mixotrophic species and biomass concentrations

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 μm), 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].



1

2

Fig 4 Techniques for microalgae harvesting

3 **3.1 Chemical treatments**

4 Owing to smaller cellular size, microalgae tend to remain suspended in aqueous solution
 5 and difficult to remove from the medium due to stable suspension. The addition of chemical
 6 flocculants can help to destabilize the cells and agglomerate them to settle down through
 7 gravitational forces. Synthetic polymers and electrolytes are used to neutralize the charge and
 8 flocculate the cells [88]. Ferric chloride and aluminum sulfate are used for neutralization of charge
 9 due to presences of +3 charges on their cations. The use of metal salts in flocculation and
 10 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 μm . 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. Petrusovski 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 ³	- Reliable - Solid concentration is high	- Fouling - High cost
Sedimentation	0.5-3	10-90	- Very high 8 kWh/m ³	- Cheap	- Time consuming process - Unreliable
Dissolved air flotation	3-6	50-90	- High dissolved air flotation 10–20 kWh/m ³	- 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 CO₂ 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 1st 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 2nd 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 crops 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 3rd generation, algae are used to produce biofuels. It is a viable alternate energy source
11 as it tackles all the major issue with 1st and 2nd 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.

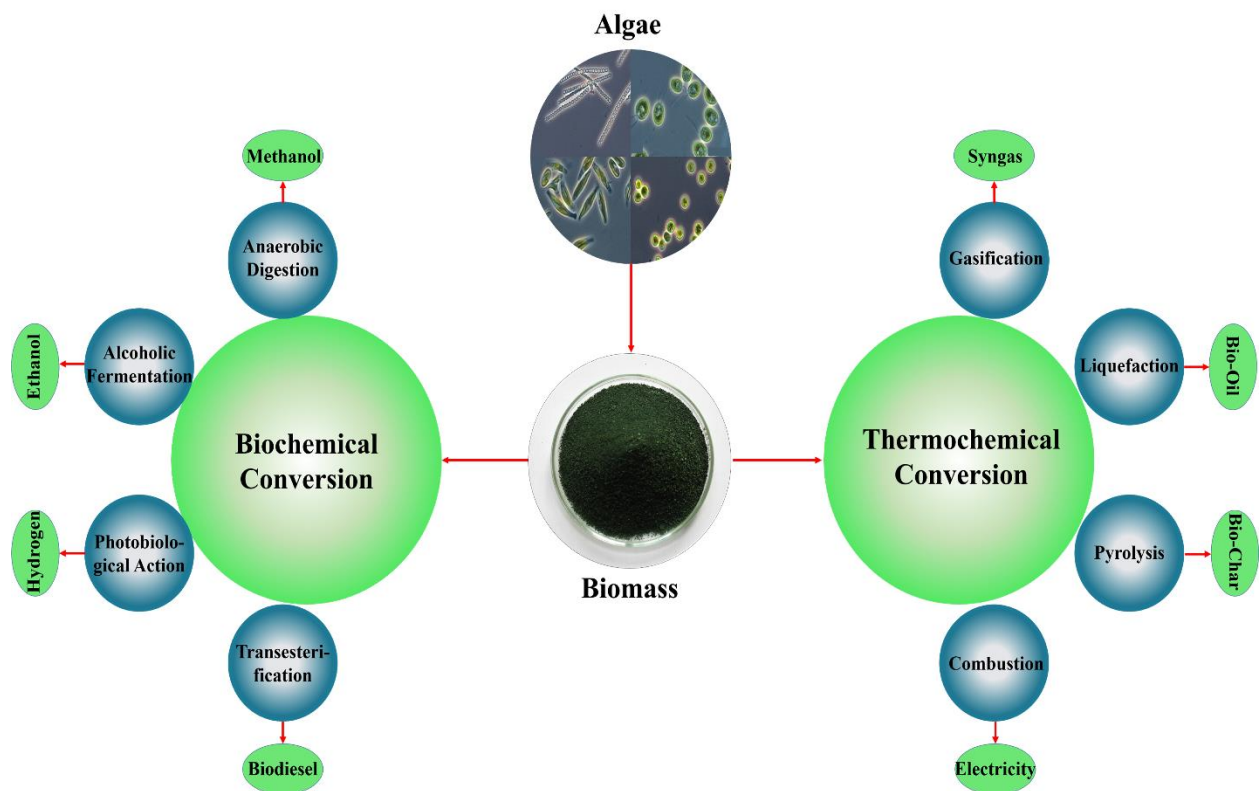
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Table 7 Comparison of all three generation biofuels

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

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].



