Sanjay Kumar Gupta · Faizal Bux *Editors*

Application of Microalgae in Wastewater Treatment

Volume 2: Biorefinery Approaches of Wastewater Treatment

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Preface

In the past few decades, algal technologies have been one of the extensively studied fields of biological sciences for numerous environmental, biological, biomedical, and industrial applications. Microalgae are one of the simplest photosynthetic life forms which have an amazing potential of growing in very harsh environmental conditions. Microalgae hold amazing potential for the sequestration of various nutrients from water as well as carbon dioxide from the air. These organisms hold great potential required for sustainable development and management of food, fodder, and fuels. Algal biomass can be used for the production of food and chemicals, bioremediation of several pollutants, and synthesis of biofuels. Further, microalgae have a capacity to produce polymers, fatty acids, enzymes, and toxins, which can be useful for pharmaceutical, nutraceutical, and cosmeceutical developments. This book *Application of Algal Technologies for Wastewater Treatment* Volume II deals with various aspects of the industrial wastewater-based algal biorefineries.

Sustainability is a key principle in natural resource management, and it involves operational efficiency, minimization of environmental impact, and socioeconomic considerations, all of which are interdependent. Wastewater-based production of algal biofuels is one of the most environmentally friendly and sustainable methods utilizing wastewater to reduce deleterious environmental impacts and recycle wastewater and nutrients. This book provides a brief introduction about the role of microalgal biotechnology in environmental sustainability. Algae-based bioremediation of wastewater, its status, and challenges toward global sustainability have been discussed. This provides insight about how developing algae production systems would help reduce greenhouse gas emissions by capturing carbon dioxide and simultaneously producing an alternative for fossil fuels in the form of biofuels.

High-rate algal ponds (HRAPs) are an effective system to remove several pollutants and nutrients from wastewater and to generate a substantial amount of biomass. A chapter in the book discusses the design and mechanisms of pollutants removal from wastewater in HRAPs. A comparative account is presented thereafter which highlights the factors affecting the production of biomass in HRAPs and removal of pollutants from wastewater. Subsequently, economic and environmental aspects are discussed to assess the sustainability of HRAPs. Thereafter, strategies for further improvements for enhanced treatment and biomass production for biodiesel have been proposed and discussed, in an attempt to reduce the cost gap for biodiesel commercialization. The knowledge and literature presented here may help to design and improve HRAP wastewater treatment and biomass production.

Economics remains the single most significant hurdle for large-scale production and commercialization of algal products. Coupling wastewater treatment and microalgae cultivation combine the prospects of nutrient remediation and biomass production which could lead to economic savings through avoidance of costly wastewater treatment approaches and producing high-value algal biomass. Considering the current and projected demand for algae-based products, careful and strategic valorization of algal biomass depending on the composition of biomass and processing strategies is critical and needs to be evaluated. Few chapters of the book exclusively provide an economic perspective of phycoremediation for nutrients from wastewater and valorization of microalgal biomass. Authors have analyzed the economic dimensions of coupling wastewater treatment and production of algal biomass and their subsequent valorization to value-added products.

The concomitant generation of renewable energy and material resources with distinct environmental applications for CO_2 mitigation and wastewater treatment is one of the hallmarks of microalgal research. Since microalgae have the potential to utilize CO_2 as well as N, P, and K from wastewater, high-density cultivation of microalgae can be accomplished by utilizing wastewater and CO_2. Wastewater generated from domestic, agricultural, and industrial activities contains a variety of ingredients which can be utilized as a cultivation medium for microalgae. The use of wastewater with co-utilization of CO_2 for microalgae cultivation is beneficial since it reduces the requirements of freshwater and essential nutrients (N, P, and K). Microalgae biomass produced through CO_2 fixation and wastewater treatment can potentially be used for the production of biofuels, pharmaceuticals, and feed grade products. Selected chapters have been included in the book which mainly focus on the potential of microalgae for integrated biomass production utilizing CO_2 and food industry wastewater. The challenges and future needs for the cultivation of microalgae in wastewater have also been reviewed.

Two chapters in the book discuss the industrial wastewater-based microalgal biorefinery and the production of biofuels and valuable compounds from microalgal biomass in a cost-effective manner by utilizing various types of industrial effluents and wastewaters. In addition, techno-economic analysis of the wastewater-cultivated microalgae-based biorefinery is also discussed to determine its feasibility on a large scale. A separate chapter elaborates the applications, constraints, and future prospects of industrial wastewater-based microalgal biorefinery. Another chapter describes the potential of algal biomass production in conjunction with wastewater treatment and power generation in a microbial fuel cell (MFC). It also discusses the factors governing the performance of microbial carbon-capture cell (MCC) and its applications.

The significance of biogas generation is analyzed based on the technical aspects of processing. A comprehensive overview of the biomethane production potential of algal biomass cultivated in wastewater is summarized in a separate chapter.

A detailed analysis on the importance of environmental conditions on the foreseen composition of biofuel is presented. Along the efficiency of various technologies, the assessment of processing parameters is done by which the energetic value of biomethane is evaluated. A comparative survey on the conversion of algal biomass into biomethane fuel emphasizes the pivotal roles of raw material digestion and the energetic potential of harvesting and processing.

The book titled *Application of Microalgae in Wastewater Treatment* Volume II is mainly based on integrated and biorefinery approaches for the treatment of domestic and industrial wastewater as well as the production of biomass for numerous applications. This book is intended to be a practical guide for scholars and experts from academic and industrial institutions working on the application of algal technologies for bioremediation. This book is divided into two volumes. The first volume contributes significant knowledge about various algal technologies using microalgae, diatoms, and blue-green algae applied for the treatment of domestic and various types of industrial wastewater as well as phycoremediation of emerging pollutants. Whereas, the second volume comprises of various aspects of water- and wastewater-based algal biorefineries. This book is based on various scientific viewpoints and field experiences and shares the fascinating compilation of recent innovations in wastewater-based algal biorefineries. This volume provides a realistic assessment of various techno-economical perspectives of integrated wastewater treatment, production of biomass for various types of biofuels, and its potential for commercialization.

This book comprises of 22 chapters contributed by 74 authors from 16 countries, namely, India, the USA, South Korea, Iran, Malaysia, Chile, Oman, Romania, Brazil, South Africa, Egypt, China, Canada, Greece, Japan, and Spain. All the chapters were selected logically and arranged to provide comprehensive state-of-the-art information on practical aspects of domestic and industrial wastewater-based algal biorefineries. Each chapter discusses topics with simplicity and clarity. All the chapters and their contents are supported by extensive citations of available literature, calculations, and assumptions based on realistic facts and figures on the current status of research and development in this field.

In summation, this edited volume provides a wealth of information based on realistic evaluations of contemporary developments in the application of algal technologies in wastewater treatment. The main focus of this volume is wastewater-based algal biorefineries with an emphasis on pilot-scale studies. Prospects for the commercialization of algal biofuels are another highlight of the book.

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Sanjay Kumar Gupta is Technical Superintendent, Environmental Engineering, Department of Civil Engineering at the Indian Institute of Technology, Delhi, India. Dr. Gupta started his research carrier in 1999 at CSIR-Indian Institute of Toxicology Research, Lucknow India. His PhD was awarded in 2010 from Dr. Ram Manohar Lohia Avadh University, Faizabad. Later, he did his post-doctoral research from Durban University of Technology, South Africa. He has been recognized thrice as "One of the Top Publisher Post-Doc Fellow" in 2014, 2016 and 2017 for his active research contributions. Dr. Gupta has authored 65 articles in peer-reviewed journals and books, and has presented 20 papers in national and international conferences. He was an Editor for Journal of Ecophysiology and Occupational Health, and is a life member of many professional societies including the International Society of Environmental Botanists, Society of Toxicology, Academy of Environmental Biology, and the Indian Network for Soil Contamination Research. His research interests include ecotoxicological risk assessment, bioremediation of wastewater and industrial effluents, and algal biotechnology.

Faizal Bux is Director of the Institute for Water and Wastewater Technology at Durban University of Technology in South Africa. He received his B.Sc. from University of Durban-Westville in 1986, his M. Tech. in Biotechnology from Technikon Natal in 1997, and his Ph.D. in Biotechnology from Durban institute of Technology in 2003. He has edited 5 books, written 121 articles in peer-reviewed journals, authored 17 book chapters, and is an Editor for the journals Environmental Science and Health, Biofuels Research Journal, and Water Science and Technology. His research interests include biological nutrient removal in wastewater treatment, algal biotechnology, and bioremediation of industrial effluents. He has more than 20 years of experience at higher Education Institutes and has received numerous institutional awards including the Vice Chancellor's and University Top Senior researcher awards. Prof Bux is ranked as the most published researcher at Durban University of Technology. He has supervised over 50 Masters and Doctoral students, and 10 Post doctoral fellows served their tenure under his guidance. He was an editor for CLEAN–Soil, Air, Water (John Wiley & Sons, Germany), Environmental Science and Health Part A (Taylor Francis, USA), and served as a reviewer for 28 International Journals. His citations are in excess of 5159 with an H Index of 33. Prof Bux is an invited Member of the Management Committee of the International Water Association IWA) specialist group (Microbial Ecology and Water Engineering, MEWE) and is actively involved in coordinating activities of MEWE globally.

Phycoremediation of Nutrients and Valorisation of Microalgal Biomass: An Economic Perspective

Dipesh Kumar, Bhaskar Singh, and Ankit

1 Introduction

Wastewater includes all the discharges from commercial establishments, household, and institutions, industries, hospitals, etc. It also envisages urban runoff and storm water, as well as horticulture, aquaculture and agricultural effluents. Effluent implies liquid or sewage waste that is discharged into water streams either from treatment plant or direct sources. Using macro- or microalgae for biotransformation or removal of pollutants, including toxic chemicals and nutrients from wastewater, is known as phycoremediation (Mulbry et al. 2008). Algae have developed broad tolerance to environmental conditions including high nutrient levels. This advantage has led to the wide use of the algae in bioremediation of wastes, resulting in treated waters as well as the production of useful biomass which can serve as feedstock for several valuable products, including food, feed, fertiliser, pharmaceutical and, of late, biofuel.

Microalgae can remove environmental toxicants such as heavy metals, hydrocarbons, and pesticides through various mechanisms, ranging from bio-sorption, bioconcentration, biotransformation to volatilisation.

Nutrient removal by algae is economical, sustainable, simple and beneficial for the environment because it can be used as feedstock for production of biofuel and also as fertiliser or animal feed (Filippino et al. 2015). An advantage of using algae is that it does not require supplementary organic carbon additions. Another benefit of algal remediation processes is removal of phosphorus during their growth. Both aerobic and anaerobic media are conducive for microalgae for the treatment of industrial effluents, municipal wastewater and solid waste (Oswald and Gotaas 1985).

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Using algae for sewage treatment is cost-effective. Coupling wastewater treatment and biomass production leads to economic savings regarding avoidance of costly wastewater treatment approaches and produces high-value algal biomass. Microalgae have been found to be promising in the removal of nutrients primarily nitrogen and phosphorus (Aslan and Kapdan 2006; Lebeau and Robert 2003). Wastewater is an available source of water as well as nutrients that are important for algae cultivation. Microalgae provide a sustainable as well as economical means for the treatment of wastewater along with the production of substances that are commercially valuable. Microalgae exhibit higher efficiency in nutrient removal as compared to another microorganism because nutrients like nitrate, ammonia, phosphate and other trace elements are found in wastewater that is essential for the growth of microalgae. Significant progress in the field of cultivation of microalgae coupled with treatment of wastewater has resulted in the improvement in production of algal biomass (Salama et al. 2017).

There is a possibility of assimilating nitrogen and phosphorus into algal biomass that ultimately can be used as fertiliser thereby putting a check on the discharge of oxygenated effluent into the water stream and removing sludge handling problems. Moreover, the process does not require carbon for phosphorus and nitrogen removal which is a sustainable way of sewage treatment (Olguı´n 2003). Algae can grow luxuriously in wastewater due to the availability of all necessary nutrients. Wastewater treated by algae can be used for irrigation, or they may be released into waterbodies. In developing countries, algae-based bioremediation of wastewater can be useful because chemical and physical remediation is costly. Moreover, remediation of toxic substances is essential before discharging of wastewater as they may pollute natural waterbodies. It must be noted that all species of microalgae cannot tolerate the wastewater environment, so, proper screening is required for algae-based wastewater phycoremediation. Strain selection is most likely to be contingent upon the biological and chemical composition of wastewater (Singh et al. 2017).

Phycoremediation is not a new concept, but the hurdle lies in searching for a suitable species of algae which can yield higher biomass and its subsequent application like the production of biodiesel. Studies conducted in the past have revealed that both marine and freshwater algae are suitable for phycoremediation of several types of wastewater like industrial, municipal and agricultural wastewater (Van Den Hende et al. 2014).

The bitter truth is that the market for the algal product is still in the naïve stage, so, uncompetitive economics remains the single most significant hurdle for large-scale production and commercialisation of algal products. A solution in this regard is Integrated Multi-Trophic Aquaculture (IMTA). In IMTA process, microalgae are used as feed for other aquatic organisms; in this way biomass that is obtained from microalgae can be valorised; this is one of the principal advantages associated with the use of microalgae. Microalgae that have been cultured can be used to feed herbivorous fish, low trophic level fish and molluscs; these can then be sold to market at a reasonable price. Both macroalgae and microalgae can be employed in IMTA systems because they have fundamental properties which make them beneficial vis-à-vis bacterial processes because they together remove excess nutrients from the effluents. Microalgae are considered to be the best because they have promising

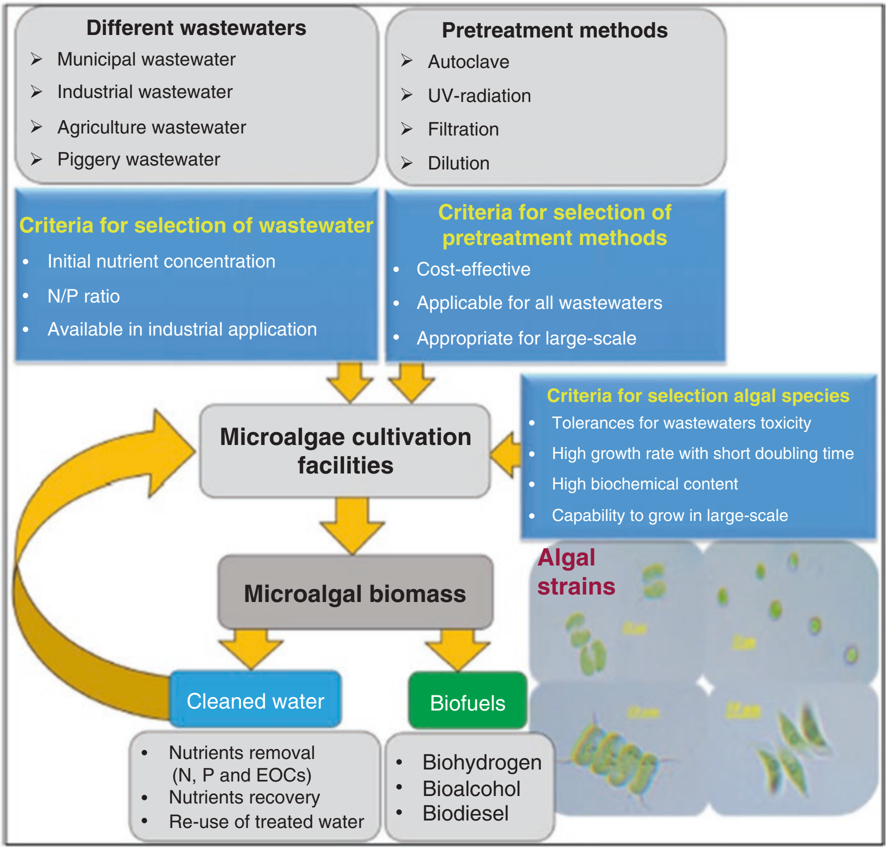

Fig. 1 Schematic representation of wastewater treatment simulations with microalgal biomass cultivation. (Reproduced by permission of Elsevier; Salama et al. (2017))

potential to remove excess nutrients as compared to macroalgae and bacteria and also because the biomass obtained from microalgae can be utilised for various other purposes (Milhazes-Cunha and Otero 2017). Figure 1 provides a schematic representation of wastewater treatment simulations with microalgal biomass cultivation.

Culturing the microalgae at a commercial scale using wastewater would be an environment-friendly approach to check cultural eutrophication (Mehrabadi et al. 2016). Various studies that have been done in the past in this regard unequivocally state that without the treatment of wastewater as the primary goal, the near-term result for scale-up microalgal fuel generation is not promising. Pittman et al. (2011), in their study, found the potential of the production of microalgal biofuel production and also established that by using current knowledge, culturing the microalgae without using wastewater will be a costly affair. Lundquist et al. (2010) looked into various approaches to coupling biofuel production with wastewater treatment using microalgae and found that coupling can fetch cost-competitive microalgal biofuel.

2 Phycoremediation

Industrialisation, population rise, urbanisation and irresponsible usage of natural resources are among the prominent factors which have severely degraded our environment particularly regarding the colossal amount of waste generation. In the quest of attaining more and more luxury, we have polluted our streams and groundwater resources, and consequently, the per capita availability of potable and safe water is declining at an unprecedented rate (Shafik 1994). To maintain the sanctity and also to ensure the adequate availability of freshwater resources, efficient, economical and environment-friendly approaches to wastewater treatment are urgently required. Several advanced physical and chemical treatment techniques are available, but the requirement of excess energy and chemical input makes these processes economically unattractive (Libralato et al. 2012).

Algae have shown tremendous potential as an alternative source of bioenergy (biofuels, heat, and electricity), protein-rich food/feed, nutraceuticals and industrially relevant materials. Aquatic species of algae capable of growth in freshwater, brackish water, and saline water are well known. Additionally, several native algal species have exhibited growth with high accumulation of biomass in wastewater, and the process has shown the capability to reduce the pollution load of the effluent. Compared to the physical and chemical operations, utilisation of algae to remediate pollution load of domestic effluents is economically and ecologically appealing as they also offer the potential of resource recovery, recycling and biomass production (Oswald 2003).

The quantum of water used in industries and households globally between 1987 and 2003 was ≈990 billion m^3, and 90% of it emerged as polluted effluent. Assuming realisable biomass productivity of 0.5 g $^{L-1}$ and oil content of only 20%, if 50% of these effluents are used to support algal culture, around 50 million tonnes of oil could be produced which can displace a substantial quantum of fossil fuels currently in use (Bhatnagar et al. 2011). The quality of municipal wastewater and the Indian standard for discharge of treated effluents into inland waters are listed in Table 1.

Phycoremediation is defined as the application of algal cultures for the removal and transformation of pollutants present in soil, water, and air. Phycoremediation can be thought of as an algae-mediated process to (a) remove the organic load of polluted wastewater, (b) uptake xenobiotics and pollutants using algae-derived biosorbants, (c) treat acidic and metal-laden waste media, (d) sequester CO_2 from gaseous waste streams, (e) degrade and transform xenobiotic compounds and (f) detect pollution using algae-based biological sensors (Olguı´n 2003).

High photosynthetic efficiency, high areal productivity, less nutrient demand, ability to utilise waste CO_2 rich streams and biomass readily amenable to processing are some of the prominent advantages which give phycoremediation an edge over phytoremediation. Algae have been identified as one of the most promising feedstocks for the mass scale production of low-value but high-volume products (e.g. biofuels) and low-volume but high-value products (e.g. nutraceuticals). Currently, there is extensive research attention towards mass scale production of algal biomass.

Table 1 Quality of municipal wastewater and the Indian standard for discharge of treated effluents

S.No	Pollutant	Reported value[a]	Indian standards for discharge of treated effluent into inland surface waters[b]
1	pH	8.39	5.5–9.0
2	Dissolved oxygen (mg L^{-1})	2.42	*Na*
3	Biochemical oxygen demand (mg L^{-1})	620.27	30
4	Chemical oxygen demand (mg L^{-1})	1420.54	250
5	Total Kjeldahl nitrogen (mg L^{-1})	84.99	100
6	Total suspended solid (mg L^{-1})	1824	*Na*
7	Phosphate (mg L^{-1})	124.42	*Na*
8	Electrical conductivity (dS m^{-1})	2.84	100

Na Not available
[a]Kumar and Chopra (2012)
[b]CPCB (1986)

Although the potential of algae as a feedstock for biofuel production is enormous, algal biomass production in a cost-competitive and environmentally sustainable manner is currently a matter of extensive investigation. Since freshwater is a scarce resource, algal species capable of sustenance and growth in wastewater are more suited for the purpose. Using wastewater as culture medium can lower the water footprint of algal biomass production by 90% (Yang et al. 2011).

The current production of agricultural fertilisers cannot sustain the demand for large-scale algae cultivation (Chisti 2013), and if the required nutrients are to be supplied from an external source, the cost of biomass production can be prohibitively high (US DOE 2010). The ultimate aim of wastewater treatment is to make the water fit for disposal in surface water resources/soil or to facilitate its recycling/reuse. Municipal wastewaters often contain an excessive amount of nitrogen and phosphorous, and when these nutrient-laden wastes find their way to lakes, they promote algal bloom which can eventually kill a lake. Mass scale production of algal biomass is dependent on the availability of moisture, nutrients, space, and sunlight. Production of synthetic nitrogenous fertilisers such as urea, mono-ammonium phosphate (MAP), di-ammonium phosphate (DAP), etc. is not only expensive but is also known as a source of greenhouse gases (GHGs). Municipal wastewater can supplement the nutrient demand for algae by reducing the external input of synthetic fertilisers. In addition to the macronutrients such as nitrogen and phosphorous, algae also require several other micronutrients such as silica, magnesium, potassium, calcium, iron, manganese, zinc, copper, sulphur and cobalt. These micronutrients are rarely limiting the growth of algae in wastewaters (Christenson and Sims 2011).

Algae can utilise nitrogen in various forms, but algae prefer NH_4^+ over other forms of available nitrogen and have shown excellent potential to remediate NH_4^+ load of wastewater. Nitrogen is an integral component of amino acids, structural and signalling proteins and nucleic acids, and its starvation can affect the overall growth of algae.

However, several studies have enunciated the role of limiting few of the nutrients (mainly nitrogen) on the algal biomass composition. Such manipulations of the culture media are aimed at overexpressing some of the genes involved in biomolecule synthesis and are ultimately dependent on the desired concentration of individual biomolecules in the produced biomass. Although, nitrogen starvation in culture media is known to promote lipid accumulation the overall growth, development and biomass productivity are often hampered in the process.

One of the earliest studies on using algae to effect wastewater treatment came in the 1960s in which Oswald (2003) evaluated the potential of algae to bring about the tertiary treatment of municipal wastewater. Since then, numerous studies assessing the potential of algae to remediate municipal wastewater (Wang et al. 2010), animal wastewater (Park et al. 2009), industrial wastewater (Azarian et al. 2007), biotransformation of xenobiotics (Thies et al. 1996), heavy metal pollution (Yu et al. 1999), flue gas (de Godos et al. 2010) and other emerging contaminants (Matamoros et al. 2016) have been reported.

Activated sludge process is the most commonly employed secondary treatment process for reducing the organic load of wastewater. An interesting study (Su et al. 2012), assessed the effectiveness of synergistic co-operation between photosynthetic algae, and native aerobic bacteria present in activated sludge for the treatment of domestic wastewater. The highest nitrogen removal efficiency of 91 ± 7%, and phosphorous removal efficiency of 93.5 ± 2.5% were reported within 10 days, at an algae sludge ratio of 5:1. The maximum settleability of biomass was found to be at an alga to sludge ratio of 1:5. Algal culture improved the content of dissolved oxygen (DO) in wastewater which was utilised by aerobic bacterial consortia in the decomposition of organic load. The bacterial respiratory release of CO_2, in turn, can be used by algae during photosynthesis. Further, increase in temperature, pH and DO associated with algal photosynthesis are known to check the growth of pathogenic bacteria and viruses.

The removal of phosphorous from wastewater is comparatively tricky, and it is either removed as precipitate using chemicals or through the activated sludge process. These approaches somewhat hamper the complete recyclability of P, and the precipitate is either disposed of in landfills or are converted to sludge-based fertilisers. The algae-based operations match the performance of chemical treatments regarding removal efficiency particularly during the tertiary treatment operation (Ahluwalia and Goyal 2007; Hoffmann 1998).

Algae are more efficient in converting photosynthetically active radiation than C3 plants and can sequester up to 1.83 kg of CO_2 in a kg of biomass (Brennan and Owende 2010). However, the very low concentration of CO_2 in the atmosphere (≈ 0.04 vol. %) is at times a limiting factor for the unhampered growth of algae which is primarily attributed to the low solubility and mass transfer limitations (Kumar et al. 2010). Under low CO_2 levels, competitive inhibition of photosynthesis by photorespiration can take place, and in the process, 20–30% reduction in carbon fixation is likely (Zhu et al. 2008). Although algae a have carbon-concentrating mechanism in place, the current levels of CO_2 in the atmosphere hamper the high rate of growth. CO_2 is a well-known GHG, and tremendous efforts are underway to limit its concentration beyond certain levels to prevent the catastrophic effects of climate change. Increasing levels of CO_2 in the atmosphere may promote the growth of algae but at the expense of irreversible ecological damage. The flue gases emanating from coal-fired thermal power plants (15–20% CO_2 by volume) and cement industry (15% CO_2 by volume) are one of the attractive alternative sources of CO_2 and consequently have been examined as a source of CO_2 for biomass production, for the decarbonisation of flue gas and also for earning carbon credits. The reduction in the concentration of CO_2 present in flue gases from concentrated sources can be around 80–90% if flue gas is diverted towards algal biomass production facility. Unavailability of concentrated point sources of CO_2, the high temperature of the exhaust gas, the presence of noxious and toxic pollutants and difficulties and cost relating to pumping and mixing of CO_2 are some of the impediments capable of restricting this synergy (Van Den Hende et al. 2012). Doucha et al. (2005) investigate the utilisation of flue gas from natural gas combustion containing 6–8% vol. CO_2 for the cultivation of *Chlorella* sp. in a photobioreactor. They maintained pCO2 > 0.1 kPa, and decarbonisation to the tune of 50% was achieved. The presence of NOx ($\approx$ 45 mg m^{3-}) and CO (3 mg m^{3-}) did not have any adverse effect on the growth.

In addition to atmospheric CO_2 and flue gas, a number of species have also shown the ability to uptake Na_2CO_3 and $NaHCO_3$ dissolved in water. High activity of extracellular carboanhydrase which converts Na_2CO_3 and $NaHCO_3$ to free CO_2 facilitates these processes (Huertas et al. 2000). Further, direct uptake mechanism for Na_2CO_3 and $NaHCO_3$ has also been found in some species (Merrett et al. 1996).

The techno-economic viability of heterotrophic mode of algal growth has been shown, and hence several types of organic wastes can be efficiently transformed into algal biomass with a high content of lipids. Algae during the heterotrophic mode of growth have been identified to uptake pentose and hexose sugars, glycerol, acetate and several other types of organic substrate (Bhatnagar et al. 2011). Thus, it can drastically reduce the BOD and COD of wastewater (Wang et al. 2010). Heterotrophic cultivation systems are capable of attaining high levels of lipids, higher cell densities, and easy harvesting and have better scale-up opportunities (Mohan et al. 2015). Compared to only autotrophic mode, combining photoautotrophic growth with heterotrophic growth (mixotrophy) can result in three to ten times more biomass production (Bhatnagar et al. 2011). The mixotrophic growth forms performed better in wastewater than the commonly used BG11 media. During mixotrophy, *S. bijuga* depicted flocculation tendency which is an encouraging sign as it would facilitate easy harvesting of biomass. Further, mixotrophy also reduces the respiratory loss of biomass during dark conditions (Sforza et al. 2012). Some of the potential opportunities involved in algae cultivation are shown in Fig. 2.

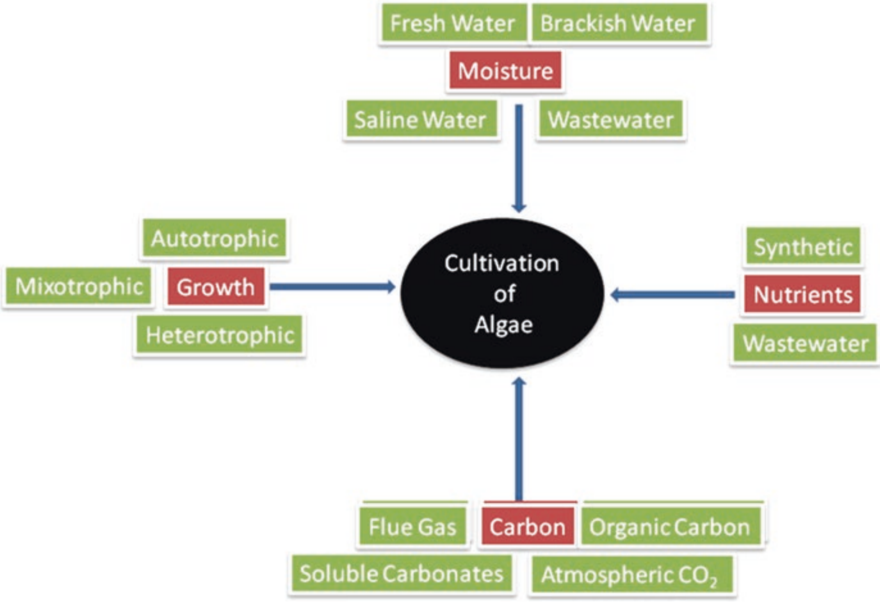

Fig. 2 Potential opportunities in cultivation of algae

3 Valorisation of Algal Biomass

Different biomass processing strategies are available, and the selection of a given route over others should be judiciously determined. The prospects of algal biomass valorisation are tremendous. The potential avenues from algal biomass can be broadly divided into bioenergy (biofuels, heat, and electricity), food/feed, chemicals, nutraceuticals and pigments (Demirbas 2007). Direct combustion, gasification, pyrolysis, hydrothermal liquefaction, transesterification, hydrotreatment, fermentation and anaerobic digestion are among the typical processing strategies for the production of bioenergy/biofuels (Demirbas 2010). Algal biomass mainly consists of lipids, carbohydrates and proteins. The relative proportions of these biomolecules vary depending mainly on the strain, culture conditions, growth mode and availability of nutrients. The typical compositional range of these biomolecules is listed in Table 2. Triacylglycerol (TAG) dominates the lipid fraction of algal biomass, and it is the most suitable feedstock for lipid-based biofuels. Biodiesel also known as fatty acid alkyl esters can be used in existing compression ignition engines. Biodiesel is produced via the transesterification of TAG in the presence of methanol as a methoxy group donor and an alkaline/basic or an acidic catalyst. Green diesel which is also known as renewable diesel is usually produced by hydrotreating vegetable oils in a pressure vessel in the presence of a catalyst. Unlike biodiesel, green diesel is a drop-in fuel, and hence it is compatible with the existing transportation, refining,

Table 2 Chemical composition of dried algal biomass (dry wt. %) (Demirbas 2010)

S.No	Alga	Protein	Carbohydrate	Lipid
1.	*Scenedesmus obliquus*	50–56	10–17	12–14
2.	*Scenedesmus quadricauda*	39–41	11–14	19–31
3.	*Scenedesmus dimorphus*	8–18	21–52	16–40
4.	*Chlorella vulgaris*	51–58	12–17	14–22
5.	*Chlorella pyrenoidosa*	57	26	2
6.	*Dunaliella salina*	57	32	6
7.	*Spirogyra* sp.	6–20	33–64	11–21
8.	*Dunaliella bioculata*	49	4	8
9.	*Euglena gracilis*	39–61	14–18	14–20
10.	*Prymnesium parvum*	28–45	25–33	22–38
11.	*Tetraselmis maculata*	52	15	3
12.	*Porphyridium cruentum*	28–39	40–57	9–14
13.	*Spirulina platensis*	46–63	8–14	4–9
14.	*Anabaena cylindrica*	43–56	25–30	4–7
15.	*Chlamydomonas reinhardtii*	48	17	12–14

Table 3 Comparison of biodiesel, green diesel and mineral diesel fuel properties

S.No	Property	Algal biodiesel[a]	Green diesel[b]	HSD diesel (Bharat Stage IV standard)[c]
1.	Cetane number (min.)	52	80–90	51
2.	Calorific value (MJ kg^{-1})	41	44	*Na*
3.	Flash point min. (°C)	115	120–138	35
4.	Kinematic viscosity (at 40 °C; $mm^2\ s^{-1}$)	3.6–5.4	2.5–3.5	2.0–4.5
5.	Density (at 40 °C; kg m^{-3})	850	780	820–845
6.	Sulphur content max. (mg kg^{-1})	50	negligible	50

Na Not available
[a]Prathima and Karthikeyan (2017)
[b]Aatola et al. (2008)
[c]Bharat Petroleum (2010)

storage, and distribution infrastructure. Green diesel is chemically more stable than biodiesel and has better cold flow properties. A comparison of diesel, biodiesel and green diesel in terms of fuel properties is listed in Table 3.

Some of the strains of algae are known to have high levels of docosahexaenoic acid (DHA) and other omega-3 fatty acids. These fatty acids command very high value in the commercial market as a dietary supplement and are known to improve the conditions of schizophrenia, arthritis, dementia, asthma, depression headaches and migraine (Ruxton et al. 2004). Unlike fish, DHAs in algae are stable and are usually devoid of toxic metals. The carbohydrate-rich fraction can be accessed for the production of sugar-based biofuels, e.g. bioethanol, biobutanol, etc.

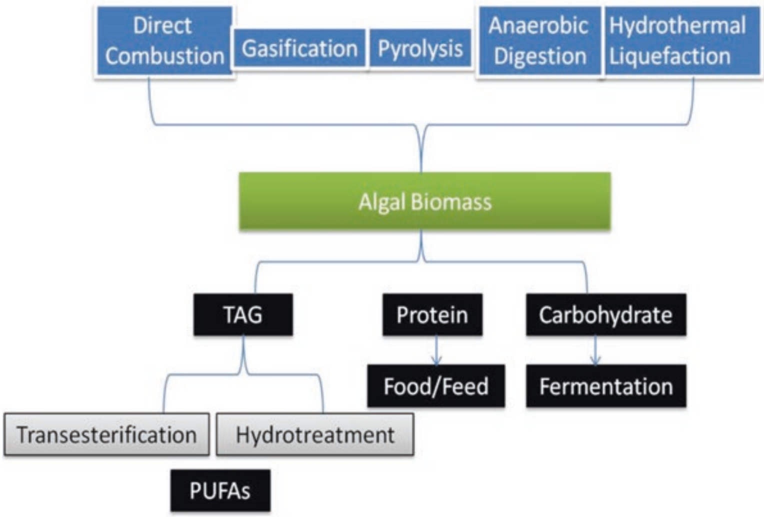

Fig. 3 Potential avenue routes from algal biomass

Fermentation is a decade's old and well-research technique. Bioethanol is the most commonly produced sugar-based biofuel, and it can be used in existing spark ignition combustion engines (petrol engine) as a substitute/supplement to petrol. In addition to carbohydrates and lipids, algal biomass also contains a significant proportion of proteins. Algal proteins can be used as a valuable dietary supplement for human and livestock. Under nitrogen-starved conditions, algae divert their metabolic machinery and energy towards the production of lipids at the expense of other biomolecules. Some of the potential routes of biomass processing are shown in Fig. 3.

