sustainability

Review

Microalgae Potential and Multiple Roles—Current Progress and Future Prospects—An Overview

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Abstract: Substantial progress has been made in algal technologies in past few decades. Initially, microalgae drew the attention of the scientific community as a renewable source of biofuels due to its high productivity over a short period of time and potential of significant lipid accumulation. As of now, a technological upsurge has elaborated its scope in phycoremediation of both organic and inorganic pollutants. The dual role of microalgae—i.e., phycoremediation coupled with energy production—is well established, however, commercially, algal biofuel production is not yet sustainable due to high energy inputs. Efforts are being made to make the algal biofuel economy through modification in the cultivation conditions, harvesting, and extraction of value added products. Recent studies have demonstrated algal biomass production with various types of wastewater and industrial effluents. Similarly, the recent advent of eco-friendly harvesting technologies—such as low-cost green coagulants, electrochemical harvesting, etc.—are energy efficient and economical. Contemporary improvement in efficient lipid extraction from biomass will make algal biodiesel economical. The absolute extraction of all the value added products from algal biomass, either whole cell or lipid extracted biomass, in a complete biorefinery approach will be more economical and eco-friendly.

Keywords: microalgae; lipid extraction; energy; biofuel; value added products; waste water treatment; CO_2 sequestrations

1. Introduction

Energy usage is dramatically increasing, and the worldwide demand is estimated to rise by more than 85% by 2040 [1]. Fossil fuel resources provide most of the world's energy demands, but are limited, and thus additional sources of renewable energy must be considered. Biofuels have significant potential to supply a possible portion of our society's energy demands. Three generations of biofuels have emerged so far. The first generation of biofuels, also known as 'conventional biofuels', are produced mainly from edible plant parts (beet sugarcane, potatoes, corn, oilseeds, grains, etc.); the second generation of biofuels refers to energy production from 'plant biomass' (non-edible plants and its parts); and the third generation of biofuel production is from unicellular photosynthetic microorganisms such as microalgae (Figure 1). However, the first and second generation biofuel feedstocks have limitations that pose new challenges such as the arable land occupation which would contribute to food crises. Competition between food and fuel is one of the serious concerns regarding sustainability today, as the need for land to produce food is more important for the increasing population instead of the production of fuels.

As per a 2003 Food and Agriculture Organization (FAO) report, on average, 25,000 people die of hunger every day in the world [2]. Third generation biofuels emerged as a viable option, with reference to maintaining the balance between both economic and environmental sustainability. Third generation biofuels are extracted from algae (especially microalgae) or other rapidly growing biomass sources which are away from the debate of food over fuels. As per estimates 20,000–80,000 L algae oil can be produced per acre which is almost 30 times higher than oil crops such as palm oil [3]. Such lipids can easily be converted into the biofuels by bio/thermochemical methods. Fourth generation biofuel production involves the use of metabolic engineering or genetically modified (GM) organisms (especially microalgae) and has great potential to achieve sustainable and clean energy through increases the photosynthetic ability of the microbial cell [4]. However, due to several practical reasons, the current GM algal culture in open ponds is ineffective for industrial scales. Microalgal biomass is considered a high-energy renewable feedstock, but pilot scale economic production is still challenging.

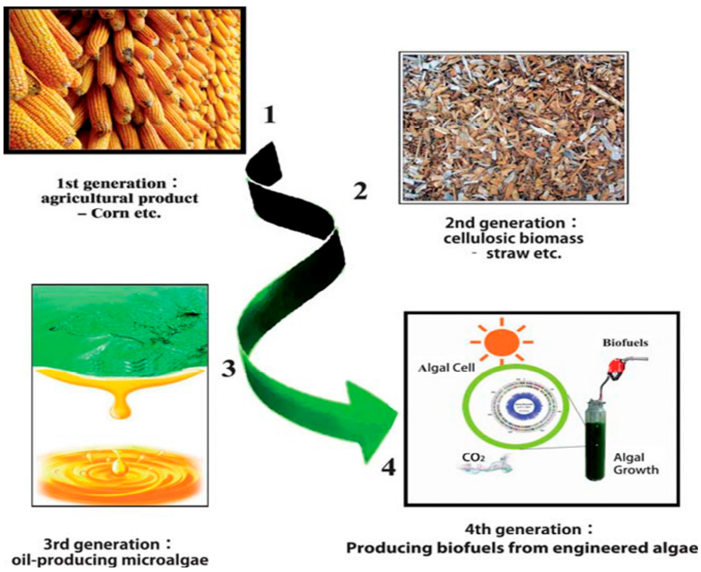

Figure 1. Four generations of biofuel production: from agricultural products to algae. Reprinted with permission from [4], copyright 2011, Royal Society of Chemistry.

2. Microalgae: As Feedstock for Biofuel

2.1. Biomass Composition of Microalgae

Microalgae are unicellular and photosynthetic microorganisms, ranging from 0.2 to 2 µm (picoplankton) up to filamentous forms with sizes of 100 µm or higher (Figure 2) [5–7]. These are unicellular organisms that consist of both prokaryotic (*Cyanophyceae*) or eukaryotic (*Chlorophyta*) organisms and they can grow rapidly in aquatic environments such as fresh water, waste water, and the marine environment.

