

Biofuel Production from Algae Utilizing Wastewater

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Abstract

The growing concern surrounding the continued use of fossil fuels and rapid depletion of fossil fuel reserves, global climate change, rising crude oil price and environmental degradation have forced scientific community and researchers to find out alternative energy sources. The potential of microalgae as a source of renewable energy has received considerable interest. However, the overall current production and harvesting techniques of microalgal biomass and downstream processing of microalgae to produce biofuels and other bioproducts of value is still too expensive to ensure a competitive production price for biofuels from algae. If microalgal biofuel production is to be economically viable and sustainable, further optimization of mass culture and harvesting conditions and biofuel processing techniques are needed. However, the technologies required for large-scale cultivation, processing, and conversion of microalgal biomass to energy products are not yet a commercial reality. Wastewaters derived from municipal, domestic, agricultural and industrial activities can potentially provide cost-effective and sustainable means of algal growth for biofuels. However, currently there are no commercial algae-to-fuels technologies that can overcome techno-economic barriers and address serious sustainability concerns. Coupling microalgae cultivation with wastewater treatment is considered as one of the most promising routes to produce bio-energy and bio-based by-products in an economically viable and environmentally friendly way. This paper reviews current research on this topic (wastewater-based algae cultivation systems), major challenges to sustainable production and harvesting of algae, compare the benefits and limitations of the different approaches to algae production, research need and future direction for sustainable microalgal biofuel production.

Introduction

Due to increasing greenhouse gas emissions, recent high prices for petroleum, declining fossil resources, energy insecurity, growing demand for transportation fuels and global warming, recently research interest has focused on searching alternative and sustainable renewable biofuels from microalgae [1-5]. However, the production of biofuels and bioproducts using algal biomass has been impeded due to lacking of a reliable and cost effective technology of producing and harvesting large quantities of algal biomass.

Various industry operations produce wastewater and if this wastewater is discharged in aquatic systems without proper treatment, excess nitrogen and phosphorus in discharged wastewater can lead to downstream eutrophication and ecosystem damage [6]. The negative effects of such nutrient overloading of receiver aquatic systems include nuisance algae, low dissolved oxygen concentrations and fish kills, undesirable pH shifts, and cyanotoxin production. While chemical and physical based technologies are available to remove these nutrients, they are yet to be cost effective [7].

Microalgae are photosynthetic microorganisms that grow utilizing solar energy and fertilizers and the amount of fertilizer required for their production are enormous. One alternative to the use of synthetic fertilizers is to use domestic, municipal, agricultural, industrial, aquaculture wastes and wastewaters, which are rich in organic and inorganic pollutants such as nitrogen and phosphorus [8-13]. Simultaneously, with the cultivation of microalgae using wastes and wastewaters for biomass production these pollutants could be removed from the aquatic environment. Thus, treatment of the wastes and wastewaters occur through removal of the pollutants. Compared to physical and chemical treatment processes, algae based treatment can potentially achieve nutrient removal in a less expensive and ecologically safer way with the added benefits of resource recovery and recycling [14]. However, acceptable nutrient levels in the effluent can be achieved only through large scale production and harvesting of the algal biomass. Unfortunately, producing biofuel by the large scale cultivation of microalgae is not commercially fully viable. The overall production process is still too expensive to ensure a competitive production price for biofuels from algae. Nevertheless, coupling microalgae culture with wastewater treatment is considered one of the most promising routes to produce biofuel and bio-based by-products in an economically viable and environmentally friendly way since large quantities of freshwater and nutrient required for algal growth could be saved [15]. This paper reviews current research on this topic (wastewater-based algae cultivation systems), major challenges to sustainable production and harvesting of algae, compare the benefits and limitations of the different approaches to algae production, research need and future direction for sustainable microalgal biofuel production.

History of Wastewater-Based Algal Research

In early 1950s the first research on using micro-algae for wastewater treatment was started. The cultivation of algae on wastewaters evolved from the use of algae in wastewater treatment [15-16]. The nutrients were removed efficiently in such a symbiotic system. It was demonstrated that algae-based wastewater treatment could remove the nutrients (e.g., N and P) from settled domestic sewage more efficiently than traditional activated sewage process [17-18], indicating a great potential of algae-based wastewater treatment system.