4 Economic Perspective

Although algae as a bioenergy feedstock offer several environmental advantages over fossil fuels, cost-competitive production of algal biomass is critical for its commercialisation and acceptability. There is substantial cost involved in the management and treatment of wastewater as the operations involved are energy and material intensive. Culturing algae in wastewater reduces the water footprint of algal biomass production, and in the process, the capital associated with the treatment of wastewater using traditional approaches is saved. Further, the utilisation of wastewater could eliminate or minimise the input requirements of synthetic fertilisers, and with a high degree of nutrient recycling, the system can be self-sustained in terms of nutrient demand. The current global production of synthetic fertilisers cannot absorb the demand for large-scale algal biomass production. The synthetic nutrients (particularly nitrogenous fertilisers) are the expensive and excessive amount of pollutants having high global warming potential released during their synthesis.

Different components of biomass supply chain exert different demands for resources, and the processes downstream of algae cultivation account for roughly 50–60% of the total cost. Several studies have highlighted the importance of wet biomass processing techniques for the cost-competitive production of algae-derived products (Demirbas 2011). Harvesting and biomass drying are highly energy-intensive operations, and therefore self-flocculating strains and in situ processing of biomass are attractive. Hydrothermal liquefaction, anaerobic digestion, gasification, and pyrolysis are suitable for processing of wet biomass and have higher-energy return and improved cost-competitiveness. Further, stand-alone production of biofuels is unlikely to achieve substantial cost reductions, and processing of biomass into a spectrum of products using advanced techniques and equipment is required for better economic gains. It is essential to take into consideration the current and projected demands of bioproducts during the conception and designing of a biorefinery. There is a growing awareness among the public about the adverse effects of fossil resources and the advantages of organic and environmentally benign products.

Slade and Bauen (2013a, b) suggested that a substantial cost reduction (>50%) is attainable if cheap sources of nutrients, CO_2 and water are available. An economic analysis of algal biomass production was performed for a base and a projected scenario in open raceway and photobioreactors (Figs. 4 and 5). Only the cultivation and harvesting stages of the biomass supply chain were assessed, and the coproducts

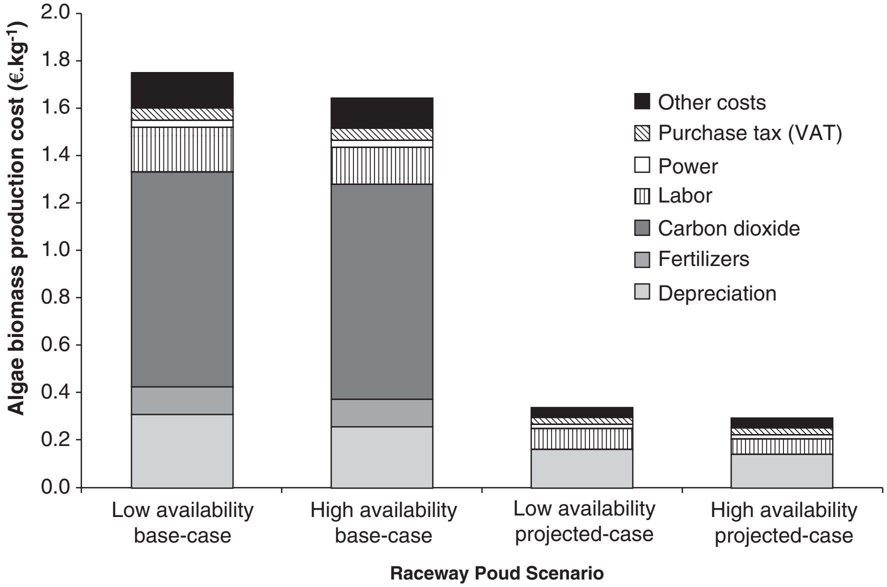

Fig. 4 Cost of algal biomass production in raceway pond. (Reproduced by permission of Elsevier; (Slade and Bauen 2013b))

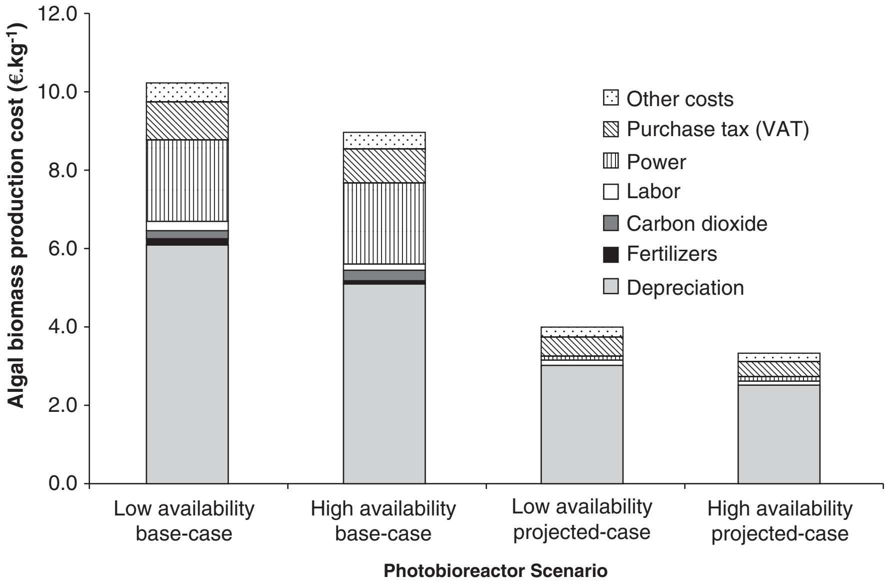

Fig. 5 Cost of algal biomass production in photobioreactor. (Reproduced by permission of Elsevier; (Slade and Bauen 2013b))

remained unallocated. The projected scenario assumed that the freely available wastewater caters all the demand for water and nutrients and industrial flue gas free of cost meets CO_2 demand. Further, no credits were included for the treatment of wastewater. The base case mainly differed from the projected scenario in terms of CO_2 which is the case of the later incurred expenditure. Appropriate assumptions regarding the requirements and the performance of photobioreactors were made. The projected scenario consistently performed better than the base case, while the raceway pond cases were comparatively very cheap. Moving from the base case towards the projected case, almost 50% reduced the cost of biomass production in open raceway. The cost of biomass production in open raceway was estimated to be 1.6–1.8 € for the base case, while in the projected case, the cost was only 0.3–0.4 € kg^{-1}. Incorporation of coproduct allocation and wastewater treatment credit in calculations could have further reduced the cost of biomass production. Compared to raceway pond, the biomass production cost for the photobioreactor was five times higher for the base case and 9–12 times higher for the projected case (Slade and Bauen 2013a). From the preceding discussion, it is clear that the raceway ponds are more appealing than the photobioreactors and for the cost-competitive production of biomass, free/cheap sources of nutrients and water are valuable. The cost of algal biomass production in the absence of cheap and abundant sources of water, nutrients, and CO_2 will be prohibitively high. Utilisation of wastewater and flue gas CO_2 for the mass production of algal biomass has economic and environmental desirability. Considering the current and projected demand for algae-based products, careful and strategic valorisation of algal biomass depending on the composition of biomass and processing strategies is critical.

5 Conclusions

The attractiveness of algal biofuels is constrained due to the prohibitive cost of biomass production. Low concentration of CO_2 in the atmosphere, low availability and the high cost of synthetic fertilisers and high water footprint are among the significant hurdles in the cost-competitive production of algal biomass. Several studies have highlighted the utility of wastewater as a source of nutrients and moisture for the mass scale cultivation of algae. Coupling phycoremediation with biomass production offers immense potential for the remediation of wastewater and economical biomass production. Utilisation of concentrated sources of CO_2 is equally attractive for the enhanced rate of biomass production and decarbonisation of the flue gases.

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Astaxanthin Production by Microalgae *Haematococcus pluvialis* Through Wastewater Treatment: Waste to Resource

Md Mahfuzur Rahman Shah

1 Introduction

Astaxanthin, known as "super antioxidant," can be obtained from synthetic and natural sources. Natural astaxanthin *can be found in fishes (*salmon), crustaceans (shrimp), *Phaffia* yeast and *Paracoccus* bacteria, zooplankton (krill), and some microalgae (e.g., *Haematococcus pluvialis*) (Higuera-Ciapara et al. 2006; Ranga Rao et al. 2014). *H. pluvialis is produced commercially as the richest source of natural* astaxanthin which has 20 times stronger antioxidant capacity than the synthetic astaxanthin (Lorenz 1999; Ranga Rao et al. 2010). Astaxanthin can be extensively applied in human nutrition, animal and aquaculture feed, and cosmetics industry.

Astaxanthin has high market value (\$2500–7000/kg), and its market potentiality is estimated to increase from 280 metric tons, \$447 million (in 2014), to 670 metric tons, \$1.1 billion, by 2020 (Koller et al. 2014; Pérez-López et al. 2014; Industry Experts 2015). Presently, only <1% of the commercialized quantity is produced from *H. pluvialis* (Koller et al. 2014), and the interest of producing astaxanthin from *H. pluvialis* is increasing. Different approaches of production system have been reported such as photoautotrophic, heterotrophic, mixotrophic, indoor, outdoor, open raceway, photobioreactors, batch, fed-batch, two-stage mixotrophic, and attached biofilm-based system (Kang et al. 2005, 2010; Kaewpintong et al. 2007; Ranjbar et al. 2008; García-Malea et al. 2009; Issarapayup et al. 2009; Li et al. 2011; Han et al. 2013; Wang et al. 2013a, b; Park et al. 2014; Zhang et al. 2014).

The astaxanthin accumulation is controlled by various physicochemical factors such as temperature (Yoo et al. 2012), pH (Hata et al. 2001), light (Saha et al. 2013; Park et al. 2014), salinity (Kobayashi et al. 1993), plant hormones (Yu et al. 2015), and nutrient stress (Boussiba et al. 1999; Chekanov et al. 2014). Since wastewater

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contains (in)organic compounds, it can be a potential asset for different living creatures (Rogers et al. 2014). Microalgae have the ability to metabolize and eradicate pollutants, and also they predominate in breaking and separating resistant organic molecules from wastewater (Matamoros et al. 2016 a, b). Various bioproducts have been produced from microalgal biomass harvested during wastewater treatment (Woertz et al. 2014). Different investigations have verified the viability of utilization of microalgae in the treatment of wastewaters (municipal, agricultural, and industrial) (Chinnasamy et al. 2012; Fenton and hUallachain 2012; Dickinson et al. 2013; Neveux et al. 2016). Among them, municipal wastewater has the best potentiality for microalgae cultivation. Commonly, various culture media (BM, BG11, and M1B5) are used for cultivation of *H. pluvialis*, and for transformation of vegetative cells into cyst cells, different chemical additives such as ferric or acetate anions are used (Kobayashi et al. 1997; Ruen-ngam et al. 2010; Solovchenko 2013). Numerous experiments have been reported on the development of best synthetic medium (e.g., Gong and Feng 1997; Fábregas et al. 2000), but, as far as we are aware, a limited number of studies are accessible on the likelihood to use wastewaters for *H. pluvialis* cultivation and astaxanthin accumulation (Kang et al. 2006; Wu et al. 2013; Wang 2014; Sato et al. 2015; Ledda et al. 2015; Haque et al. 2016a; Liu 2018).

Recently, phyco-valorization (nutrient removal from wastewater and simultaneous by-product generation by microalgae) has gained great attention (Querques et al. 2015). *H. pluvialis* was explored for cultivation in diluted primary-treated sewage and primary-treated piggery wastewater which demonstrated better growth and successful uptake of nitrate and phosphorus (Kang et al. 2006). There are a lot of advantages of using wastewater such as reduction of costs and natural resource inputs and simultaneously obtainment of high-value bioproducts (Farooq et al. 2013), but there are a number of challenges involved too. The main challenges include the following:

Harvesting of the algae.
The control of biomass composition is complicated by the selection of the desired species.
Bacterial contamination.
Micro-pollutant removal.
The conceivable requirement for external CO_2.

In this chapter, *H. pluvialis*-derived astaxanthin, its application and market potential, and culture conditions and nutritional requirements of *H. pluvialis* cell growth and astaxanthin formation have been discussed. The potentiality of microalgae cultivation using various wastewater streams and integration of *H. pluvialis* culture in different wastewater streams and nutrient removal and biomass production efficiency are also discussed. Furthermore, the challenges associated with coupling *H. pluvialis* cultivation in wastewaters and possible ways to overcome such challenges have been highlighted.

2 The Green Microalga *H. pluvialis*-Derived Astaxanthin

In *H. pluvialis*, maximum accumulation of astaxanthin can reach up to 5% DW (Wayama et al. 2013). Health food supplements consisting astaxanthin from microalgae considered as safe and broadly utilized as a nutraceutical supplement (Capelli and Cysewski 2013; Yang et al. 2013). *H. pluvialis*-derived astaxanthin can be used for health benefit in dosages from 3.8 to 7.6 mg per day (Yang et al. 2013). Due to structure, function, application, and security, *H. pluvialis* astaxanthin appears to be more effective than the synthetic one (Capelli and Cysewski 2013; Pérez-López et al. 2014; Shah et al. 2016).

2.1 Applications of Astaxanthin

Astaxanthin in Medical and Nutraceutical

Many published reports are available on human health and nutraceutical applications of astaxanthin (Guerin et al. 2003; Chew et al. 2004; Higuera Ciapara et al. 2006; Palozza et al. 2009; Yuan et al. 2011). It works as an antioxidant (Hussein et al. 2006; Liu and Osawa 2007; Ranga Rao et al. 2010), protects peroxidation of membrane lipids (Naguib 2000), terminates the induction of inflammation, helps in ulcer disease (Liu and Lee 2003), enhances human digestive health (Nishikawa et al. 2005; Kamath et al. 2008), and deals with treatment of gastrointestinal pain (Andersen et al. 2007; Kupcinskas et al. 2008).

Astaxanthin can be helpful for reduction of risk for heart attacks (Iwamoto et al. 2000), increment of basal arterial blood flow (Miyawaki et al. 2008), and reduction of blood plasma level (Karppi et al. 2007). It can also reduce the effects of Alzheimer's and neurological disorders; hinder fibrosarcoma growth, cancer cells (breast and prostate), and embryonic fibroblasts (Palozza et al. 2009); and improve respiratory and sympathetic nervous system (Nagata et al. 2006) and mammary tumor (Nakao et al. 2010).

Astaxanthin also helps to protect the skin from photooxidation by UV induction and has antiaging effects (Seki et al. 2001; Yamashita 2002; Tominaga et al. 2012; Ranga Rao et al. 2013). In the case of human, astaxanthin can improve semen quality, pregnancy rate, and sperm velocity (Elgarem et al. 2002; Comhaire et al. 2005) and decrease unexplained infertility (Andrisani et al. 2015).

Astaxanthin in Aquatic Animal and Poultry Diet

Haematococcus-derived astaxanthin can provide essential nutrient for body weight increment and breeding of economically important fishes such as salmonid, red sea bream, rainbow trouts, and shellfish (shrimp). It has been proved as important compound for improvement of pigment in the fish flesh (Torrissen and Naevdal 1984; Tolasa et al. 2005). Use of *H. pluvialis* biomass has shown to enhance egg quality, growth, and rate of survival of fish (salmonid, sea bream, and rainbow trout, ornamental fish), fry (Arai et al. 1987; Ako and Tamaru 1999; Sommer et al. 1991;

Choubert and Heinrich 1993; Sheikhzadeh et al. 2012a, b), and shrimp (Arai et al. 1987; Parisenti et al. 2011). It has been demonstrated that the diet containing *H. pluvialis* improved the growth of adult yellow croaker fish (Li et al. 2014). *H. pluvialis* appeared to be effective in egg yolk coloration, improving egg-laying capacity in hen (Elwinger et al. 1997), muscle in meat-producing chicken (Inborr and Lignell 1997; Inbbor 1998), and fertility and decreasing mortality of chicken (Lignell and Inborr 1999, 2000).

2.2 Market Potential of Astaxanthin

Recently, there has been increasing pattern toward utilizing organic in food, feed, and cosmetic products. The interest for *H. pluvialis* astaxanthin in the international market worldwide has been "emerging" as a result of expanding customer attention to its medical advantages. Worldwide market for astaxanthin (synthetic and natural) is assessed in 2014 at 280 metric tons which is anticipated to achieve by 2020 at 670 metric tons (Industry Experts 2015; Panis 2015). The market value of astaxanthin is about \$2500–7000/kg, and in some cases for *H. pluvialis* astaxanthin, it goes up to \$15,000/kg (Borowitzka 2013; Koller et al. 2014; Pérez-López et al. 2014; Industry Experts 2015). Natural astaxanthin is 3–4 times more expensive than the synthetic one (Han et al. 2013). Considering the increasing market potentiality for natural astaxanthin for industrial utilization, large-scale production of *H. pluvialis* has great prospects and appealing commercial possibility. However, contemporary market requirement for astaxanthin from *H. pluvialis* is not fulfilled. Once the production technology is optimized, the production costs *H. pluvialis* astaxanthin would be comparable to the artificial astaxanthin (Pérez-Lópezetal et al. 2014).

3 Culture Parameters for *H. pluvialis* Growth and Astaxanthin Production

Improvement of culture conditions is important to accomplish greater yield and astaxanthin generation. These conditions have diverse optimum level for cell growth and pigment production. Different kinds of media such as BG-11, BBM, OHM, and KM1-basal medium (Bischoff and Bold 1963; Rippka et al. 1979; Kobayashi et al. 1993; Fábregas et al. 2000) are used for cultivation. At nutrient-deficient conditions, astaxanthin accumulates inside the cells (Saha et al. 2013). In nitrogen-deficient condition, the production rate of astaxanthin is twice than the limitation of phosphorus. Micronutrients (selenium and chromium) play important role to increase yield and astaxanthin formation (Tripathi et al. 1999; Fábregas et al. 2000; Domínguez-Bocanegra et al. 2004). Astaxanthin generation can also be accelerated by incorporating 0.25–0.5% w/v of NaCl or combining 2.2 mM sodium acetate to the media (Sarada et al. 2002b).

The appropriate temperature for *H. pluvialis* ranges from 20 to 28 °C (Fan et al. 1994; Hata et al. 2001; Lababpour et al. 2005; Kang et al. 2010; Yoo et al. 2012; Wan et al. 2014a). However, >30 °C temperature triggers a transition from green to red stage (Tjahjono et al. 1994). pH also can significantly have an effect on the growth and synthesis of carotenoids. The optimum pH ranges from 7.00 to 7.85 (Hata et al. 2001; Sarada et al. 2002a). The optimal light irradiation ranges from 40 to 50 μmol photons m^{-2} s^{-1} (Hata et al. 2001; Chekanov et al. 2014; Park et al. 2014). Optimum light intensity to accomplish better growth rates inclines to be greater such as 70 (Zhang et al. 2014), 80 (Saha et al. 2013), 90 (Fan et al. 1994), or 177 μmol photons m^{-2} s^{-1} (Domínguez-Bocanegra et al. 2004). During green stage, the regular photoperiod (12:12 or 16: 8 h) is frequently maintained (Saha et al. 2013; Park et al. 2014) but higher growth obtained with continuous light (Domínguez-Bocanegra et al. 2004).

Culture Systems *H. pluvialis* can be grown indoor and outdoor and in open or closed system; batch, fed-batch, semicontinuous, or continuous system; and photoautotrophic, heterotrophic, or mixotrophic modes.

Photoautotrophic Culture This type of culture is generally performed in ponds/raceways or photobioreactors. Typically tubular, bubble column and airlift photobioreactors are used for cultivation. Since circumstances for maximum cell yield and astaxanthin concentration are usually incompatible, a double-step production policy is frequently followed for the industrial cultivation. The step one is to maximize vegetative growth in optimum conditions (e.g., less light intensity and with nitrogen) (Boussiba 2000; Aflalo et al. 2007; Del Rio et al. 2007). Once maximum growth is achieved, in the second step, the cells moved to stress situation (e.g., strong light and nitrogen limited, pH or salt manipulation, phosphate depletion, etc.). These stress conditions either individually or in combination with others can stimulate astaxanthin formation (Fábregas et al. 2001; Torzillo et al. 2003; Orosa et al. 2005; He et al. 2007; Hu et al. 2008; Li et al. 2010; Choi et al. 2011). The biomass production in vegetative and red stage varied from 0.01 to 0.5 g L^{-1} d^{-1} and 0.01 to 4.8 g L^{-1} d^{-1}, respectively. In terms of astaxanthin production and content, it varied from 0.44 to 21 mg L^{-1} d^{-1} and 0.8 to 4.8% of DW, respectively (Table 1). Attached cultivation strategy is utilized in the initiation of astaxanthin formation in *H. pluvialis*. In this system, the biomass and astaxanthin productivities were 2.8- and 2.4-fold greater than those of the suspended cultivation system, respectively (Wan et al. 2014b). Additional researches that used the same techniques have shown increased astaxanthin production: 124 mg m^{-2} d^{-1} (Yin et al. 2015) and 164.5 mg m^{-2} d^{-1} (Zhang et al. 2014). Attached induction system can be a potential way to enhance commercial profit and significantly lower cultivation cost (Zhang et al. 2014; Wan et al. 2014b). Park et al. (2014) invented "perfusion culture" system coupling it with stepwise increase associated with light intensity. This culture can offer greater cell growth of 0.18 g L^{-1} d^{-1}. Under stepwise improved light irradiance (150–450 μE/m2/s), cell growth of 12.3 g L^{-1} can be achieved. This cell growth is usually 3.09 and 1.67 times greater than batch and fed-batch processes, respectively (Park et al. 2014).

Table 1 Biomass production and astaxanthin content of *H. pluvialis* grown in various studies

PBRs type	Outdoor/ indoor	Mode	Biomass productivity in green stage ($g\ L^{-1}\ d^{-1}$)	Biomass productivity in red stage ($g\ L^{-1}\ d^{-1}$)	Astaxanthin content (%, DW)	Astaxanthin productivity ($mg\ L^{-1}\ d^{-1}$)	Reference
Airlift column (30 L)	Indoor	Batch	0.03	0.01	2.7	0.44[b]	Harker et al. (1996)
Tubular/open pond (25,000 L)	Outdoor		0.036–0.052	N/A	2.8–3.0	N/A	Olaizola (2000)
Tubular (50 L)	Indoor	Semicontinuous	N/A	0.05	3.6	7.2 [c,d]	Torzillo et al. (2003)
Bubbling column (1.8)	Indoor	Batch	N/A	0.6	0.8	5.6 [a]	Del Rio et al. (2005)
Airlift tubular (55 L),			N/A	0.41	1.1	4.4[a]	Lopez et al. (2006)
Bubbling column (0.5 L)	Indoor	Batch	0.5	0.21	4	11.5 [b]	Aflalo et al. (2007)
Tubular (200 L)	Outdoor	Batch	0.37	0.21	3.8	10.1[b,c]	Aflalo et al. (2007)
Bubbling column (1.8 L)	Indoor	Batch	N/A	1.9	1.1	21 [a]	Del Rio et al. (2007)
Bubbling column (1 L)	Indoor	Batch	0.36	0.14	3.6	12 [b]	Ranjbar et al. (2008)
Tubular (1.8 L), outdoor	Outdoor	Continuous	N/A	0.7	1	8 [a]	Garcia-Malea et al. (2009)
Open pond	Indoor	Batch	N/A	0.15	2.79	4.3[a]	Zhang et al. (2009)
Flat type (1 L)	Indoor	Fed-batch	0.33	0.44	4.8	14[b]	Kang et al. (2010)
Airlift column	Indoor	Batch	N/A	0.14	N/A	3.3[a]	Choi et al. (2011)
Bubbling column V-shaped bottom (6 L)	Indoor	Batch	N/A	0.047	N/A	1.4[a]	Yoo et al. (2012)
Bubbling column (0.6 L)	Outdoor	Batch	N/A	0.58	2.7	17.1[b,d]	Wang et al. (2013a)

[a]One-step culture
[b]Two-step culture. Productivity value calculated based on total time required by the “green” and “red” stage
[c]Induction of astaxanthin performed outdoors
[d]A two-step process where astaxanthin productivity was calculated based on time spent on the “red stage” only

Heterotrophic and Mixotrophic Culture In heterotrophic growth, organic compounds act as carbon and energy for cell propagation and secondary metabolite construction with the absence of light. Different sources of organic carbon have been utilized in this culture. It was shown that acetate helped effectively to cyst formation and astaxanthin formation (Kobayashi et al. 1991; Kakizono et al. 1992; Orosa et al. 2000; Hata et al. 2001; Kang et al. 2005). These microalgae can be also cultured mixotrophically utilizing acetate or carbohydrates (Kobayashi et al. 1993). It is proved that biomass and astaxanthin accumulation can be improved following this culture system. For example, cell density of 0.9–2.65 g L^{-1} and astaxanthin content of 1–2% DW were achieved (Chen et al. 1997; Wang et al. 2003). A sequential, heterotrophic-photoautotrophic culture strategy was also investigated. Biomass was produced by utilizing heterotrophic culture, but for astaxanthin production, photoautotrophic culture was applied under nitrogen depletion, with bicarbonate or CO2 as carbon sources. In this system, a superior astaxanthin content (7% DW) was obtained which is 3.4-fold higher than heterotrophic induction (Kang et al. 2005).

4 Wastewater as a Resource for Microalgae Cultivation

Microalgae cultivation and biomass production require huge quantities (for 1 gram dry biomass >1 kg water) of water (Burlew 1953; Shen 2014). Wastewater (cheap and readily available) provides appropriate atmosphere (pH, dissolved CO_2, and HCO_3^-) and macronutrients (nitrate, ammonia, phosphate) and micronutrients that support for microalgal growth (Abdel-Raouf et al. 2012; Ji et al. 2013; Ajayan et al. 2015; Ding et al. 2015). Wastewater-grown microalgae biomass can be used to extract the accumulated nutrients (Mehta et al. 2015; Gouveia et al. 2016). Three nutrients (carbon, nitrogen, and phosphorus) are of most interest during evaluating a wastewater for microalgae growth enhancement (Kabra et al. 2014).

4.1 Macroelements and Microelements

The cell growth and biochemistry of microalgae require the receptiveness of 15–20 essential elements. The macronutrients consist of C, N, P, H, O, S Mg, K, Na, and Ca, and the micronutrients include Fe, Cu, Mn, Zn, Cl, V, Mo, B, Co, and Si (Eyster 1964). The macroelements are typically utilized as development materials, and the microelements are involved in biological reactions (Arnon 1961). Five microelements (Mn, Zn, Cu, Ca, and Fe) are directly associated with microalgal photosynthesis. Microelements (Cl and Mn) play an important role in O_2 evolvement. The supplementation of macroelements (C, N, and P) with essential microelements (Si, Mg, Ca, Fe, P, S, Mn, Zn, Cu, and Co) is needed for continuous microalgae growth. In case of application of wastewater for microalgae cultivation, the supply of essential microelements such as Si, Mg, Ca, Fe, P, S, Mn, Zn, Cu, and Co rarely limits microalgal growth.

4.2 Composition of Wastewater

Wastewater is a compound mixture of organic, inorganic, and artificial elements. Three quarters of organic carbon in sewage are proteins, carbohydrates, fats, amino acids, and volatile acids. The inorganic parts include large amount of calcium, potassium, sodium, magnesium, chlorine, sulfur, phosphate, bicarbonate, ammonium, and heavy metals (Lim et al. 2010). Wastewaters from various sources (municipal, agricultural, and industrial) can be treated efficiently by microalgae. The typical N:P features of different wastewaters and the feasibility of cultivation of microalgae are shown in Table 2.

In the wastewater influent, nitrogen is present in the form of ammonia (NH_4^+), nitrite (NO_2^-), or nitrate (NO_3^-). Phosphorus is present as phosphates ($PO4_3^-$) in wastewater. Municipal wastewater contains several heavy metal pollutants such as arsenic, cadmium, chromium, copper, lead, mercury, and zinc (European Commission on Environment 2002). It contains comparatively lower amounts of total N and P (10–100 mg L^{-1}) (Dela Noue et al. 1992). Once the secondary treatment is done, total N and P decrease to 20–40 mg L^{-1} and 1–10 mg L^{-1}, respectively (McGinn et al. 2011), which is very suitable for microalgae growth. The N and P ration in municipal wastewater is about 11 to 13 (Christenson and Sims 2011). The widely accepted N:P ratio for microalgae growth is 16 (Larsdotter 2006; Christenson and Sims 2011; Park et al. 2011; Cai et al. 2013), on the basis of empirical formula $C_{106}H_{181}O_{45}N_{16}P$ (Stumm and Morgan 1970). The typical microalgae cell biomass contains 6.6% N and 1.3% P in dry weight (Chisti 2013) with a molar N:P ratio of 11.2, which is similar to that found in wastewater.

Agricultural wastewater derived from animal manure contains N and P concentrations of >1000 mg L^{-1} (Dela Noue et al. 1992). Agricultural runoff consists of herbicides, fungicides, and insecticides.

Industrial wastewater contains less N and P compared to agricultural and municipal wastewater. It has high levels of heavy metal pollutants such as Cr, Zn, and Cd and organic chemical toxins such as hydrocarbons, biocides, and surfactants (Chinnasamy et al. 2010). Textile, tanning, leather, and electroplating and related metal processing industry effluent possess considerable amounts of toxic metal ions (Salama et al. 2017).

4.3 Treatment of Wastewaters

Municipal Wastewater Treatment

Increasing urbanization and population expansion have resulted in large quantities of municipal wastewaters produced every day. Physical and chemical treatment methods are commonly used for removing buoyant, non-buoyant, and dissolved organic materials from wastewaters (Ruiz-Marin et al. 2010). Microalgae cultivation into the municipal wastewater treatment systems for nutrient removal has been widely studied. For example, Pittman et al. (2011) reported that *Chlorella* sp. and

Table 2 Comparison between the physicochemical characteristics of wastewaters and a common synthetic medium

Properties	Unit	Municipal wastewater	Concentrated municipal wastewater	Anaerobic digestion wastewater	Piggery wastewater	Bold's basal medium
pH	–	8.10	7.28	7.30–7.50	7.97	6.80
Alkalinity (total CO3)	mg CO_3/L	272	–	–	–	–
Salinity	g/L	1.03	–	–	–	–
TSS	mg/L	50	–	59.35–85.26	-	-
Conductivity	mS/cm	2.29	–	–	–	–
COD	mg/L	31	–	1572.45–2265.37	37,643	–
TOC	mg/L	9	180.6	–	–	–
TIC	mg/L	–	80.9	–	–	–
TN	mg/L	27	56	537.26–702.73	2055	41.01
TP	mg/L	5.04	15.8	72.62–111.58	620	53
Microbes						
E. coli	cfu/100 mL	5.4×10^6	–	–	–	E–
P. aeruginosa	cfu/100 mL	0.2×10^6	–	–	–	–
Fecal coliforms	cfu/100 mL	6.2×10^6	–	–	–	–
Total coliforms	cfu/100 mL	75.0×10^6	–	–	–	–
Metals						
Magnesium	mg/L	0.088	16.5	23.83–58.26	213	7
Manganese	mg/L	0.09	0.4	0.96–1.91	4.1	0.23
Zinc	mg/L	0.009	–	–	28.9	3.93
Copper	mg/L	–	–	0.31–0.92	10.6	0.63
Calcium	mg/L	29	65.6	–	437	7
Cobalt	mg/L	–	–	0.02–0.06	3.8	
Iron	mg/L	0.12	0.05	6.83–15.35	169.2	4.2
Aluminum	mg/L	0.04	0.02	–	–	–
Sulfate	mg/L	–	40.4	–	–	43.2
Sodium	mg/L	–	39.5	–	772	68
Potassium	mg/L	20	45.7	22.38–68.15	2524	34
Chloride	mg/L	–	–	–	–	12
Barium	mg/L	–	–	0.74–1.67	–	2.0

Adapted from Salama et al. (2017)

Scenedesmus sp. performed with very high efficiency on nutrient removal in sewage wastewater after secondary treated, particularly ammonia, nitrate, and total P, ranging from 80% to 100% removal rates in many cases. Another study indicated that *C. vulgaris* could remove more than 90% of N and 80% of P from primary-treated municipal wastewater (Lau et al. (1995). *Dunaliella salina* showed the capacity for removing nitrate, ammonia, and phosphorous in the range of 45–88% from municipal wastewater after a 6-day cultivation (Liu and Yildiz 2018).

Agricultural Wastewater Treatment

Agriculture is another major wastewater-producing sector. Agricultural wastewater is frequently derived from livestock production and contains high levels of N and P (Wilkie and Mulbry 2002). Generally, livestock manure is often treated and used as fertilizer. However, nutrients may not be completely consumed due to the various ratios of N:P requiring by the crops. As a result, excess nutrients find their ways to the surrounding aquatic systems and cause eutrophication significantly reducing water quality (Cai et al. 2013). Piggery wastewater is typically treated with anaerobic bacteria for reduction of nutrient. However, the nutrient removal capacity of anaerobic bacteria is comparatively lower than microalgae and some cyanobacteria (Markou and Georgakakis 2011). As in the case of municipal wastewaters, previous researches have also demonstrated that microalgae can significantly assimilate N and P from manure-based wastewaters. For example, An et al. (2003) reported that 80% of nitrate content was effectively removed from piggery wastewater by *Botryococcus braunii*. Moreover, compared with microalgae that were cultivated in municipal wastewaters, Wilkie and Mulbry (2002) indicated that higher microalgae growth rates and equivalent nutrient removal efficiencies were observed in manure-added recycling wastewater.

Industrial Wastewater Treatment

The traditional methods of industrial wastewater treatments include electrowinning, precipitation, and ion exchange. Since industrial wastewaters contain lower N and P contents and greater levels of toxic elements, most microalgae cannot grow well. It is necessary to select specific strains that have high metal absorption capacities to handle industrial wastewater remediation. So far, only a few strains have been explored for metal removal capacity research. One study using carpet mill wastewater, which has relatively lower toxins and higher N and P contents, reported that *Botryococcus braunii*, *Chlorella saccharophila*, and *Pleurochrysis carterae* grew well in untreated wastewater with large amounts of biomass generated (Chinnasamy et al. 2010).

5 Integrating *H. pluvialis* Cultivation in Wastewater Treatment and Nutrient Removal

The growth rate of *H. pluvialis* is slow, and its cultivation is a highly sensitive process due to its susceptibility to contamination by other algae and microbes (Orasa et al. 2000). Generally, BM, BG11, and M1B5 media used for cultivation of *H. pluvialis* and chemical additives such as ferric or acetate anions are added to stress the cells (Kobayashi et al. 1997; Ruen-ngam et al. 2010; Solovchenko 2013).

Table 3 Research highlights on integration of different wastewaters with *H. pluvialis*

Wastewater type	Removal efficiency of TN (%)	Removal efficiency of TP (%)	Biomass production g/L	Astaxanthin production mg/L	Culture volume (L)	Culture days	Reference
Primary-treated sewage	100%	100%	0.78	39.7	130 ml	18	Kang et al. (2006)
Primary-treated piggery wastewater	100%	100%	1.43	83.9	130 ml	18	Kang et al. (2006)
Domestic secondary effluent	(93.8%	97.8%)	0.20	–	200 ml	20	Wu et al. (2013)
Coagulated wastewater	90% ± 8%	99% ± 1%		3.26	200 ml	25	Sato et al. (2015)
Piggery wastewater	99%	98%	1.31	–	300 ml	20	Ledda et al. (2015)
Bioethanol plant wastewater	91.7%	100%	4.37	–	2.2 L	16	Haque et al. (2016b)
Minkery wastewater	100%	100%	0.90	67.95	2.25 L	6	Liu (2018)

Although various researches have been performed on the development of optimal synthetic growth medium (e.g., Gong and Feng 1997; Fábregas et al. 2000), only few studies focused on the possibility to use wastewaters for *H. pluvialis* and succeeding astaxanthin production (Table 3). For instance, Kang et al. (2006) reported *H. pluvialis* cultivation in primary-treated wastewater and piggery wastewater. They showed that the cell growth rate in primary-treated wastewater was 0.24 day^{-1}, which was comparable to 0.23 day^{-1} in artificial medium; the cells were composed of 5.1 and 5.9% astaxanthin content using the two-step process; and the cells yielded 43 mg L^{-1} nitrogen and 2.6 mg L^{-1} phosphorus.