Among various microalgae, a few selected species—such as *Scenedesmus*, *Chlorella*, etc.—have the ability to survive in the most extreme environments (e.g., high temperature and high CO_2) [8]. Marchetti et al. [9] reported that the biochemical composition of microalgal cells varies with species and depends on culture and geographical conditions. The growth, biomass yields, as well as the micro and macro metabolites of microalgae are mainly affected by environmental factors such as light, pH, temperature, nutrients, etc. The typical major metabolites of an algal cell—proteins, carbohydrates, lipids, and other chemical compounds—are shown in Figure 3 [10]. The biomass composition of various microalgae species is presented in Table 1.

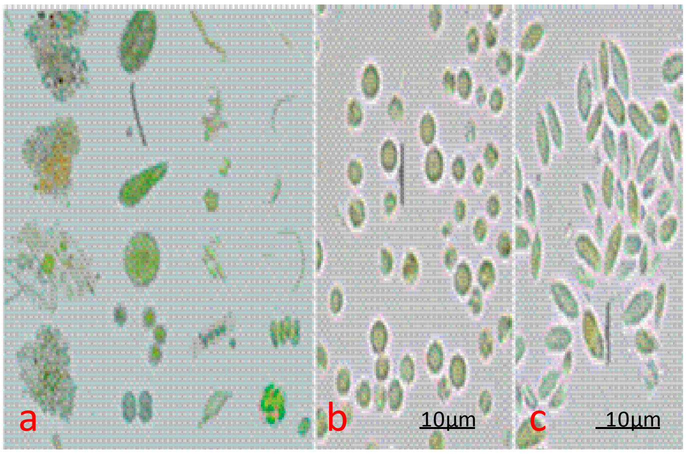

Figure 2. Microscope images of various microalgae species. (**a**) Overview of the different microalgae presents in an inoculums; (**b**) *Scenedesmus*; (**c**) *Chlorella*. Reprinted with permission from [5], copyright 2013, Royal Society of Chemistry.

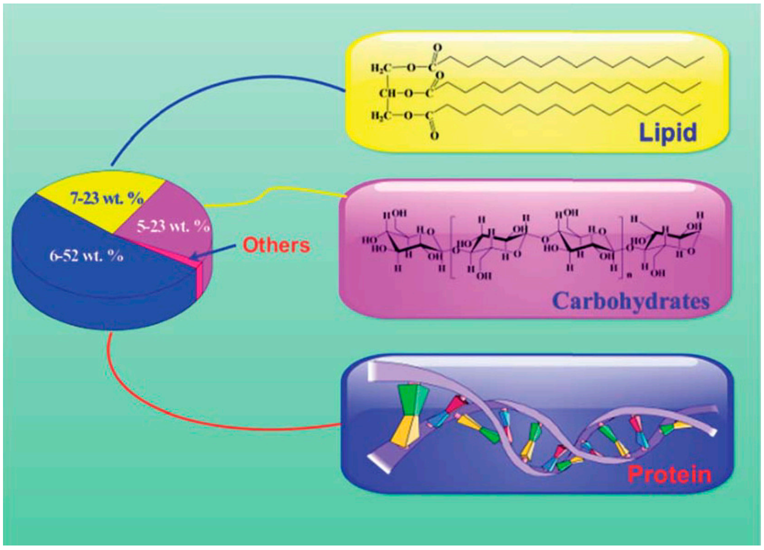

Figure 3. Components of typical microalgae. Reprinted with permission from [10], copyright 2015, Royal Society of Chemistry.

2.1.1. Carbohydrates

Microalgae contain carbohydrates, a wide category encompassing sugars (monosaccharides) and their polymers (di-, oligo-, and polysaccharides), and they serve both structural and metabolic functions. The most abundant carbohydrates are mainly glucose (21%–87%), galactose (1%–20%), and mannose (2%–46%) and varying amounts (0%–17%) of arabinose, fucose, rhamnose, ribose, and xylose [11]. In algal cells, these carbohydrates are synthesized inside the chloroplast, whereas in the case of prokaryotes, carbohydrates are synthesized in the cytosol. In algal cells, the most abundant carbohydrates are glucose, rhamnose, xylose, and mannose. However, the carbohydrate percentage of the cells depends on the microalgal species, cultivation, and environmental conditions. Several microalgal species possess high carbohydrate contents, such as *Spirogyra* sp. (33%–64%), *Porphyridium cruentum* (40%–57%), *Chlorella emersonii* (37.9%), *Chlorogloeopsis fritschii* (37.8%) [12,13].

Table 1. Biomass composition of microalgae expressed on a dry matter basis [12,13].