7

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/m³. 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 H₂ 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 prothothecoides* and *Microcystis*
16 *aeruginosa* through fast pyrolysis [143].

17 **4.1.1.1.4 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 CO₂, H₂O, 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 CO₂ 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 prothothecoides</i>	Pyrolysis	450	0.101	57.9	
<i>Chlorella prothothecoides</i>	Pyrolysis	450	0.101	16.6	[143]
<i>Chlorella prothothecoides</i>	Pyrolysis	500	0.101	18	
<i>Chlorella prothothecoides</i>	Pyrolysis	500	0.101	24	
<i>Chlorella prothothecoides</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 $\mu\text{mol/g}$ 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 H₂O oxidation electron is released. The electron is
3 directly fed into the hydrogenase-mediated H₂-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].

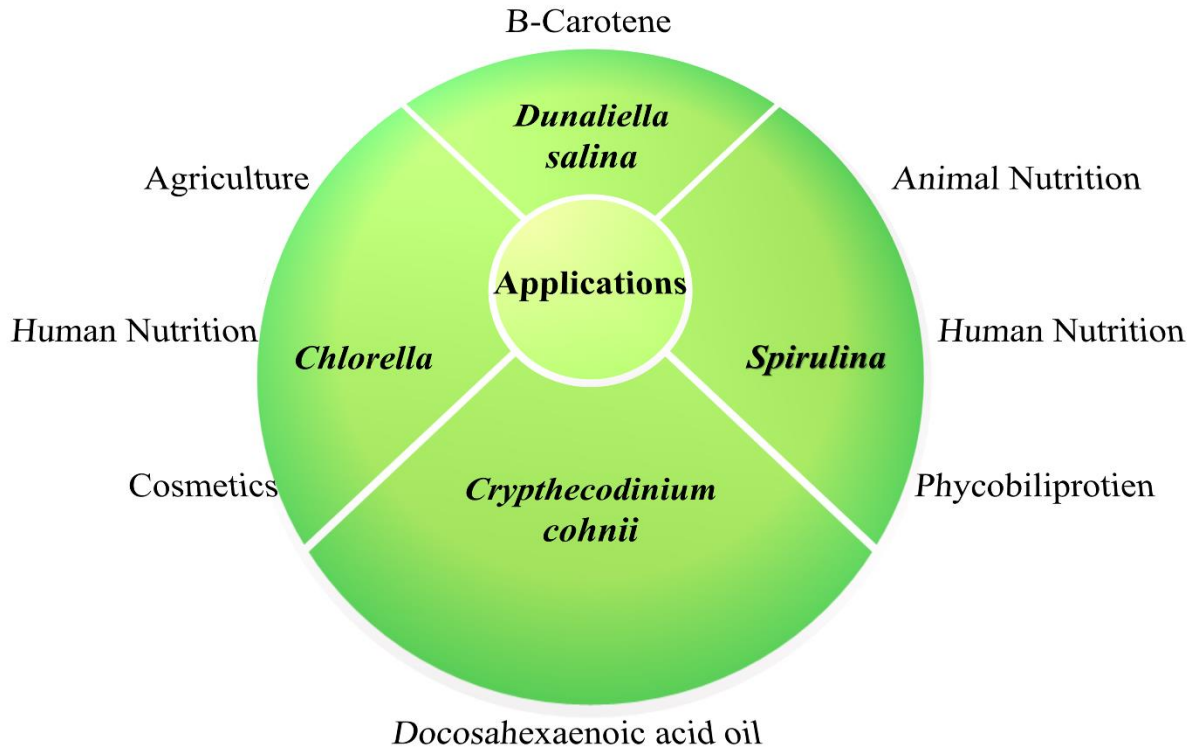
16 **4.2 Commercial applications of algal biomass**

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].