Compared with most microalgal species reported in the literature, *H. pluvialis* attained highest biomass production (27.8 mg L^{-1} d^{-1}), efficient nutrient removal (both nitrogen (93.8%) and phosphorus (97.8%) were removed efficiently), and highest lipid accumulation (43%) in unsterilized domestic secondary effluent (Wu et al. 2013).

Sato et al. (2015) reported a new wastewater treatment process that involves coagulation, ozonation, and microalgae *H. pluvialis* cultivation. *H. pluvialis* grew well in the supernatant of coagulated wastewater, and the astaxanthin yield was 3.26 mg/L, and total phosphorus and nitrogen contents decreased to 99% and 90%, respectively.

In another study, wastewater treatment and astaxanthin production were conducted by a primary treatment filtering system: culture and subsequent carotenogenesis induction of *H. pluvialis* on piggery wastewater.

In this study, a drastic reduction in macro- and micronutrient concentration (up to 99% for NO_3-N and NH_4-N, 98% for TP) and astaxanthin accumulation of 1.27% on a dry weight were observed (Ledda et al. 2015). This method showed potentiality as biological wastewater treatment process since it can combine inorganic waste removal without any additives and the simultaneous production of astaxanthin.

Since *H. pluvialis* can use CO_2, CO_3, and carbohydrates as carbon sources, its production cost can be reduced by utilizing waste sources like flue gasses or waste containing carbon and nutrient compounds (Wu et al. 2013).

The required energy and nutrients in auto-, hetero-, and mixotrophic cultivation can be recycled from anaerobic digestion. Based on the culture system, carbon sources can vary. The recycled CO_2 from energy production at anaerobic digestion can be used in photoautotrophic cultivation. The required carbon (carbohydrates or acetate) can be provided from alternative source in heterotrophic cultivation. These carbon sources can be produced from waste (carbohydrate-rich food waste from food industry can be used in heterotrophic cultivation) (Wang 2014). In mixotrophic cultivation both carbon sources can be utilized. After concurrent extraction of astaxanthin and triglycerides, algal cake is used as a feedstock for biogas production through anaerobic digestion that helps in the extraction of residual energy from this integrated bioprocess (Shah et al. 2016). The biorefinery strategy is shown on Fig. 1.

In a recent study by Haque et al. (2017), high-density (4.37 g/L) *H. pluvialis* culture was obtained using the bioethanol plant waste stream as the growth media and resulted in 91.67% total nitrogen and 100% total phosphorous removal. The residual microalgal biomass, obtained after astaxanthin extraction (1.109 mg/g DW), was characterized as a potential bioenergy feedstock. This production process could be environmentally friendly and economically viable, compared to conventional astaxanthin production processes, due to integrating culture in an existing bioethanol plant and using the waste product produces in the plant. Culturing *H. pluvialis* in bioethanol wastewater streams can be a greener alternative to conventional media. The maximum vegetative growth of *H. pluvialis* was obtained in 60× diluted thin stillage, and maximum astaxanthin production was obtained in GroAst media (60% 60× thin stillage and 40% acetate-rich process condensate). The GroAst media appeared to be not only a cheaper media, compared to the chemically synthesized media, but it is also a "greener" sustainable alternative to conventional growth media (Haque et al. 2016b).

Minkery wastewater contains extremely high level of ammonia, which is a different N source from BBM. *H. pluvialis* grew well in the appropriately diluted minkery wastewater (MW) media, and a higher biomass production was realized as compared with conventional culture medium under optimal growth condition. *H. pluvialis* achieved maximum biomass at 1.5% MW cultures, yielding 906.03 ± 34.0 mg L^{-1}, with a successful removal of total nitrogen and phosphorus in a 6-day culture. The optimal initial cell density and volume ratio between microalgae and MW were

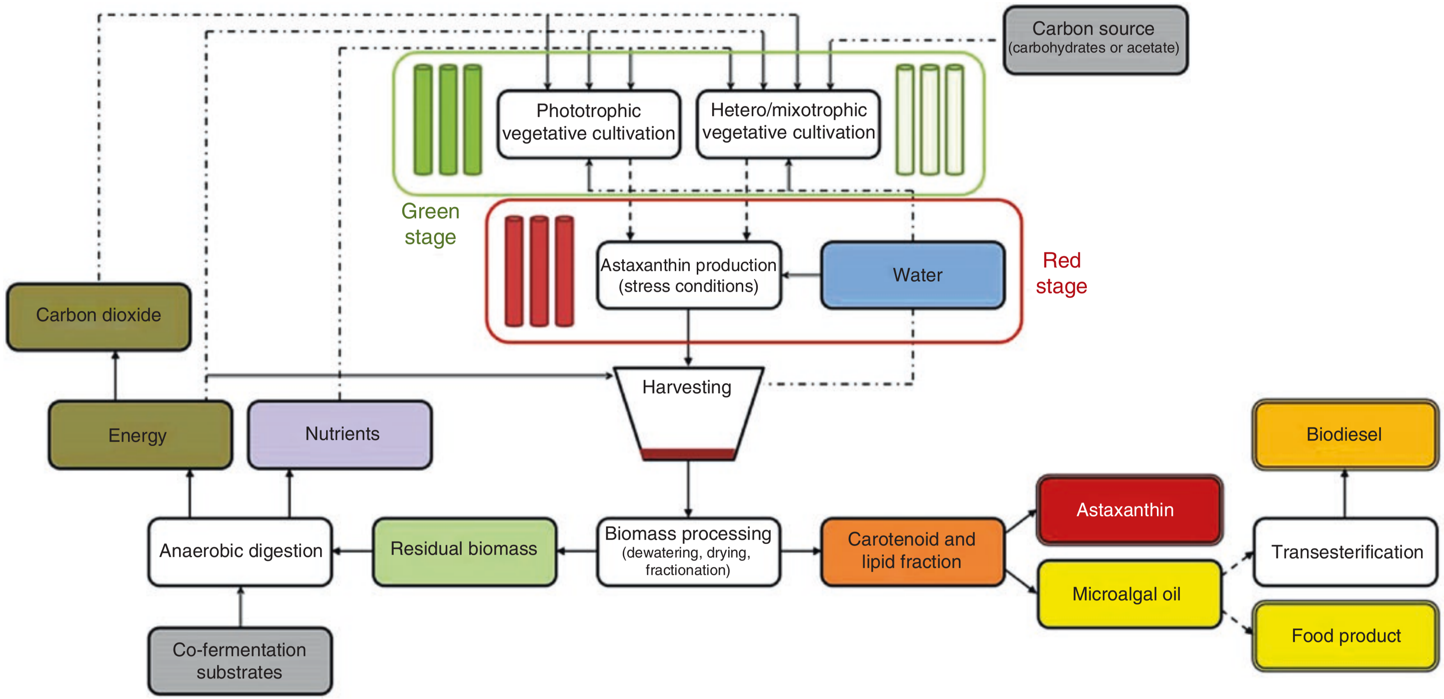

Fig. 1 Schematic diagram of two-stage cultivation *H. pluvialis* biorefinery producing astaxanthin and either edible oil or biofuel compound-biodiesel. (Adapted from Shah et al. 2016). Green stage: performed using either photoautotrophic (deep green section) or hetero−/mixotrophic (pale green section) systems. Red stage: (red section) takes place after green stage to maximize astaxanthin content. Recycling of waste is performed through an aerobic digestion process. The following annotations are used: solid arrows, subsequent steps; dashed arrows, optional steps; double lines, final products; double arrows, inputs; dotted lines, opportunities for recycling resources

also determined to have great help on maximizing the biomass yield. The findings support the claim that integration of wastewater into microalgae cultivation has the advantages of reducing cultivation costs and natural resource inputs and simultaneously obtaining high-value bioproducts (Liu 2018).

Considering the above facts, *H. pluvialis* can be considered as a promising candidate for integrated systems of wastewater treatment and microalgae cultivation while producing high-value bioproducts.

6 Challenges Associated with Growing *H. pluvialis* in Wastewater Streams

Despite the promising features of microalgae, there are huge challenges to overcome before this route can be exploited in commercially and environmentally sustainable manner. The following points are considered as the most important challenges for the cultivation of microalgae in general and *H. pluvialis* using wastewater:

- The cultivation of *H. pluvialis* in wastewater can be susceptible to contamination by fungus, zooplankton (rotifer), protozoans (e.g., amoebas, ciliates), and other microalgae due to its relatively slow growth (Han et al. 2013; Orasa et al. 2000).
- Abiotic contaminants in wastewater such as CO_2, NOx, SOx, O_2, and NH_3^+ and heavy metals can also inhibit microalgae like *H. pluvialis* growth (Kumar et al. 2010).
- In case of low concentration of trace mineral nutrients in the wastewater, it can result in poor growth, low biomass, and low lipid productivity (Christenson and Sims 2011). However, Kang et al. (2007) and Hata et al. (2001) indicated that high concentration of nutrients would also cause inhibitory effects on *H. pluvialis* growth, and thus, the suitable concentration of wastewater must be determined for *H. pluvialis* cultivation.
- Due to the lack of carbon sources in most domestic wastewater, the growth of the microalgae can be inhibited which might eventually affect the treatment of the wastewater (Craggs et al. 2011).
- High concentration of oxygen in wastewater can induce oxidative damage to microalgae cell and inhibit photosynthesis (Christenson and Sims 2011).
- The cost and energy demand of harvesting microalgae in general and *H. pluvialis* from wastewater either by flocculation or centrifugation are still very high (Razon and Tan 2011; Acién et al. 2012; Shah et al. 2016).

7 Conclusion and Prospects

This chapter provides perception regarding the recent scientific and technical improvement in different areas of *H. pluvialis*-derived astaxanthin, its application and market potential, and culture conditions and nutritional requirements of this microalgal cell growth and astaxanthin formation. It also scans a broader image including the potentiality of microalgae cultivation using various wastewater and integration of *H. pluvialis* culture in different wastewater streams and nutrient removal and biomass production efficiency to the challenges associated with growing *H. pluvialis* in wastewater streams.

Recently the demand from *H. pluvialis* is increasing. A number of developments have been obtained concerning production and processing to achieve astaxanthin during the last decade. Still its large-scale cultivation is very expensive for mass adoption of natural astaxanthin compared to the synthetic one. *H. pluvialis* has been cultured in different ways. Various studies have been focused on optimization of parameters (media, light, pH, temperature, etc.) for maximum growth. For biomass accumulation and astaxanthin production, most of these parameters found different.

There is not much can be done to solve this challenges since it is fundamentally connected with the whole life cycle of *H. pluvialis*. We believe that integration of *H. pluvialis* cultivation with wastewater treatment could be a great option to produce astaxanthin effectively in large scale. Research in the use of wastewater for cultivating *H. pluvialis* is still very limited as compared to research for growing other microalgae species. Therefore, further clarifications are needed to prove the feasibility of *H. pluvialis*-based systems in full scale. A number of wastewater types are encouraged to be investigated in *Haematococcus* cultures. Moreover, the relevant optimal production routes and advances in technologies are needed. The improvements in integration processes, harvesting, and extraction technology will contribute to accelerate the speed of the *Haematococcus*-derived astaxanthin production from laboratory scale to commercial scale. Further study in these areas can have a profound influence on the market of natural astaxanthin from *H. pluvialis*.

The challenges of wastewaters directly for *H. pluvialis* culture should be addressed since they restrict the utilization of the easily accessible and low-cost wastewater. There are a number of areas that can improve the integrated *H. pluvialis* cultivation using wastewater for nutrient removal and efficient astaxanthin production. These include the following:

Consideration of sterilized wastewater for microalgal cultivation to prevent biotic contamination.
Coupling of immobilize or attached cultivation of *H. pluvialis* in wastewater stream to maximize the biomass production.
In cases of low concentration of nutrients, there is a need to supplement these nutrients in wastewater to achieve high productivity.
Bubbling of CO_2 can improve algae growth while using domestic wastewater with lack of carbon source for *H. pluvialis* cultivation.
Technological advancement in cost-effective *H. pluvialis* biomass harvesting from large-scale wastewater culture to make it more economically attractive.

Future improvements in these fields can have a thoughtful effect on the commercial implementation of *H. pluvialis* astaxanthin products. Finally, global microalgae industry can be benefited in the near future.

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Potential of Microalgae for Integrated Biomass Production Utilizing CO_2 and Food Industry Wastewater

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1 Introduction

The requirement of energy throughout the world is increasing continuously, and the International Energy Agency (IEA) has predicted it to rise by approximately 50% by the year 2030. Since the eighteenth-century industrial revolution, huge quantities of fossil fuels are exploited to meet the energy demands and to deliver unprecedented comfortable circumstances to the increasing population. Fossil fuels are primarily exploited to cater about 80% the total energy need of mankind all over the world. However, the burning of fossil fuels is one of the main causes for emissions of greenhouse gases (GHGs). Majorly, the increasing population, industrialization, and economic growth are the primary causes of enhanced anthropogenic CO_2 emissions. The exponential increase of energy consumption has resulted in the average atmospheric CO_2 concentration to increase from 280 ppm in the year 1750 to ≥ 410 ppm as of mid 2018. Despite of various climate change mitigation policies and strategies, the largest absolute increase in CO_2 concentration was observed between the decade spanning from the year 2000–2010. The average yearly concentration of CO_2 in the atmosphere (Mauna Loa Observatory, Howaii 2018) was noted to be 398.55 ppm in the year 2014. In an earlier decade to the year 2014 (i.e., 1995–2004), the average annual increase was noted to be 1.9 ppm per year. In the last decade (year 2005–2014), the average annual increase in CO_2 was 2.0 ± 0.1 ppm/year.

Continued emission of CO_2 will contribute further to the global warming. This will lead to the adverse impact on mankind and the environment, including the disruption of ecosystems and floods in the coastal areas due to rising sea levels (IPCC 2014). Currently, about 50% of the CO_2 emitted from the fossil fuel burning remains in the

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atmosphere without being absorbed by vegetation and the oceans. Therefore, GHGs mitigation actions are being taken particularly for large point source emissions. Carbon capture and storage (CCS) technologies are the important part of these procedures considering their GHGs abatement potential (Gough 2008). A number of CO_2 utilization and abatement methods have been studied and evaluated, which can be generally classified based on their chemical and biological nature. The chemical reaction-based approach is achieved by cyclic carbonation/decarbonation reactions in which CO_2 reacts with solid metal oxide and generates metal carbonate; this is a widely studied chemical approach for CO_2 mitigation (Gupta and Fan 2002). Since the chemical methods for CO_2 capture are relatively cost-expensive and energy-consuming, the mitigation benefits remain marginal. Mitigation of CO_2 by biological means is primarily carried out by plants and other organisms having photosynthetic ability like microalgae. Biological CO_2 mitigation has been considered as one of the best alternative CO_2 mitigation approaches due to CO_2 utilization and concomitant biomass production through photosynthesis (Shekh et al. 2013b).

2 Modes of Microalgae Cultivation for CO_2 Utilization and Wastewater Treatment

Practically, algal biomass can be produced in large scale by cultivation using open pond systems and closed photobioreactors.

2.1 Open pond systems

Large open ponds, paddle wheel-operated circular ponds, raceway ponds, and large bags are the most common and well-established commercial microalgae cultivation systems in practice (Borowitzka 1998; Uchiyama et al. 2008; Kunjapur and Bruce 2010). Outdoor open ponds can easily be constructed on nonagricultural land and wastelands. Further, the capital expenditure can be reduced by constructing plastic-lined compact earthen raceway ponds (Chanakya et al. 2012). Since the open ponds are dependent on natural light for photo-illumination, construction, and operation, the maintenance of open pond system is relatively cheaper than the closed photobioreactors. The most widely used microalgae in an open pond system are *Spirulina* (biomass, protein, and phycocyanin), *Chlorella* (biomass, lipids, and proteins), *Haematococcus* (astaxanthin), *Nannochloropsis* (biomass and lipids), *Dunaliella* (carotenoids), *Nostoc*, *Anabaena*, *Scenedesmus*, and *Cyclotella* (Chisti 2007, 2008; Griffith and Harrison 2009; Chanakya et al. 2012). Though the various shapes and sizes of the open ponds can be chosen depending upon cultivation location, the raceway ponds and circular pond are the most frequently used designs. In the CO_2

fed ponds, CO_2 can be supplied as a sole carbon source by sparging CO_2 at the bottom of the raceway pond with homogeneous mixing by using the paddle wheels (Stephensons et al. 2010).

The biomass productivity of 10–50 g m^{-2} d^{-1} can be achieved in open pond systems irrespective of raceway or circular ponds (Sheehan et al. 1998). The open ponds are favored due to lesser capital requirement for establishment than closed photobioreactors (Stephenson et al. 2010). The hindrances of establishing the open system include availability and cost of the land, availability and the quality of the water, and non-conducive climatic conditions since it is challenging to keep optimal cultivation conditions in an open pond system (Lam and Lee 2012). Loss of water due to evaporation remains one of the major limitations of open pond cultivation (Sheehan et al. 1998). On the other hand, lower atmospheric CO_2 concentration (0.04–0.06% CO_2), high surface tension of water, and poor mixing result in CO_2 mass transfer limitations which ultimately lower the algae biomass productivities (Uchiyama et al. 2008; Mata et al. 2010). Microalgal cultivation can be integrated with nitrogen- and phosphorus-rich wastewater treatment using high-rate algal ponds where concomitant microalgae biomass production and nutrient removal from wastewater can be targeted (Sheehan et al. 1998). The integrated use of wastewater can reduce the overall biomass production cost by compensating the requirement of costly nutrients (Christensen and Sims 2011).

2.2 Closed Photobioreactors

Closed photobioreactors were used to surmount the limitations of open pond cultivation. These were selected over open systems as they can be sustained at indoor as well as outdoor conditions. Mainly, closed photobioreactors enable axenic microalgae cultivation for longer period in controlled conditions to get consistent biomass and lipid production (Carvalho et al. 2006). Various species such as *Chlorella vulgaris*, *Chlamydomonas*, *Scenedesmus* sp., *Cyclotella cryptica*, *Monoraphidium minutum*, and *Tetraselmis suecica* have been effectively cultivated in closed systems (Fulke et al. 2010; Sheehan et al. 1998). Many types of photobioreactors like plate reactors, tubular photo bioreactors, bubble column reactors, and less commonly used semi-hollow spheres are used for microalgae cultivation. The closed photobioreactors apart from being helpful in maintaining axenic and monoalgal cultures (Jorquera et al. 2010) also provide the appropriate conditions for enhanced CO_2 mass transfer, efficient nutrient mixing, and temperature and light intensity control (Lam and Lee 2012). Optimization of operating conditions is being given special attention for implementing the closed photobioreactor-based cultivation process on a commercial basis. High capital expenditure and operating expenditure remain the major drawback with these reactor systems. Efficient mixing and optimum gas-liquid mass transfer in tubular photobioreactors require a significant amount of energy (Lam and Lee 2012).

3 Microalgae Cultivation for CO_2 Sequestration Using Food Industry Wastewater

3.1 Wastewater Generation in the Food Industry

The life on earth entirely depends on the water, and it is one of the major resources on this planet. The worldwide scenario of increasing population and rapid industrialization is deteriorating the freshwater resources and the quality of water. The rate of water pollution has considerably increased due to rapidly expanding industrialization and urbanization. The water supplies from the natural resources have declined over the time which is limiting the industrial growth and also hampering the standard of urban living. The chemical contaminants released from various types of wastewater (domestic/municipal/industrial) pollute the natural water bodies, reducing the integrity of freshwater.

According to a US geological survey, as the global population has exploded even with the limited freshwater resources, the availability of water to individuals has seen drastic reduction. Annual per capita water availability has fallen from 3300 m^3 in the year 1960 to about 1250 m^3 in the year 1995 (60% decrease), the lowest in the world. Further, by the year 2025, it is expected to decrease by another 50% to about 650 m^3. It is anticipated that near the year 2025, the international water demand will be more than the supply by 56%. It was estimated that in India the rate of sewage production was 120 liters/day in metropolitan cities and 60 liters/day per person in cities (Bhuvaneshwari and Devika 2005). Various food processing companies, regardless of their sizes, have contributed to strengthen the economy of the worldwide nations. To achieve the overall economic growth and food security, most of the countries emphasize on the development of food processing industries. Of all the industries, the food processing industries have one of the highest water consumptions, effluent, and sludge generation per unit of production (Ramjeawon and Cleaner 2000). Most of these effluents are contributed by various food processing industries like dairy, sugar mills, brewing, distilleries, oil mills and sweet manufacturing industries, and slaughterhouses.

The quality of water resources usually depends on its physical, chemical, and biological characteristics. The wastewater from food industry is characterized by higher levels of COD and BOD, oil and grease (dairy effluent), nitrogen, and phosphorus than the specified discharge limits. The physicochemical characteristics of food processing industry wastewater are given in Table 1.

In the recent past, scientists across the globe have special emphasis on reuse and recycling of various industrial effluents including the dairy industry effluents (Balannec et al. 2005). Mostly, these effluents remain untreated and discharged, where these effluents contributed to the eutrophication of various surface water bodies. Treatment of wastewater effluents is important not only to prevent eutrophication of surface water bodies but also to reuse it for industrial processes. The physicochemical processes of water treatment suffer the disadvantage due to high reagent costs and low soluble COD removal (Demirel et al. 2005). In addition, chemical treatment processes

Table 1 Composition of various wastewater used for microalgal growth in terms of nitrogen, phosphorus, and carbon

Wastewater type	Source/industry	N ($mg\ l^{-1}$)	P ($mg\ l^{-1}$)	C ($mg\ l^{-1}$)	Reference
Agri-industry	Effluent (potato processing)	NO_3-N – 54 NH_4-N – 12	PO_4-P – 48	COD – 745	Goncalves et al. (2017)
	Effluent (rice processing)	TN – 25–95	PO_4-P – 12–94	COD – 2578–6480	
Anaerobically digested	Dairy industry (manure)	NO_3-N – < 1 NH_4-N – 1279–1961	TP – 240	COD – 4855–4945	
	Domestic sewage	NH_4-N – 646	PO_4-P – 101	TOC – 76	
	Swine waste	NH_4-N – 303–495	Not specified	Not specified	
Food industry	Brewery	NO_3-N – 2–11 NH_4-N – 3–106	TP – 57–326	COD – 565–7837	
	Carpet mill	NO_3-N – 0–28 NH_4-N – 18–26	PO_4-P – 20–35	COD – 1412	
	Dairy	NO_3-N – <1 NH_4-N – 120–350	TP – 35–350	COD – 2000–20213	
	Starch	NH_4-N – 49–115	TP – 50–385	COD – 2000–20213	
	Food processing	NO_3-N – 30–52	PO_4-P – 16–32	COD – 3890–5190	Gupta et al. (2016)
	Olive oil mill	TN– 58.9	TP – 43.1	COD – 5839	Hodaifa et al. (2009)
	Sugar industry	TN – 232.45	TP – 54	COD – 11333	Hodaifa et al. (2009)
Municipal	Domestic sewage	NH_4-N – 25–66	PO_4-P – 7–12	COD – 400–500	Goncalves et al. (2017)
	Landfill leachate	NH_4-N – 112–192	PO_4-P – 7–9	COD – 3725–4861	
	Sewage	NO_3-N – 1 NH_4-N – 23–219	PO_4-P – 1–12	COD – 183–380	

NO_3-N – nitrate-nitrogen ($mg\ N\ l^{-1}$); *NH_4-N* ($mg\ N\ l^{-1}$) – ammonium-nitrogen; *TN* – total nitrogen; *PO_4-P* – phosphate –phosphorus ($mg\ P\ l^{-1}$); *TP* – total phosphorus; *TOC* – total organic carbon ($mg\ C\ l^{-1}$); *COD* – chemical oxidation demand ($mg\ C\ l^{-1}$)

could lead to a secondary pollution by contributing chemicals to the treated water. Dairy wastewater is a complex wastewater containing various contaminants through treated raw materials, inorganic or organic chemicals, and residues from each operation. Regarding the food industry wastewater, treatment methods must ensure to fulfill the required quality of discharged effluents. The commonly used primary and secondary treatment methods of the wastewaters are known to remove the easily settleable materials and to degrade the organic matter present in wastewater. Though the secondary treated effluent looks clear and clean, the secondary effluent is characterized by organics, heavy metals, inorganic nitrogen, and phosphorus. The release of these secondary effluents into the environment may also cause long-term adverse effects on living beings. Microalgae with their unique characteristics of removing nitrogen and phosphorous from wastewater can be used for the tertiary treatment of wastewater with concomitant microalgal biomass production, which can be further exploited for various applications. Microalgae, due to their ability to remove inorganic nitrogen, phosphorus, heavy metals, and some toxic organic compounds, offer a pragmatic solution to tertiary and quaternary wastewater treatment.

3.2 CO_2 Utilization and Wastewater Treatment by Microalgae: Concept and Application

Microalgae-based wastewater treatment system represents a cost-effective and eco-friendly alternative to conventional wastewater treatment processes (Aziz and NG 1992). Microalgal ability to utilize carbon, nitrogen, and phosphorus offers opportunities for their application in advanced phycoremediation and in concomitant high biomass production. Microalgal cultivation with integrated CO_2 sequestration and wastewater treatment systems utilize CO_2 and removes nutrients like N and P from wastewater, which is essential for the growth of microalgae for biomass production. Integrated CO_2 sequestration and wastewater treatment using microalgae are known to have advantages like (1) microalgae act as the effective CO_2 scavengers, which utilize CO_2 sustainably from flue gas for growth, (2) advanced and eco-friendly tertiary wastewater treatment process to remove N and P from wastewater, (3) high biomass production, and (4) exploitation of biomass for various other applications such as feed, fertilizer, and fuel grade products. In wastewater, nitrogen is mainly available in the forms of nitrates, nitrites, and ammonia. Phosphorous is available as phosphates and orthophosphates which plays a crucial role in metabolism upon assimilation by microalgae. The adequate quantities of these major nutrients in the waste water are helpful for the growth of microalgae. Phosphorous is a vital macronutrient that supports the growth of microalgal cell being the constituent of nucleic acids, lipids, and ATP. It is also known that microalgae can reduce the organics and heavy metals from polluted water, thereby preserving the freshwater resources. In addition, it is also reported that when microalgae are cultivated along with the conventional activated sludge systems, the algae-bacteria symbiotic association can reduce the electrical energy demands from aeration, which represents more than 50% of the total energy of wastewater treatment plant. A microalga through photosynthetic activity provides oxygen, which is utilized by aerobic bacteria to deteriorate organic pollutants, and CO_2 released is in turn fixed by algae as a carbon source. The biomass produced thus can be used for several applications including substrate for biogas production, lipids, biodiesel, fertilizers, and biopolymers, which can be converted into packaging materials, and have the advantage of being renewable.

3.3 Cellular Mechanism of Carbon, Nitrogen, and Phosphorus Utilization by Microalgae

Carbon

Microalgae are capable of fixing inorganic carbon into organic energy molecules through uptake of gaseous CO_2, soluble carbonates (HCO_3^-, CO_3^{2-}) as a source of inorganic carbon. These molecules are transported across the membrane via various transporters, channels, and the enzymatic systems. At low pH values (pH 5–7), CO_2 fixation occurs through the diffusion, and at pH 7–9, bicarbonate (HCO_3^-) remains as the most dominant form of inorganic carbon. As an anion, HCO_3^- is impermeable to the lipid bilayer of biological membranes, it is utilized through exchange across the membrane mainly by the membrane transporter proteins and/or extracellular and transmembranous carbonic anhydrase (Goncalves et al. 2017). HCO_3^- inside the cells gets converted into the CO_2 by carbonic anhydrase to be utilized by enzyme RuBisCO for fixation into sugars (Picardo et al. 2013; Sydney et al. 2014).

Nitrogen

Microalgae are capable of fixation as well as assimilation of nitrogen as a nutritional component. The most common inorganic nitrogen forms which are utilized by the microalgae are nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), nitrogen dioxide (NO_2), ammonium (NH_4^+), and molecular nitrogen (N_2). However, nitrate is the most oxidized, stable, and the most preferred form of nitrogen for the cultivation of algae (Barsanti and Gualtieri 2006). The NO_3^- is actively taken up by algae via NO_3^-/H^+ cotransporters, which is maintained by the proton-motive force generated by H^+-ATPases. Cellular nitrate is reduced to ammonium (NH_4^+) to assimilate into organic nitrogenous compounds. Initially, nitrate reductase catalyzes the reduction of NO_3^- to NO_2^-. Further, NO_2^- is reduced to NH_4^+ by the activity of nitrite reductase. Finally, NH_4^+ with 2-oxoglutarate is converted to glutamate via reductive amination by the enzyme glutamate dehydrogenase (GDH). Also NH_4^+ resulting from NO_3^- and NO_2^- reduction and actively incorporated into microalgal cells is directly converted into amino acids via the glutamine synthetase (GS)-glutamate synthase (GOGAT) pathway, where GS catalyzes the conversion of glutamate to glutamine with the incorporation of NH_4^+ with the expense of ATP. GOGAT then catalyzes transamination of glutamine to 2-oxoglutarate, forming two molecules of glutamate. While one of these molecules is recycled back by GS, the other is at the base of the formation of amino acids by transamination of the amino nitrogen of glutamate to various alpha-keto acids (Barsanti and Gualtieri 2006; Crofcheck et al. 2012; Hellebust and Ahmad 1989). Since NH_4-N assimilation does not require previous reduction steps, it is thought that this is the preferred nitrogen form for microalgae. However, according to Grobbelaar, the effect of both NO_3-N and NH_4-N on microalgal growth is not clear, since no considerable differences were found in microalgal biomass productivity. In addition to microalgal uptake, NH_4-N removal may occur in response to an increase in pH and temperature, where large amounts of NH_4-N can be volatilized (Grobbelaar 2004).

$$NO_3^{\ -} + 2H^+ + 2e^- \xrightarrow{\text{Nitrate reductase}} NO_2^{\ -} + H_2O$$

$$NO_2^{\ -} + 8H^+ + 6e^- \xrightarrow{\text{Nitrate reductase}} NH_4^{\ +} + 2H_2O$$

$$\text{Glutamate} + NH_4^{\ +} + \text{ATP} \xrightarrow{\text{Glutamine Synthetase}} \text{Glutamine} + \text{ADP} + \text{Pi}$$

$$\text{Glutamate} + H_2O + \text{NAD}(\text{P})^+ \xrightarrow{\text{GDH}} 2\ \ \text{Oxoglutarate} + NH_4^{\ +} + \text{NAD}(\text{P})\text{H} + H^+$$

$$\text{Glutamine} + 2\ \ \text{Oxoglutarate} \xrightarrow{\text{NADH+GOGAT}} 2\,\text{Glutamate} + \text{NAD}^+$$

Cyanobacteria can fix N_2 into ammonia (NH_3), which can either be incorporated into nitrogenous compounds like amino acids or excreted outside.

$$N_2 + 8H^+ + 8e^- + 16\,\text{ATP} \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16\,\text{ADP} + 16\text{Pi}$$

Phosphorus

Energy transfer and nucleic acid synthesis are mediated by phosphorus assimilation. Phosphorus enters microalgal cells through active transport across the plasma membrane in the forms of $H_2PO_4^-$ and $HPO_4^{2\,-}$. Incorporation of PO_4^{3-}-P into organic compounds occurs through phosphorylation at the substrate level, oxidative phosphorylation, and photophosphorylation (Martinez et al. 1999). Moreover, the pH of the medium defines the availability of phosphorus for utilization by microalgae. It is known that pH > 9 and high dissolved oxygen concentration results in phosphate precipitation (Cai et al. 2013).

4 Application of Microalgae Grown in Wastewater

Though the use of microalgae for the first time by humans was about 2000 years ago, when *Nostoc* was used by the Chinese to survive during the famine, applications of microalgae began to prosper in the middle of the last century (Spolaore et al. 2006). The "Aquatic Species Program" was one of such developmental programs launched in 1978, funded by the US Department of Energy. This program aimed at looking into the production of energy using aquatic plants and algae over a period of nearly two decades (1978–1996). However, the July 1998 closure report from the program stated that even with elevated lipid yield, the algal biodiesel production would be feasible only if petrodiesel prices increased to double the year 1998 levels (National Algal Biofuels Technology Review 2016). Fossil fuels are the non-sustainable, conventional source of energy used worldwide. Fossil fuel combustion results in the emission of GHGs. Microalgae have received growing attention as a renewable source of energy with CO_2 mitigation potential. Microalgal cultivation using wastewater is increasing its value, as the cultivation can be done on nonagricultural/arid land providing an optimum yield. Carbon, Nitrogen, Phosphorus and other nutrients essential for the growth of algae can be obtained from wastewater. Algae also contains high oil and starch, which favors the high-quality biodiesel production. Therefore, many researchers are considering algae cultivation using wastewater as a cost-effective and eco-friendly approach for obtaining high biomass along with wastewater treatment. The biomass produced can be exploited for various applications. Application of wastewater-grown microalgae is discussed below.

4.1 Biodiesel

The mono-alkyl esters of fatty acids obtained from renewable oil feedstocks using alcohol and a catalyst (acid or a base) can be defined as biodiesel (Mata et al. 2010). The worldwide studies on biodiesel production as an alternative to petrodiesel have been conducted mainly due to rising crude oil prices. Biodiesel produced from oilseed crops like soybean oil, palm oil, and rapeseed oil cannot meet the existing demand of fuel because of their low reliability and less acceptability due to food versus fuel debate. Therefore, microalgae due to their high biomass productivity ($0.02\ gl^{-1}\ d^{-1}$ to $7.7\ gl^{-1}\ d^{-1}$) and high lipid content (30–70%) are considered as the renewable alternative of oil for biodiesel (Mata et al. 2010). Microalgal lipid productivity is reported to be more than the best oilseed-producing crops. Lipid content in microalgae is considered as the primary criteria for the selection of microalgal species to produce biodiesel (Griffith and Harrison 2009). In a research conducted by Hempel et al. (2012), several microalgal species (*Chlorella* sp., *Cosmarium* sp., *Spirulina* sp., etc.) were screened for fatty acid profiling. Reports showed that major fatty acids like palmitic acid, oleic acid, and linolenic acid, which are suitable for the production of biodiesel, account for about 90% of total fatty acid content in the screened microalgal species (Hempel et al. 2012). Microalgae for the purpose of biodiesel can be sustainably grown in wastewater, considerably reducing the wastewater's nutrient load (N, P, K) (Christenson and Sims 2011). Studies on microalgal lipid production and fatty acid methyl ester profiling have been widely carried out (Shekh et al. 2013a, 2016a, b; Fulke et al. 2010).