Strain	Protein (%)	Carbohydrates (%)	Lipid (%)
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	41–58	12–17	10–22
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	29	14	11
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Prymnesium parvum	28–45	25–33	22–39
Scenedesmus dimorphus	8–18	21–52	16–40
Scenedesmus obliquus	50–56	10–17	12–14
Scenedesmus quadricauda	47	-	1.9
Spriogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechoccus sp.	63	15	11
Tetraselmis maculata	52	15	3
Pseudochoricystis ellipsoidea	10.2	34	38
Chlorogloeopsis fritschii	41.8	37.8	8.2
Chlorella emersonii	9.03	37.9	29.3
Chlorella zofingiensis	11.2	11.5	56.7
Chlorella FC2 IITG	10.4	24.5	37.3

Such high carbohydrate contents can be converted into biofuels by various biochemical or thermochemical processes. Markaou et al. [14] suggested that the high innate carbohydrate content helps to maximize the production of biofuels. As such, carbohydrates can be converted to various forms of biofuels by several biomass conversion technologies such as anaerobic digestion, anaerobic fermentation, and biological biohydrogen production.

2.1.2. Proteins

Microalgae have the ability to synthesize all essential amino acids within their cell, thus possesses high levels of proteins. The amino acid pattern of the algae compares positively with that of other proteins [15]. Moreover, proteins also have both structural and metabolic functions, and the cellular proteins are the major constituent of the photosynthetic apparatus, cell growth machinery and CO_2 fixation pathways. [16]. Very high protein content has been reported in some of microalgae species such as *Spirulina maxima*, (60%–71%), *Synechoccus* sp. (63%), *Anabaena cylindrical* (43%–56%), and *Chlorella vulgaris* (41%–58%). Therefore, all algal species with high protein contents can serve as an ideal source of nutrients for functional foods, nutraceuticals, and food additives. However, the high protein content implies high nitrogen content, which is undesirable for biofuel production [17].

2.1.3. Lipids

Microalgal lipids occurred in the range of 20%–70%, and the fatty acid composition of algal cells depend upon genetic and phenotypic factors, including environmental and culture conditions [18]. The algal lipids can be divided into two categories; (a) the polar lipids and (b) non-polar lipids. Polar lipids are also known as structural lipids containing maximum content of polyunsaturated fatty acids (PUFAs). These PUFAs are essential for the nutrition of humans and aquatic animals. Sterols and polar lipids are the key structural components of cell membranes, providing the matrix for different metabolic processes. It also acts as key intermediates in cell signaling pathways. On the Other hand, Nonpolar lipids also known as storage lipids or neutral lipids. These storage lipids mostly include triacylglycerols (TAGs), are predominantly saturated fatty acids, and some unsaturated fatty acids that can be converted to energy (biodiesel) by transesterification [19]. Profiling of lipids in biomass feed stocks is critical for the production of quality biodiesel as well as other algal biofuels. Lipids are mostly accumulated in microalgae under specific environmental stress conditions, such as phosphate or nitrogen limitation [20]. Fatty acids composition and the profile of a particular species, ranging from the length of 12 to 22 carbons, is affected by the various factors such as growth medium composition, aeration rate, temperature, the ratio of light/dark cycle, and illumination intensity [21].

2.2. Biofuel Production from Microalgae

In recent years, microalgae are seen as an alternative biodiesel feedstock and have attracted tremendous interest. Microalgae are considered living cell factories for the production of bio-fuels. The initial stage of microalgae biofuel production is the cultivation process, either by open ponds/raceways or through photobioreactors. The open ponds/raceways, are shallow circular big ponds varying in forms and shapes. Whereas, the photobioreactor cultivation systems are horizontal tubular reactors or external tubular loop reactors used for microalgae cultivation in controlled conditions. After cultivations, microalgal cells are harvested by various techniques such as centrifugation [21], filtration, and/or flocculation [22,23] with the help of flocculants. The harvested biomass can be extracted to obtain its oil, which is converted into biodiesel and bioethanol through biochemical and thermal conversion processes [24]. Biochemical conversion utilizes microorganisms [25] whereas thermochemical conversion utilizes heat to decompose organic components [26] to produce biofuel from the biomass. At the same time, transesterification and photosynthetic microbial fuel cell processes are also used to produce biodiesel and bioelectricity, respectively. These overall processes have been established so that algal biomass is a comparatively better biofuel feedstock than the traditional feedstock. Chisti [27] and Deng et al. [28] reported up to 70% oil content available in some species of microalgae such as *Botryococcus braunii* and *Schizochytrium* spp. and it could reach up to 121,104 kg/ha per year biodiesel productivity. Marine microalgae bear potential resources for biofuel purposes and can be used to produce biogas, including hydrogen and methane via an anaerobic digestion process [29]. An overview of microalgal biofuel transformation processes is provided in Figure 4 [30].