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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.

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**

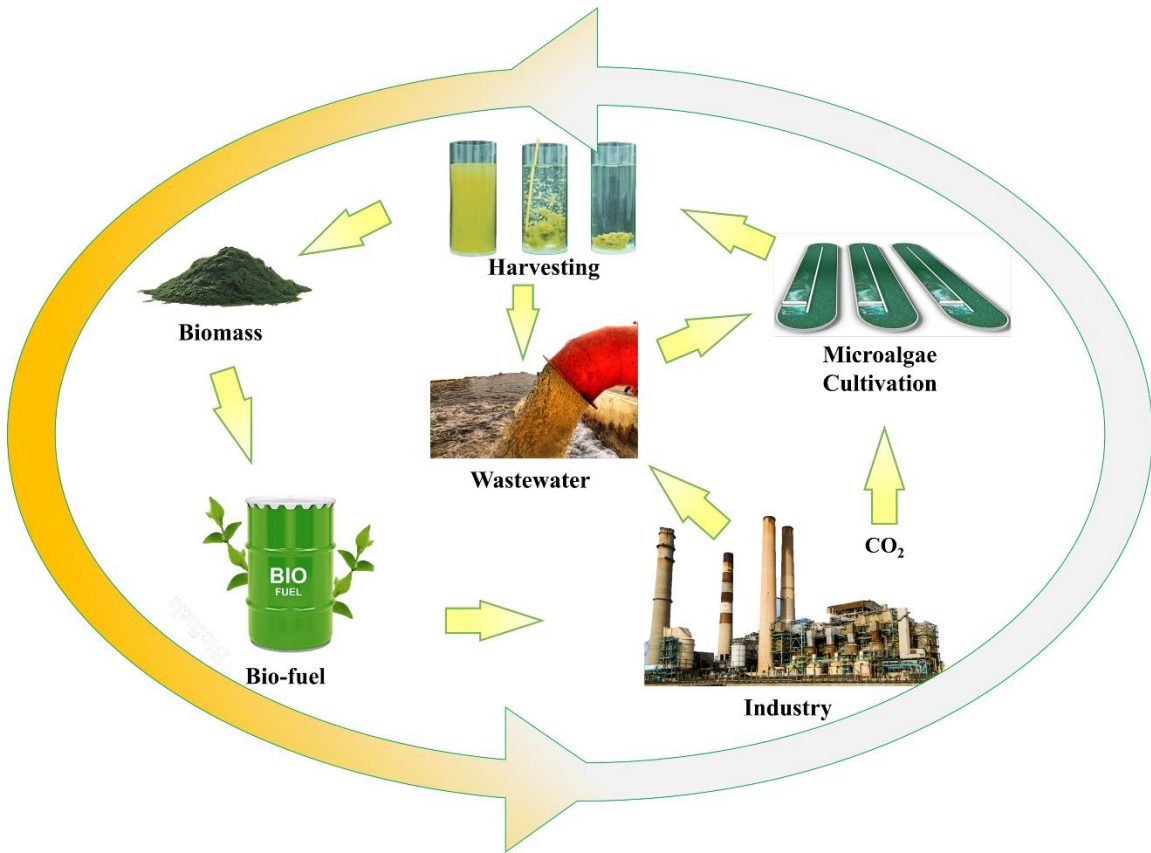
10 There are many challenges for the commercialization of algal biomass for bioenergy
 11 production such as high operating and capital cost. To understand the current scenario, many
 12 studies have been concluded for both open pond and photobioreactors. To find the way for
 13 reducing costs, a brief economic analysis was performed by Davis et al. [168]. \$8.52/gal for 25
 14 g/m²/day were achieved for open system and cost for photobioreactor achieved \$18.10/gal for 1.25
 15 kg/m³/day, which provided biodiesel cost of \$9.84/gal, \$20.53/gal for open pond 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.



3
4 **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.

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