Various microalgal species have been studied for wastewater treatment and concomitant biomass production. Majority of fatty acids in *Chlorella* sp. were identified to be short-chain fatty acids (C14–C18) which form a major component of biodiesel; and, hence, these species are considered to be better for biofuel production (Gulyurt et al. 2016). He et al. (2013) cultivated *Chlorella vulgaris* in wastewater containing elevated concentrations of ammoniacal nitrogen (NH_4^+-N). It was found that increase in NH_4^+-N concentration from 17 to 207 mg l^{-1} enhanced short chains and saturated fatty acids. The productivity of lipid also increased up to 23.3 mg l^{-1} d^{-1} at 39 mg l^{-1} d^{-1} NH_4^+-N. The C_{16} and C_{18} fatty acids comprised of about 80% of total fatty acids in the algal biomass (He et al. 2013). The biomass and lipid productivity of a consortium of ten native microalgal strains (isolated from dairy wastewater) cultivated in treating dairy wastewater at 10% CO_2 supply. The lipid content of 16.89% and biomass production of 153.54 t ha^{-1} y^{-1} was reported. Also, more than 98% COD as well as nutrients were efficiently removed (Hena et al. 2015). Studies by Malla et al. (2015) showed that algal species *C. minutissima* removed a major nutrient load from partially treated wastewater with the specific lipid productivity of 132–171 mg g $cells^{-1}$ d^{-1}. The oleic acid content in algal cells was also seen to be augmented when cultivated using wastewater from the common effluent treatment plant. It was also characterized by the removal of 60% BOD and 75% COD. *C. minutissima* removed about 70–80% N, 90–98% TDS, 45–50% K, and 60–70% P within 12 days of the cultivation period (Malla et al. 2015). A study by Kothari et al. (2013) demonstrated that *C. polypyrenoideum*, when cultivated in dairy industry wastewater, substantially reduced N (90%) and P (96%) content which can otherwise contribute to eutrophication of water. It is also reported that oil yield of microalgal biomass cultivated on dairy wastewater was found to be comparatively better than that of microalgal biomass cultivated in the standard BG-11 medium. Lipid content of 42% (w/w) clearly delineated the potential of algal biomass for biodiesel production (Kothari et al. 2013). Studies on several microalgae species for enhancement of lipids by employing various strategies (Adenan et al. 2016; Huang et al. 2010) for biodiesel production are reviewed by Chisti (2007), Mata et al. (2010), and Griffith and Harrison (2009), which can be referred for further more insights (Yellapua et al. 2018; Makareviciene et al. 2013; Hosseinia et al. 2018). Considering these advantages, microalgae can be used as a sustainable source of oleaginous biomass for biodiesel production (Table 2).

4.2 Biomethane and Syngas

Biomethane

Direct utilization and/or co-digestion of wastewater grown microalgae biomass to enhance biomethane production is considered as an attractive economic integration of microalgae cultivation for treating wastewater and bioenergy generation. The suitability of the microalgal biomass depends on its ability to produce biomethane and

Table 2 Potential microalgae species studied for integrated biomass and lipid production with wastewater treatment and nutrient removal

Algal species	Cultivation strategy	Cultivation parameters				Cultivation output		Nutrient removal rate			References
		Nutrient loading	Light intensity	CO_2	T (°C)	Biomass prod.	Lipid (%)	COD	TN	TP	
Chlorella sp.	Indoor, bench scale (25% RDW)	COD – 600 mg l^{-1} TN – 283 mg l^{-1} TP – 29 mg/l	3000 ± 10 lux	No	25	338.8 mg l^{-1} d^{-1}	–	88.38 mg l^{-1} d^{-1}	37.28 mg l^{-1} d^{-1}	2.03 mg $l^{-1}d^{-1}$	Weidong et al. (2015)
Chlorella sp.	Outdoor pilot scale (10% RDW)	COD – 600 mg l^{-1} TN – 283 mg l^{-1} TP – 29 mg l^{-1}	123–1418 μmol m^{-2} s^{-1}	No	14–38	160 mg l^{-1} d^{-1}	–	41.31 mg l^{-1} d^{-1}	6.58 mg l^{-1} d^{-1}	2.74 mg $l^{-1}d^{-1}$	Weidong et al. (2015)
Rhizoclonium hieroglyphicum	Lab-scale algal turf scrubber (raw dairy manure effluent)	TN – 0.3–2.3 g m^{-2} d^{-1}	240–633 μmol m^{-2} s^{-1}	Yes	18–28	8.8–20.4 g m^{-2} d^{-1}	5.3–7.5	–	–	–	Mulbry et al. (2008a, b)
Rhizoclonium hieroglyphicum	Lab-scale algal turf scrubber (raw dairy manure effluent)	TN – 0.3–2.3 g m^{-2} d^{-1}	240–633 μmol m^{-2} s^{-1}	No	18–28	8.3–18.6 g m^{-2} d^{-1}	5.3–7	–	–	–	Mulbry et al. (2008a, b)
Rhizoclonium hieroglyphicum	Lab-scale algal turf scrubber (anaerobically digested dairy manure effluent)	TN – 0.38–1.56 g m^{-2} d^{-1}	240–633 μmol m^{-2} s^{-1}	Yes	18–28	10.3–21 g m^{-2} d^{-1}	3.5–7.7	–	–	–	Mulbry et al. (2008a, b)

(continued)

Table 2 (continued)

Algal species	Cultivation strategy	Cultivation parameters				Cultivation output		Nutrient removal rate			References
		Nutrient loading	Light intensity	CO_2	T (°C)	Biomass prod.	Lipid (%)	COD	TN	TP	
Rhizoclonium hieroglyphicum	Lab-scale algal turf scrubber (anaerobically digested dairy manure effluent)	TN – 0.38–1.56 g $m^{-2} d^{-1}$	240–633 μmol $m^{-2} s^{-1}$	No	18–28	10.5–17.3 g $m^{-2} d^{-1}$	4.2–6.9	–	–	–	Mulbry et al. (2008a, b)
Rhizoclonium hieroglyphicum	Pilot-scale algal turf scrubber (raw dairy manure effluent)	TN – 0.39–1.9 g $m^{-2} d^{-1}$	–	No	20–25	5.3–14.6 g $m^{-2} d^{-1}$	6.5–9.9	–	–	–	Mulbry et al. (2008a, b)
Rhizoclonium hieroglyphicum	Pilot-scale algal turf scrubber (anaerobically digested dairy manure effluent)	TN – 0.43–0.68 g $m^{-2} d^{-1}$	–	No	20–25	4.3–7.6 g $m^{-2} d^{-1}$	6.0–8.0	–	–	–	Mulbry et al. (2008a, b)
Chlorella sp.	Shake flask (20% raw dairy farm wastewater)	COD – 2310 mg l^{-1} NH4-N- 73 mg l^{-1} TP- 58.5 mg l^{-1}	5000 lux	No	27	95 mg $l^{-1} d^{-1}$	NA	89.7%	83.2% (NH_4-N)	91.97%	Ding et al. (2014)
Neochloris oleoabundans	Shake flask (2% anaerobically digested dairy manure)	TN – 120 mg l^{-1}	200 μmol $m^{-2} s^{-1}$	Yes (3%)	23–25	90 mg $l^{-1} d^{-1}$	9.9 ± 2.7	–	7 mg $l^{-1} d^{-1}$	–	Levine et al. (2011)
Chlorella sorokiniana	Shake flask	COD – 1074 mg l^{-1} TN – 5.27 mg l^{-1} TP – 6.41 mg l^{-1}	80 μmol $m^{-2} s^{-1}$	–	–	230.71 mg $l^{-1} d^{-1}$	22.91	90%	99%	98%	Hena et al. (2015)

Chlorella vulgaris	Glass photobioreactor (UV treated)	COD – 1505 mg l^{-1} TN – 92.5 mg l^{-1} TP – 62.9 mg l^{-1}	300 µmol m^{-2} s^{-1}	Yes (5%)	25	287 mg l^{-1} d^{-1}	14.38	74%	96%	96%	Qin et al. (2013)
Chlorella vulgaris	Glass photobioreactor (sodium hypochlorite)	COD – 1364 mg l^{-1} TN – 70.5 mg l^{-1} TP – 60.9 mg l^{-1}	300 µmol m^{-2} s^{-1}	Yes (5%)	25	441 mg l^{-1} d^{-1}	10.50	57%	73%	92%	Qin et al. (2013)
Chlorella sp.	Flask (5% anaerobically digested dairy waste)	COD – 3850 mg l^{-1} TN – 349.8 mg l^{-1} TP – 349.8 mg l^{-1}	1100 µmol m^{-2} s^{-1}	Yes	25	340 mg l^{-1} d^{-1}	10	11%	71.40%	86.20%	Wahal and Viamajala (2016)
Chlorella pyrenoidosa	Flask	–	55 µmol m^{-2} s^{-1}	No	25	–	7	92%	97%	99%	Yadavalli et al. (2014)
Euglena gracilis	Flask	–	55 µmol m^{-2} s^{-1}	No	25	–	11	86%	95%	97%	Yadavalli et al. (2014)
Chlorella vulgaris	Dairy manure with polystyrene foam support	TN – 118 mg l^{-1} TP – 77 mg l^{-1}	110–120 µmol m^{-2} s^{-1}	No	20	2.6 g m^{-2} d^{-1}	9	–	77.16%	90.04%	Johnson and Wen (2010)
Chlorella vulgaris	Pilot-scale open raceway pond (aerobically digested tofu wastewater)	BOD – 246.34 mg l^{-1} TN – 5.02 mg l^{-1} TP – 10.4 mg l^{-1}	–	Yes	28–32	175 mg l^{-1} d^{-1}	–	80.62%	85.47%	65.96%	Choi (2016)

its influence on the overall anaerobic digestion process. Non-processed whole algae biomass is not considered as a superior substrate for biomethane production as the high amount of microalgal lipids results in the generation of the volatile fatty acids (VFAs) and is inhibitory for anaerobic digestion. However, the lipid-extracted biomass acts as a good substrate for biomethane production.

Microalgae as an alternative feedstock for biomethane production are receiving great attention mainly due to its high carbohydrates and lipids with easy digestibility. Various studies have been conducted for exploiting microalgal biomass for biomethane production. Microalgae-bacterial biomass grown in high-rate algal ponds for the treatment of wastewater were evaluated for the effect of microwave pretreatment on the solubilization and anaerobic digestion with varying exposure times. The maximum biogas production rate was observed with the biomass pretreated at 65,400 KJ/kg TS, followed by biomass, which was pretreated at lower specific energies and untreated biomass. The biogas production rate of treated biomass was 88 ml g^{-1} VS d^{-1}, whereas the control produced biogas of 50 ml g^{-1} VS d^{-1} which is 57% of it (Passos et al. 2013). The potential of *Diplosphaera* sp. MM1 for biomethane production through dairy and winery wastewater remediation was also studied. Anaerobically digested microalgae grown in BG-11 medium (control), 50% winery effluent, and 33% dairy effluent resulted in a biomethane production potential of 197.39, 218.51, and 129.75 ml g^{-1} VS (Liu et al. 2016). In the most recent study, a hydrogen and methane co-production system using *Laminaria digitata* and *Arthrospira platensis* biomass at a C/N ratio of 20 was established. The first-stage H_2 reactor and the second-stage CH_4 reactor showed a hydraulic retention time (HRT) of 4 days and 12 days, respectively. At an organic loading rate of 6.0 g VS l^{-1} d^{-1}, the maximum specific hydrogen yield of 55.3 ml g^{-1} VS was obtained, and a specific methane yield of 245.0 ml g^{-1} VS was achieved at a subsequent organic loading rate of 2.0 gVS$L^{-1}d^{-1}$ (Ding et al. 2018). Lavric et al. (2017) has tested the microalgal biomass generated in thermophilic anaerobic digestate as a substrate for the production of biogas in thermophilic anaerobic digestion. The results of the pilot experiment suggest that the microalgae produced by thermophilic biogas digestate can be utilized as a potential feedstock with biomethane production potential of 157.5 ± 18.7 ml CH_4 g^{-1} VS. Concomitantly, microalgae were also capable of reducing the digestate nitrogen (nitrite max 10 mg l^{-1}, ammonia-nitrogen max 200 mg l^{-1}) and COD (Lavric et al. 2017). Though the studies on dairy wastewater-grown microalgal biomass utilization for biomethane production are scarce, it has huge potential for integrated wastewater treatment and biomethane production considering the high amount of carbohydrates.

Syngas

Syngas is also called synthetic gas, synthesis gas, or producer gas. It comprises a mixture of carbon dioxide, carbon monoxide, and hydrogen (Azadia et al. 2014). At industrial scale currently, the major supply of syngas is from fossil fuels, which is obtained mainly from natural reforming processes and coal gasification (Popp et al. 2014). Hence, there is a necessity to promote more alternative resources that are sustainable, for syngas production. After thermochemical gasification of biomass, under partial oxidation, syngas is obtained containing H_2, CO, CO_2, and CH_4. Microalgae are also considered as the sustainable source for syngas production mainly because of their high content of protein, carbohydrates, and lipid. Microalgal biomass also has a high calorific value of 30–35 KJg^{-1} in *C. vulgaris* (Ghayal and Pandyaa 2013). The type and composition of biomass are known to influence the process conditions for the optimization of syngas production and its quality. Raheem et al. (2015) has employed central composite design for syngas production using high-temperature horizontal tubular furnace to optimize the microalgal gasification. Their results indicate that an optimal H_2 yield of 41.75 mole% was obtained at a heating rate of 22 °C min^{-1}, a temperature of 703 °C, a biomass loading of 1.45 g, and an equivalent ratio of 0.29 (Raheem et al. 2015). Microwave-induced pyrolysis is one of the beneficial methods for the production of syngas when compared to the conventional electric furnace. Benerso et al. (2014) studied the microwave-induced pyrolysis (400 °C and 800 °C) of microalga *Scenedesmus almeriensis*. A considerable syngas yield (c.a. 94 vol.%) was reported at the pyrolysis temperature of 800 °C (Benerso et al. 2014). Hu et al. (2013) conducted studies on fast pyrolysis of microalga *C. vulgaris* in a quartz tube reactor at varying temperatures (500–900 °C). Increased pyrolysis temperature (800–900 °C) showed a higher biofuel yield (91.09 wt.%) which contains syngas (Hu et al. 2013). In another study by Hu et al. (2014), an investigation was done for the pyrolysis of microalgae *C. vulgaris* to produce syngas in the presence of different catalysts and varying amount of activated carbon. Results prove that activated carbon of 3% is the most favorable content of activated carbon for the production of syngas (Hu et al. 2014). Ebadi et al. (2017) reported the enhancement in the yield of syngas (H_2) by steam gasification of algal biomass (*Cladophora glomerata L.*) using alkali and alkaline earth metal compounds (NaOH, $KHCO_3$, Na_3PO_4, and MgO) as catalysts. Results indicated that NaOH comparatively gave a high yield of syngas (H_2) (Ebadi et al. 2017). Hence the production of syngas from the microalgal biomass is a valuable contribution with several commercial applications. Syngas is an eco-friendly fuel with a potential for several commercial applications. It can be effectively used as a fuel for transportation and in the generation of heat/electricity (Yoo et al. 2010). Ethanol is also a desirable product which can be formed from syngas. The catalytic reaction of CO and CO_2 with H_2 (syngas component) produces methanol. Syngas can be used as an intermediary source for the production of hydrogen, industrial chemicals, and ammonia using chemical and biological processes like syngas catalytic reforming and syngas fermentation, respectively (Raheem et al. 2017).

4.3 Bioethanol and Biobutanol

Bioethanol

Bioethanol is a product obtained by the fermentation of sugars, which are derived through hydrolysis of starch. Potential of ethanol as a biofuel was tested early in the year 1800. At present, the USA and Brazil are the leading producers of bioethanol using corn and sugarcane as the feedstock, while potato, wheat, and sugar beet are used for bioethanol production in Europe (Jambo et al. 2016). Microalgae can be grouped as the third-generation feedstock, which can overcome the challenges (food scarcity, cost ineffectiveness) of conventional bioethanol sources. Carbohydrate accumulation of about 40% of the dry biomass has been observed in several microalgal strains (Chaudhary et al. 2014) mainly as insoluble starch and cellulose (Hernandez et al. 2015). Therefore, due to considerable carbohydrate content and higher ethanol yields, the microalgae could be an apt substrate for commercial production of bioethanol (John et al. 2011). The microalgae, viz., *Dunaliella*, *Chlorella*, *Chlamydomonas*, *Sargassum*, *Spirulina*, *Gracilaria*, *Prymnesium parvum*, *Euglena gracilis*, and *Scenedesmus*, have been used for bioethanol production (El-Sayed et al. 2016). Hwang et al. (2016) reported the combined effect of sonication, heat, and enzymatic hydrolysis (SHE) on a biomass containing various microalgal populations, for ethanol production. SHE treatment of mixed algal biomass had optimum carbohydrate conversion efficiency, raising the concentration of dissolved carbohydrate increasing from 0.02 to 0.07 gg^{-1}. A higher ethanol yield (1.4 fold) was observed through alcohol fermentation by *Dekkera bruxellensis* (yeast). These results conclude that mixed algal biomass, when subjected to a combined SHE treatment, can be utilized as a sustainable substrate for continuous ethanol production when fermented by yeast (Hwang et al. 2016). Further, wastewater-grown microalgae have also been studied for bioethanol production through carbohydrate estimation. Reyimu and OzcImen (2017) has evaluated the carbohydrate and the bioethanol production potential of *N. oculata* and *T. suecica* cultivated in varying concentrations of municipal wastewater. 75% and 25% of wastewater were found to be ideal for the cultivation of *N. oculata* and *T. suecica*, respectively. Microalgae, which were grown on 100% of wastewater, accumulated 6.52% carbohydrate. *N. oculata* gave a bioethanol yield of 3.68% when cultivated in 75% wastewater. The highest bioethanol yield ozf 7.26% was reported by the control group of *T. suecica* which had a carbohydrate content of 27% (Reyimu and OzcImen 2017). After oil extraction, the residual algal biomass rich in carbohydrates can be utilized as a substrate for ethanol fermentation. Harun et al. (2009) reported the potential of the residual microalgal biomass of *Chlorococcum* sp., as a substrate for the production of bioethanol through fermentation by the yeast *Saccharomyces bayanus* (bioethanol yield, 3.8 g l^{-1}; substrate concentration, 10 g/L) (Harun et al. 2009). Rizza et al. (2017) reported two new strains, *Desmodesmus* sp. FG and a green microalgae (SP2–3), which is unidentified, producing high carbohydrate content of 57% and 70% (of DCW), respectively. For these species, proficient biomass to ethanol conversion (0.24 g ethanol per gram of algal biomass) was observed (Rizza et al. 2017). Report by Ho et al. (2013) showed the prospective bioethanol production by microalgae *C. vulgaris* FSP-E as a feedstock. After enzymatic hydrolysis, these algal genera gave a 90.4% glucose yield. The microalgal acidic hydrolyzate *C. vulgaris* FSP-E biomass was assessed by separate fermentation and hydrolysis (SHF), and concurrent fermentation and saccharification (SSF) processes. About 11.7 g l^{-1} of ethanol was produced by SHF process which was 87.6% of theoretical yield (Ho et al. 2013).

Biobutanol

Biobutanol is a fairly non-polar, four-carbon, long-chain alcohol produced by fermentation of biomass. Biobutanol can be substituted for diesel, gasoline, and kerosene (Bharathiraja et al. 2017). Microalgae-based carbohydrates are a sustainable source for biobutanol production. Furthermore, the commercial production of algal biobutanol was reported to be economically feasible (Lee 2016). Wang et al. (2014) studied the feasibility of biobutanol production by using *C. vulgaris* JSC-6 which contains glucose and xylose (carbon source) in the ratio of 5:1–6.5:1 and a total of 50% carbohydrate content. Biobutanol production was carried out with the hydrolyzed biomass of *C. vulgaris* JSC-6 C using (acetone-butanol-ethanol) fermentation by *Clostridium acetobutylicum* ATCC824. The butanol production rate was found to be 0.89–0.93 g/h/L (Wang et al. 2014). Wang et al. (2017), microalga *Neochloris aquatica* CL-M1 was grown in swine wastewater; the results illustrated 81.7% of COD removal and 96.2% of NH_3-N removal with a carbohydrate concentration of 50.46%. The obtained carbohydrate-rich biomass (48.7 g l^{-1} glucose and 3.4 g l^{-1} xylose) was used as a feedstock for the production of butanol after pretreatment. The butanol yield, concentration, and productivity were 0.60 mol mol^{-1} sugars, 12.0 g l^{-1}, and 0.89 g l^{-1} h^{-1}, respectively (Wang et al. 2017). Batch experiments were conducted by Cheng et al. (2014) for the production of butanol from biodiesel residues of microalgae (*Chlorella sorokiniana* CY1) using *C. acetobutylicum*. The concentration of glucose in the substrate to be used was optimized. Also, the butyrate addition effect of on the production of butanol was studied. ABE fermentation by *C. acetobutylicum* produced 3.86 g l^{-1} of butanol. Results indicated that the optimal butanol yield of 0.4 g/g glucose was seen with 18 g l^{-1} of butyrate and 60 g l^{-1} of glucose (Cheng et al. 2014).

4.4 Animal Feed

Microalgae as an ingredient in animal feeds are gaining popularity due to their high nutritional values. Many studies regarding the toxicological and nutritional assessments have proved the aptness of microalgal biomass as a feed supplement. Currently, the global animal feed market accounts for about $20 billion and is likely to grow at a CAGR of 3–4%. At present, world's 30% algal production is being sold for applications in animal feed, and more than 50% of the world's *Spirulina* production is used for animal feed (Becker 2004). Microalgae for the purpose of animal feed can be cultivated in food industry wastewater as it has low heavy metal load and is less toxic (Maizatul et al. 2017). Microalgal biomass with long-chain omega-3 fatty acids and high digestible protein content is preferred for animal feed. Spirulina is widely used to feed animals like cats, breeding bulls, dogs, ornamental birds, aquarium fish, cows, and horses. Algae when given as animal feed show positive effects on the physiology and the external appearance in animals (Chen 2003). The most commonly used species of algae in aquaculture are

Chlorella, *Skeletonema*, *Tetraselmis*, *Isochrysis*, *Skeletonema*, *Pavlova*, *Chaetoceros*, *Phaeodactylum*, *Nannochloropsis*, and *Thalassiosira* (Spolaore et al. 2006). Considering these advances, several researchers have further explored the potential of microalgae to be used as animal feed. A study by Evans et al. (2015) reported that when broilers were given about 16% of the dried *Spirulina* in their diet, they showed a considerable impact on the growth performance of the chicks (Evans et al. 2015). In another report by Bruneel et al. (2013), increase in DHA content was found in the egg yolk of hens fed with *Nannochloropsis gaditana*. Therefore, this microalga could be used to produce eggs enriched with DHA (Bruneel et al. 2013). Studies by Bhalamurugan et al. (2018) showed that there was improved growth performance when 1% of fresh liquid *Chlorella* was introduced into the diet of chicks. Improved immune system functioning and an increase in the intestinal microflora was also noted (Bhalamurugan et al. 2018). In another study by Sugiharto and Lauridsen (2016), dietary *Chlorella* administration (0, 5, and 10 g kg^{-1}) was given to the chicks. As a result *Chlorella* supplementation increased the IgA concentration in the intestinal mucosa when tested after 35 days of administration (Sugiharto and Lauridsen 2016). It is estimated that about 4.4–11 million tons of soybean meal can be saved for the use of humans if 20–50% of the soybean meal fed to animals are replaced by microalgal biomass (Gatrell et al. 2014). Thus, microalgal biomass can be a very useful source of feed in nurturing the animals, poultry, and marine life.

5 Challenges for Cultivation of Microalgae in Food Industry Wastewater

Recent research has manifested the potential of microalgae for integrated wastewater treatment, CO_2 utilization, and concomitant production of high biomass for biofuel and other application (Mobin and Alam 2014). However, algae cultivation using food industry wastewater still encounters challenges mainly due to varying composition of wastewater. Apart from this various environmental, biological, and operational problems persist for mass cultivation of microalgae. The challenges in large scale using CO_2 and wastewater include land availability, CO_2 mass transfer and solubility, photosynthetically active radiation (PAR) delivery, supply and recycling of nutrients, the integrity of the high-performing culture, environmental control, and harvesting.

The growth rate of algae and cyanobacteria solely depends on physical, chemical, biological, and operational factors throughout their cultivation (Becker 1988). The suitability of wastewater for microalgal cultivation is dictated by the source and composition of wastewater, pH, intensity of light, temperature, concentrations of phosphorus, nitrogen, and organic carbon as well as its ratio (C: N: P), intensity of light, and availability of CO_2 and O_2 (Pittman et al. 2011). Moreover, the microalgae growth rate in wastewater is highly influenced by the composition of the bacteria-algae population, competition between species, and the presence of grazing organisms and viruses (Kotasthane 2017).

The elemental composition of an average algal cell is $C_{106}H_{181}O_{45}N_{16}P$. To maintain this composition, the required elements should be present in the requisite proportions in the growth medium so as to obtain optimal growth (Oswald 1988). For optimal growth and maximum efficiency of the culture, Redfield (N: P) ratio of 16:1 is required in the growth medium (Rhee 1978). A molar ratio of the nutrients in the wastewater should be such to maintain the stoichiometric ratio of the elements in the algal cell. Therefore, in case of insufficient nutrients, the nitrogen or phosphorus should be externally added to maintain the proper N to P ratio of the wastewater media. A major difference between food industry wastewater media and other standard growth media is the presence of high concentration of N and P in the food industry wastewater. The wastewater containing nutrients like NH_4^+ and PO_4^{3-} is suitable for microalgae growth; the NH_4^+ is actively taken up by the algal cell as compared to other sources of nitrogen, i.e., nitrates and nitrite. However, high concentrations of NH_4^+ and PO_4^{3-} in food industry wastewater can have a negative effect on the growth (Borowitzka 1998). It has been studied that the metabolic activities of microalgae increase the pH of the medium. However, the elevated pH could result in ammonia striping and precipitation of phosphates in the form of calcium phosphate (Guldhe et al. 2017). The pretreatment of wastewater should be done in order to lower COD, BOD, turbidity, suspended solids, microorganisms, etc., which are necessary to optimize the utilization of wastewater for microalgal growth.

5.1 Robust Microalgal Strain Selection

Over the years, several studies have been conducted to grow microalgae using various wastewaters. The fluctuating wastewater composition influences the growth, biomass productivity, as well as lipid quantity and quality of microalgae (Chinnasamy et al. 2010). The performance of microalgal strains differs when used in different types of wastewaters. This is mainly due to variation and the imbalance in nutrient profile, lack of some or all required trace elements, and occurrence of growth inhibiting/toxic non-biodegradable chemicals in wastewater. Only some of the strains of few species (e.g., *Scenedesmus* sp. and *Chlorella* sp.) are known to acclimatize well in different wastewaters. Therefore, it is essential to screen and characterize the wastewater as well as local climate specific robust microalgal strains. Locally isolated microalgae strains are known to perform better than those purchased from algae banks. Further, strain improvement to develop high-performance resistant strains can be done through genetic and/or breeding manipulations (Zhou et al. 2014). For the efficient wastewater treatment, there is a need for selecting the wastewater-specific algal strain which could decrease the COD of wastewater effectively with simultaneous biomass and high-quality lipid production (Zhou et al. 2014). Monocultures of high lipid-producing strains can also be used for wastewater treatment (Tian et al. 2014) with careful maintenance. Thus, when varied wastewaters are used, domination by locally occurring mixed cultures of algae is always expected.

5.2 Effect of Grazer Organisms and Viruses

In wastewater, there is a complex population of microorganisms that compete with each other for nutrients and survival. Microalgae are sensitive and can be affected by many kinds of parasite, bacteria, fungi, and viruses present in the wastewater. Due to the high sensitivity of microalgae to ecological changes, they have been used as sensitive indicators in toxicity tests or in the study of microbial ecology (Day et al. 1998). Microalgae cultivated in the wastewater are often negatively affected by different kinds of predators such as protozoa and unicellular organisms that feed on algae. The predation of the microalgae is hard to avoid, but it is possible to control by maintaining the appropriate environmental and operational conditions. Some of the methods that have been developed to prevent microalgae infection are as follows: Acidification of the wastewater to a pH of 2 for a short period of time, this can help in killing most rotifers and protozoan. Daily removal of particulate matter larger than 100 μm (Grobbelaar 1982) or dosing of wastewater with high ammonia concentration (Becker 1994) can help in removing zooplankton. Using a system that has a short period of anaerobic stages before microalgae treatment can help to reduce fungal development (Borowitzka 1998). However, when wastewater is used as a growth medium, the issues remain unresolved: (1) high nutrient (N, P, K) concentration, which could considerably cause algal growth constraints; (2) poor availability of a large share of the carbon sources due to its association with insoluble organic compounds; (3) high demand of freshwater to dilute the concentrated wastewater; and (4) development of high-performance robust microalgae strains are still in progress.

5.3 Land Requirement

The major shortcoming of microalgae cultivation for biomass production through wastewater treatment is the requirement of the algal pond system since it demands for higher land footprint than other forms of sewage treatment. Therefore, design engineers must take into consideration the local land prices and soil suitability to arrive at the least cost method of wastewater treatment. Most of the times, open ponds are the favored treatment system, as required land is generally available at the cheapest cost.

5.4 pH

The pH of the wastewater can impact the metabolism of various organisms including microalgae. All microalgae are known to have their own optimum range of pH. The pH can play a critical key role in the biochemical composition of the wastewater. For instance, Hodaifa et al. (2009) found that pH has great influence on the growth and biomass composition of the microalgae *Scenedesmus obliquus*. They observed that high reduction of BOD occurs at elevated pH and the highest protein and chlorophyll contents in the cell occur when algae cultivated under pH of 7 (Hodaifa et al. 2009). pH can affect the solubility of the CO_2, O_2, and the essential mineral salts required for microalgae growth in wastewater. It influences the equilibrium as well as the dissociation of different ionic species. It is also known to cause toxic effects to the organism through inhibition of metabolic processes (enzymes) and/or creating deficiencies in some nutrients in the culture medium (Rubio et al. 1999). It affects the availability of inorganic carbon with respect to the concentration and the proportion of the various carbon species such as CO_2, HCO_3^-, and CO_3^{2-} (Niess et al. 1981). As a result, pH determines which form of inorganic carbon is available for assimilation by microalgae. High levels of pH alter the physicochemical environment of the wastewater and cause phosphate and metals precipitation, ammonia stripping, and disinfection.

5.5 CO_2 Availability

The availability of dissolved CO_2 in wastewater varies greatly with pH. At lower pH values, CO_2 is abundant, while at higher pH of greater than 8, there will be a deficit in CO_2 concentration, and most of the carbon will be in the form of carbonate (CO_3^{2-}) which cannot be uptaken by microalgae (Borowitzka 1998) unless converted to CO_2 which is catalyzed by carbonic anhydrase (Badger and Price 1994). Another challenge is the optimization of CO_2 delivery to the cultivation system. The direct bubbling of CO_2 in the pond comes with an engineering challenge. It is directly related to CO_2 supply and removal of excess dissolved oxygen. Oxygen saturation in the cultivation system is known to inhibit photosynthesis through photooxidative damage. Open pond productivities potentially suffer from carbon limitation mainly arising due to CO_2 mass transfer limitations. It is reported that CO_2 supply in shallow cultures results in high CO_2 losses due to the insufficient bubble residence time required for CO_2 absorption (Richmond 2013). However, at alkaline pH, the CO_2 absorption is better, mainly due to faster CO_2 hydration followed by acid-base reaction to form HCO_3^- and direct conversion of CO_2 to HCO_3^- upon reaction with OH^- ions. It has been noted that below pH 8, the rate of the former reaction is faster, while the second reaction dominates above pH 10 (Weissman et al. 1988).

6 Future Needs

Advances in research clearly delineated the potential of microalgae for integrated biomass and lipid production with concomitant wastewater treatment and CO_2 utilization. However, considerable research efforts are needed to enhance CO_2 solubility in ponds, wastewater treatment efficiency, biomass, and lipid productivity. To overcome the mass transfer limitations of CO_2, compressed CO_2 can be one of the viable options to enhance availability of dissolved CO_2 into the open ponds. However, the use of compressed CO_2 can add on to the final cost of pond operation. Further, the use of concentrated CO_2 supply can result in the significant CO_2 loss into the atmosphere. Therefore, to address the issues associated with CO_2 losses, it is vital to devise and optimize an effective CO_2 delivery system for open ponds.

Various researchers are still working to enhance the robustness of the microalgae for wastewater treatment. The isolation, characterization, and use of local, stress tolerant microalgae for CO_2 sequestration and wastewater treatment remains the key. It is necessary to screen more robust microalgae for growth in wastewater and to evaluate their biomass and lipid productivity. In addition, adaptation capability of microalgae growing in one type of wastewater to the other wastewater remains to be explored. Further, combining the screening of the robust microalgal species and molecular phylogeny will be significant to identify the suitable species and their molecular traits governing better performance for wastewater treatment.

The varying efficiency of removal of various forms of nitrogen, especially considering the possible nitrogen fluctuations in wastewater, is a major concern. It is known that there is a dearth of studies on combining microalgal screening and Omics tools to use it for microalgae-driven wastewater treatment. Deciphering the molecular level information of microalgal growth and nutrient removal via Omics approaches and applying genetic modification techniques to improve concomitant biomass production and nutrient removal abilities will also be the attention of the future research. Although the suitably screened and acclimatized microalgae have shown reliability, the research should be done to overcome difficulties in scaled cultivation of these microalgae with real wastewater (secondary effluent) in open ponds subjected to strong competition (from local microalgal and zooplanktons) and predation. Also, understanding of the interaction between algae and other biota and the contributing factor of biota to algae remediation output in wastewater treatment remains to be studied in the future.

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Microalgae: A Biorefinary Approach to the Treatment of Aquaculture Wastewater

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1 Introduction

The aquaculture industry is one of the fastest growing food sectors around the globe and has long been growing at 10% per annum. Aquaculture provides feed for 47% of worldwide human fish consumption and its production is continuously growing (Khatoon et al. 2016). The rapid expansion of aquaculture has contributed to the excessive increase of nutrients, especially nitrogen and phosphorous, in aquatic ecosystems. These nutrients generally originate from pond fertilization, feed, and metabolic residues of the cultivated animals (Ansari et al. 2017; Guldhe et al. 2017). To maintain the rapid growth of aquaculture requires huge amounts of freshwater and economic and feasible technology to treat aquaculture wastewater. The production of 1 kg of penaeid shrimp requires approximately 20,000 L of water (Timmons and Losordo 1994). Direct discharge of aquaculture wastewater into a water body can cause eutrophication and associated serious environmental problems. Therefore, it is very important to treat wastewater generated by aquaculture companies prior to its reuse or discharge. Many traditional techniques have been applied to treat aquaculture wastewater such physical, chemical, and biological. All these techniques have their own advantages and disadvantages. Microalgae are photosynthetic organisms and are well known as third-generation feedstock for biofuel production (Chisti 2007; Rawat et al. 2011). The application of aquaculture wastewater as a nutrient medium for microalgae cultivation could be a promising biorefining approach.

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During cultivation, microalgae utilize nutrients available in aquaculture wastewater and subsequently produce biomass that contains lipids, proteins, carbohydrates, and other value-added products. Microalgal biomass (whole algae or lipid extracted algae) can be supplemented with conventional feed ingredients to improve the growth performance and nutritional quality of fish (Ju et al. 2012). In conventional feed, different ingredients (e.g., soybeans, maize meal, feather meal, gluten) are used to meet the associated requirements.

The major benefits from using microalgae in aquaculture are that they are natural food for fish, provide protein with a balance of essential amino acids, essential fatty acids, pigments, and vitamins, among others (Becker 2007). The integration of microalgae in aquaculture has the potential to make the aquaculture industry economical, viable, and sustainable.

2 Aquaculture Industry

2.1 Types of Fish and Cultivation Conditions

There are two types of fish farming: (a) intensive aquaculture and (b) extensive aquaculture. In intensive aquaculture, fish are reared in artificial tanks in high densities. Such a system makes it very easy to control all water quality parameters such as temperature, dissolved oxygen (DO), pH, and nutrient concentration. It is also easy to set stocking densities and optimum feed level to improve growth, control disease, and lower the mortality rate. In intensive systems, a monoculture of microalgae is used in particular for larval stages of bivalves, shrimp, and other species. The technology of these systems can be developed throughout the year, which makes it easy to optimize most of the required conditions for the fish, and is easy to operate. The main disadvantages of this system are high start-up costs, large amounts of nutrient-rich aquaculture wastewater generated.