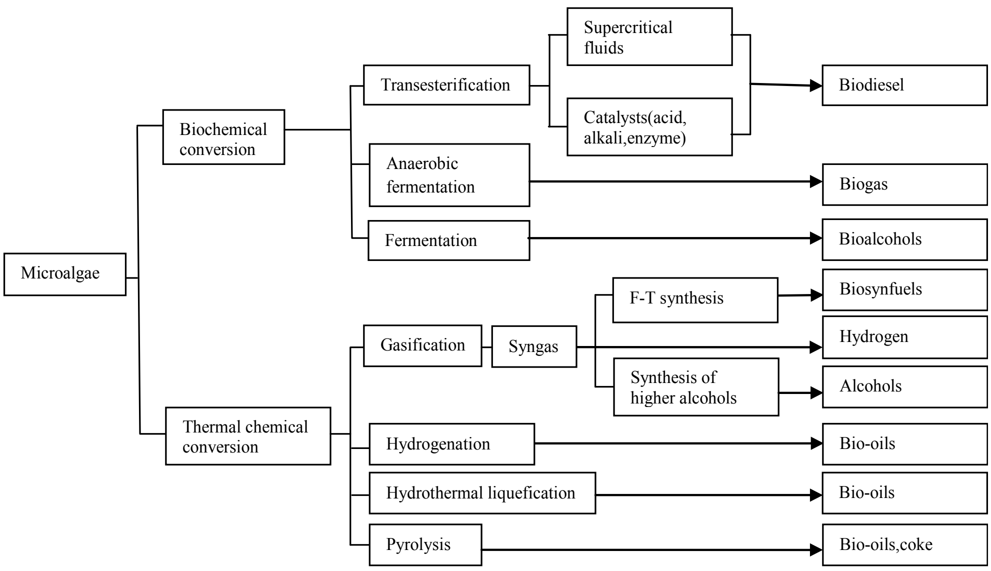

Figure 4. The flow diagram of potential energy conversion processes from microalgae. Reprinted with permission from [30], copyright 2014, Royal Society of Chemistry.

3. Microalgae Cultivation Techniques

The cultivation of microalgae is one of the most important aspects of algal biofuels. Numerous types of algal cultivation systems are in practice. However, most of them are mainly based either on open ponds or raceways and closed bioreactor systems.

3.1. Open Pond Culture Systems

Open pond systems are the first and most common oldest system for mass cultivation of micro algae. The open pond is usually between 1 and 100 cm deep, from about one acre to several acres in size. Major pond systems are circular ponds, shallow big ponds, and raceway ponds [31]. Borowitzka [32] suggested that these systems are depending upon types of algal species, climatic conditions, and the cost of lands and water. The most popular type is the paddle-wheel raceway pond, because its shape resembles a race track and the liquid is circulated around the pond by a paddle wheel [27]. Raceway ponds are utilized mostly for algal cultivation and wastewater treatment [33]. The major advantage of open ponds is that they are easier to construct and operate than closed cultivation systems. However, major drawbacks include high land requirements, poor light utilization by the cells, contamination issues, diffusion of CO_2 to the atmosphere, and water loss due to evaporation. Due to such drawbacks, closed photobioreactors are preferred for cultivation of microalgae over the open pond.

3.2. Closed Photobioreactors

Closed photobioreactors (PBR) are very versatile systems and can be located both indoors and outdoors with artificial light and natural light, respectively. These systems have overcome the major issues associated with open-pond cultures. Mainly photoautotrophic algae are cultivated in open systems, whereas closed cultivation systems are used for both photoautotrophic and heterotrophic cultivations. The closed photobioreactors are tubular transparent vessels of varying shape and sizes. The most popular of these systems are tubular PBR, helical PBR, airlift PBR, and flat panel PBR (flat plate). However, due to more advantages, tubular PBR is widely used in this field and these can be run either vertically or horizontally. It has a number of clear transparent tubes, composed of either glass or plastic measuring 10 cm or less in diameter, which allows for sufficient light penetration. Moreover, algal biomass are prevented from settling by maintaining highly turbulent flow within the reactor with either a mechanical pump or an airlift pump [27].

In algae cultivations, the cost of the nutrient media is one of the major hurdles for economic biomass production. Therefore, efforts are being made to substitute the costlier nutrient media with comparatively cheaper nutrient sources. Among various options nowadays the use of various types of wastewater is in practice which serves dual advantages of biomass production along with waste water treatments. Other cultivation practices are also in use and some of the examples are as follows.

3.3. Culture Using Deep Sea Water

Deep-sea water (DSW) utilization has also received substantial attention from past few decades due to its vastly available quantity and potential for recycling energy. DSW contains traces of various elements and nutrients that could be stimulating the production of specific components/metabolites in the microalgae [34]. Tan et al. [35] reported that the oil-rich microalga *Chlorella sorokiniana* CY1 was cultured in 50% DSW in BG-11 medium to determine its growth and oil production and this culture method achieved a comparatively higher biomass (2.4 g/L) along with a higher oil yield of 176.6 mg/L/day. Various other studies also demonstrated that with minor modifications of composition or the addition of a comparatively smaller amount of nutrients, DSW could be used for high biomass yield of various marine microalgae species [36].

3.4. Co-Culture Methods

Algal-bacterial consortia are getting tremendous attention nowadays due to their high phycoremedial potential and biomass yield. Therefore, co-culture methods are being considered for improving microalgal cultivation and harvesting process. Gonzalez and Bashan [37] reported the *Chlorella vulgaris* was successfully co-immobilized and co-cultured with plant growth promoting bacteria (*A. brasilense*) in small alginate beads. The results revealed that *A. brasilense* boosted the growth of *C. vulgaris* through indole acetic acid (IAA) utilization of the amino acid tryptophan conversion process [38]. The *Chlorella vulgaris* exhibits poor harvesting due to flocculation efficiency of 0%–2% [39], but its co-culturing with bioflocculant-producing bacteria improved the harvesting. This highlights cost-effective production of microalgal biomass for biofuels. Use of fungi (*A. fumigatus*) cells in co-cultivation of algae, showed synergistic and additive effects on wastewater bioremediation, biomass production, and lipid yield efficiency [40]. Such insights are promising with respect to sustainable as well as economic microalgae cultivation and harvesting.