Wastewater Resources for Algal Biofuel Production

Algae can grow in various aquatic environment, such as fresh, brackish and marine water, municipal wastewaters, industrial wastewaters, aquaculture wastewaters, animal wastewaters, domestic wastewaters as long as there are adequate amounts of carbon (organic or inorganic), N (urea, ammonium or nitrate), and P as well as other trace elements are present. Waste waters are unique in their chemical profile and physical properties as compared with fresh and marine waters. Recent researches indicated the great potential of mass production of algal biomass for biofuel and other applications using wastewaters [4, 15, 24]. However, wastewater- based algae cultivation still faced many

uncertainties and challenges including variation of wastewater composition.

Industrial Wastewater

The composition of wastewater discharged from industrial facility is complex. Carbon is deficient but nitrogen and phosphorus are two main components in industrial wastewater, which are capable of supporting algae growth. However, cultivation of microalgae in industrial water may face many challenges as this water contains variable constituents.

Although industrial wastewaters are commonly considered unsuitable for algae cultivation due to their intrinsic properties of relatively unbalanced nutrient profile and high toxic compounds, some studies demonstrated the potential of microalgae grown on different industrial wastewaters for algal biomass production. For example, wastewater from carpet mill effluent contained process chemicals and pigments used in the mills, plus a range of inorganic elements including low concentrations of metals, and relatively low concentrations of total P and N. This type of wastewater was shown to be low enough in toxins and high enough in P and N to support the growth of two freshwater microalgae *B. braunii* and *Chlorella saccharophila*, and a marine alga *Pleurochrysis carterae* [19]. Wu et al. [20] investigated nitrogen and phosphorus assimilation and lipid production of microalgae in industrial wastewater. They evaluated the biomass growth and lipid production of two strains of freshwater microalgae in modified BBM medium. They observed that *Chlamydomonas* sp. TAI-2 had better biomass growth and higher lipid production than *Desmodesmus* sp. TAI-1. They tested optimal growth and lipid accumulation of *Chlamydomonas* sp. TAI-2 under different nitrogen sources, nitrogen and CO₂ concentrations and illumination period in modified BBM medium. They found that the *Chlamydomonas* sp. TAI-2 achieved maximum lipid accumulation under continuous illumination when optimal CO₂ aeration was 5%. They observed that when industrial wastewater was used as the medium, *Chlamydomonas* sp. TAI-2 removed 100% NH₄⁺-N (38.4 mg/L) and NO₃⁻-N (3.1 mg/L) and 33% PO₄⁻³-P (44.7 mg/L) and accumulate the lipid up to 18.4%. Over 90% of total fatty acids were 14:0, 16:0, 16:1, 18:1, and 18:3 fatty acids, which could be utilized for biodiesel production.

Municipal Wastewater

Integrating intensive, large-scale microalgal cultivation with traditional municipal wastewater treatment may provide the means by which significant quantities of biofuel and/or bioenergy could be generated. Because most municipal wastewater are rich in ammonia (NH₃), phosphate (PO₄⁻), and other essential nutrients that are required to support microalgal biomass production, as well as trace metals essential for photosynthesis such as Fe, Cu, Mn and Zn. Zhou et al. [21] reported that using wastewater to grow algae is probably the most promising route to reduce production costs associated with nutrients and water. In that study, they examined algal growth, wastewater nutrient removal efficiency, and lipid accumulation of *Auxenochlorella protothecoides* UMN280 in batch and semi-continuous cultivation with various hydraulic retention time using concentrated municipal wastewater as a culture media. The results of the 6 day batch cultivation of *Auxenochlorella protothecoides* UMN280 showed that the maximal removal efficiencies for total nitrogen, total phosphorus, chemical oxygen demand (COD) and total organic carbon (TOC) were over 59%, 81%, 88% and 96%, respectively, with high growth rate (0.490/day), high biomass productivity (269 mg/L/day) and high lipid productivity (78 mg/L/day). Further fatty acid methyl ester (FAME) analysis showed that the microalgal lipids were mainly