Extensive aquaculture is another type of fish cultivation system. This system can be used for the proper growth of bivalves, carp, and shrimp. The most common genera include *Chaetoceros, Thalassiosira, Tetraselmis, Isochrysis,* and *Nannochloropsis*, those fed directly or indirectly through artemia, rotifers, and daphnia to the cultured larval organisms (Guedes and Malcata 2012b). This cultivation system is used in oceans and artificial lakes, rivers, and so forth. Fishes reared in these habitats have different mesh enclosures for harvesting purposes. Moreover, extensive systems depend on the surrounding area for optimum water quality, increased survivorship, and decreased mortality, and so forth. The most common fish raised in extensive systems include prawn, carp, tilapia, tuna, and salmon.

2.2 Aquaculture Wastewater Generation and Challenges

In 2009, annual worldwide fresh water consumption was approximately 3908.3 m^3, and most of the water consumed was converted into wastewater (Chen et al. 2015). Different types of wastewater (e.g., domestic, industrial, dairy, piggery, brewery, agricultural) may require treatment before discharge into water bodies (Abou-Shanab et al. 2013; Gupta et al. 2016; Hena et al. 2015; Mata et al. 2012; Ruiz-Martinez et al. 2012). Due to rapid growth aquaculture will meet almost 50% of all fish demand for human consumption by 2030 (Andreotti et al. 2017). Fish rearing requires huge amounts of water, which can become polluted after a certain period, and such water is known as aquaculture wastewater. Aquaculture wastewater is rich in nutrient sources, especially nitrogen, because of the high protein content of aqua feed. Fish requires three times more protein than humans, and their low digestion efficiency produces five times more waste than humans (Milhazes-Cunha and Otero 2017). The production of 1 t of live channel catfish produces 1190 kg dry matter, 60 kg nitrogen, and 12 kg phosphorus in aquaculture wastewater (Hu et al. 2017). The composition of aquaculture wastewater is related to feed types and quantity fed to fish and the type of fish rearing being practiced. The main sources of waste from aquaculture are untreated water with fish excreta, fecal matter, and uneaten feed. After aqua feed, water is the second most important part of a successful aquaculture operation. Many key parameters, such as DO, temperature, pH, salinity, hardness, and ammonia, are vital for fish survival. Therefore, it is imperative to maintain most of these parameters within the range required to maintain an optimum growth rate and productivity of fish.

The aquaculture industry is one of the major contributors to increasing levels of DO and particulate nutrients in aquatic systems, which represent a serious challenge to aquatic systems (Lamprianidou et al. 2015). The nutrients found in aquaculture wastewater are ammonia (3–7 mgL^{-1}), nitrate (2–110 mgL^{-1}), phosphate (2–50 mgL^{-1}), and chemical oxygen demand (COD) (100–150 mgL^{-1}) (Guldhe et al. 2017). The nutrient concentration varies by fish species and cultivation conditions (Table 1). It is important to treat aquaculture wastewater prior to reuse or discharge in water bodies to avoid eutrophication.

3 Conventional Techniques for Removing Nutrients from Aquaculture Wastewater

Many techniques have been used to treat aquaculture wastewater prior to reuse or discharge (Guldhe et al. 2017). The selection of economical and environment friendly treatment techniques is vital for industrial progress. Many chemical and biological techniques are used to treat aquaculture wastewater to obtain a satisfactory quality of aquaculture effluent. Chemical precipitation with ferrous chloride is used for the removal of phosphorus (Mook et al. 2012; Kiran et al. 2014).

Table 1 Nutrients available in aquaculture wastewater

Type of fish	Common name	NO3 (mgL^{-1})	NO2 (mgL^{-1})	NH3 (mgL^{-1})	PO4 (mgL^{-1})	References
	Tilapia	259	5.6	13.3	62.8	Attasat et al. (2013)
	Tilapia	40.67	5.52	5.32	8.82	Guldhe et al. (2017)
	Tilapia	110	5	7	6.60	Ghaly et al. (2005)
L. calcarifer	Silver sea bass	12.22	0.12	5.59	6.75	Lananan et al. (2014)
	Tilapia	17.6	–	–	16.9	Halfhide et al. (2014)
Oncorhynchus mykiss	Rainbow trout	0.66–0.7	–	0.58	0.34–0.37	Schulz et al. (2003)
D. labrax	–	4.87	–	–	0.76	Porrello et al. (2006)
Penaeus vannamei		2.00	0.13	–	0.42	Gao et al. (2016)
	Shrimp	3.47	3.80	6.12	7.20	Khatoon et al. (2016)

The direct bacterial anaerobic conversion of ammonia (NH_3) into nitrogen (N_2) and biomass (ANAMMOX) requires sophisticated and expensive systems to operate that are not economical (Milhazes-Cunha and Otero 2017). Most of these methods are neither economical nor environmentally friendly and result in the formation of byproducts that are considered secondary pollutants (Nasir et al. 2015).

3.1 Microalgae

Microalgae are unicellular photosynthetic organisms that utilize solar light energy, inorganic nutrients, and environmental CO_2 (carbon dioxide) to generate biomass (Rawat et al. 2011; Bhola et al. 2016). Microalgae are biofactories of several high-value products such as pigments, polyunsaturated fatty acids (PUFA), and others. In particular, omega 3 fatty acids are recognized as essential dietary supplements required for the growth and development of higher eukaryotes (Mitra and Mistra 2018). Microalgae possess many advantages over terrestrial plants, such as a high rate of CO_2 sequestration and faster growth rate, and can grow in different types of wastewater while requiring minimal arable land (Chisti 2007; Gupta et al. 2016). The cultivation of microalgae in wastewater has gained prominence due to their potential for nutrient uptake for growth and for the generation of biomass and other products (Rawat et al. 2011; Gupta et al. 2016). Microalgae in phycoremediation processes have a 70–90% removal efficiency rate of nitrate, sulfate, and phosphate (Maizatul et al. 2017). Phycoremediation is associated with high and valuable biomass production, which contains lipid proteins, carbohydrates, and other valuable products. The yield of major metabolites largely depends on the algal strain and cultivation conditions. The generated biomass could be used as an important aqua feed ingredient (e.g., protein, oil, pigments). Moreover, the application of microalgal biomass as an aqua feed ingredient might be more applicable without more extraction and preparation because microalgae are natural feed for fish in water bodies (Maizatul et al. 2017).

3.2 Microalgae for Aquaculture Wastewater Treatment and Biomass Production

Microalgae have great potential and can be used for the treatment of different types of wastewater. The microalgal based bioremediation of wastewater is very efficient, effective, environmental friendly, economical (Sirakov et al. 2015). The use of microalgae in aquaculture wastewater can be explored in a biorefinery concept in which the production of microalgae and their uses in aquaculture can be integrated together for various products in an economically feasible manner. The cultivation of microalgae in aquaculture wastewater has three benefits: (1) it can be used to treat aquaculture wastewater, (2) the treated wastewater can be reused for fish cultivation, and (3) microalgal biomass could be potentially used in aqua feed. During the cultivation process, microalgae remove nutrients from wastewater to aid their growth. After the harvesting of the biomass, the treated water can be redirected to fish farming or other activities depending upon its suitability, and the biomass can be used in aqua feed. This cultivation approach can reduce the cost of feed preparation and provide environmental benefits. Guldhe et al. (2017) studied the cultivation of *Chlorella sorokiniana* heterotrophically in tilapia aquaculture wastewater. The results showed that nutrient removal efficiencies were 75.56% for ammonium, 84.51% for nitrates, 73.35% for phosphates, and 71.88% for COD (Table 2). The biomass produced during cultivation showed high lipid (150.19 $mgL^{-1}d^{-1}$), carbohydrate (172.91 $mgL^{-1}d^{-1}$), and protein productivity (141.57 $mgL^{-1}d^{-1}$). Wuang et al. (2016) used *Spirulina platensis* biomass for aquaculture wastewater treatment and observed excellent remediation results for ammonia and nitrate. The chemical composition of *S. platensis* was 16.8% carbohydrate, 48.5% protein, and 4.7% lipid. Nasir et al. (2015) cultivated *Chlorella* sp. in Africa catfish wastewater and their results showed 63.1–92.2% phosphate removal. Gao et al. (2016) cultivated *Chlorella vulgaris* in aquaculture wastewater and found 42.6 $mgL^{-1}d^{-1}$ biomass productivity and 82.7% phosphate removal. Cultivation of various microalgae in aquaculture wastewater and nutrient removal or biomass production have been widely studied, but the biochemcial composition of the biomass has not been elucidated. The integration process such as microalgae cultivation in aquaculture wastewater can be used to enhance biomass productivity. The biomass produced can be simultaneously used in aquaculture feed to improve feed quality. The integration of aquaculture with microalgae will lead to the expansion of a biorefinery concept for sustainable and economical aquaculture and microalgae development.

Table 2 Nutrients removal efficiency of microalgae cultivated in aquaculture wastewater

Aquaculture wastewater	Microalgae	Nitrate (mgL^{-1})	Nitrite (mgL^{-1})	Ammonia (mgL^{-1})	Phosphate (mgL^{-1})	COD (mgL^{-1})	References
Tilapia	*C. sorokiniana*	84.51	96.38	75.56	73.35	71.88	Guldhe et al. (2017)
Tilapia	*S. obliquus*	77.7	73.83	88.71	~100	42	Ansari et al. (2017)
Tilapia	*C. sorokiniana*	75.76	81.79	98.21	~100	69	Ansari et al. (2017)
Tilapia	*A. falcatus*	80.85	99.73	86.45	98.52	61	Ansari et al. (2017)
–	*Chlorella sp.*				63.1–92.2		Nasir et al. (2015)
–	*Platymonas subcordiformis*				98–99		Guo et al. (2013)
–	*Chlorella sp.*			97.71	49.73		Lananan et al. (2014)
–	*Gracilaria birdiae*	~100		34	93.5		Marinho-Soriano et al. (2009)

4 Microalgae Biochemical Composition

4.1 Protein

Proteins are among the major metabolites in microalgae biomass, and their yield largely depends on several factors, including species types, growth phase, and light quality and can be further modified through nutrient stress and environmental stress (Table 3). For example, *Spirulina* sp. is known to contain about 50–70% protein depending on the strain (Plaza et al. 2009). The microalga *Dunaliella* can produce 50–100 times more protein per unit area than traditional plants and animals currently grown for food. Nitrogen is the most important nutrient ingredient for higher protein yield and productivity. Microalgae cultivated in higher nitrogen concentration show higher protein yield. Microalgae species depend on the cultivation conditions and often include all essential amino acids, which are important for humans and animals. The ratio of amino acids differs from species to species; freshwater microalgae have more sulfur-containing amino acids than marine algae. Amino acids present in microalgae are very close to in terms of quantity and quality, amino acids present in microalgae are very similar to egg protein, soybean, and fish, for example (Becker 2007). Microalgae protein may

Table 3 Biochemical composition of microalgae and different fish diet ingredients

Ingredient/Algae	Protein	Lipid	Carbohydrate	References
Fishmeal	63	11	–	Shields and Lupatsch (2012)
Soybean meal	44	2.2	39	–
Wheat meal	12.2	2.9	69	–
Corn gluten	62	5	18.5	–
C. vulgaris	35.13	9.81	16.82	Zhao et al. (2014)
C. vulgaris	64.1	13	15	Aziz (2015)
Nannochloropsis sp.	34.03	10.65	7.64	–
N. salina	17.21	37.16	11.52	–
Scenedesmus sp.	56	13	25	Vardon et al. (2012)
A. falcatus	45.02	26.37	15.98	Guldhe et al. (2016)
Scenedesmus sp.	20.88	23.62	42.68	Pancha et al. (2015)
Scenedesmus obliquus	50–56	12–14	10–52	Becker (2007)
D. tertiolecta	61.32	2.87	21.69	Shuping et al. (2010)
A. platensis	38.62	6	23.22	Markou et al. (2013)
D. tertiolecta	27.2	22	40.5	Kim et al. (2015)

have lower biological value in terms of, for example, digestibility, protein efficiency value, and net utilization, than the casein and egg (Becker 2007). Significant amounts of fish are produced in the aquaculture industry, and almost 40% of all aquaculture production is now firmly dependent on commercial feeds. In aquaculture, feed protein is one of the most important ingredients. Because of their high digestibility, protein, balanced amino acids, oil, and essential fatty fishmeal are widely accepted as the most vital ingredients in aqua feed. Fishmeal as a fish feed ingredient has been exploited from wild stocks for years and is currently being fished at a close to maximum sustainable level (FAO 2016). As the aquaculture food industry has expanded, the amount of farmed species fed commercial feeds has also increased, which represents approximately 70% of worldwide aquaculture production (Sarker et al. 2018). Unfortunately, due to progressive depletion of global fish stocks as well as various associated constraints, global fishmeal demand is not fulfilled. To fulfill protein requirements, conventional protein sources are added to aqua feed. Many conventional protein sources provide basic nutrition but do not fulfill specific nutritional requirements such as essential amino acids (methionine), long-chain polyunsaturated fatty acids (LC-PUFAs), and pigments (carotenoid) for fish. Furthermore, these supplements are expensive as raw materials, which adds significantly to the cost of fish feed preparation and the price of fish feed (Hass et al. 2016). The use of microalgae as protein sources in aqua feed has many advantages, including balancing nutrition, coloring the flesh, and other biological activities (Hemaiswarya et al. 2011). Microalgae are natural feed for zooplankton in the food chain, which provide natural sources that are important for biological processes. Many studies have been done on the application of different algae (*Arthospira* sp., *Chlore*lla sp., *Scenedsmus* sp., *Nannochloropsis* sp. and *Tetraselmis suecica*) as protein source in fish feed of different types of fish species (Badwy et al. 2008; Shah et al. 2018). The selection of the right microalgae as aqua feed ingredient is vital.

4.2 Lipid

Lipids are other major metabolites of microalgae cells. Microalgal lipids contain both essential and nonessential fatty acids in which most species contain high amounts of eicosapentaenoic acid (EPA) (Brown 2002). The lipid content of microalgae significantly depends on cultivation conditions and types of species (Table 3). In stress nitrogen conditions, microalgae accumulate higher lipid content in their cell. Microalgae are rich in lipids, which store energy, and in stress conditions accumulate more than 70% lipids (Stephenson et al. 2011). However, without stress, rapidly grown algae accumulate 14–30% lipids, which is a very suitable level for aquaculture diets, and microalgae accumulation of nutrients is species specific. Moreover, microalgae lipids contain both essential and nonessential fatty acids. Some microalgae are rich in essential fatty acids (EPA, docosahexaenoic acid (DHA), alpha-linolenic acid (ALA), which can be potentially used as alternatives to fish oil (FO) in aqua feed. Marine microalgae are usually higher in DHA content, for example, *Crypthecodinium cohnii* contains approximately 30–50% of its constitutes as fatty acids which are DHA. Heterotrophic algae such as *Schizochytrium mangrove* (33–39%), *Amphidium caryerea* (17%), and *Thrautocytrium* (16.1%) are also rich in DHA content (Yaakob et al. 2014).

4.3 Carbohydrates

Carbohydrates are third major metabolites present in microalgae cells and represent the main energy source for animals including fish feed. Carbohydrates are structural components in cell walls and serve as energy storage for the metabolic process for microalgae. The content of carbohydrates in algae species depends on the mode of cultivations, for example (Table 3). The common sugars found in polysaccharides in some microalgae used in aquaculture, viz. *Pyramimonas virginica, Pseudoisochrysis paradoxa, C. vulgaris, P. lutheri*, and *Isochrysis galbana*, are glucose, mannose, ribose/xylose, rhamnose, and fructose, with glucose being the major constituent and accounts for 28–86% of the total carbohydrates, while mannose is a substantial component in all cases (Roy and Pal 2015). Apart from aquaculture, microalgae carbohydrates can be used for several purposes such as biofuel production via anaerobic digestion, anaerobic fermentation, and biological biohydrogen production.

4.4 Other Value-Added Products

In addition to lipids, proteins, and carbohydrates, microalgae biomass is also known to contain considerable amounts of other products such as carotenoids (astaxanthin, β-carotenes, and xanthophyll). Carotenoid and pigments are largely used as bulk commodities in different industrial and commercial sectors (Usher et al. 2014). These products hold promise as alternatives to chemical-based commercial pigments used in different industries as a raw material (Kothari et al. 2017). Organic metabolites, such as sporopollenin, scytonemin, and mycosporine, show a wide range of applications in different sectors like pharmaceutical, therapeutics, human nutrition, food technology, functional food, antibiotics, and green plastics (Kothari et al. 2017). The appropriate algal species could have potential for use as feed additives to provide natural pigments in low- or high-nutritional-value farmed fish (Tilapia, Catfish, Trout, Salmon etc.).

5 Integration Process of Microalgae Cultivation in Aquaculture Wastewater

The integrated process of microalgae cultivation in aquaculture wastewater is an economically viable and sustainable way forward (Fig. 1). The aquaculture wastewater produced during fish rearing contains microalgae with essential nutrients such as nitrogen, phosphorous, and organic carbon. Thus, the application of such wastewater can be used as a growth medium to cultivate microalgae and generate

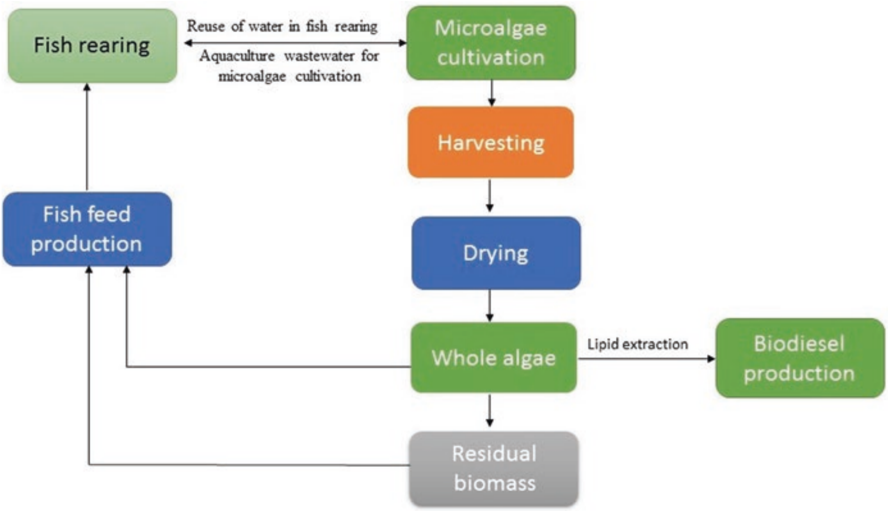

Fig. 1 Schematic diagram of microalgae and aquaculture integrated process

microalgal biomass. The microalgal biomass generated can be effectively used as either whole algae or lipid-extracted algae as a protein source in aqua feed diets. The biochemical composition of some algae is comparable to available aqua feed ingredients that are widely used at the industrial level (Table 3) and can be potentially be used in aqua feed. Algae species vary significantly in their biochemical compositions, and this may change with cultivation conditions (Brown et al. 1997; Singh et al. 2016). Microalgae must have a good nutritional composition without any toxic substances that might be transferred up the food chain. Approximately 30% total production of microalgae biomass is used in aquaculture feed applications (Sirakov et al. 2015). However, the appropriate algal species can be excellent aqua feed ingredients and be used directly or with supplementation by conventional feed ingredients. Many factors make algae suitable for aqua feed, including size, palatability, shape, and digestibility, for example (Ju et al. 2012).

Microalga cultivation in aquaculture wastewater removes nutrients from the wastewater to support microalgae growth. After harvesting of algal biomass, the treated water can be potentially reused in fish-rearing tanks. The application of microalgae in aquaculture wastewater treatment and recycling has been given impetus in the past few years. The integrated approach can improve aqua feed quality, reduce the cost of feed preparation, and confer environmental benefits. The most common microalgae genera used in aquaculture wastewater treatment are *Chlorella, Ankisterodesmus, Scenedesmus, Euglena*, and others (Palmer 1974). Cultivation of various microalgae and nutrient removal efficiency from aquaculture wastewater have been widely studied; however, the integration process, such as high-quality biomass production and simultaneous application in aquaculture feed, needs to be investigated further. Studies need to identify suitable and robust microalgae (high in PUFAs, proteins, and essential amino acids) to cultivate in aquaculture wastewater.

6 Application of Microalgae Aquafeed

Microalgae are natural food for fish, and their use at the industrial level has the potential to provide important ingredients (protein, pigments, oil, energy) in fish diets. Some microalgae species have comparable nutrient profiles with conventional feed ingredients that are widely used in fish feed. The nutritional value of any ingredient is determined by the protein-content profile of essential amino acids, the presence of polyunsaturated fatty acids, digestibility, palatability, and other factors. Several factors such as size, shape, palatability, digestibility, biochemical composition, and others, make microalgae suitable ingredients in aqua feed (Guedes and Malcata 2012a). There are three modes (live, whole algae as supplement, and lipid-extracted algae as supplement) of microalgae use in aquaculture feed. Microalgae are used as live feed for all growth stages of bivalve mollusks for the juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food chains (Sirakov et al. 2015). The most common microalgae genera used in larval feeds are *Chaetoceros*, *Thalassiosira, Tetraselmis, Isochrysis*, *and*

Nannochloropsis, which are used directly or through artemia and rotifers. Whole and lipid-extracted algae (LEA) used as supplements in aqua feed have shown better growth performance than a feed composed of algae alone (Spolaore et al. 2006). Whole microalgae inclusion in fish feed can provide proteins with balanced amino acid profiles, oils rich in essential unsaturated fatty acids, pigments, antioxidants, vitamins, and minerals. Various studies have demonstrated that the inclusion of a small proportion of algae improved growth performance and nutritional quality as measured by the weight and length of fish, protein retention, and omega-3 fatty-acid contents and provided well-balanced essential amino acids in final products (Abdulrahman et al. 2014; Radhakrishnan et al. 2014). The replacement of fishmeal (FM), pigments, and fish oil (FO) by microalgae can decrease the price of fish feed. Whole algal biomass of *Spirulina* has been used in feed for giant freshwater shrimp, *Penaeus japonicas*, and improved growth, survival, and feed utilization were observed (Nakagawa and Gomez-Diaz 1995). Kousoulaki et al. (2016) reported that the inclusion of 5% whole heterotrophic microalgae (*Schizochtrum* sp.) in the extruded meal of salmon successfully replaced FO without any adverse effects on morphological characteristics. Supplementation of *Schizochtrum* sp. in salmon diets has promising potential to improve the preservative capability of nutritional quality. Vizcaíno et al. (2014) incorporated five different levels (0%, 12%, 20%, 25%, and 39%) of *S. almeriensis* in the diets of sea bream (*Sparus aurata*). After a 45-day trial, they observed that that incorporated *S. almeriensis* showed no negative effect on fish growth or nutrient utilization efficiency. Fish feed with 12% *S. almeriensis* incorporated into diets showed higher trypsin than the control.

The LEA biomass obtained after lipid extraction is rich in proteins, carbohydrates, and other important components such as minerals, water-soluble vitamins, and bioactive compounds (Ju et al. 2012). The proteins remaining in LEA have promising potential to be used in aquaculture feed because they can replace FM in aquaculture feed (Sørensen et al. 2017). Ju et al. (2012) used the LEA biomass of *Haematococcus pluvialis* as a protein source and prepared four test diets to partially replace FM at 12.5%, 25%, 37.5%, or 50% in diets of Pacific shrimp. After 8 weeks of feeding trials, they observed that feed with 12.5% replacement of FM showed significantly higher weight, percentage weight gain, and specific growth rate in shrimp than the control diet. Patterson and Gatlin (2013) used three different LEA biomasses of *Naviculla* sp., *Chlorella* sp., and *N. salina* as alternative protein sources for red drum diets. In their first experiment, the LEA of *Naviculla* sp. were used to replace 5% and 10% of crude protein. The result showed that the inclusion of 10% LEA negatively affected the protein value and energy retention value. In their second experiment, *Chlorella* sp. was used to replace 5%, 10%, 20%, and 25% of crude protein in the reference diets. The results showed that replacement of 20% and 25% crude proteins by *Chlorella* sp. significantly reduced growth and protein efficiency ratio but without changing the whole body composition. In their third experiment, small inclusion levels of *N. salina* were made to replace 5%, 7.5%, 10%, and 15% of the crude protein in reference diets. Results showed that LEA of *N. salina* could not be used at more than 10% to replace crude proteins in juvenile red drum diets due to a negative effect. The use of whole algae and LEA in aqua feed depends on the biochemical composition of the microalgae and fish species. Whole algae supplementation provides proteins, lipids, pigments, and other ingredients. High supplementation of whole algae and LEA in aqua feed may negatively affect the growth performance of fish. If LEA is used for partial replacement of conventioal protein and carbohydrate source of aquaculture feed, then it will not only reduce feed prices, it will also reduce captive fishing of wild fish species used in aquaculture feed production as well as it will substaintially reduce the production cost of algal biofuels.

7 Conclusions

Aquaculture wastewater has the potential to be used as a nutrient source for the cultivation of microalgae. Application of microalgae in aquaculture industry to treat wastewater prior to its being release to the environment is an economical and feasible technology. The treated wastewater can be reused for fish rearing or other suitable purposes and microalgal biomass, which can be potentially used in aqua feed as a protein (whole or LEA), carbohydrate, or lipid source. This integrated technology for aquaculture wastewater treatment and microalgae's subsequent use as feed is a promising direction that will help in the development of a biorefinery concept for sustainable and economical aquaculture and microalgae sectors.

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Dual Role of Microalgae in Wastewater Treatment and Biodiesel Production

Hamed Eladel, Sivakumar Esakkimuthu, and Abd El-Fatah Abomohra

1 Introduction

Microalgae are photosynthetic aquatic microorganisms which are widely attracted in recent years. It is one of the most important bioresources; they can serve a double role of treating the wastewater and simultaneously producing biomass for fuel production along with sequestering carbon dioxide (Mulbry et al. 2008). Microalgae are photoautotrophic microorganisms that require nutrients, present naturally in wastewater or seawater. Thus, wastewater can act as excellent nutrient medium for microalgal growth and leads to consequential removal of wastes from water. In addition to nutrient removal, microalgae can assimilate organic carbonaceous matter and convert it into cellular constituents especially the macromolecules like fats and sugars (Wang et al. 2010) making microalgae a better alternative to conventional wastewater treatment. Phycoremediation is an effective process without any eventual pollution by the produced biomass and altogether leads a nutrient recycling (Mulbry et al. 2008).

Urbanization leads to a serious environmental deterioration as a result of huge release of domestic municipal wastewater. The release of organic compounds along with phosphates and nitrates leads to eutrophication. This severe issue can be solved by using these wastes as nutrients to grow microalgae. Thus, dual advantages of

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wastewater treatment and microalgal biomass production can be achieved. The produced biomass can be used for producing biofuels such as biodiesel, bioethanol, biogas, etc. (Pizarro et al. 2006; Godos et al. 2009; Uludag Demirer et al. 2013; Abomohra et al. 2016).

Fossil reserves supporting the conventional fuel will be depleted in less than 50 years which urge the whole world for finding cost-effective energy source (Barsanti and Gualtiery 2006; Briens et al. 2008). The options of inventive ways and the relevant day-to-day innovations bear the potential of overcoming these challenges (Park et al. 2011). Algal culture collections around the world contain thousands of diversified microalgal species and strains which help in making all kind of innovative efforts in biofuel production along with wastewater treatment. Uptake of nutrients from the wastewater consequently improves the quality of the water and acts as an effective tertiary treatment of wastewater (Costa and Morais 2011). As a result, microalgae can help with wastewater treatment and biofuel production in a sustainable way (Rittmann 2008; Pienkos and Darzins 2009; Shao et al. 2018). However, nutrient removal and recycling is still a significant issue for wastewater treatment in many countries because of the elevated cost and environmental impacts. Therefore, effective, eco-friendly, and low-cost technologies for nutrient removal from wastewater are in a great demand.

2 Wastewater and Its Characteristics

Wastewater is that water negatively impacted by human activities as a result of domestic, industrial, commercial, or agricultural uses. It could be a complex blend of common organic and inorganic substances, which discharged to the environment driving to genuine contamination. In sewage, natural carbon can be found in three forms such as carbohydrate, lipids, and proteins or amino acids. Sewage also contains variety of inorganic nutrients which includes calcium, potassium, sodium, magnesium, phosphate, sulfur, chorine, bicarbonate, ammonium, and some heavy metals (Tebbutt 1983; Horan 1990; Lim et al. 2010). The composition of wastewater reflects the anthropogenic activities, and especially it is controlled by the lifestyles exhibited by the particular society of the location (Gray 1989). Pollutants released from sewage and mechanical plants cause numerous environmental problems, so that an arrangement is important to look for (Horan 1990). On the other hand, lack of water is driving scientists to investigate the possibility of wastewater reusing and seawater utilization (de la Noue and Pauw 1988; Tu et al. 2015; Abomohra et al. 2017).

2.1 Physical Characteristics

Wastewater temperature is a critical factor because it influences the responses of aquatic life-forms. Temperature is exceptionally critical too to decide the values of other different factors like pH of the water, conductivity, gases concentrations in the water, and various alkalinity ranges. The color of a household wastewater is as a rule demonstrative of its age. Odors display in wastewaters due to its state because of the living and rotting aquatic organisms and rising of gases. The odor emitted by septic wastewater is because of hydrogen sulfide production by anaerobic microorganisms.

2.2 Chemical Characteristics

Carbon, hydrogen, and oxygen constitute organic compounds with other critical components such as sulfur, phosphorus, irons, and ammonia which are present in wastewater. The presence of ammonia within the wastewater can be acknowledged as the chemical proof of natural contamination (Barlow et al. 1975). Like organic substance, there were considerably larger proportions of nitrogenous matter present in sewage water along with the components of carbohydrates, fatty acids, and urea. There are some inorganic compounds like nitrogen, phosphorous, sulfur, and certain heavy metals (Hu et al. 2012)

2.3 Biological Characteristics

Wastewater harbors plenty of organisms including macro- and microorganisms. The amount of any species of these organisms in a wastewater body dictates the treatment requirement. Wastewater gives a perfect medium for potential microbial development (Hu et al. 2012).

3 Wastewater Treatment

Wastewater treatment must be taken more seriously for obtaining clean environment. Characterizing the wastewater is highly essential to design and operate efficient treatment processes. Treating wastewater usually has series of steps including physical, chemical, and biological treatments of the wastewater for successive removal of solid substances, organic matter, and nutrients from wastewater. Such successive steps are conventionally named as preliminary, primary, secondary, and tertiary treatment. Disinfection step was usually carried for highly contaminated wastewater as a last treatment step.

Wastewater treatment aims to reduce COD and BOD_5 as well as nutrient concentration (mainly N and P) to reduce the contaminants level and to have eventual betterment of the water quality (Carey and Migliaccio 2009). BOD misuses the capacity of microorganisms to oxidize the natural materials to CO_2 and water utilizing oxygen. Subsequently, BOD can exhaust the dissolved oxygen of wastewater driving to death of aquatic creatures; consequently, its diminishment may be an essential point of wastewater treatment. The problem with the conventional treatment practices which are varying removal efficiency depends upon the nutrient to be removed, expensive methods, consequential pollution because of the chemicals associated with treatment process, and less utilization of natural resources (de la Noue et al. 1992; Guterstan and Todd 1990).

Treatment operation is carried out through collection of raw wastewaters and moves to treatment plant and then subjected for the sequential steps such as preliminary, secondary, tertiary, and disinfection processes. In wastewater treatment frameworks planned to evacuate mineral elements, basically dissolved nitrogen and phosphorus, are getting to be a critical step of treatment.

3.1 Preliminary Treatment

It is the first process that the wastewater encounters and is achieved by removing of any materials that are rigid which can clog, block, destruct pumps, or hinder the successive steps. These materials are composed of floating objects; therefore, preliminary treatment devices designed to:

(i) Remove large, floating solids (Tebbutt 1983; Abdel-Raouf et al. 2012).
(ii) Remove mainly larger solids like sand and small broken pieces of stones and glass. The grit (loose particles of stones) is usually removed by subjecting to flow with optimum velocity at which the grit is able to settle leaving the other matter suspended for the subsequent treatment (Gray 1989).
(iii) Remove excessive amounts of oils or greases.

3.2 Primary Treatment

Primary treatment process can be defined as the settling of suspended solids physically. The basic objective of primary treatment for wastewaters is removing the solids that are suspended to make the treated water bit clear than the earlier. Then, the water is fed into sedimentation tanks, which point to eject the heavier settleable solids (this prepare evacuates from 50% to 75% of the entire suspended solids) by gravity. It carried out through devices which decrease the speed and scatter the stream of wastewater. A fine constructed sedimentation tank can be reduced about 40% of BOD by just settling the suspended solids (Horan 1990). The traces of remaining solids in wastewater will be subjected to next phase for the secondary treatment.

3.3 Secondary Treatment

Secondary treatment mainly performs the reduction of BOD produced by the reduction of organic substance present in wastewater by a mixed population of heterotrophic bacteria used for their growth. To achieve good treatment operations, microbes are provided with the suitable optimum conditions to grow. Secondary treatment can be carried out via two systems: aeration tanks and sedimentation tanks. In aeration tanks, oxidation of carbonaceous organic matter takes place by bacterial population in the presence of oxygen forming nitrogenous organic matter and CO_2. Then, biological treatment using bacteria to remove nitrogenous has two steps such as nitrification and denitrification. In the step of nitrification, conversion of ammonia to nitrite and finally to nitrate takes place with the help of autotrophic bacteria. The best examples of autotrophic bacteria are *Nitrosomonas* and *Nitrobacter.* In denitrification step, reduction of nitrate to gaseous nitrogen (N_2) is achieved in the absence of oxygen by heterotrophic bacteria like *Flavobacterium* and *Pseudomonas*. The secondary treatment plant has another part of settling tanks for separating, clear effluent water from the biomass that has grown during biological treatment (sludge), which pumps to drying tanks.

3.4 Tertiary Treatment

Tertiary treatment (advanced treatment) is defined as a treatment following the primary and secondary processes. It aims to remove all ions, while primary and secondary treatments have been introduced to dispose the sediment materials and oxidize the organic substance, respectively. This clear water can be released into the water bodies; however the presence of inorganic nitrogen and phosphorous can result in eutrophication in freshwater bodies like lake which in turn can cause destructive microalgal flora (Sawayama et al. 1998). Subsequently, advance treatment is essential to avoid eutrophication of water environment (Sawayama et al. 2000).

Tertiary treatment of wastewater is performed when the secondary treatment is failed to remove particular contaminant which is essentially to be removed. The tertiary treatment usually includes supplementary procedures to classical biological treatment. The main resistant substances are the compounds of nitrogen and phosphorus that cause increase of planktonic algae in water. Advanced or tertiary treatment depends on technologically complex techniques which incorporates steps planned to evacuate nutrients, such as phosphorus or nitrogen. Some of the industrial and agricultural wastewaters possess significant proportions of nitrogen and phosphorous which are threefolds greater than normal water bodies (de la Noue et al. 1992).

Tertiary treatment is usually achieved either biological or chemical means. Bioremediation can be defined as the process of applying microorganisms for the removal of the pollutants which is the biological mean of tertiary treatment. The mechanism of bioremediation depends on CO_2 and N_2 release because of oxidation of carbonaceous organic substances and reduces in nutrient content such as nitrogen and phosphate. The biological wastewater treatment shows to be better than physical or chemical means since the latter one leads to other sort of contaminations. The total tertiary wastewater treatment which intends to reduce ammonium, nitrate, and phosphate is approximately fourfolds higher in expense than basic treatments (de la Noue et al. 1992).