4. Novel Approaches for Harvesting of Microalgal Biomass

Microalgal harvesting refers to the concentration of the dilute culture suspension to paste or slurry and it contains 5%–25%, or more, total suspended solids (TSS). It means, 2%–7% TSS can be achieved using flocculation, flotation, and/or sedimentation and 15%–25% TSS can be achieved with filtration or centrifugation [6]. Sedimentation is one of the simplest ways of process to harvest microalgae through solid–liquid separation. However, there are several techno-economic hurdles in the harvesting of algae due to the negatively charged, very small cell size of individual oleaginous cells (<30 μm). The cell densities are similar to the negative water surface charges. So, due to low settling velocities (10^{-5}–10^{-6} $m \cdot s^{-1}$), the gravity settling is ruled out which is a cheaper harvesting method and this adds an additional 20%–30% harvesting cost to the other operation cost [30]. Centrifugation is a process of recovery of algal biomass by using centrifugal force to accelerate the rate of sedimentation. This separation process is based on algal cell size and density difference between the algal biomass and the medium. The main advantage of this process is that it is easy to apply to all strains and able to recover/concentrate at a high rate, and the harvested biomass is free from flocculants or any other chemical contamination due to the absence of chemical addition process. Although it has some advantages, this process is energy intensive and requires higher maintenance costs, which are major disadvantages of this process. Moreover, the filtration process is time-consuming and requires the backwash process for membrane filter systems. For the primary concentration, flocculation is seen as a promising, low-cost harvesting method able to harvest small-sized microalgae cells through the aggregate steps.

To date, the flocculation is one of the easiest and most cost effective methods of harvesting of microalgae and developments of various new flocculants are in practice [23,41]. To increase the particle size, some of other harvesting methods (centrifugation or gravity sedimentation) can be applied. However, harvested biomass quality is affected by the nature of the flocculant. Ultrasound technique for microbial harvesting is a comparatively younger technique and currently under development [42]. Electrochemical harvesting (ECH) is also getting popular. Misra et al. [43] reported that the ECH process can be an advantageous process for harvesting different species of microalgae. However, the cost of the electrodes are an economic concern. To reduce the processing cost of microalgal biofuels or other products some of novel methods were introduced for microalgae harvesting.

Cell pelletization with an attached microalgal growth system has been found to be a novel method of trapping the microbial cells and cultivating the microalgae on supporting structures in the photobioreactors [35]. In the cell pelletization process, filamentous fungi have great potential to form into large pellets, trapping the micro algal cells. This process enables easy separation through simple filtration due to higher density pellets than water [44]. In the attached microalgal growth system, the tendency of microalgal cells is to be suspended (planktonic) in stagnant waters, but attached (benthic) in lotic (high current velocity) conditions. The shaking mechanism is used to promote strong binding of the microalgal cells onto the submerged state support structures in the culture medium [45]. These methods significantly decrease the processing cost of microalgal biomass production for biofuel or other products [46].

5. Technologies for Effective Lipid Extraction from Microalgae

Algal biodiesel production includes five major processes—i.e., cultivation, harvesting, drying, cell disruption, and oil extraction (lipid extraction)—and transesterification of extracting lipids from microalgae. Among all these methods, cell disruption processes are of immense significance, as it is very important for determination of the quality and quantity of the microalgae cellular extracted lipids for biofuel production [16]. Therefore, the suitable cell disruption method and device is a key factor for improving the efficiency of lipid extraction. Presently, various methods—i.e., autoclaving, microwaves, ultra-sonication, bead-beating, osmotic shock, etc.—are used for the microalgal cell disruption. In the autoclaving process, the algal dried biomass is added with ultrapure water and autoclaved at 121 °C and 15 lbs for 5 min followed with extraction with solvent mixtures for removing a lipid layer [22]. However, the energy inputs in autoclaving are high and the solvents are also very costly [47]. Moreover, solvent recoveries are not in practice, which adds additional cost while its disposal presents serious environmental concern. Bead beating involves direct mechanical cell disruption based on high-speed spinning of microalgal biomass with fine beads. Bead beating is considered to be easily scalable, but this technique is always energy intensive [21]. Yu et al. [48] reported bead beating is less effective and only up to 51.2% lipid contents can be extracted compared to the autoclaving and microwaving methods. Another disadvantage is that it is time consuming for separating the culture from beads.