composed of C16/C18 fatty acids (accounting for over 94% of total fatty acid), which are suitable for high-quality biofuel production. Wang et al. [22] investigated the growth of *Chlorella* sp. on four different types of wastewater for their abilities to utilize and remove N, P, COD, and other trace elements and concluded that algae growth profile and nutrient removal efficiencies were proportional to the nutrient concentration of municipal wastewaters derived from different process stages of municipal wastewater treatment plant. It was found that the algal growth was significantly enhanced (more than 10 times higher) in the centrate wastewater probably due to its much higher levels of COD, N and P compared with other wastewater streams [22]. Similar research was conducted by Li et al. [23] to evaluate the feasibility of growing *Chlorella* sp. on centrate wastewater and the results showed that the algae removed ammonia, total N, total P, and COD as high as 93.9%, 89.1%, 80.9%, and 90.8%, respectively. Woertz et al. [34] treated municipal wastewater in semi-continuous indoor cultures with 2-4 day hydraulic residence times (HRTs). Maximum lipid productivity for the municipal wastewater was 24 mg/day/L, observed in the 3-day HRT cultures. Over 99% removal of ammonium and orthophosphate was achieved in this experiment. They suggested that CO₂-supplemented algae cultures can simultaneously remove dissolved nitrogen and phosphorus to low levels while generating a feedstock potentially useful for liquid biofuels production.

All above studies suggested that growing algae in nutrient-rich municipal wastewater was a new option to enhance algal biomass productivity and serve the dual roles of nutrient reduction and cost-effective biofuel feedstock production.

Agricultural Wastewater

A comparison of the mineral composition of several classic mass culture media and animal manure wastewaters shows that animal manure wastewater appears to be a suitable medium for the growth of microalgae [24-26].

Numerous researches reported that microalgae are efficient tiny cell factory for removing N and P from manure-based wastewater [5, 24-25, 27]. For example, the green alga *Botryococcus braunii* grew well in swine manure wastewater containing 788 mg/L NO₃ and removed 80% of the initial NO₃ content [51]. Studies of nutrient recovery from dairy manure using benthic fresh water algae have been considered to be very effectively due to the significantly higher nutrient uptake rates in some species of benthic algae than those in planktonic suspended algae [28-29].

However, there are some major issues when animal manure wastewater was used for algae cultivation, which include: (1) high turbidity due to presence of solid particles, which would affect light penetration significantly; (2) high nutrient concentration especially high ammonia concentration, which could inhibit algal growth considerably; (3) a large portion of the carbon sources is locked in the large insoluble organic compounds and unavailable for algae to assimilate; (4) a large quantity of freshwater is necessary to dilute the concentrated animal wastewater unless water recycling and reuse is enabled; and (5) high performance algae strains adapted to the adverse environment in animal wastewaters have not yet been developed [15]. In order to address above issues, numerous methods and strategies were developed and adopted.

Wastewater-Based Algal Production Technologies

Over the past decades numerous studies have been conducted on growing microalgae on different types of wastewaters (e.g., agricultural run-off, concentrated animal feed operations, and industrial and municipal waste streams). It has been reported that the successes of such studies was depended on the performance of the selected microalgae strains. Many micro-algae species

such as *Chlorella* sp., *Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Heynigia* sp., *Hindakia* sp., *Pediastrum* sp., *Chlamydomonas* sp., *Dictyosphaerium* sp., *Botryococcus* sp. and *Coelastrum* sp. have been tested and were proved to be able to utilize and remove N and P as well as other trace elements in the wastewaters [15]. In addition, the harvested low-cost algal biomass could be used as an ideal feedstock for production of biofuels and other by-products such as drugs, foods, fertilizers, and animal/fish feed supplements [15]. All these studies will be of great importance to the development of wastewater based microalgae cultivation system.

Strains

Microalgal strains are generally sensitive to different types of wastewaters due to the imbalance in nutrient profile, deficiency of some important trace elements, and presence of inhibiting/toxic compounds in wastewater streams, and only limited number of strains within a few species (e.g., *Chlorella* sp. and *Scenedesmus* sp.) could adapt well in different waste water environments [15]. There is a great need to select more robust microalgal strains that are tolerant to specific type of wastewater of interest. Numerous researches demonstrated that microalgae adapted to culture conditions similar to where they were found and generally grew better than those purchased from algae banks [15]. Resistant strains can be obtained through genetic engineering and/or breeding manipulation in order to obtain extra resistance to environment stress and/or improve oil synthesis [15]

Cultivation Systems

There are different ways microalgae can be cultivated. Efficient and cost-effective large-scale cultivation of microalgae is essential for the success of microalgal biomass as a candidate in renewable energy. Many designs for mass algal cultivation have come forward and can be generally separated into a) suspended cultures, including open ponds and closed reactors, and b) immobilized cultures, including matrix-immobilized systems and biofilms. The most common large scale production systems in practice are high rate algal ponds or raceway ponds. Raceway ponds (range of volumetric capacities 102–106 L) are open and shallow with paddle wheel to provide circulation of the algae and nutrients. Raceways are relatively inexpensive to build and operate, but often suffer low productivity for various reasons [35, 36]. Tubular photobioreactors (range of volumetric capacities 101–104 L) are the only type of closed systems used at large scale production of algae [35, 52].