3.5 Disinfection

There are many microorganisms still left over even after three stages of treatments, and hence to prevent the disease spread or infection, these effluents are essentially treated for the killing of the pathogens. Disinfection is the term used for process of killing these pathogens. In common, there are many ways to kill the pathogens either by chemical or physical means. The effluent after tertiary treatment will be subjected for this disinfection step. One conventional chemical method of pathogen destruction is chlorination since chlorine is an excellent disinfecting agent. Chlorine was cheaper before, and now the cost of chlorine has been raising, and associated side effects of chlorine in exerting toxicity to fishes by the formation of chlorinated hydrocarbons become the serious concern. This reduces the chlorine use, and now the usage of ozone (ozonation) or ultraviolet radiation for pathogen killing is widely encouraged. Thus, ozone and ultraviolet radiation in application for pathogen killing becomes successful without any toxic to the environment (Abdel-Raouf et al. 2012). In addition, ozone increases the dissolved oxygen level, and economy of ozone production is still on its way to compete with chlorination. The disinfection of the effluent can be usually measured with the help of microbiological test for the total coliform bacteria estimation in the treated effluent (Sebastian and Nair 1984).

3.6 Wastewater Treatment Efficiency

Wastewaters harbor various constituents which can be divided as suspended and dissolved solids and inorganic and organic substances. The treatment efficiency can be defined as the percentage of removal of these constituents from the wastewater. The parameters that are measured are displayed as follows:

(i) *BOD*: biochemical/biological oxygen demand (measuring unit in mgL^{-1})
(ii) *COD*: chemical oxygen demand (measuring unit in mgL^{-1})
(iii) *TSS*: total suspended solids (measuring unit in mgL^{-1})
(iv) *TDS*: total dissolved solids (measuring unit in mgL^{-1})
(v) *Nitrogen forms*: including nitrate and ammonia (measuring unit in mgL^{-1})
(vi) *Phosphate*: measured (measuring unit in mgL^{-1})
(vii) *pH*: measured in number from 1 to 14
(viii) *Coliform bacteria count*: measured as most probable number (MPN) per 100 mL (*Escherichia* and fecal coliform bacteria are most common indicators)

4 Wastewater Remediation Using Microalgae (Phycoremediation)

Increased concerns of worldwide warming, exhaustion of conventional fuels derived from fossils lead to greenhouse gas outflows, make investigation of the possibility of biological wastewater treatment by microalgae coupled with biofuel generation necessary. There has been enormous attempts, discussions, and suggestion for using microalgae as potential candidate for cheaper and efficient treatment process (Chinnasamy et al. 2010a, b; Wu et al. 2012; Han et al. 2016). In general, the amount of the total nitrogen and phosphorous present is between 10 and 100 mg L^{-1} and more than 1000 mgL^{-1} in agricultural effluents. Microalgae are exemplary in treating wastewater by utilizing the nutrients efficiently from the wastewater. Sustainable low-cost wastewater treatment has been strongly proven by using microalgae (de-Bashan and Bashan 2010). In addition, microalgal biomass produced from wastewater can be finely used for energy production which is a promising route for dual use of microalgae (Zhou et al. 2012a, b; Abomohra et al. 2018a, b).

Microalgae as a remediation candidate are much higher effective than terrestrial plants in terms of photosynthetic activity, and therefore algal biomass can be produced effectively using the nutrients from organic wastewater. Oswald et al. 1957 suggested an alternative wastewater treatment approach using algal-bacterial system grown together in the wastewater. In such system, bacterial cells use oxygen produced by microalgal photosynthesis to remove the organic matter from wastewater, and microalgal cells spontaneously remove the inorganic nutrients using CO_2 released by bacterial respiration (Fig. 1). Therefore, pollutants as nutrients can be removed at relatively low energy consumption in a cost-effective eco-friendly technique, without using mechanical aeration or chemical additives as in the case of conventional aerobic wastewater treatment. The concept was later expanded to use this system producing energy which can be achieved through harvesting of biomass followed by utilization (Mutanda et al. 2011; Zhou et al. 2014; Abomohra et al. 2018a). Algae can assimilate nutrients and perform photosynthesis, where the oxygen released in the wastewater which is organically enriched and will facilitate the

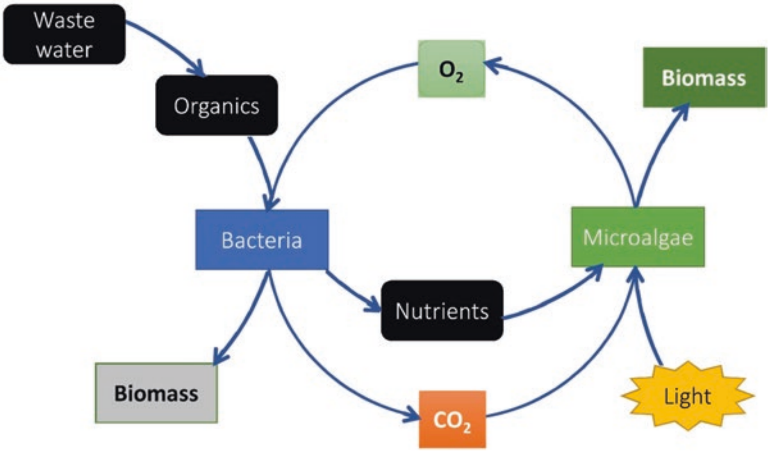

Fig. 1 Microalgal utilization of wastewater nutrients and biomass production in algal-bacterial systems

aerobic degradation processes by heterotrophic microorganism. The key act of microalgae is the utilization of nutrients from wastewater for biomass generation which in turn can be used for biofuel generation.

4.1 Microalgae Used in Wastewater Treatment

The most common microalgal strains used in wastewater are *Chlorella* and *Scenedesmus*. *Chlorella* species are reported to be tolerant and can survive under different wastewater conditions (Garcia et al. 2000; Wiley et al. 2009). Wastewater characteristics and seasonal environmental conditions are some of the factors that affect the characteristics and the predominance of microalgae (Fukami et al. 1997). *Chlorella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp. have been utilized in numerous research experiments and found to be effective in removal of nitrogen and phosphorous of different concentrations from various wastewaters. Microalgae are reported to possess high ability of removing heavy metals and harmful compounds (Matamoros et al. 2015; Shao et al. 2018).

The choice of microalgal species to be used for wastewater treatment mainly depends on the ability to grow and uptake nutrients from wastewater (Olguin 2003). There are plenty of advantages associated with bioremediation of wastewater over conventional methods. They are as follows:

(i) Nutrient removal is fairly high.
(ii) No toxic by-product (sludge) production.
(iii) Cost-effective.

(iv) An eco-friendly process.
(v) High growth rate when fed on wastewater nutrients.
(vi) Tolerance level for the seasonal variation in outdoor conditions is high enough.
(vii) Ability to form aggregates which enables the settling and harvesting easy.
(viii) Many value-added compounds can be produced (e.g., lipid for biodiesel).

Strain selection, which allows the usage of water as growth medium with varying nutritional quality based on types of wastewater altogether, is a crucial step for microalgal-based biofuel production. Many microalgal species were reported to treat wastewater from different sources.

4.2 Industrial Wastewater Treatment

In many research studies, industrial wastewater was used as the growth medium for microalgae to remove nutrients and accumulate lipids with fatty acids ranged C14–C18, which could be utilized for biodiesel production (Table 1).

Table 1 Reported studies on industrial wastewater treatment and biofuel generation by microalgae

Microalgae	Types of wastewater	References
Four marine microalgae	Industrial (Soy sauce effluent)	Shirai et al. (1998)
Botryococcus braunii; Chlorella saccharophila; Dunaliella tertiolecta; Pleurochrysis carterae	Industrial (untreated carpet mill)	Chinnasamy et al. (2010a, b)
Chlorella pyrenoidosa	Azo dyes wastewater	Jinqi and Houtian (1992)
Ankistrodesmus and *Scenedesmus* *Scenedesmus quadricauda*	Wastewaters from olive oil mill and from paper industry	Narro (1987), Pinto et al. (2002), Tran et al. (2010)
Prototheca zopfii	Degraded the hydrocarbons discharged from Louisiana crude and motor oils	Walker et al. (1975)
Scenedesmus obliquus *Micractinium reisseri*	Industrial wastewater from salt and soda companies	Abo-Shady et al. (2017)
Scenedesmus obliquus	Brewery effluent	Mata et al. (2012)
Arthrospira pcrtenix	Olive oil mill wastewater	Markou et al. (2012)
Scenedesmus sp.	Tannery wastewater	Ajayan et al. (2015)
Chlamydomonas sp. and *Desmodesmus* sp.	Modified BBM medium as industrial wastewater	Wu et al. (2012)

4.3 Municipal Wastewater Treatment

Treatment of wastewater by using microalgae has been an attracting research area for decades (Mata et al. 2010). Table 2 reveals several studies which reported that culturing of microalgae in municipal wastewater is nutritious for microalgae. In addition, it is a great option to induce the biomass productivity, which leads to the dual advantages of utilizing pollution elements as nutrients and cost-effective biofuel feedstock generation. Further fatty acid methyl esters (FAMEs) investigation appeared that the microalgal lipids were basically consisting of fatty acids such as C16 and C18, which are appropriate for high-quality biofuel production.

4.4 Agriculture Wastewater Treatment

Numerous researchers reported that microalgae are effective minor cell manufacturing plant for evacuating nitrogen and phosphorous from manure drained wastewater (Han et al., 2016) as shown in Table 3. However, a few major issues obstruct utilizing manure-based wastewater for microalgal growth, and that are as follows:

Table 2 Some studies on municipal wastewater treatment and biofuel production by microalgae

Microalgae	Types of wastewater	References
Chlorella, Scenedesmus; Phormidium; Botryococcus; Chlamydomonas	Domestic wastewater	Wang et al. (2010)
Auxenochlorella protothecoides	Municipal wastewater	Zhou et al. (2012a)
Chlorella sp.	Centrate wastewater	Li et al. (2011)
Chlamydomonas reinhardtii	Municipal (centrate)	Kong et al. (2010)
Scenedesmus obliquus	Municipal (secondary treated)	Martinez et al. (2000)
Botryococcus braunii	Municipal (secondary treated)	Orpeza et al. (2009)
Mix of Chlorella sp.; *Micractinium* sp.; *Actinastrum* sp.	Municipal (primary treated + CO_2)	Woertz et al. (2009)
Scenedesmus sp.	Artificial wastewater	Voltolina et al. (1999)
Spirulina platensis	Domestic wastewater treatment	Laliberte et al. (1997)
Chlorella sorokiniana	Wastewater treatment	Ogbonna et al. (2000)
Botryococcus braunii	Secondarily treated sewage	Sawayama et al. (1992) and Sawayama et al. (1994)
Scenedesmus obliquus	Municipal wastewater from students' dormitory	Han et al. (2016)
Chlorella sorokiniana; Scenedesmus obliquus	Domestic wastewater	Gupta et al. (2016)
Chlorella minutissima	Primary- and tertiary-treated wastewater	Malla et al. (2015)

Table 3 Some studies on agricultural wastewater treatment and biofuel production by microalgae

Microalgae species	Wastewater type	References
Botryococcus braunii	Agricultural (piggery manure with high NO_3–N)	An et al. (2003)
Chlorella sp.	Agricultural (dairy manure with polystyrene foam support)	Jacobson and Alexander (1981)
Scenedesmus sp.	Agricultural (fermented swine urine)	Kim et al. (2007)
Mix of Microspora willeana; Ulothrix zonata; Ulothrix aequalis; Rhizoclonium hieroglyphicum; Oedogonium sp.	Agricultural (anaerobically digested dairy manure)	Wilkie and Mulbry (2002)
Rhizoclonium hieroglyphicum	Agricultural (swine effluent, maximum manure loading rate)	Mulbry et al. (2008)
	Agricultural (daily effluent + CO_2. maximum manure loading rate) Agricultural (digested dairy manure, 20 dilution)	
Botryococcus braunii	Swine manure wastewater	Mulbry et al. (2008)
Mix of Chlorella sp.*; Micractinium* sp.*; Actinastrum* sp.	Agricultural (dairy wastewater, 25% dilution)	Woertz et al. (2009)
Chlorella sp.	Anaerobic digested dairy waste	Wang et al. (2010)
97 microalgal strains from algae bank and 50 microalgal isolated from local waters	Swine manure wastewaters	Zhou et al. (2012b)
Benthic freshwater algae	Dairy manure	Lau et al. (1995)
Chlorella zofingiensis	Dairy wastewater	Huo et al. (2012)
Chlamydomonas polypyrenoideum	Dairy wastewater	Kothari et al. (2013)

(i) Turbid enough and hence significantly influences light penetration.
(ii) Higher level of ammonia prevents algal growth.
(iii) A huge amount of the carbon sources is insoluble and unavailable for algae.
(iv) A huge amount of freshwater is necessary to dilute concentrated animal manure.
(v) Animal manure is unfavorable for algal growth, and the strategies are underdeveloped (Moreno-Garrido 2008).

5 Potential of Microalgae for Wastewater Treatment

Algae farming is a promising mean for wastewater bioremediation because of their ability to uptake, assimilate, and thereby remove a wide range of pollutants (Matamoros et al. 2016). Microalgae have recently become attractive due to their important role in uptake of pollutants from water and their ability to produce valuable biomass. The first purpose of utilizing microalgae in treating wastewater is the removal of contaminant, and the associated benefit is biomass production for biofuel generation. This coupling of wastewater treatment and biomass production is a successful implementation of strategy which may reduce the usage of freshwater which is precious for water-scarce countries for microalgal cultivation (Lima et al. 2003). Generally, quick advancement all over the world has expanded the utilization of freshwater sources, driving to depletion of freshwater sources. Thus, treating wastewater and utilizing the treated water as like freshwater have been broadly investigated to secure the attainable freshwater (Pescod 1992).

During photosynthesis, microalgae require energy and carbon which can be obtained through light and CO_2, respectively, with the uptake of nitrogen and phosphorus to build cellular components. Thus, this process plays a crucial role in CO_2 mitigation and significantly reduces the concentration of nutrients in wastewater. In addition, production of oxygen by microalgae as a by-product from photosynthesis process can be utilized by aerobic bacteria for the biodegradation of pollutants (organic compounds). In fact, microalgae can help to reduce the need for mechanical aeration during the process of wastewater treatment. Some heterotrophic microalgae can also grow in the absence of light using oxygen to assimilate organic carbon. Moreover, treatment using microalgae provides unfavorable environment for the growth of pathogenic organisms due to the optimum microalgal elevated pH and antibacterial substances that may be secreted by algal cells (Polprasert et al. 1983).

Wastewater could be a nutritious source for various living organisms because of its organic and inorganic constituents which feed the corresponding organism for growth (Rogers et al. 2014). Microalgal cultivation for biofuel production, feed supplements, or even fine chemicals using wastewater could easily be coupled with reduction in BOD, removal of nitrogen and phosphorus compounds. Researches have shown that microalgae have very good ability to assimilate nutrients (N and P) from wastewater. Microalgae can reduce the nutrient concentration in wastewater by direct removal and indirect removal. Direct removal involves assimilation of these nutrients by microalgal cells, while indirect removal involves removal of nutrients by precipitation and elimination because of elevated pH levels caused by microalgal activity. Therefore, longer retention times improve nutrient removal. There are some suggested mechanisms in which microalgae can help to reduce nutrient concentration in wastewater as follows:

(i) Diffusion of substances through the cell wall is one direct mechanism that helps microalgae in nutrient assimilation. The diffusion rate may depend on type and thickness ranges of the membrane directly surrounding the cell. Turbulence is essential to improve mass transfer rate of the nutrients (Mostert and Grobbelaar 1987; Borowitzka 1998).
(ii) Precipitation because of high pH level, such as phosphorus precipitation. Microalgae normally consume CO_2 as a carbon source, but if it is not available, it will consume bicarbonate (HCO_3^-) that needs carbonic anhydrase enzyme to

convert it to CO_2 and the pH will increase; this increase in pH can alter the water chemistry and cause phosphorus to precipitate.

(iii) Stripping of ammonia because of the elevated pH as previously explained.

Microalgae have significant affinity toward polyvalent metals and very efficient in phosphate and nitrate removal. Such removals are achieved in economic way as compared to other treatment especially (mechanical treatment) (Owen 1982; Craggs et al. 1999). The rate of nutrient assimilation can react up to 24 kg N ha^{-1} day^{-1} and 3 kg P ha^{-1} day^{-1} which mainly depends upon the biochemical composition, and a maximum biomass productivity can be achieved up to 30 g m^{-2} day^{-1}. The wastewater-grown microalgae can be harvested and converted to various value-added products (Fig. 2, Woertz et al. 2014).

The microalgal photosynthesis gives an opportunity of recapturing the nutrients to biomass. Many studies shown the effectiveness of utilizing microalgae for treating wastewater that contains pollutants of various origins like municipal, agricultural, and industrial activities (Fenton and Huallachain 2012; Neveux et al. 2016). A few microalgal species can utilize organic contaminants such as tannins and detergents and, therefore, can be used for treatment of effluents produced from anaerobic digestion, handling of olives and swine excrement, pulp/paper plants, and others (Gruber-Brunhumer et al. 2015; Matamoros et al. 2016). Algae are known to treat human sewage effectively (Shelef et al. 1980; Mohamed 1994; Ibraheem 1998), animal waste (Lincoln and Hill 1980), agro-industrial wastes (Ma et al. 1990; Phang 1990 & Phang 1991), and industrial wastes (Kaplan et al. 1988). Moreover, microalgae can be utilized for remediating various wastewaters like piggery wastewater, dairy wastewater (Craggs et al. 2004; Kebede-Westhead et al. 2006; Mulbry et al. 2008), and the effluent from food manufacturing plants (Rodrigues and Oliveira 1987).

Removal of nutrients at high pH in high rate algal pond (HRAP) and thereby ammonia volatilization and phosphate precipitation along with cations can be greatly reduced by CO_2 addition to the pond (Nurdogan and Oswald 1995; Garcia et al. 2000; Craggs et al. 2003; Heubeck et al. 2007). A recent report shows that complete removal of nutrients was achieved with the addition of CO_2 to wastewater HRAP. Park and Craggs (2010) investigated the control of pH below 8 during daytime reduced the vitalization of ammonia by 24%.

5.1 *Nitrogen Removal by Microalgae*

Recent reports shown that microalgae are highly potential enough in removing nitrogen and phosphorous from wastewater (Blackall et al. 2002; Mallick 2002; An et al. 2003; Orpeza et al. 2009; Abomohra et al. 2018b). Nitrogen is widely available in wastewater in many forms. However, microalgae can assimilate only the inorganic nitrogen; and ammonia is the most favorable nitrogen form for assimilation. After depletion of ammonia (NH_3-N) in wastewater, microalgae may start to

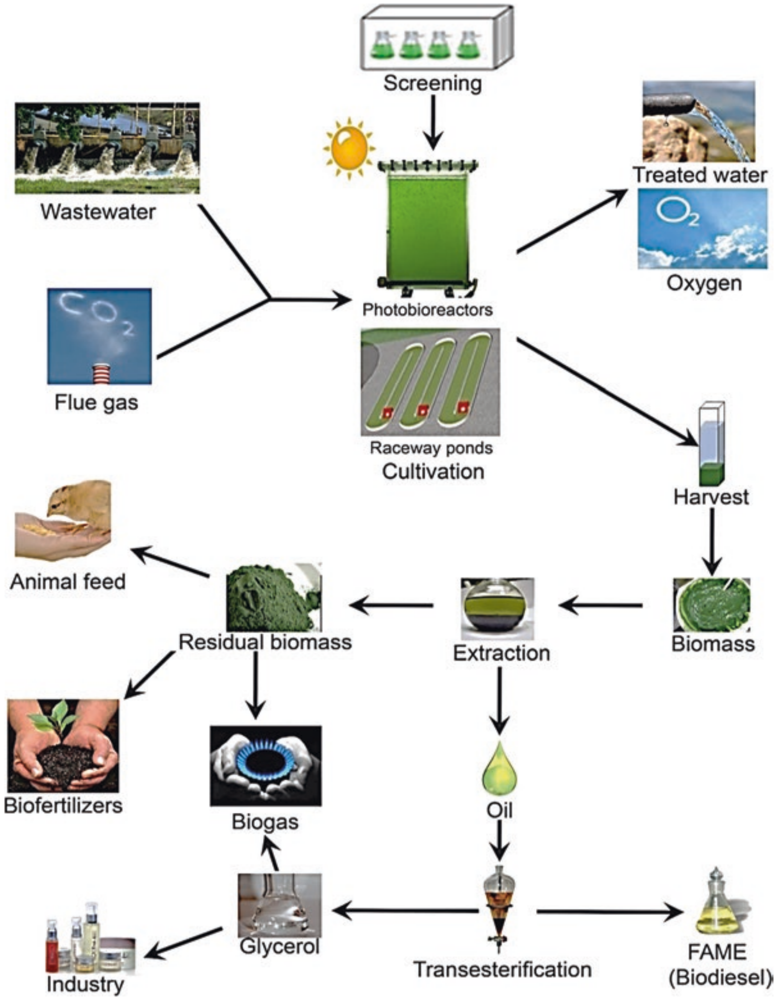

Fig. 2 Schematic diagram showing the different products available from microalgal biomass produced by microalgal cultivation on wastewater

assimilate nitrate (NO_3^-) as a nitrogen source for their cells. Even though nitrogen is the principle element for microalgae (Morris 1974) and ammonia is a form of nitrogen, free ammonia is toxic to many microalgae due to its uncoupling effect in photosynthetic process (Crofts 1966). Rise of pH above 9.0 hinders the microalgal growth as release of ammonium is highly dependent on pH Azov and Goldman (1982). For instance, *Chlorella* was inhibited at higher pH values not at neutral pH (Mayo and Noike 1994).

5.2 Phosphorus Removal by Microalgae

Phosphorus is the essential nutrient next to nitrogen for microalgal growth. It is an important element that comprises 1% of the cellular dry weight (Brown and Shilton 2014). However, microalgae can uptake more than what they need through their ability to synthesize and accumulate polyphosphate in their cells. One important factor that affects phycoremediation is the hydraulic retention time (Larsdotter 2006; Woertz et al. 2009). Phosphate can be depleted through different steps starting with adsorption on the surface of cells, followed by partial intracellular assimilation and/or chemical precipitation. Phosphorus has been widely reported to be removed by biotic processes through assimilation into the biomass (Wilhelm et al. 2006; Su et al. 2011), by abiotic processes such as adsorption (Martinez et al. 2000), or by chemical precipitation (Larsdotter et al. 2010).

5.3 COD and BOD Removal by Microalgae

COD is the measure of oxygen required to oxidize the organic substance in a water sample. It provides an index to measure the effect of discharged wastewater on the environment, and, therefore, it is used as an important water quality parameter. By increasing of COD value, it means increase of oxidizable organic material in the sample, which will reduce the dissolved oxygen (DO) levels. The COD test is often used as an alternate to BOD due to shorter length of testing time. On the other hand, BOD can be defined as the amount of dissolved oxygen (DO) required by the aerobic microorganism to decompose the organic matter in water. It is another important water quality parameter because it also measures the effect of discharged wastewater on the environment. Increasing of BOD means higher amount of organic matter. If the consumption rate of dissolved oxygen by microorganisms exceeds the dissolved oxygen supply from aquatic plants, algae, or diffusion from the air, it leads to unfavorable conditions. Depletion of DO causes stress on aquatic organisms, making the environment unsuitable for life.

Microalgae normally use inorganic carbon source, and CO_2 is the first choice for microalgae, and bicarbonate (HCO_3^-) is the second choice in the absence of CO_2. However, HCO_3^- needs the enzyme carbonic anhydrase to convert it into CO_2 leading to pH elevation. This increase in pH can alter the water chemistry and lead to phosphorus precipitation due to reaction with the available cations and also elimination of ammonia because of elevated pH (Oswald 1988; Borowitzka 1998). Some microalgae such as *Chlorella* species can function differently in response to the environmental conditions. They can grow under phototrophic conditions in which they use CO_2 as a carbon source. In addition, they can grow under heterotrophic conditions in which it uses dissolved carbons like acetate, sugars, and organic acids as a carbon source (Borowitzka 1998). Microalgal heterotrophic growth can be classified into two categories:

(i) Chemoheterotrophic: in the absence of light and at low CO_2 concentration, microalgae can use the dissolved carbon for energy source and as feed which is causing the concentration of COD to decrease.
(ii) Photoheterotrophic: under illumination and depletion of CO_2 concentration, microalgae can use light and carbon as energy and nutrient source.

BOD removal in open ponds is high with higher microalgal growth and productivity because of bacterial decomposition of organic substance promoted with more release of oxygen by microalgae (Oswald et al. 1957). Aeration is one of the expensive procedures in wastewater treatment which can cost about 50% of the total cost of the treatment (Tchobanoglous et al. 2003). It was generally evaluated that each 1 kWh of electricity can support the elimination of 1 kg of BOD (Rosso et al. 2008), whereas 1 kg BOD is eliminated due to no requirement of energy since photosynthetic oxygenation results in biomass production and provides sufficient biogas to produce 1 kWh of electricity (Oswald 2003). Hence, utilizing microalgae while treating wastewater could be an environmental-friendly aeration strategy which can minimize the requirement for the mechanical aeration.

5.4 *Oxygen Release and CO_2 Uptake by Microalgae*

By utilizing light as a power source, microalgae uptake CO_2 from the environment as a carbon source to synthesize sugars for their biomass and release O_2 as a by-product. Oxygen released by microalgae can assist oxygen consuming heterotrophic microscopic organisms to decay the complex organic compounds in wastewater (i.e., lipids, proteins, carbohydrates). In addition to that, it can encourage the nutrient elimination through nitrification and denitrification practicability.

Microalgae can assimilate CO_2 that's emitted by bacterial breath and consequently assist to diminish its release to the air. Microalgae have a capacity to fix CO_2 utilizing solar energy that can be 10% more than that of terrestrial plants (Singh and Ahluwalia 2013). Dry biomass of microalgae contains up to 46% carbon, 10% nitrogen, and 1% phosphorus. Microalgae uptake 1.8 Kg of CO_2 to grow and gain 1 Kg of dry weight biomass (Chisti 2007; Hu et al. 2008; Rodolfi et al. 2009). As a result, utilizing microalgae in wastewater treatment contributes to decrease the CO_2 emissions from plants. Microalgae have more potential to resolve the challenges associated with energy and ecology which may be a more eco-friendly approach to decrease nitrogen and phosphorus from wastewater. Biotechnological utilization of microalgae is preferable as it can perform the dual functions of remediation and biofuel production. Microalgae became one of the prominent feedstocks for biodiesel production in conjugation with wastewater treatment (Campbell 1997; Chisti 2007; Huntley and Redalje 2007; Schenk et al. 2008; Rodolfi et al. 2009; Khan et al. 2009; Abomohra et al. 2013).

6 Microalgal Mass Cultivation Systems

To perform microalgal cultivation in a considerable scale along with the treatment of wastewater, there are different culture systems available such as photobioreactors, open raceway ponds, oxidation ponds, polybags, and vertical reactors (Munoz et al. 2006). Open ponds are found to be cost-effective method which can be used for nutrient removal from wastewater through microalgal cultivation. Even though the productivity is high for photobioreactors, remediation could not be encouraged in photobioreactors because of the cost associated with it. Cost-effectiveness is one key phenomenon for making microalgal-based biomass production as successive form of renewable energy.

6.1 Open Raceway Ponds

High rate algal ponds (HRAPs) or open raceway ponds were firstly developed in the 1950s. HRAPs were shallow of about 30–60 cm depth in a shape like raceway containing a large paddle wheel to generate a flow of water and gentle mixing (Fig. 3). HRAPs are designed for increasing algal growth which are shallow and provide maximum light penetration. It has short hydraulic retention time (HRT) with the range of 4–10 days depending on the weather conditions and required area. Constant mixing is required to prevent cells from settling and to get enough light penetration. HRAPs are most cheap system for wastewater treatment and for efficient solar capturing (Oswald 1995). Open raceway pond may be a cost-effective microalgal-growing method for nutrient elimination in domestic wastewater and biomass generation more than 0.5 g L^{-1} in commercial scale (Grobbelaar 2007; Wijffels 2007). However, biomass concentration remains low since raceway ponds are inefficient in mixing and not able to maintain an active photic zone for photosynthesis. The operation of this outdoor culture open system for biomass generation is simple

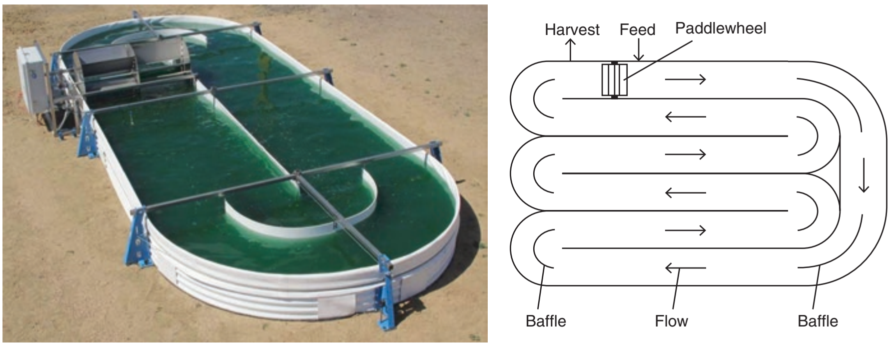

Fig. 3 Photographic illustration of the cascading raceway pond, simple (*left*) and compound (*right*)

and requires minor maintenance. The method has few working costs, minimal power utilization, and small overheads as compared to photobioreactors (Pushparaj et al. 1997). The major merit of this pond is that wastewater can be used as media along with the cheaply available CO_2 from closely located power plant to enhance the photosynthetic rate and, in case of not having nearby power plant, pure CO_2 in a certain concnetration can be used (Huber et al. 2006).

To avoid impurity from rainfall, raceway ponds can be covered as agricultural greenhouse. Water depth of the pond ought to not exceed of 30 cm height to permit adequate penetration of light. Another effective system is cascading system which found to be effective than single channel raceway pond due to long retention times and extensive mixing. To examine the cell's physiological status, few parameters should be monitored such as pH, conductivity, temperature, light intensity, evaporation rates, dissolved oxygen, salinity, dissolved CO_2, oxidation reduction potential, TDS, phosphate, and nitrate within the raceway ponds (Mutanda et al. 2011). Microalgal cells are harvested based on dewatering followed by microfiltration system and through flocculation by flocculants addition or by centrifugation, etc. Among these, centrifugation was found to be costlier. Drying after collection can be achieved using sunlight which is a cost-effective approach.

6.2 Advantages and Disadvantages of High Rate Algal Pond

High rate algal ponds are the moderately controlled setup in which microalgae exist with heterotrophic bacteria which in turn degrade organic substance of the sewage. This method of coexisting is termed as HRAP symbiosis and reflects the concept of Oswald for using HRAP as an integrated secondary/tertiary system for sewage treatment. These are the most effective reactors for wastewater management and solar capturing in cheaper way. It is cheap, simple maintenance, utilization of non-agriculture land and low energy inputs. HRAPs for wastewater treatment face issues like a weak control of culture conditions, rich microalgal biomass, climate conditions, appropriate mixing, poor efficiency, and restricted to few strains (Fallowfield and Garret 1985). Another restriction of HRAPs wastewater treatment is that cultures can be contaminated with other invaders like protozoan which can feed on microalgae and affects the growth. Fungal and viral infection can moreover altogether decrease the algal growth (Kagami et al. 2007).

6.3 Closed Photobioreactors

Photobioreactors (PBRs) allow the microalgal growth in monoculture for expanded cultivation period compared to open raceway ponds which are subjected to contamination. The preferences of photobioreactors are as follows: moderately cheap, huge light area, appropriate for open air cultures and for generation of larger biomass

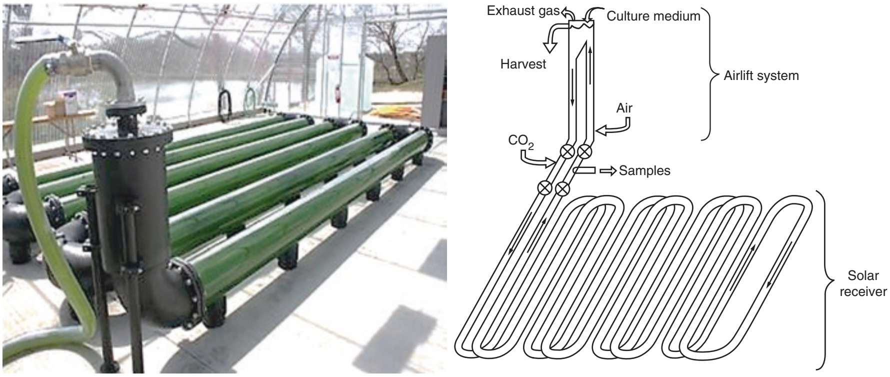

Fig. 4 Real view (*left*) and schematic representation (*right*) of the horizontal tubular photobioreactor

(Harun et al. 2010). The tubular PBR is one commonly used PBRs which is continuous system. It has arrangements of glass tubes either vertically or horizontally for maximum solar capture (Munoz and Guieysse 2006). PBRs are not meant for remediation since it can harbor only smaller volume and can be fit for small scale (Garcia et al. 2006). Figure 4 shows the basic design of a horizontal tubular photobioreactor which includes airlift section and solar receiver. The airlift allows the passage of CO_2 and also helps in biomass harvesting. It provides a larger surface area for alae to grow through high surface area to volume ratio.

7 Biodiesel

The shortage in fossil fuels has been the driving forces for humans to investigate sources of renewable energy that could provide an alternative to fossil fuels (Barbara 2007). There are three main generations of biodiesel feedstocks.

7.1 First-Generation Feedstocks

Feedstocks such as rapeseed (Lang et al. 2001), soybeans (Celikten et al. 2010), palm oil (Canakci et al. 2009; Kansedo et al. 2009), and sunflower (Rattanaphra and Srinophakun 2010) are classified as first-generation feedstock for biodiesel since they are the first considered crops for biodiesel production. Because about 95% of this biodiesel feedstock generation is made from food oils, relying on first-generation biodiesel feedstocks has created many troubles; basically it is because of worldwide food markets and food safeness (Brennan and Owende 2010). As an example, soy and palm are crops whose oils are an essential part of human nourishment;

redirecting these food crops to create large-scale generation of biodiesel may result in imbalance to the food market. Biodiesel production from edible oil has a serious disadvantage of arable land requirement to be used to produce biodiesel.

7.2 Second-Generation Feedstocks

To overcome the challenges associated with edible oil, second-generation feedstock is concentrated which uses nonedible feedstock for biodiesel production. Wastes of energy crops such as the stalks of wheat straw, corn stover, rice straw, rice husk, and tobacco seed (Usta 2005) were used as second-generation biodiesel feedstocks. In addition, restaurant grease and animal fat are also called as second-generation feedstock (Canakci 2007). Nonedible oils are extensively attracted and investigated for a decade. These feedstocks showed the following advantages:

(i) Less arable land is required, and a mixture of crops can be used.
(ii) They remove competition for food (Leung et al. 2010).
(iii) They are more effective and more ecofriendly.
(iv) Crops (nonedible) are developed in nonarable land or land that is not suitable for food crops (Leung et al. 2010).
(v) Valuable by-products are created, which can be utilized in other chemical processes or burned for its power.
(vi) Diversion of nonedible oil into biodiesel is more acceptable than edible oils in terms of generation and quality (Pinzi et al. 2009).
(vii) Animal fats have a few preferences over first-generation feedstocks, such as a better cetane number, noncorrosive qualities, and clean and renewable properties (Guru et al. 2009).

Though the second-generation feedstocks do not commonly affect the human food and can be developed in nonarable lands, they are not abundant enough to cover much of the total transportation energy. As illustrated formerly, biodiesel produced from these terrestrial crops is not potential enough for ultimate replacement, and such seek for alternative resulted in biodiesel production from microalgae. Biodiesel such produced is expected to replace petroleum-based transport fuels, and production can be attained without any competition to food crops.