Ultrasonication is another process that has been intensively used for microalgal cell disruption through extremely localized shock waves [49]. The major advantage of this method is the low power input, thus making it comparatively economical [47]. In addition, this technology can be employed as a flow system which means there are no issues with a fixed treatment volume. However, it is not well understood if this can sufficiently disrupt microalgal species with extremely thick cell walls such as *Nannochloropsis* or *Scenedesmus*. Günerken et al. [50] reported the lack of very high disruption efficiency in ultra-sonication method. Osmotic shock, which leads to bursting of cells and release of their contents through abrupt lowering of osmotic pressure, is considered as an option for an effective cell disruption process. Rakesh et al. [51] reported high lipid yield in the microwave-pretreated microalgae biomass among other methods in terms of relative percent of the fatty acids (up to 71.08%) in comparison to 55.52% in the control, in *Botryococcus* sp. This microwave method also achieved five-fold enhancement in unsaturated fatty acid (UFA) content, over osmotic treatment. Especially, 77% of oil was recovered from microalga *Scenedesmus obliquus* through continuous microwaving at 95 °C with the help of solvent-hexane [52]. This microwave treatment advantage is rapid heating that leads to assuring a high internal temperature and pressure gradient acting on the microalgal cell wall to enhance mass transfer rates. Additionally, there is no thermal degradation of lipids [51]. Lee et al. [16] also suggested the microwave oven method is the most effective and simple for microalgal lipid extraction.

6. Microalgae as a Feedstock for Value Added Products

Microalgae synthesize several compounds, such as pigments, enzymes, sugars, and lipids—along with fatty acids, sterols, antioxidants, and vitamins—which can be used as food and feed additives and cosmetics [53,54]. Microalgal pigment, i.e., carotenoids, phycobiliproteins, peptides, etc. are used as natural food colorants, additives for animal feed and aquaculture, and cosmetics and have nutritional and therapeutic use as well [55,56]. Garofalo [57] reported that microalgae contain some bioactive compounds which have antimicrobial and various neurological properties. Some peptides from microalgae can also be used for pharmaceuticals by acting on body enzymes. After lipid extraction from microalgae, the residual biomass contains a high amount of nitrogen and phosphorus contents, therefore, it can be used as fertilizer. Some marine algae such as *Nannochloropsis salina*, *Isochrysis galbana*, *Arthrospira* (Spirulina) *Phaeodactylum tricornutum*, *Platensis* (*cyanobacterium*) are rich in protein and essential lipid content (ω-3 and ω-6 fatty acids). Microalgae lipid (oil) content used as an alternative to fish oil and flaxseed as a source of omega-3 (n-3) polyunsaturated fatty acids (PUFA) including docohexaenoic acid (DHA), arachidonic acid, γ-linoleic acid, and eicosapentaenoic acid (EPA). These omega-3 long chain polyunsaturated fatty acids are dietary nutrients offering multiple health benefits for both humans and animals [58].

7. Environmental Applications of Microalgae

Biofuel and other bio-products production from microalgae can be more cost-effective, profitable, and environmentally sustainable, if these are coupled with processes such as wastewater treatment (Figure 5) and flue gas treatment.

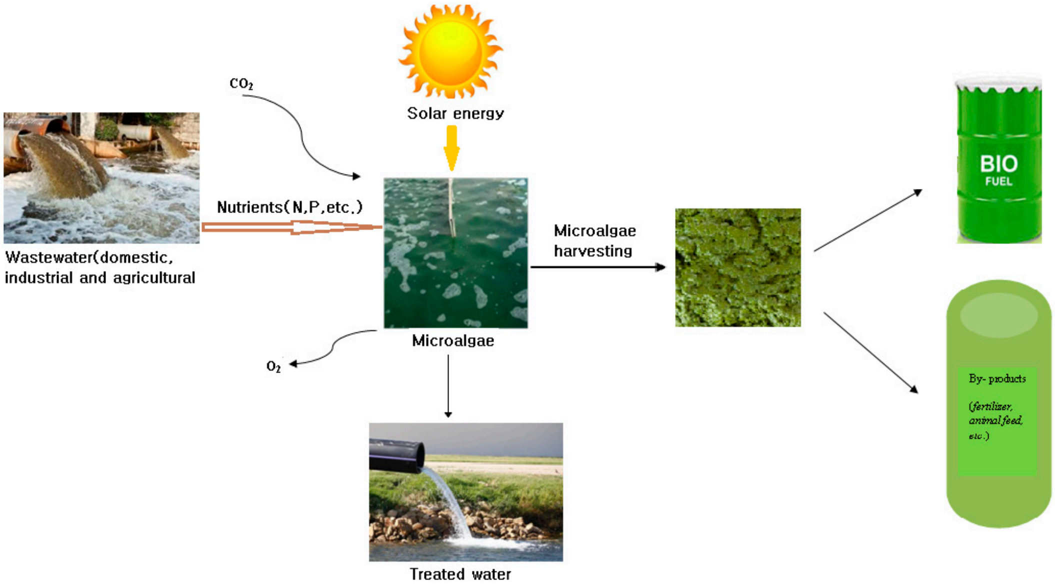

Figure 5. Principles of the microalgae production integration with wastewater treatment.