Open raceway ponds have been used for over 60 years and there is extensive knowledge and experience in their operation [37]. The raceway pond culture is usually no more than 30 cm deep to allow for efficient penetration of sunlight [35]. Although open systems are generally easy to operate and use sunlight as an energy source, they have several disadvantages. The principal drawback is the lack of any real control over the environmental conditions encountered. For instance, temperature is not controlled and will vary seasonally and diurnally. There can be significant water loss due to evaporation and the distribution of light and CO₂ through the culture is much less efficient than in Photobioreactor systems. In open systems, contamination by competing algal strains and by bacteria is difficult to avoid. In many cases, it is necessary to use extremophilic organisms that can grow under conditions that other strains will not tolerate (high salinity for instance), which places a severe limit on the number of different strains which can be cultivated for mass production. Due to these limitations, open pond productivities are typically fairly low. Closed Photobioreactors are more expensive to construct and operate but offer more control over culture conditions. The photoreactor system can be sub-classified as: a) vertical photoreactor, b) flat or horizontal photoreactor, and c) helical photoreactor. The helical photoreactor is considered the

easiest to scale up production. Compared to open ponds, tubular photobioreactors can give better pH, nutrient dosing and temperature control, better protection against culture contamination, better mixing, less evaporative loss and higher cell densities [36]. However, each system has relative advantages and disadvantages. One of the significant challenges of using raceways and tubular photobioreactors is biomass recovery. Likewise, due to their low construction costs and ease of operation, open raceway ponds will likely be the systems of choice for mass microalgae cultivation. Figure 1 illustrates various microalgae cultivation systems.

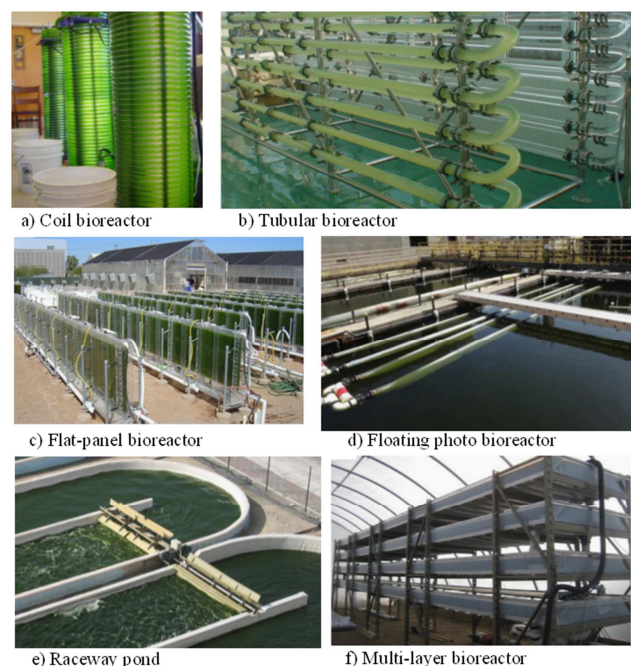


Figure 1. Microalgae cultivation systems, adapted from [15].

Environmental Factors

The key environmental parameters includes light, temperature, pH, predation by zooplankton, pathogens (including bacteria, fungi and viruses) and invading species competition. A major problem limiting algal productivity in mass cultivation settings is the availability of sufficient quantities of light energy to drive photosynthesis. This limitation arises mainly from the phenomenon known as shelf-shading due to the 'light-saturation' effect. As a culture of microalgae grows and cell density increases, a higher proportion of the photosynthetically active radiation is intercepted by algal cells close to the surface of the cultivation vessel or pond before it can penetrate more deeply into the culture. Different strains respond to light intensity differently.

Another important factor for the successful mass cultivation of microalgae is temperature control. It is well known that temperature exerts strong control over metabolic rate processes. Maintaining proper control of culture temperature within a fairly narrow range of only a few degrees C is therefore critically important to optimize biomass productivity. Variation of temperature can have deleterious effects on algal growth rates and productivity.