7.3 Third-Generation Feedstocks

The biodiesel cost remains the major hurdle for large-scale commercial applications, basically due to the high cost of the oils (Lang et al. 2001). In contrast, third-generation feedstocks, derived mainly from algae, are discussed as the prominent alternative source for oil to produce biodiesel which has several advantages of lipid for use in biodiesel production:

(i) The microalgal growth period varies between 24 h and several days, and doubling time of microalgae varies within hours during their exponential phase of growth (Mata et al. 2010; Chisti 2007).
(ii) Most microalgae have high lipid content in the range of 15 to 70% compared to terrestrial crops that are used for biofuel extraction (Sheehan et al. 1998; Singh et al. 2011).
(iii) Microalgae are an ideal source of biodiesel as they are having the fastest photosynthetic rate and the ability to produce high quantities of lipids (Minowa et al. 1995; Braun 1996; Miyamoto 1997).
(iv) No dispute between food and fuel for land occupancy and hence non-fertile land can be used (Huang et al. 2010).
(v) Ability to grow on the environment that is not suitable for any kind of farming (Patil et al. 2008; Costa and Morais 2011).
(vi) Microalgal culturing are easy to achieve and large scale are feasible for higher biomass production using different varied cultivation systems (Janaun and Ellis 2010).
(vii) Generally, neutral lipids of microalgae have a high degree of saturation, which makes microalgal lipids a potential diesel fuel substitute (Mcginnis et al. 1997; Danquah et al. 2009).
(viii) Microalgal biomass production will not require any pesticide or herbicide and hence no such pollutions (Rodolfi et al. 2009).
(ix) Microalgal biomass can be harvested almost any time of the year, and annual productivity is far ahead of terrestrial plants.
(x) No lignin and hence easily degradable.
(xi) Microalgae are able of fixing CO_2 of air, reducing the CO_2 level by sequestration (1 kg of biomass would have 1.8 kg of fixed CO_2) (Chisti 2007; Rodolfi et al. 2009).
(xii) Processing of algal biomass to synthesize biofuel is more environmentally friendly since it uses less chemicals and emits less CO_2 (Sheehan et al. 1998).
(xiii) They produce high amount of sugars and proteins along with lipids, and hence lipid-extracted residual biomass can be used for production of bioethanol and biogas and as animal feed.
(xiv) Microalgae are essential for nutrient cycling and fixing inorganic carbon into organic molecules. A feasible source of nutrient is the waste water from the treatment of sewage or agricultural runoff.

However, the most disadvantage of utilizing microalgae for oil production is the harvesting of such tiny organisms in suspension. Moreover, extended water prerequisite for microalgae and complex lipid extraction methods are still at a developmental stage representing the most challenge.

8 Microalgal Strains Used for Biodiesel Production

Microalgal biomass has diverse biofuel potential and can be used for several types of renewable biofuels including methane, biocrude oil, and biodiesel (Brennan and Owende 2010). Microalgae are reported to provide about 25% of global energy requirements and also act as source for various value-added products (chemicals, pharmaceuticals, and food additives) (Briens et al. 2008). Microalgal species can produce magnificent quantities of lipids, ranging between 15% and 70% (~30%, when grown in wastewater) as shown in Table 4, which can be transformed to biodiesel through operation of transesterification.

9 Fatty Acid Profile of Microalgae

Fatty acids are synthesized by microalgae as a building blocks of lipids. Fatty acids are synthesized through lipid biosynthetic acetyl CoA pathway as shown in Fig. 5 (Petkov and Garcia 2007). The common fatty acids have a chain length ranging from C16 to C18 similar to higher plants. These fatty acids in microalgae are of saturated or unsaturated. Unsaturated fatty acids may vary based on the number and position of double bonds (Borowitzka 1988). Main fatty acids are C16:0 and C18:1 in the *Chlorophyceae*; C16:0, C16:1, and C18:1 in the *Chrysophyceae*; C16:0 and C18:1 in the *Eustigmatophyceae*; C16:0 and C20:1 in the *Cryptophyceae*; and C16:0 and C16:1 in the *Bacillariophyceae* (Cobelas and Lechado 1989).

Fatty acid esters largely determine the biodiesel properties. The main properties are ignition, cold flow, and oxidative stability, and all of them depend upon the fatty acid profile of microalgae. Though fatty acid profile does not show up to have much influence on transesterification operation, they do influence on fuel properties. For instance, saturated fatty acids produce biodiesel with fine oxidative stability and cetane number but with poor cold flow properties. Biodiesel created utilizing these saturated fats is more likely to solidify at natural temperatures. On the other hand, biodiesel created from sources that are high in PUFAs has great cold flow properties. Hence a proper blending of fatty acid composition is essential to produce biodiesel with desirable properties (Knothe 2005).

10 Algal Biomass Production from Wastewater

Large-scale production of biomass was essential for product generation as well as for wastewater treatment. Microalgae are known since 1950 for its excellent nutrient removal ability and growing by utilizing carbon, nitrogen, and phosphorous. Generation of algal biomass gives an effective strategy of nutrient reusing not available through ordinary treatment of wastewater. Generated biomass can be

Table 4 Lipids of various microalgal species in percentage to the dry biomass

Species	Lipid content (% dry weight)
Spirulina maxima	6–7
Spirulina platensis	4–9
Anabaena cylindrica	4–7
Botryococcus braunii	25–80
Phaeodactylum tricornutum	20–30
Chlorella emersonii	28–32
Nannochloropsis sp.	31–68
Chlorella pyrenoidosa	2
Euglena gracilis	14–20
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella bioculata	8
Dunaliella primolecta	23
Dunaliella salina	6
Dunaliella tertiolecta	35.6
Chlorella vulgaris	14–22
Chlorella protothecoides	57.9
Hormidium sp.	38
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	30–50
Chlamydomonas reinhardtii	21
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Pleurochrysis carterae	30–50
Porphyridium cruentum	9–14
Prymnesium parvum	22–38
Tetraselmis maculata	8
Tetraselmis suecica	15–23
Schizochytrium sp.	50–77
Spirogyra sp.	11–21
Scenedesmus dimorphus	16–40
Scenedesmus obliquus	12–14
Synechococcus sp.	11

Adopted from Becker (1994, 2004), Chisti (2007), Illman et al. (2000), Moheimani and Borowitzka (2005) and Wu et al. (2012)

effectively used for various purposes like biofuels and residual biomass as fertilizer and animal feed. Biomass production from wastewater has been excellent at times than produced from synthetic media. Park et al. (2011) observed following advantages of microalgae in HRAPs:

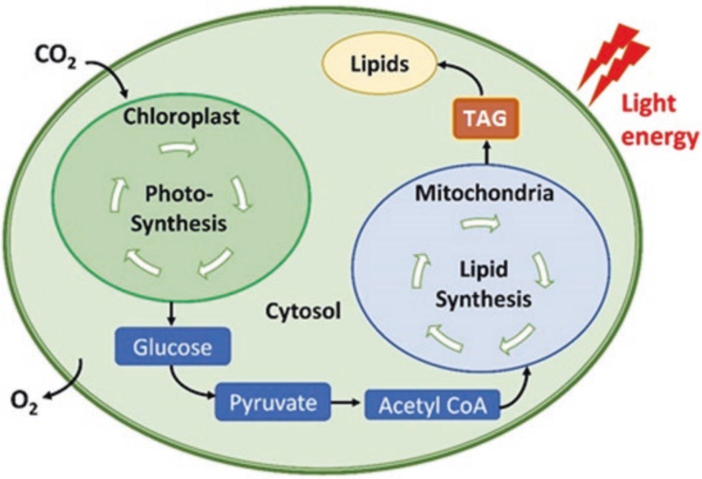

Fig. 5 Schematic diagram showing photosynthesis and lipid biosynthetic simplified pathway in microalgal cell. (Modified from Abomohra et al. 2018b)

(i) Large amount of biomass generation on wastewater culturing
(ii) Resistance to various robust conditions especially seasonal fluctuations
(iii) Aggregation and thereby easy harvesting
(iv) High productivity of lipids or other valuable products

11 Harvesting and Dewatering Methods of Microalgal Biomass

Microalgal biomass production and conversion to biofuel with effective wastewater treatment require efficient cellular separation from the medium or water. Commercialization of microalgal-based biodiesel heavily depends upon cost-effective separation. The harvesting cost can occupy 20–30% of the total cost, and hence the choice of selection in harvesting is very important. Even though there are plenty of extraction methods, efficiency and cost-effectiveness are hard to achieve at a time. The common methods include sedimentation, flocculation, floatation, and coupling of these processes (Munoz et al. 2006; Abomohra et al. 2018a).

11.1 Sedimentation

The separation of particle from suspension based on gravity is called as sedimentation. Microalgal species have diverse settling speeds. Milledge and Heaven (2013) prescribed this as prior methodology before subjecting for any other methodologies.

11.2 Flocculation

Flocculation of microalgae occurs as its cells aggregate together to flocs either by itself or due to the addition of a flocculant. Flocculation happens by chemical or physical ways. Commonly utilized flocculants include alum which is an inorganic flocculant and chitosan or starch which are organic flocculants (Vandamme et al. 2010). Ordinary flocculation works by scattering of charge. Microalgal cell surface is negatively charged which can be neutralized by applying positive charge to flocculate the microalgal biomass, hence permitting more effective sedimentation or filtration.

Alum, lime, and multivalent metal salts are widely used removing suspended solids during wastewater treatment. It is also proven to be effective in removing microalgae from suspensions (Papazi et al. 2010). Alum poses serious concern on affecting the microalgal viability and altering the composition of growth medium (Milledge and Heaven 2013). Microbes that are coexisting with microalgae can speed up flocculation by forming bioflocculants. For instance, *Paenibacillus* sp. AM49 bacterium has successfully bioflocculated *Chlorella vulgaris* (Oh et al. 2001). There are many different types of flocculants that are in use for algal separation, and no universal flocculant is equally efficient for all microalgal species. The major advantage of this harvesting method is its less energy usage.

11.3 Flotation

Usage of air bubbles in floating microalgae over the surface of water is called floatation. Dissolved air flotation is a common process in which algal cells are initially flocculated with flocculants and then brought to the surface with bubbling of air. This is often favored strategy of wastewater treatment ponds that collect microalgal biomass. The basic principle includes hydrophobic interaction and surface charge of the microalgae. A drawback of utilizing DAF is energy intensive in pressurizing the water.

11.4 Filtration

Filtration implies filters for harvesting biomass which separates algae as thick paste in the filters and lets the water pass through. Filtering microalgal cells are termed as microfiltration, and filtration of large flocculated cells is called as macrofiltration (Milledge and Heaven 2013). Different filtrations are available such as dead-end filtration, microfiltration, ultrafiltration, pressure filtration, vacuum filtration, and tangential flow filtration. Highly expensive and time consuming are the demerits of this process (Harun et al. 2010).

11.5 Centrifugation

Centrifugation can efficiently collect microalgae and is most commonly used practice for harvesting foremost (Sharma et al. 2013). Even though it is expensive, it is attractive and efficient because of its rate of separation. However, this methodology is not suitable for large-scale system because of its expensiveness. After gathering of microalgae, drying is required for biomass. Common strategies are flash drying, drum drying, freeze-drying, and sun drying. Solar drying is very economic, whereas splash and solid drying are expensive.

12 Biodiesel Production

The word “biodiesel” refers to any diesel-equivalent biofuel made from renewable biological feedstock by using special processes to convert the biomass into fuel. Biodiesel has gotten much consideration around the world. Biodiesel is the formation of monoalkyl esters of fatty acids with or without catalyst called transesterification. The process can be used to produce biodiesel from the oil obtained from any of the renewable feedstock such as oil crops or microalgae.

12.1 Lipid Extraction

Lipid extraction is a basic process to generate biodiesel from microalgal biomass. It is performed by chemical methods utilizing organic solvents, physical methods, or a combination of the two. Extraction process utilized must be quick, effective, and non-damaging to lipids and easily scaled up (Medina et al. 1998). Extraction using a modified Bligh and Dyer strategy is the foremost commonly utilized (Mutanda et al. 2011). The choice of solvent for lipid extraction depends on microalgae grown. Other favored characteristics of the solvents are that they must be cheap, nontoxic, nonpolar, volatile, and poor extractors of other cell components.

Lipids are associated inside the cells and need to be extracted without much loss. Hydrophobic interaction for nonpolar lipids and hydrogen bonding for polar lipids are two key processes of lipid extraction achieved with solvents of respective polarity. Alkalinity is used to disturb the internal association of lipids in the cell (Cerniglia et al. 1980). The pretreatment of samples before oil extraction includes homogenization, sonication, grinding, dot beating, osmotic shock, microwaving, and freeze-drying to destruct the cells that are in use (Mutanda et al. 2011). The choice of strategies in terms of extraction depends upon the type and scale of biomass (Uduman et al. 2010).

12.2 Lipid Measurement

Storage of lipids in algae varies among diverse strains and indeed inside a single strain beneath diverse cultivation conditions. Lipid divisions and types of lipids are essential for managing the biodiesel to be produced. Lipid quantification can be done through Nile red spectrofluorometry, Nile red fluorescence microscopy, Fourier transform infrared microspectroscopy (FTIR), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography (GC), or any other chromatography with mass spectrometry. Nile red staining technique is mainly for detecting lipid bodies inside the cell and a mean of screening lipid accumulation and storage in the cell. Lipid profile analysis can be usually done through gas chromatography with flame ionization detector (CG-FID) which analyzes the methyl esters of fatty acids produced through transesterification (Mutanda et al. 2011).

12.3 Transesterification

Transesterification is the process of lipid-alcohol esterification for producing alkyl esters along with a by-product glycerol. It proceeds as follows such as triglycerides (TAG) to diglycerides and diglycerides to monoglycerides and finally to biodiesel. This reaction changes the high viscous oil into low viscous biodiesel similar to diesel in fluidity for successful application in engines (Fig. 6).

The transesterification in this way needs an abundance of alcohol to preserve balance flow toward product with increasing response rate (Gultom and Hu 2013). Base catalysis could be a quicker reaction but is restricted by the free fatty acids (Harun et al. 2010). Acid catalysis is preferred for biodiesel production for oils rich in free fatty acids (Sharma et al. 2013), but the reaction is slow. Speeding up the acid catalysis requires the application of temperature and pressure which will be costly at large scale. Hence, chemical-catalyzed transesterification has its demerits, and above all separation of catalyst from product is of greater challenge (Kim et al. 2013). Enzymatic-catalyzed esterification is a reasonable strategy for oils possessing which can be largely converted to alkyl esters (Fig. 7).

Enzyme catalysis occurs within the form of immobilized lipase, and immobilized form has advantages like higher stability and reusability nature. Other benefits incorporate direct reaction conditions, lower alcohol to oil ratio, less energy intensive are required, and simpler product recovery (Kim et al. 2013). However, enzyme production costs at present make it unsuitable for large scale.

The most productive oil crops like palm oils are not close enough for biodiesel produced from microalgae. The oils are usually comprised of triacylglycerols (TAG) which are the esters of fatty acids with glycerol. Each oil varies in fatty acid proportions, and hence it is very important to select the appropriate oil having preferable

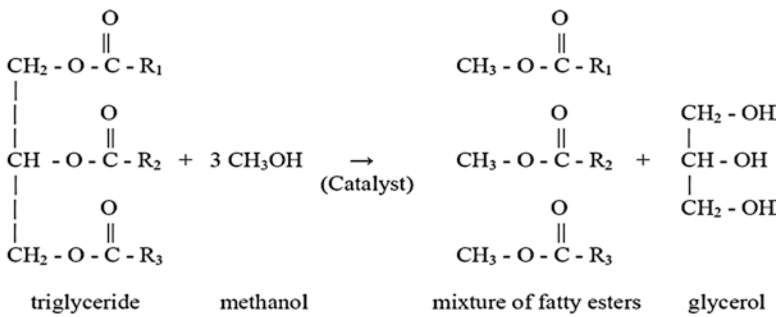

Fig. 6 Chemical reaction of transesterification of triglycerides for biodiesel (fatty esters) production

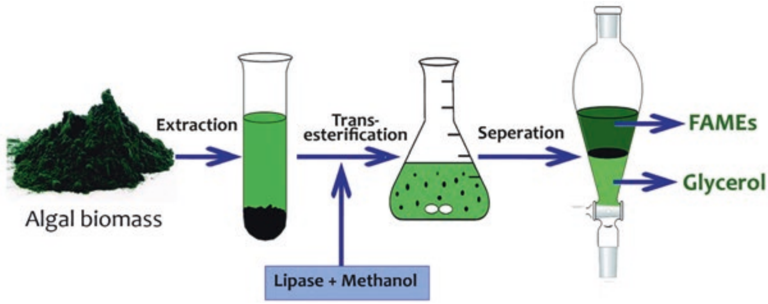

Fig. 7 Diagram showing the enzyme intermediated alcoholysis for biodiesel (FAMEs) generation

fatty acid composition for efficient conversion as well as for better properties in biodiesel produced.

Biodiesel has several advantages such as lower emissions of CO, hydrocarbons, and particulate matters as compared with conventional diesel-fueled engines. Hence biodiesel is an attracted alternative fuel with lots of advantages over conventional diesel. Since biodiesel is produced from biological origin, it emits the carbon dioxide that is taken from the atmosphere for growing the biological source, and thereby it acts as carbon neutral. The biodiesel characteristics are similar to those of petroleum diesel, and, therefore, the biodiesel is rated as a strong potential alternative to conventional diesel. In case of biodiesel, because the cost of raw material accounts about 75% of the entire cost of production, selection of a suitable feedstock is essential to ensure the low biodiesel production cost.

13 Conclusion

Benefits of an ecofriendly approach are within the front of sustainable development subjects. Hence, this chapter included the most recent advancements on exploiting microalgae for dual utilization such as wastewater treatment and biodiesel generation. Recently, wealth from waste or waste management in any of the lucrative way is increasingly attractive because of abundance of wastes. In such perspective microalgae can grow well by utilizing the nutrients of wastewater and thereby nullify the nutrient cost for biomass production. Thus, microalgae can be the excellent candidate for satisfying dual purpose of waste management and energy production, and thus finding suitable conditions to make it more applicable and economically feasible is the path to be walked on.

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A Biorefinery from *Nannochloropsis* spp. Utilizing Wastewater Resources

Madhusree Mitra and Sandhya Mishra

1 Introduction

1.1 Reciprocity Between the Microalgal Biorefineries and Wastewater Treatment

Among the several potential applications of microalgae, their utilization as a biofuel feedstock seems to be the most promising future scheme on a large-scale basis (Wijffels et al. 2013). Microalgae-based products are currently subjugating a considerable portion of the high-value markets, for instance, as human dietary supplements (nutraceuticals), pharmaceuticals, cosmeceuticals, and animal feed (Milledge 2011). Microalgae, being one of the essential bioresources, are gaining a lot of research attention nowadays, but the open pond cultivation of microalgae requires huge amount of water (11–13 million L ha^{-1} $Year^{-1}$). Thus, depleting water resources have put the feasibility of microalgal cultivation under the scanner.

The potential of microalgae to utilize wastewater nutrients and seawater for their growth not only slash down the production cost by substitute the commercial growth medium but also purify the wastewater feed for reuse in irrigation and other commercial purposes. Oswald (1963) honed this phycoremediation process of wastewaters and proposed utilization of several by-products obtained from the grown biomass.

Under the aegis of their various nutritional growth mode (phototrophic, heterotrophic, and mixotrophic), microalgae can efficaciously pull out the organic and

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inorganic wastewater nutrients to produce valued biomass (Oswald and Gotaas 1957; Martinez et al. 2000; Mallick 2002; Park et al. 2011). Microalgae-mediated wastewater treatment (WWT) process is a sustainable, ecofriendly, and cost-effective means of achieving bioremediation by substituting the traditional and comparatively costlier processes. In the process of wastewater treatment, microalgal cells tend to accumulate wastewater nutrients and tailor the biosynthesis pathway of cellular components in order to produce valuable end products. Amidst the profitable strategies involved in mass cultivation of microalgal biomass, an integrated approach of utilizing "algal farming" for wastewater treatment is inevitably one of the pragmatic perspectives. The aforesaid approach causes the annihilation of the dismissive ecological footprint that would else ways emerge from the pollution linked to the conventional WWT process (Fig. 1).

1.2 Microalgae-Assisted WWT Process

The concern for the occurrence of "pharmaceutical and personal care products" (PPCPs; which include pharmaceutical drugs, ingredients used in food supplements, cosmetics, and other personal care), endocrine disrupting compounds (EDCs), and heavy metals in the water bodies has grown immensely over the last few years, owing to their involvement in various health issues. The US Environmental Protection Agency (US EPA) has enlisted 12 PPCP and EDC material to appraise their level of extant and related safety concerns. In search of a sustainable and economically viable pollution control technology, the bioremediation efficiency of microalgae has taken into consideration since they are the known scavengers of water pollutants. Microalgal cells not only assimilate the organic and inorganic compounds (including nitrates, phosphates, ammonium, etc.) of wastewater but also known to break down the pertinacious molecules such as antibiotics, hydrocarbons, PPCPs, EDCs, and heavy metals. Although, the ability of microalgae to degrade these micropollutants has been confirmed by several researchers (Subashchandrabose et al. 2013), the available knowledge on the magnitude of degradation and the effectivity of the microalgal cell to degrade micropollutants remain obscure (Schwarzenbach et al. 2006).

1.3 Current Scenario of Microalgae-Based WWT

Microalgae-mediated wastewater treatment has been conducted through three different strategies: (I) treatment of urban wastewater in the microalgae-based high rate algal ponds (HRAPs), (II) treatment of specific (industrial, municipal, agricultural, and so forth) wastewater, and (III) degradation of specific pollutants.

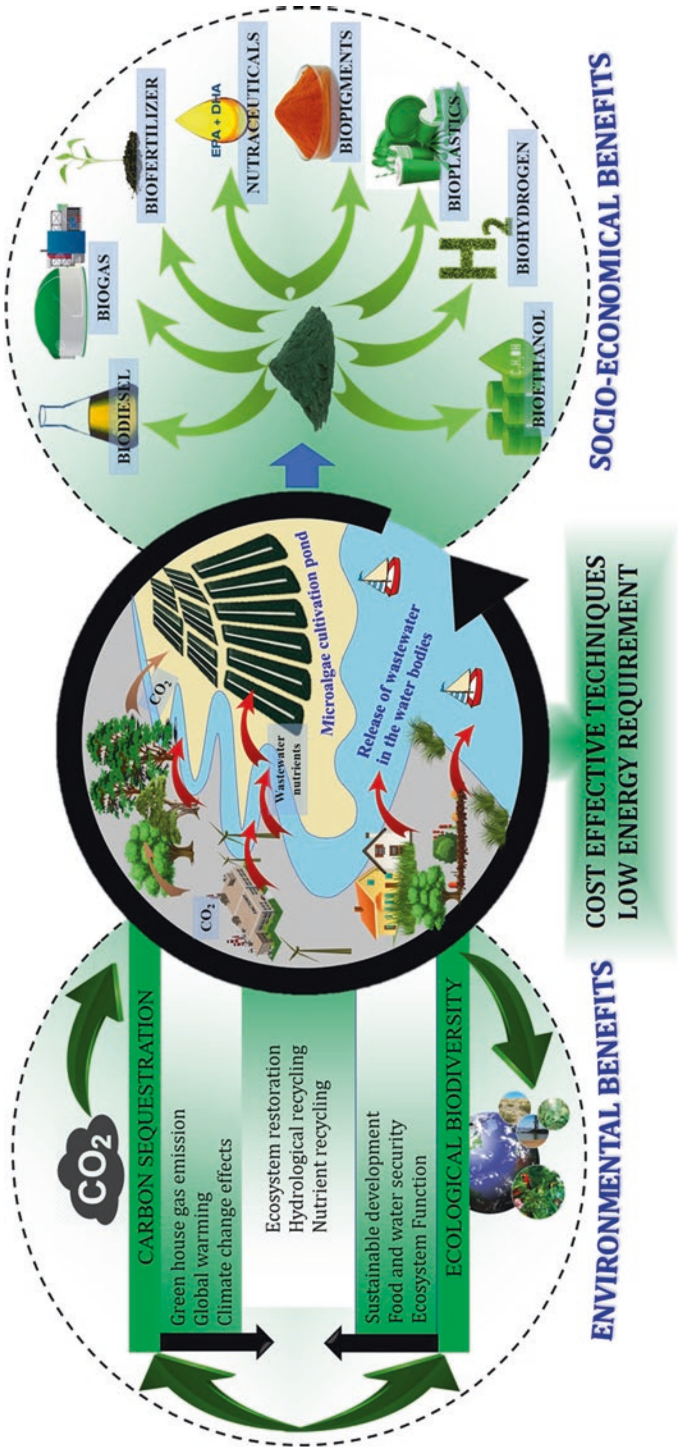

Fig. 1 Integrated approach of wastewater treatment and microalgal biorefinery and its benefits

1.4 Microalgal Product-Based Industry: Requirement of Wastewater Nutrients

Employing microalgae for the wastewater bioremediation and simultaneous production of lucrative by-products is a sustainable approach and has gained substantial research recognition over the decades (Oswald 1988; Rawat et al. 2011; Mitra et al. 2016). Wastewater-grown microalgae may offer lipid-rich biomass (as an energy feedstock) and other nonfuel products (pigments, nutraceuticals, biofertilizers, and so forth) besides its bioremediation potential. Previous research activities revealed that microalgae are efficient in unloading the excess nutrient and heavy metals from the effluents (Tam and Wong 1989; Olguın 2003; Li et al. 2011; Zhu et al. 2013; Cai et al. 2013a, b). Thus, wastewater treatment with microalgae proposes an opportunity for effectual reutilization of wastewater nutrients surpassing the need of expensive chemical treatment, particularly at the tertiary stage. In a study conducted by Tam and Wong (1989), *Chlorella pyrenoidosa* and *Scenedesmus* sp. successfully attained removal of secondary effluent nutrients in a tertiary treatment process to prevent eutrophication upon the discharge of wastewater nutrients into the water bodies. Apart from nutrient removal, microalgae help in plummeting the BOD and growth of coliform bacteria in the wastewater stream by increasing the pH of the wastewater as a consequence of their photosynthetic activity.

This chapter addresses the wastewater treatment capability of *Nannochloropsis* spp. while attaining valorization of resulted biomass with a biorefinery point of view.

Marine eustigmatophyte *Nannochloropsis* was first designated by Hibberd (1981). These organisms are unicellular, nonflagellated, small (<5 μm) coccoid, mixotrophic organisms (Van den hoek et al. 1995). Species belonging to the genera of *Nannochloropsis* are an excellent source of omega-3 polyunsaturated fatty acids mainly eicosapentaenoic acid (C20:5n3; EPA). Because of their nutritive value, they have found extensive applications in the field of food aquaculture, pharmaceutical, nutraceutical, and cosmeceuticals (Wang and Chai 1994; Srinivas and Ochs 2012).

2 Role of *Nannochloropsis* spp. in Wastewater Treatment

Researchers advocated that employing microalgae including *Chlorella*, *Scenedesmus*, *Spirulina*, *Chlamydomonas*, *Phormidium*, and so forth for treating wastewater is an efficient and promising approach. Besides, this known microalgae species, *Nannochloropsis* spp., is presently acquiring recognition for phytoremediation of wastewater, while utilizing wastewater nutrients to produce biomass rich in valuable compounds. This marine eustigmatophyte is favored due to its inflated lipid content and being exploited as a prospective biodiesel feedstock and/or as a reservoir of high-value lipids, viz., EPA. Targeting single or multiple products (in a biorefinery)-based approach, so far *Nannochloropsis* has been cultivated utilizing nutrient-rich effluents from pesticide industry, pharmaceutical industry, municipal effluents, petroleum (oil) industry, dairy industry, pulp and paper industry, and anaerobic digestion effluent (Mitra et al. 2016).

3 Utilization of Wastewater Nutrients for the Biomass Production of *Nannochloropsis* spp.

Aiming at reducing the cost of upstream and downstream processing associated with microalgal cultivation, a cost-effective biomass production process is an exigency. Production of algal biomass for valuable compounds intigrated with wastewater treatment would open up a new revenue that eventually brings about an effective process of producing high-value and low-value products of microalgae. Downing et al. (2002) specified that a microalgae-based wastewater system is 40% more profitable as compared to the best known conventional techniques. Till date, numerous research activities were executed on phycoremediation of wastewater howbeit the consumption of generated biomass for producing the high-value products was not being studied in depth. Various wastewater streams (petroleum, pharmaceutical, pulp and paper, municipal, dairy, agriculture, and piggery farm) (Fig. 2) have been evaluated as nutrient sources for cultivating *Nannochloropsis* spp. (Cai et al. 2013b; Sirakov and Velichkova 2014; Sirin and Sillapaa 2015; Polishchuk et al. 2015; Gupta et al. 2016; Galindro et al. 2016; Mitra et al. 2016).

Nannochloropsis salina grown in municipal wastewater was reported to remove nitrogen and phosphorous successfully at the rate of 35.3 mg L^{-1} day^{-1} and 3.8 mg L^{-1} day^{-1}, respectively, while achieving highest lipid productivity of 38.7 mg L^{-1} day^{-1} with 50% harvesting ratio and 2 days harvesting hiatus (Cai et al. 2013b), while *N. oculata* achieved ~74–90% removal of total nitrogen and total phosphate when grown in municipal wastewater (Sirin and Sillapaa 2015). Gupta et al. (2016) have affirmed that *N. oculata*, mixotrophically grown in municipal wastewater supplemented with 0–5 g L^{-1} glycerol, are capable of removing nitrate and phosphate from the wastewater streams. *N. oculata* grown in wastewater having 1, 3, and 5 g L^{-1} glycerol efficaciously eliminate 96.3% COD, 80.72% total nitrogen, and 60.72% total phosphate, respectively. The nutrient-rich mixotrophic conditions exhibited a 30-fold upsurge in the biomass productivity in contrast to wastewater lacking glycerol (Gupta et al. 2016). Sirakov and Velichkova (2014) utilized aquaculture wastewater for growing *N. oculata* for biomass production and proposed that the studied microalgae efficiently remove 78.4% of total nitrogen and 92% of nitrate from the wastewater stream. In a similar study carried out by Galindro et al. (2016), the nutrient removal efficiency of *N. oculata* grown in treated effluent from superintensive shrimp cultivation was relatively lower than the control (f/2 medium).

Until now different microalgal strains have been successfully assessed for their nutrient from efficiency from different wastewater streams. Even if some in-depth

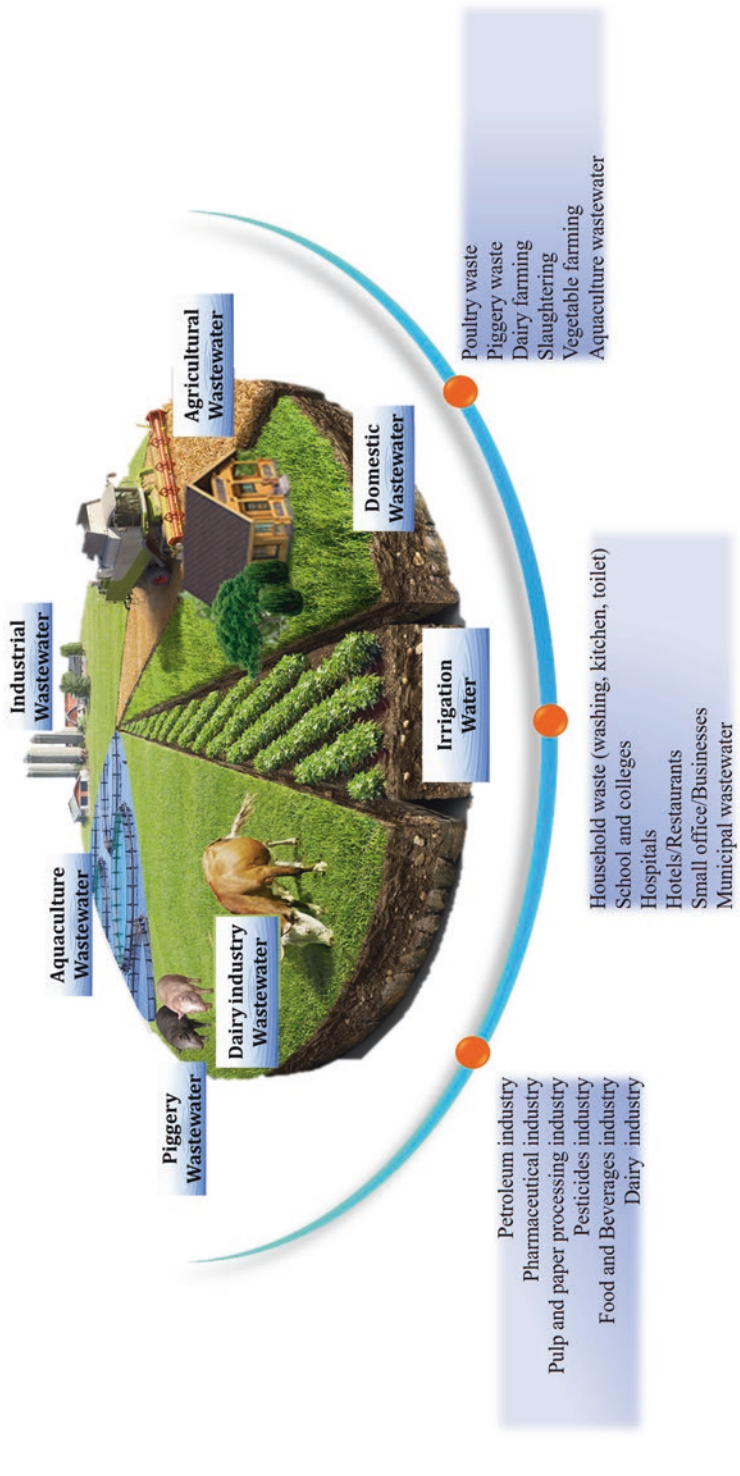

Fig. 2 Wastewater sources used for the generation of microalgal biomass

studies are yet to be done to fit *Nannochloropsis* under the same roof, the aforesaid experimental evidence envisaged that *Nannochloropsis* spp. could take an integral part in supplying treated water for reuse in agricultural, industrial, and recreational purposes.

4 Growth-Inhibiting Substances in Wastewater and Its Impact on *Nannochloropsis* spp.

One cannot deny the fact that all the available wastewater streams are not appropriate (even in their modified form) for the growth of microalgal species. Since wastewater nutrients not only include the essential growth-promoting nutrients (e.g., nitrogen and phosphate) but also contain some growth-inhibiting contaminants. Sporadically, these contaminants get hoarded within the microalgal cell and diminish the product value of the enriched biomass.

Nicholson et al. (1999) corroborated the presence of substantial amount of pollutants (e.g., heavy metal) in the industrial wastewater. Furthermore, domestic discharge and manure from animal husbandry may also contain a considerable number of surfactants and heavy metals. Available literature bespeaks the growth inhibiting the effect of surfactants (toxicity ranges from 0.003 to 17,784 mg L^{-1}) on microalgae albeit it may vary within species. As of heavy metal, members come under the genus *Nannochloropsis* showed susceptibility toward these toxic compounds including Cu, Cd, Mn, Ni, Pb, Zn, and so forth. Table 1 summarizes the toxic concentrations of heavy metals to *Nannochloropsis* spp.

Table 1 The toxic concentrations of heavy metals to *Nannochloropsis* spp.