7.1. Wastewater Treatment and Nutrient Removal

Microalgae is a good sequester of heavy metals and nutrients from wastewater. Pan et al. [59] suggested that the accumulation of heavy metals by algae can be used extensively for biomonitoring or bioremediation purposes. The big advantages of this process are using low cost raw material, big adsorbing capacity, and no production of secondary pollution. Moreover, algae can be used to treat different industrial effluents and wastewater containing heavy metals through a sequester process. The economic viability of algae cultivation is questionable due to abundance requirements of nutrients such as carbon, nitrogen, and phosphorus and water. For example, 6–8 tons·ha^{-1}·year^{-1} nitrate is required as the nitrogen resource, comparatively 55–111 times more than the nitrogen required for field crops [60]. Similarly, the global fresh water requirement was estimated around 3908.3 billion m^3 for the culturing of microalgae [61]. Therefore, tremendous efforts are being made to microalgal biomass production economically viable by using wastewaters instead of fresh water. This includes the use of substitutes for culturing and dewatering of microalgal biomass. In this regard, use of industrial and domestic wastewater for microalgae cultivation has come into practice which is rich in nutrients. The findings of the earlier studies on the use of wastewater and domestic sewage as culture media have shown promising results [34,62]. Conventionally, biological methods and chemical treatment methods are used to remove N and P nutrients from wastewater to reduce the pollution. However, high cost and more sludge production are the disadvantages to this process. Whereas, the use of microalgae for the removal of nutrients from wastewater reduces the cost and sludge production. Microalgae are well capable of removing/utilizing N and P efficiently from various types of wastewater, such as agricultural wastewater and municipal wastewater and therefore reduce eutrophication and ecosystem damage in downstream watersheds [63,64]. However, the application of industrial wastewater in microalgae based biofuel production is limited due to the high level of toxicants, nutrients, turbidity, and sometimes color. Attasat et al. [65] suggested that during storage, wastewater should be diluted for algal cultivation and treatment process. Various research studies have shown promising phycoremediation potential of algae for olive mill wastewater, palm oil mill effluent, and carpet mill wastewater [66]. As most of the wastewater pollutants are ideal nutrients for inducing algae growth [67]. Nitrogen (N) is the most significant nutrient for the production of microalgal biomass and its cellular components—i.e., proteins, amino acids, amides, DNA, RNA, alkaloids, and enzymes [68].

Phosphorus (P) is also one of the most significant nutrient for microalgal growth and also contain 1%–3% P in the dry weight of microalgae. Phosphorus plays major role in algal cellular metabolic processes such as energy transfer, DNA, and nucleic acid synthesis, and forms many structural and functional components required for microalgae growth and development [69]. Microalgae grown in phosphorus-rich wastewaters assimilate phosphorus as inorganic orthophosphate, preferably as H_2PO_{4-} or $HPO_4{}^{2-}$. These are stored within the cells in the form of polyphosphate (volutin) granules and this can be enough for prolonged growth in the absence of phosphorus [70,71]. Zhou et al. [72] reported removal of 76.7%–92.3% of total nitrogen (TN) and 67.5%–82.2% of total phosphorus (TP) by *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella pyrenoidosa*, and *Chlorella vulgaris* during wastewater treatment.

Fecal coliforms are indicators of water, fecal pollution, and the pathogenic organisms of concern are *Salmonella* and *Shigella*, viruses and protozoa [73]. Algal growth indirectly reduced the bacterial growth through competitive utilization of nutrients and carbon sources from waste water. Kiso et al. [74] reported the microalgal photosynthesis induces the oxygen and pH variation in effluent which helps to reduce coliform and other pathogenic bacteria. The light attenuation and pH increases algal density and turbidity, and the increased algal growth results in a decreased and destruction of fecal coliforms [75]. Ansa et al. [76] reported the algal presence include starvation, sedimentation, and photo-oxidation leads indirectly to removal of the coliforms. Microalgae releases some exudates which inhibit the growth of pathogenic microorganisms. Microalgal treatment is sustainable and efficient in the reduction of coliforms in effluent before being discarded from the sewage treatment plants [77].

7.2. CO_2 Sequestrations

The emission of greenhouse gases like carbon dioxide (CO_2) into the environment are mainly from burning of fossil fuels and this has contributed substantially to climate change and caused a serious global warming effect. The global energy-reacted CO_2 emissions are expected to rise two-fold by 2035 and the emissions rate is expected to rise by approximately 1.6% per year. Several sectors including industrial, power generation, and transportation generated around 70 billion metric tons and it has reached 110 billion metric tons in the year 2000. Emissions are forecasted to reach over 140 billion metric tons of CO_2 by 2035 [78]. Microalgae and green plants can fix CO_2 from different sources for the formation of complex sugars through photosynthesis [79]. Certain microalgae species have faster growth rates and greater CO_2 fixation efficiency compared to C4 plants. These processes are also known as algae-based carbon capture (CO_2 sequestration) technology to reduce CO_2 in the atmosphere. This CO_2 sequestration technology has several advantages such as (1) mitigating CO_2 as the main cause of global warming and (2) biofuel and valuable secondary metabolite production. Bhola et al. [79] also reported that some of the green microalgae genera such as *Dunaliella*, *Chlorella*, *Euglena*, *Botryococcus*, *Scenedesmus*, and *Chlorococcum* are known as effective carbon sequesters. Brennan and Owende [80] reported that around 1.83 kg of CO_2 utilizes by 1 kg of algal dry cell weight. Annually, around 30–37 tons per hectare of dry weight microalgal biomass can be sequestered and around 54.9–67.7 tons of CO_2/year from raceway ponds. Several industrial plants such as electric power and steel plants produce flue gases that are also responsible for global CO_2 emissions [81]. Nevertheless, some of selected microalgal strains (e.g., *Chlorella* sp.) can assimilate CO_2 from industrial flue gas within different concentration ranges from ambient (0.036% v/v) to extremely high (100% v/v) [79]. Such findings clearly indicate that a substantial amount of the CO_2 can be sequestered by operating pilot scale microalgae based cultivation systems. Though such systems will not be capable of mitigating the global CO_2 problem but can contribute substantially.