It has been reported that the diurnal variation can have wastewater-based algae cultivation susceptible to other factors such as grazing by herbivorous protozoa and zooplankton (e.g., rotifers and cladocerans) which can reduce algal concentration and even cause culture crashing 2–3 days [40–41]. Fungal parasitism and viral infection can also significantly reduce the

algal population in a pond within a few days and trigger changes in algal cell structure, diversity and succession [42-43].

Overall, influence of the microbial community in different types of wastewaters is complex and deserves further investigation. Agitation, exogenous carbon supplementation, and harvest frequency or hydraulic retention time (HRT) are key operational parameters which affect algal growth, biomass productivity, and nutrient removal significantly. The traditional agitation methods for algae culture include bubbling, rotation, pumping and paddle-wheel based mixing, depending on bioreactor type.

Major Challenges

Algal biofuel production industry is facing two major challenges, which are large-scale production of algal biomass and harvesting of algae in a way that allows for downstream processing to produce biofuels and other by-products of value. Nutrient supply and recycling, gas transfer and exchange, photosynthetically active radiation (PAR) delivery, culture integrity, environment control, land and water availability and harvesting are the challenges of large-scale production of algae. Algae growth requires the availability of primary nutrients and micronutrients. These nutrients can be costly if they need to be added in great amounts. When gas exchange is insufficient, the algae culture can become carbon limited, and the oxygen by-product of photosynthesis can reach inhibitory levels [30]. Delivery of light in the form of photosynthetically active radiation (PAR) can also be the limiting factor at high culture densities [31-32]. Contamination can be difficult to avoid in open culture systems.

Increasing control of the growth environment can enhance productivity but involves additional costs. Sufficient land and water must also be available. The most important challenge, however, lies not in the production of the algae crop, but in the harvesting and downstream processing of it in a manner suitable for the production of bioproducts [33].

Nutrient Supply and Recycling

Three primary nutrients (carbon, nitrogen, and phosphorus) and a number of micronutrients such as silica, calcium, magnesium, potassium, iron, manganese, sulphur, zinc, copper, and cobalt are required for producing algal biomass. However, micronutrient required in trace amount for algal growth and these essential micronutrients rarely limits algal growth when wastewater is used [44]. If the water source lacks macro and micronutrients or sufficient amount of nutrients are not present in the algal culture water, the addition of commercial fertilizers can significantly increase production costs, making the price of algae derived fuel cost prohibitive. For this reason, wastewater is an attractive resource for algae production. Pittman et al. [45] reviewed the potential of algal biofuel production and concluded that, based on current technologies, algae cultivation for biofuels without the use of wastewater is unlikely to be economically viable or provide a positive energy return. Nitrogen and phosphorous contents in different types of wastewaters are shown in Table 1.

Wastewater type	Nitrogen ^a (mg/L)	Phosphorus ^b (mg/L)	Reference	N:P (molar ratio)	Theoretical algae biomass production ^{c,d}
Weak domestic	20 ^e	4	Tchobanoglous and Burton (1991)	11	0.3 g
Medium domestic	40 ^e	8	Tchobanoglous and Burton (1991)	11	0.6 g
Strong domestic	85 ^e	15	Tchobanoglous and Burton (1991)	13	1.4 g
Beef cattle feedlot	63	14	Bradford et al. (2008)	10	1.0 g
Dairy	185	30	Bradford et al. (2008)	14	2.9 g
Poultry feedlot	802	50	Bradford et al. (2008)	36	5.7 g
Swine feedlot	2430	324	Bradford et al. (2008)	17	37.1 g
Swine feedlot	895	168	Vanotti and Szogi (2008)	12	14.2 g
Coffee production	85	38 ^f	Olguin et al. (2003)	5	1.3 g
Coke plant	757	0.5 ^f	Vazquez et al. (2007)	3352	0.1 g
Distillery	2700 ^e	680 ^f	Basu (1975)	9	42.8 g
Paper mill	11 ^e	0.6	Pokhrel and Viraraghavan (2004)	41	0.1 g
Tannery	273	21 ^f	Durai and Rajasimman (2011)	29	2.4 g
Textile	90	18	Fongsatitkul et al. (2004)	11	1.4 g
Winery	110	52	Mosse et al. (2011)	5	1.7 g

^a Total Kjeldahl nitrogen (TKN) unless specified.

^b Total phosphorus unless specified.

^c Based on limiting nutrient assuming a formula of C₁₀₆H₁₈₁O₄₅N₁₆P.

^d Based on the nutrients (N and P) contained in one litre of wastewater.

^e Total nitrogen.