8. Future Prospects

Microalgae have tremendous potential for food, fodder, and fuel production. The bottleneck of the algal technologies is the economic production of biomass due to the high recurring cost of nutrient media required for the cultivation of microalgae and energy intensive methods of harvesting and oil extraction. Substantial improvization is required in various areas of algal technologies. For example, there is a scope of improvization of solar energy conversion of the algae cultivation systems which is around 1%–4% in open algal ponds and raceways whereas the theoretical conversion efficiency is around 8%–12%. The photosynthetic efficiency of microalgae can be improved by genetic, molecular, and metabolic engineering. Improvements in the cost effective mechanical mixing of avoiding photo-inhibition will also improve the economic production of algal biomass in open cultivation systems. Similarly, the existing design of photobioreactors also needs more improvements for utilization of solar energy and high biomass yields. The CO_2 supplementation and utilization depend on algae species and ranges from 1% to 20%, however, this process itself is energy intensive and consequently uneconomic for pilot operations. Therefore, the way forward is to set up the algae cultivation systems in close proximity to the industries and use flue gasses to overcome the cost of CO_2 procurement and transport. However, as the industrial flue gasses contain various other toxic gasses, therefore, the selection of the registrant species and optimization of their growth is required. More importantly, the CO_2 diffusion in the culture is also energy intensive, therefore, this needs mechanical improvizations. Microalgae are an excellent sequester of micronutrients from the culture media, therefore, the requirement of the growth media and nutrients are high which add cost to the produced biomass. The use of various types of wastewater and industrial effluents as culture media will substantially reduce the cost of biomass production, however, this needs elaborated research and optimization studies for commercial applications. This has the dual advantages of wastewater treatment and biomass production. Such biomass can be used for the production of various types of biofuels such as bioethanol, biodiesel, biomethane, and biohydrogen etc. Being comparatively economic, such concepts have elaborated the application of algal technologies from wastewater treatments to biofuels.

The biomass concentration of the culture approximately ranges between 0.5 and 5 g/L, therefore, the harvesting of the tiny algal cells is another costly affair and often accounts for 20%–30% of the cost of the biomass production. Various present technologies being used for the dewatering and recovery of the biomass are energy intensive hence uneconomic. Technological innovations for improvization of conventional harvesting techniques and or establishing new technologies are required to overcome the recurring cost of the harvesting. The microalgae accumulate substantially higher oil contents than the oil seed plants and various other compounds of economic interest. However, the extraction of oil for biofuels and other compounds is quite tricky and presents several challenges. None of the present extraction techniques are commercially feasible. The harvested biomass (wet slurry) contains more than 75% water, which is extracted either directly (wet extraction) or after drying (dry extraction). The extraction yield and the fatty acid profile are directly influenced by the cell disruption techniques, hence it is important to optimize the extraction procedures for the selected species of algae. The production of biomass only for biofuels may not be sustainable, therefore coupling biomass production with oil extraction and use of the residual biomass for various other co-products in a biorefinery concept will substantially improve the economics. This shows that algal technologies have a promising future for both bioremediation and biofuels in a sustainable manner theoretically. However, practically, there are numerous obstacles still to overcome.

9. Conclusions

In the past few decades, tremendous advances have been made in the field of algal technologies for combating numerous techno-economic hurdles and improving biomass production. The major limitations with the use of algal biomass as an alternative feedstock for biofuels are the cost involved in its cultivation and harvesting as well as extraction of value added products. Most of the technologies—i.e., cultivation, harvesting, and extraction—have their own pro and cons. Majorly, two techniques such as photobioreactors and open ponds/raceways are commonly used for cultivation, however the initial cost, requirement of suitable growth media, and energy inputs substantially add cost. Moreover, even with most advance harvesting, lipid extraction, and its conversion to biofuels, present techniques are not economically lucrative. In the current scenario, the major challenge of algal biofuels is not yet met due to higher production cost compared to the low market price of fossil fuels. In this regard, the need of the day is to invent an economical and sustainable biofuel production technique for wide acceptability. The introduction of genetically modified microalgae for increasing the energy efficiency with the utilization limited nutrients and avoiding field contamination with resistant genes could be an option. Similarly, economical harvesting and ecofriendly optimum extractions also need the attention of researchers and the scientific community. Though the economic viability of algal metabolites, byproducts, and biofuel production depends on various human and environmental variables, however, an extrapolation of bench and pilot scale production of algal biofuels is promising. The global energy requirement in 2035 is expected to be 812 quadrillion kJ (AEO, US) and as per estimates. Commercial production of algal biofuels using photobioreactors or open raceway ponds could reduce the cost by about \$3–\$4 per gallon [28]. Therefore, algal biofuels could be the most prolific photosynthetic biomass as an alternative renewable resource which can be produced with limited natural resources with the added advantages of phycoremediation along with CO_2 sequestration.

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