^f Phosphorus as phosphate (PO₄-P).

Table 1. Table 1: Nutrient contents of various types of wastewaters suitable for algae cultivation, adapted from [49].

Gas transfer and Exchange

Algal growth requires proper gas exchange that includes both sufficient transfer of carbon dioxide to the cells and sufficient removal of oxygen gas. Some algae can be grown heterotrophically but for making the process environmentally and economically viable algae's autotrophic ability needs to be

utilized by using inorganic carbon as the carbon source. Among the three principle forms of dissolved inorganic carbon, algae can directly utilize carbon dioxide and often bicarbonate, but generally can't utilize carbonate [46-47].

Contamination

Unless additional means of control are utilized, algal cultures are susceptible to contamination when we want to do monoculture for nutritional supplements or other bioproducts. Monoculture of algae almost impossible in wastewater treatment systems and when wastewater resources are used for algal culture, naturally occurring mixed culture of algae dominates.

Environmental Control

If environmental parameters such as temperature and pH can be controlled significantly, biomass production and nutrient removal will be optimized but it will add up additional production cost [48]. Finding ways to achieve proper control of the growth environment without adding unreasonable costs remains a challenge.

Land and Water Availability

Large scale production of algal biomass requires a large expense of land with an available water source. This challenge can be met up if algae can be cultured in wastewater treatment facilities.

Harvesting

The potential oil yields (litre/hectare) for algae are significantly higher than yields of oil seed crops (approximately 20 times higher than soybeans). Therefore, a smaller area is potentially required to produce triglyceride-rich oil from microalgae than from other types of biomass. However, harvesting of algae in an economically sustainable manner is a major challenge. Separating the algae from water remains a major hurdle to industrial scale processing partly because of the small size of the algal cells. Various methods are currently used for harvesting algae, which includes chemical based, mechanical based, biological based and to a lesser extent, electrical based operations. However, various combinations or sequence of these methods are also commonly in use. The cell size of algae is very small. Therefore, chemical flocculation is often performed as a pre-treatment to increase the particle size of algae before using another method such as flotation to harvest the algae. In mechanical based process, centrifugation process, which is the most reliable and rapid method, is used for recovering suspended algae [49]. In electrical based method, negative charge properties of algal cells are used for separating the cells [50]. These cells can be concentrated by the movement in an electric field. Low cost algal harvesting options for biofuels applications do not currently exist. Recovery has been estimated to contribute 20–30% of the total cost of producing the biomass [51]. The initial harvesting step is not only costly, but also affects any later processes downstream [51]. Lowering the cost of harvesting algae and harvesting in a way that allows for the creation of bioproducts remains a challenge.

Discussion and Conclusion

Microalgae offer great potential as a sustainable feedstock for the production of third generation biofuels. Much of the research addressing algae production and harvesting is currently confined to the laboratory. Microalgal production in wastewater treatment is potentially an economically viable feedstock for biofuel production. However, increasing biomass is still regarded a top priority to make microalgal biofuel a commercial reality. Microalgal light absorption and photosynthesis are limited in wastewater pond and the improvement of these will undoubtedly increase biomass yield. The technical feasibility of many algae production technologies has been extensively investigated and demonstrated. However, the economic viability and environmental sustainability remain the key obstacles to the commercialization of these technologies. Many of these challenges are cost-associated, and cannot be overcome without technical breakthroughs and innovative system integration. Using wastewater as a resource and combining wastewater treatment

with the production of algae based bioproducts can overcome several of the major challenges identified. However, several important scientific and technical challenges need to be overcome before the large-scale production of microalgae derived biofuels can become commercially viable.

Additionally, the existing infrastructure of wastewater treatment facilities can be utilized for managed algae production, thereby reducing capital costs and scalability challenges. Technological development, including advances in photobioreactor design, microalgal biomass harvesting, drying, and processing are important areas that may lead to enhanced cost-effectiveness and therefore, effective commercial implementation of the biofuel from microalgae strategy.

In order to fully utilize the advanced wastewater-based algal biofuel production technologies further researches are needed on:

- Wastewater nutrients removal process by microalgae;
- Tolerance capacity of microalgae to various wastewaters and environmental stresses;
- Augmentation of environmental parameters and combined heterotrophic and mixotrophic cultivation;
- Development of innovative, efficient and cost-effective algae harvesting and conversion technologies;
- Comprehensive life cycle assessment for economic viability, carbon foot print and sustainability.

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