Element	Toxic concentration (μM)	*Nannochloropsis* species	References
Zn	36.69	*N. salina*	Debelius et al. (2009)
Zn	153	*N. oculata*	Gao et al. (2002)
Mn	182	*N. oculata*	Gao et al. (2002)
Co	88.24	*N. salina*	Debelius et al. (2009)
Al	pH dependent	*N. salina*	Rwehumbiza et al. (2012)
Cd	20	*N. oculata*	Lee and Shin, (2003)
Cu	2.2	*N. gaditana*	Debelius et al. (2009)
Cu	7.9	*N. salina*	Dong et al. (2014)
Ni	69.86	*N. salina*	Dong et al. (2014)
Pb	3.6	*N. gaditana*	Debelius et al. (2009)
Pb	32.82	*N. salina*	Dong et al. (2014)
Se	300	*N. oculata*	Gao et al. (2002)

Adapted from Mitra et al. (2016)

5 Harvesting of *Nannochloropsis* spp. Grown in Wastewater

For a couple of microalgae-based wastewater treatment system, harvesting of cell biomass is contemplated as one of the major limiting factors. Followed by the wastewater treatment, complete removal of microalgal biomass is necessary. Unless the residual microalgal biomass will nullify the entire process by releasing the absorbed nutrients in the wastewater stream. Harvesting and dewatering of the microalgal biomass can be accomplished with the aid of several harvesting procedures such as flocculation, coagulation, sedimentation, flotation, centrifugation, electrophoresis, and magnetic separation (Danquah et al. 2009; Granados et al. 2012; De Godos et al. 2011; Salim et al. 2011; Smith and Davis 2012). However successful removal of biomass from the wastewater requires a relatively low-cost technique which may involve a two-step process indulging a coagulation-flocculation method succeeded by a solid-liquid separation (e.g., filtration and centrifugation).

Harvesting of microalgal cells is considered to be one of the challenging aspects of the biorefinery process especially in the industrial scale owing to its high operational cost which is approx. 20–30% of the total cost involved in downstream processing (Kim et al. 2013). Many harvesting methods have been explored to date including centrifugation, filtration, flocculation, and flotation; among them the most promising one is the pH-regulated auto-flocculation method. Sirin and Sillanpaa (2015) reported 80% recovery of municipal wastewater-grown *N. oculata* cells in an alkali-induced flocculation process at pH value of 10.50 with a high sedimentation rate of 10 min. As reported by Ledda et al. (2015), 0.45 μm filters was used to separate N. gaditana cells from the cenrate, whereas, industrial wastewater- grown Nannochloropsis sp. was filtered through melt-blown polypropylene cartridges (80-10-1 μm) (Biondi et al. 2013). In a study conducted with *N. salina* cultivated using anaerobic digestion effluent, Cai et al. (2013b) discussed the effect of harvesting frequency and harvesting ration on the lipid productivity and nutrient removal. At a harvesting ratio of 50% and harvest interval of 2 days, highest biomass productivity (155.3 mg L^{-1} day^{-1}), lipid content (24.9% ash-free dry weight), and lipid productivity (38.7 mg L^{-1} day^{-1}) were obtained (Cai et al. 2013b). Besides, flocculation-sedimentation and filtration techniques, centrifugation was also followed to harvest *Nannochloropsis* cells from the wastewater (Mitra et al. 2016).

6 Biochemical Composition of *Nannochloropsis* and the Relevance of Its Products Within a Biorefinery

The biochemical composition of microalgal cells varies widely within species based on their cultivation condition. The cell growth, biomass productivity, cellular metabolites, and even the micro- and macroelements of microalgae get affected by various abiotic stress factors (Paliwal et al. 2017). Similar to other microalgal species, the primary metabolites of *Nannochloropsis* sp. were lipids, proteins, and carbohydrates. Typically, the lipid, protein, and carbohydrate contents of *Nannochloropsis* were 25–45%, 30–45%, and 10–35%, respectively. Considering the high lipid content, this microalga has been shown to have a great possibility to be utilized as a biodiesel feedstock. Furthermore, species belonging to these genera of microalga contains a substantial amount of long-chain polyunsaturated fatty acids, mostly in the form of 5,8,11,14,17 eicosapentaenoic acid (EPA, C20:5n3) which offers enormous health benefits to humans and has the potential of being used as a nutraceutical (Adarme-Vega et al. 2012; Mitra et al. 2015a, b). Followed by successful extraction of lipids, the residual biomass can be used as a feedstock for other fuel forms, such as biocrude oil, biomethane, biohydrogen, combustible gases, and bioethanol through the enactment of different appropriate conversion processes including hydrothermal liquefaction, anaerobic digestion, gasification, pyrolysis, and enzymatic hydrolysis (Fig. 3).

6.1 Fuel-Based Products

Biodiesel

Biomass productivity and total lipid content (% of dry cell biomass) of microalgae are considered as the two essential parameters for the selection of microalgae as a potential biodiesel feedstock. Table 2 summarizes the specific growth rate, biomass content, lipid content, biomass and lipid productivity, and EPA content of *Nannochloropsis* spp. cultivated in the presence of wastewater nutrients. Idyllic

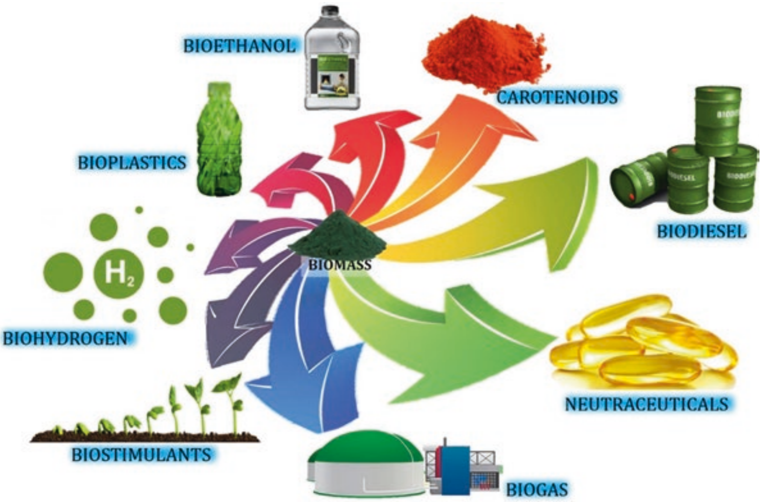

Fig. 3 Products of *Nannochloropsis* spp. within a biorefinery

Table 2 Specific growth rate, biomass content, lipid content, biomass, lipid productivity, EPA content (% of total fatty acids) of *Nannochloropsis* spp. grown in wastewater

		Specific growth rate	Biomass content	Biomass productivity	Lipid content	Lipid productivities	EPA content	
Nannochloropsis species	Wastewater sources	(day^{-1})	($mg\ L^{-1}$)	($mg\ L^{-1}\ day^{-1}$)	(% dry cell weight)	($mg\ L^{-1}\ day^{-1}$)	(% of total fatty acids)	References
N. salina	Anaerobic digestion effluent	0.3–0.6			21–36			Cai et al. (2013b)
N. salina	Anaerobic digestion effluent	0.04–0.15			26–32			Dong et al. (2014)
Nannochloropsis sp.	Municipal wastewater	0.52			33			Sheets et al. (2014)
N. oceanica	Pesticide industry effluent			27.78	24.49	6.81	29.81	Mitra et al. (2016)
N. oceanica	Pharmaceutical industry effluent			22.17	25.22	5.59	10.48	Mitra et al. (2016)
N. oceanica	Municipal sewage			21.78	26.91	5.86	32.02	Mitra et al. (2016)
N. oceanica	Petroleum industry effluent			24.78	27.4	6.79	8.56	Mitra et al. (2016)
N. oculata	Pulp and paper industry effluent		860		24.5		5.5	Polishchuk et al. (2015)

microalgal candidates for biofuel production should have apposite fatty acid composition besides high lipid content since the biodiesel properties varied substantially based on the fatty acid profile (Knothe 2009). As tabulated in Table 3, C16, C18, and C20 are the major fatty acids observed in *Nannochloropsis* sp. howbeit the fatty acid profile varies within the species. As suggested by some researchers, the theoretical and experimental properties of *Nannochloropsis* oils including cetane number, iodine value, cloud point, higher heating value, cold filter plugging point, kinematic viscosity, density, etc. meet the specification provided by both European (EN 14214) and US (ASTM D6751) standards (Ma et al. 2014; Mitra et al. 2015a, 2016).

Bioethanol

Biofuel research of *Nannochloropsis* spp. are mainly focused on biodiesel production, and with growing years, their ability to produce other fuel products including biogas, biocrude oil, and biohydrogen is also getting attention. However, only a hand full of studies are available on the bioethanol production potential of *Nannochloropsis* sp. In a recent study, Reyimu and Ízšimen (2017) evaluated the growth characteristics and carbohydrate content of *N. oculata* in municipal wastewater and also their bioethanol yield. The obtained data displayed that *N. oculata* grown in 75% municipal wastewater exhibits highest bioethanol yield of 3.68% owing to its highest carbohydrate content at the same experimental condition as compared to control (seawater grown).

Biomethane

Anaerobic digestion of microalgal biomass leads to the production of biomethane. There are few reports where *Nannochloropsis* spp. was investigated as a feedstock for methane production via anaerobic digestion (Bohutskyi and Bouwer 2013; Bohutskyi et al. 2015). Schwede et al. (2013a) reported that thermal pretreatment of the biomass followed by anaerobic digestion remarkable ameliorate the methane yield from 0.2 to 0.57 m^3 kg VS^{-1} (batch condition) and 0.13 to 0.27 m^3 kg VS^{-1} (in semicontinuous digestion). In an independent experiment by the same group, co-digestion of *N. salina* with corncob mix and corn silage resulted in a 7% and 9% increase in the biomethane content as compared to mono-digested feedstocks, respectively (Schwede et al. 2013b). The highest methane yield of 482 L CH_4 Kg^{-1} volatile solids was obtained from the deoiled biomass of *Nannochloropsis* sp. (Kinnunen et al. 2014).

Table 3 Fatty acid composition of *Nannochloropsis* spp. grown with wastewater nutrients

Nannochloropsis species	C14:0	C16:0	C16:1n7	C18:0	C18:1n9c	C18:1	C18:2n6	C18:3n6	C18:3n3	C20:3n3	C20:3n6	C20:4n6	C20:5n3	References
N. oculata	5.3	26	21.9	0.9	5.2	–	2.1	0.3	0.1	0.3	1.8	4.7	29.2	Polishchuk et al. (2015)
N. oceanica	2.87	32.43	16.8	3.84	4.47	–	7.47	–	–	–	–	2.3	29.81	Mitra et al. (2016)
N. oceanica	–	40.6	27.08	3.31	10.7	172	3.37	–	–	–	–	2.74	10.48	Mitra et al. (2016)
N. oceanica	4.74	28	18.34	4.17	4.08	0.83	3.9	–	–	–	–	3.93	32.02	Mitra et al. (2016)
N. oceanica	–	40.7	27.15	4.14	10.72	1.68	3.38	–	–	–	–	3.67	8.56	Mitra et al. (2016)

Biohydrogen

Microalgal biomass could be utilized as a substrate in a fermentation process (preferably dark and photofermentation) to produce biohydrogen. Even after the extraction of cellular lipids and pigments (mainly carotenoids) from the microalgal biomass, the defatted biomass could be further subjected to a fermentation process for the same (Nobre et al. 2013). Exploitation of wastewater-grown microalgae for biohydrogen production not only out-turn energy-efficient and economical wastewater management process but also ensure generation of a sustainable biorefinery process. Nobre et al. (2013) got successful in achieving 50–61 mL H_2 /$_{dry\ biomass}$ biohydrogen when extracted from defatted biomass of *Nannochloropsis* sp. and 47–48 mL H_2/$g_{dry\ biomass}$ from the whole biomass via fermentation, whereas, fermentation of *Nannochloropsis* sp. by immobilized cells of *Clostridium acetobutylicum* leads to the highest productivity of 0.35 mmol H_2/L medium/h (Efremenko et al. 2012). A "three-stage process" comprising dark fermentation, photofermentation, and methanogenesis leads to a biohydrogen yield of 183.9 mL/g total volatile solids along with 161.3 mg/g total volatile solids from the whole biomass of *N. oceanica* (Xia et al. 2013).

Biocrude Oil

Hydrothermal degradation of microalgal cellular components, viz., lipids, proteins, carbohydrates, and algaenans, resulted in the dark, viscous, and energy-rich biocrude oil. According to Torri et al. (2012), biocrude oils are composed of protein derivatives (mainly peptides), lipid derivatives (fatty acids and sterols), algaenan derivatives, and asphaltenes, among others. Till date, besides microalgal strains like *Dunaliella tertiolecta*, *Botryococcus braunii*, *Chlorella vulgaris*, and *Spirulina* spp.; marine eustigmatophyte *Nannochloropsis* spp. have also been used for producing biocrude oil owing to their high biomass productivity and lipid content (Rodolfi et al. 2009; Doan et al. 2011). However, their relatively smaller cell size and thick cell wall sometimes hinder the conversion process making it a bit difficult (Wijffels and Barbosa 2010). Under different experimental conditions, a biocrude oil yield of ~34–57% has been reported for *Nannochloropsis* spp. (Brown et al. 2010; Biller and Ross 2011; Biller et al. 2011; Duan and Savage 2011; Valdez et al. 2011; Toor et al. 2013).

6.2 High-Value Products

Eicosapentaenoic Acid

Nannochloropsis have emerged as a promising vegan source of omega-3 polyunsaturated fatty acids especially EPA. Several research studies have been conducted in the past year focusing on their capability to yield EPA under different abiotic stress conditions. Polishchuk et al. (2015) utilized pulp and paper industry wastewater for the production of eicosapentaenoic acid by *N. oculata* howbeit did not find any significant increase in the EPA content as compared to control condition. They have suggested a two-step process combining methane production process using the wastewater stream followed by cultivation of EPA rich *N. oculata* using wastewater nutrients. Contradictory to this study, a remarkably higher EPA yield (% of total fatty acids) has been observed in *N. oceanica* grown in municipal sewage wastewater (32.02%) followed by pesticide industry wastewater (29.81%) concerning Conway medium (21.5%) (Mitra et al. 2016), thus, signifying the probability of using wastewater nutrients for producing high-value products like eicosapentaenoic acid.

Protein

After successful removal of lipid (including EPA), the defatted biomass can be reutilized for the extraction of proteins. The utilization of the lipid-extracted biomass will also cause a value addition to the biorefinery process since the purified protein product (consists of essential amino acids) possesses a higher market value (Kent et al. 2015; Tibbetts et al. 2015). Above and beyond this, the obtained protein from *Nannochloropsis* being a vegetarian protein source can be considered as a substitute for people with soy protein allergies. In a study conducted by Samarakoon et al. (2013), ACE inhibitory peptides from *N. oculata* have been found to possess a significant role in the preclusion of hypertension. Besides the major metabolites, *Nannochloropsis* also synthesize pigments, enzymes, sterols, vitamins, and antioxidants that can be utilized as food and feed supplements and cosmetics.

7 Biomass Valorization of *Nannochloropsis* Grown in Wastewater

In view of the proximate composition, the biomass can be used in the various industrial sectors including biofuel-based industry, pharmaceutical and nutraceutical industry, agricultural sector as bio-stimulants or as a fertilizer and animal feed, and cosmeceuticals industry. Except for fuel-based by-products, to marketize other high-value food-grade products, wastewater-grown biomass should not possess a prohibitive amount of prevailing pollutants such as heavy metals and other organic pollutants, which may get transported into the animals or nature.

Concerns Related to High-Value Products in a Wastewater-Based Biorefinery of Nannochloropsis sp.

Previous research suggests a great potential for mass production of algal biomass using wastewaters (e.g., municipal and animal manure wastewaters), although, there are very few literature available focusing on the PUFA-producing potential of wastewater-grown microalgae. In a recent article, *Nannochloropsis* sp. was grown in pulp and paper industry wastewater for EPA production (Polishchuk et al. 2015). In a study conducted by Mitra et al. (2016), the EPA-producing potential of *N. oceanica* cultivated in effluents from pesticide industry, the pharmaceutical industry, oil industry, and municipal effluents was also discussed. However, the code of conduct followed by food, pharmaceutical, nutraceutical, and cosmeceutical industries would possibly incumber the use of high-value products obtained from wastewater has grown microalgal source. Therefore, regarding the regulatory issues in relation to a food-grade product deriving from wastewater resources, few possible precautionary measures have to be taken before experimenting with wastewater resources, such as low storage temperature (to prevent substrate decomposition), proper filtration and sterilization (to eliminate suspended solids which might lead to flocculation of microalgal cells), and autoclaving (to avert the peril of biotic contaminants, and also quantification of heavy metals (Mitra et al. 2016).

However, the wastewater exceeding the safe level of pesticide residues, heavy metals, or another such pollutant should not be employed for EPA production without necessary dilution and pretreatment, and, at each step of biorefinery, especially for nutraceutical separation steps, ecotoxicological pollutant concentration needs to be amended in highly safe concentrations for better safeguards.

8 Biorefinery Approaches

Figure 4 epitomizes the schematic representations of different possible biorefinery routes of *Nannochloropsis* spp. Till date, a few possible routes of *Nannochloropsis* biorefinery has been demonstrated by the researchers (Nobre et al. 2013; Ferreira et al. 2013; Adam and Shanableh 2017; Chua and Schenk 2017) as discussed below:

Lipids ➔ *Pigments* ➔ *Biohydrogen* (Nobre et al. 2013; Ferreira et al. 2013)

Nannochloropsis sp. was harvested by centrifugation and dried in an oven at 70 °C, and then the dried biomass was utilized for the extraction of lipids and pigments. 45 g lipids/100 g dry biomass (containing approx. 5% EPA) and almost 70% of the pigments (50% of which is composed of ß-carotene, canthaxanthin, astaxanthin, and zeaxanthin/lutein) were recovered using supercritical CO_2 modified with 20 wt.% ethanol at 40 °C and 300 bar pressures at a flow rate of 0.62 g/min. Remaining biomass was then utilized as a feedstock for producing biohydrogen through dark fermentation by *Enterobacter aerogenes*, and a yield of 60.6 mL/g of dry biomass was obtained (Nobre et al. 2013):

Biodiesel ➔ *Bioethanol* ➔ *Biogas* (Adam and Shanableh 2017)

With the aim of maximizing the biofuel production potential of *Nannochloropsis* species, Adam and Shanableh (2017) proposed a combinatorial strategy where the lipid and sugar content of *Nannochloropsis* sp. were utilized to generate biodiesel and bioethanol, respectively, followed by utilization of remaining biomass for the

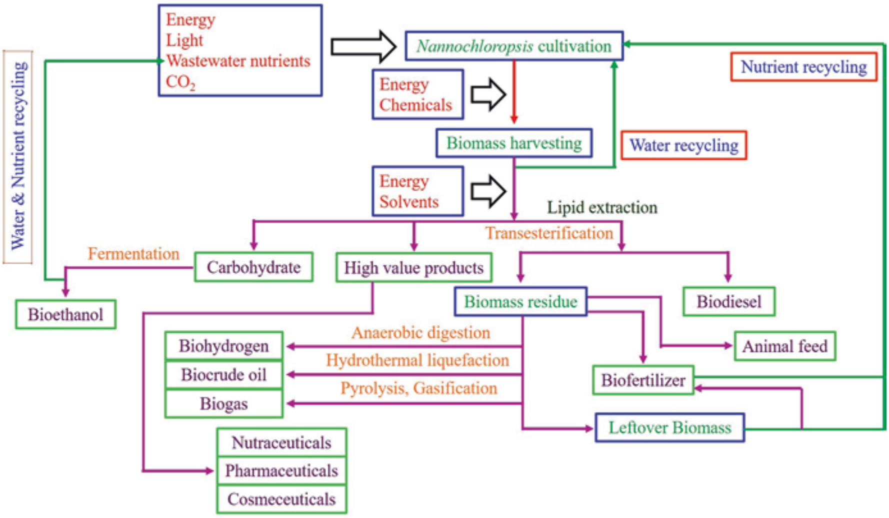

Fig. 4 Proposed biorefinery route

production of biogas. In the process mentioned above, they have applied enzymatic hydrolysis, lipid extraction, and anaerobic digestion to produce three fuel products following two possible biorefinery routes. Lipid extraction followed by enzymatic hydrolysis considered to be beneficial for this particular process:

Eicosapentaenoic acid ➔ *Food and feed supplements* (Chua and Schenk 2017)

Contrasting to the other existing biorefinery approaches, where fuel-based products were prioritized, Chua and Schenk (2017) proposed a *Nannochloropsis*-based biorefinery that initiated with the induction of EPA production. In the aforesaid process, the cells were harvested by using food-grade flocculants, followed by centrifugation and drying. Omega-3 fatty acids were then taken out from the dried biomass powder, and finally, the deoiled biomass can be further processed to be used food and feed supplement attributing to its high protein content. They also proposed that in this biorefinery approach, up to US$100 per liter of EPA rich oil may be attained.

9 Techno-economic Feasibility of *Nannochloropsis*-Based Biorefineries Using Wastewater Nutrients

Considering the possibility of contamination risk of the persisting pollutants of wastewater in the food-grade products obtained from the wastewater where microalgae has grown, the process requires the involvement of wastewater pretreatment (filtration, sterilization, etc.) prior to the experiment which in turn cause value addition in the upstream processing.

Microalgae-based biorefinery strategies involve the production of high- and low-value microalgal products aiming at making the entire process economically feasible. In comparison with the other oil crops, the biodiesel-producing potential of microalgae is enormous. However, considering the bottlenecks associated with the wastewater-based biorefinery process regarding low biomass productivity, the efficacy of harvesting, and moderately low lipid content, a careful assessment is needed prior to execution of the process on an industrial scale. Utilization of wastewater nutrients not only make microalgal biorefinery process environmentally sustainable but also helps to maintain ecological balance, reduce water footprint, and improve nutrient recycling. Techno-economic feasibility of the biorefinery process aims to understand the cost-effectiveness of the entire process and also involve in evaluating the mass and energy balance associated with downstream processing.

Lately, biofuel production from microalgae is getting established concerning energy and CO_2 assessment and also cost analysis (Campbell et al. 2010; Soratana and Landis 2011; Xu et al. 2011). In other studies which involve and optimized highly productive open pond cultivation of microalgae, the value of the life cycle of biodiesel production lies within the range of "2.8–5.4 MJ/MJ_{BD}" and "0.2–0.9 $kgCO_2/MJ_{BD}$" (Lardon et al. 2009; Stephenson et al. 2010; Khoo et al. 2011). CONCAWE (CONCAWE 2008) and Greet (Frank et al. 2011) models have been used in the Europe and USA, respectively, to evaluate energy requirement, CO_2 emissions, and cost of upstream and downstream processing for the maintenance of the database of fuel life cycle inventories (LCIs). In a much recent study with *N. oculata*, different biorefinery routes for the extraction of lipids, pigments, and bio-hydrogen production were studied by Ferreira et al. (2013). They have outlined an energy consumption of "172–239 MJ/MJ" $_{produced}$ and "12,471–14,994 gCO_2/MJ" $_{produced}$ for the first biorefinery approach, whereas, for the second route, overall energy utilization of "206–286 MJ/MJ" $_{produced}$ and "14,881–17,913 gCO_2/MJ" $_{produced}$ was achieved.

The total cost associated with the production process of microalgal biodiesel may be divided into the partial costs linked with biomass generation (82% cost associated with lighting, 13% for the water, and 4% for the nutrient consumption), harvesting of the biomass (approx.1%), lipid extraction and transesterification, and extraction of other value-added by-products. Culturing microalgae in the open pond (outdoor) utilizing natural sunlight would reduce the costs associated with lighting, however, will give rise to lower growth rate, degradation of temperature sensitive high-value products, and higher contamination risk nullifying the value addition possibility of food-grade products (EPA and carotenoids in case of *Nannochloropsis* sp.). Shorter cultivation period will also deduct the lighting cost, and as suggested by Silva et al. (2009), regular monitoring of cellular metabolites by flow cytometry would help in selecting the product-specific harvesting date that in turn shorten the algal growth period.

Although *Nannochloropsis* was getting explored for producing fuel-based and high-value by-products since long, however, the available report apropos to the cost analysis of the *Nannochloropsis*-based biorefinery strategies is insufficient. In a recent report, Ferreira et al. 2013 provided a detailed techno-economic assessment of few possible biorefinery routes of biorefinery including Path 1, Direct conversion of biomass into biohydrogen; Path 2, Soxhlet extraction of lipids with hexane; Path 3, Supercritical CO_2 (doped with 20% ethanol) aided extraction of lipids ➔ pigments; Path 4, Soxhlet extraction of lipids with hexane ➔ biohydrogen from defatted biomass; and Path 5, Supercritical CO_2 (doped with 20% ethanol) aided extraction of lipids ➔ pigments ➔ biohydrogen from defatted biomass.

Since the cost of downstream processing also involves the cost of solvents and electricity usage, they have proposed a cost of 660.56 and 365.42 €/kg of *N. oculata* oil in Path 1 and Path 2, respectively, while the cost of extracted pigments/g of biomass in Path 2 would be 0.00024 € considering the mean pigment market value of 450 €/kg biomass. They have also quantified the price of biogas resulted from both fatted (Path 1) and defatted (Path 3 and 4) biomass, and no such significant difference was observed in Path 3 and path 4. They have found that the cost of biohydrogen would be 0.00025 €, 0.00022 € and 0.00018 € considering the market value of 80 €/ kg of H_2. As bespoken by Ferreira et al. (2013), Paths 2 and 4 can be adapted as the most economically viable approach, whereas Path 1 seems to be the most expensive one. They have also mentioned that biohydrogen yield obtained in their study was relatively higher than the previously reported study of the same kind (Lakaniemi et al. 2011; Ferreira et al. 2012, 2013).

As bespoken by Thurmond (2009), the successful production of microalgal biodiesel can be in agreement with the following five keywords: (a) "fatter," (b) "faster," (c) "cheaper," (d) "easier," and (e) "fraction." The initial two keywords, i.e., "fatter" and "faster," indicate the fast-growing oleaginous microalgal species are having at least 60% of the oil content. Biodiesel production using such microalgal species may result in a substantial drop (almost half) in the size and footprint of the production plant which in turn leads to substantial dwindling of the principal and operating costs (Singh and Gu 2010).

In a much recent study, utterly different biorefinery strategy from *Nannochloropsis* (EPA ➔ defatted biomass as a food and feed supplement), Chua and Schenk (2017) has proposed that up to US$ 100/L of EPA rich oil may be attained following their strategy. After extraction of lipids, defatted protein-rich biomass can be sold as animal food.

However, all the above techno-economic assessment was based on the lab-scale data which urge for the need of pilot-scale study to achieve a commercially attainable process.

10 Final Considerations

Although the biorefinery industry promises plentiful opportunities from the economic point of view, the few existing research studies regarding the *Nannochloropsis* biorefineries have been executed at the laboratory scale only. Despite the propitious conditions for the production of biomass utilizing wastewater nutrients and valorization of the biomass with bioproducts, the process development in the industrial scale is presently far from the theoretical high-profit hypothesis. Hence, more in-depth research analysis to be done to scale up the all possible biorefinery routes from *Nannochloropsis* using wastewater nutrient which will convert the stochastic economically sustainable process into reality.

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Cultivation of Microalgae on Anaerobically Digested Agro-industrial Wastes and By-Products

Giorgos Markou, Liang Wang, Jianfeng Ye, and Adrian Unc

1 Introduction

Anaerobic digestion (AD) is a biological technology in which a consortium of microbes breaks down organic material under anoxic conditions producing biogas. Biogas is a mixture of mainly methane (45–65%) and carbon dioxide (45–35%) and is a renewable energy carrier used for the production of thermal/electric energy or as transportation fuel (Weiland 2010). AD is a mature technology and has gained interest as an approach of treating organic wastes (including wastewater) for the production of renewable energy. Worldwide tens of thousands of biogas plants are already operating, treating organic wastes, such as animal manures, crops and food residues, industrial organic wastes, sewage sludge (biosolids), etc. Some biogas plants are also integrated into the production of energy from residues or energy crops, such as maize silage (Lora Grando et al. 2017). AD is a very efficient, when compared to other treatment methods (such as aerobic treatments), at reducing high organic loads and therefore is an option of choice for high strength wastes, such as agro-industrial wastes or by-products. AD can reduce organic loads as high as 80%; however it does not remove inorganic loads, and therefore it does not achieve tertiary treatment. As a consequence, the effluents of AD, which are called digestate, are rich in inorganic nutrients that either require posttreatment

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in order to reduce their pollutant loads (organic and inorganic, and including excess forms of N and P) before disposal or used as soil amendments and nutrient source for plant production (Möller and Müller 2012).

However, since transportation of digestates over great distances and their spreading over large land areas is not always economically feasible, the extensive application of digestates to lands adjacent to the biogas plants has led to the saturation of soils in N and P, with negative impact on the environment. Moreover, the seasonal application of digestates, i.e., in seasons where crops are at the appropriate growth stages that can use fertilizer nutrients, requires long-term storage, which has its own some drawbacks and can lead to negative impacts on the environment, such as the emission of greenhouse gases like CO_2, CH_4, and N_2O (Monlau et al. 2015). In this context, further strict regulations (see, e.g., the European Union Nitrate Directives) in some jurisdictions create the need for the development of alternative valorization routes for digestate. Among the various alternatives (Monlau et al. 2015), cultivation of microalgae has gain increased attention because of the high potential of producing useful biomass with the simultaneous treatment of waste streams and recycling of valuable nutrients (Cuellar-Bermudez et al. 2017; Salama et al. 2017). This book chapter aims to give an overview on the cultivation of microalgae utilizing digestates derived from agro-industrial wastes and by-products, discussing the potentials and the drawbacks of such an approach.

2 Anaerobic Digestion Process and the Generation of Digestates

2.1 Physicochemical Characteristics and Nutrient Content of Digestates

The digestate is the residual sludge obtained at the end of the AD of organic matter of various sources. The chemistry of the ingestate, the feedstock used in the anaerobic process, does affect the chemistry of the digestate. AD degrades organic compounds and leads to significant losses of volatile solids and total carbon. This lowers the chemical and biological oxygen demands (COD and BOD), an indication of a biologically stable material. Nevertheless, post digestion storage might still lead to further mineralization and accumulation of soluble carbon (Farno et al. 2014). This is treated in more detail in the following sections. Digestion thus leads to mineralization of nitrogen and phosphorus most of which are in chemical forms relatively stable at the usual neutral to slightly alkaline pH. Digestion of animal manure such as pig slurry and cow manure may lead to more accumulation of ammonia and orthophosphate than the digestion of municipal sludge (Risberg et al. 2017; Zuliani et al. 2016), while digestion of high lignin, plant-based waste produces digestates with higher total solids (Lukehurst et al. 2010), possibly with a higher C/N ratio (Eich-Greatorex et al. 2018), and with solids often likely to be present in a hydrated form (Mudryk et al. 2016). Presence of more recalcitrant compounds, such as lignin and lipids, will lead to a more biologically stable digestate (Tambone et al. 2009) with more total solids. The solid phase may be usually separated into colloidal and large particulate matter. Any nutrient that is retained in the solid phase is usually associated with the larger particles, not in the colloidal matter (Akhiar 2017).

The utilization of digestate for algal growth is dependent on the capacity to remove the nutrients in the liquid phase. Separation of solids and liquid may lead to accumulation of mineral nitrogen in the liquid phase, as nitrate and ammonium, but a removal of P in the solid phase. Thus, the proportion of solids to liquid and the charge properties of the solids affect the separation efficiency. Most common separation procedures involve flocculation followed by centrifugation or mechanical separation. Digestates with highly hydrated solids require mechanical separation procedures. The conditions of any storage before the separation step affect the stability of the digestate and the separation efficiency (Oliveira et al. 2015). The most effective separation is a combination of flocculation followed by mechanical separation (Akhiar 2017), a common practice in municipal wastewater treatment plants. One drawback of the separation of solids is that a significant proportion of phosphorus might be retained in the solid phase (Bachmann et al. 2016; Lin et al. 2015).

Another concern when using the liquid phase of the digestate for algal growth is the color. Animal manure-based digestates are notorious for producing colored digestate difficult to remove even through significant dilution (Marcilhac et al. 2014). On the other hand, digestates produced on municipal wastewater are more transparent (Zuliani et al. 2016). For the latter the only significant interference with light is due to colloidal matter, a fraction usually removed easily through flocculation. Nevertheless, co-digestion of municipal wastewater solids with food or manure waste will still produce a highly colored liquid phase digestate difficult to treat (Akhiar et al. 2017).

AD is a highly complex and dynamic biological process, which can be divided into four stages: (i) hydrolysis of polymers into soluble monomers, (ii) acidogenesis, (iii) acetogenesis, and (iv) methanogenesis (Deublein and Steinhauser 2008). During AD the organic matter is degraded and mineralized. Macromolecules such as carbohydrates, proteins, and lipids are degraded (hydrolyzed) to smaller molecules (monomers), such as monosaccharides (simple sugars), amino acids, and long-chain fatty acids, respectively. Hydrolysis is performed by hydrolytic exoenzymes, such as cellulase, protease, and lipase, which are excreted by facultative and strict anaerobic fermentative microorganisms (Gerardi 2003). In the second process, the monomers derived from hydrolysis are fermented to volatile fatty acids (acetic acid, propionic, butyric, etc.), which then are used by microbes to produce methane at the third and fourth steps. Since during AD carbon is nearly exclusively removed as CH_4 and CO_2, the remaining inorganic elements in the feedstock are mineralized and almost fully preserved in the digestion liquor (digestate; Fig. 1). These elements are either as free ions (NH_4^+, PO_4^{3-}, K^+, SO_4^{2-}, Fe^{2+}, etc.) or as ion and/or surface bounded complexes. In general, the digestate is a very complex matrix where various free counterions and (bio-)solids interact with each other (Möller and Müller 2012). In the agro-industrial sector, AD is usually performed by mixing different substrates in order to obtain a balanced C/N ratio (around 20–30:1). Because most plant- and food-derived wastes and by-products are rich in C, thus

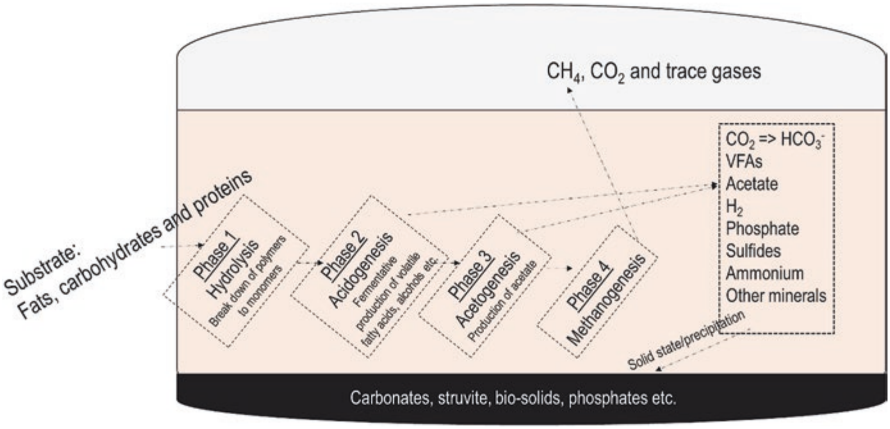

Fig. 1 Overall process of conversion of organic matter into inorganic during anaerobic digestion

having a high C/N ratio (>50:1), nitrogen-rich animal and poultry manures are typically used to adjust C/N to an optimum range (Mata-Alvarez et al. 2014). Because animal and poultry manures are also rich in other nutrients (P, K, S, etc.), digestates therefore are rich too in all of the essential nutrients required for microalgal growth (Xia and Murphy 2016).

Carbon

Carbon in digestates is either in inorganic (bicarbonate/carbonate) or in organic (volatile fatty acids, such as acetate, and undigested organic matter) forms. Regarding microalgae cultivation, bicarbonate and volatile fatty acids (mainly acetate) are the most significant C forms because they can be utilized by microalgae as C source for their growth. Bicarbonate/carbonate and volatile fatty acids (VFAs) are the end products of the acidogenic and acetogenetic stages of the digestion process, where organic polymers are biochemically broken down to monomers. The concentration of bicarbonate/carbonate and VFAs and composition of the latter can vary significantly depending on the substrate characteristics and AD parameters and on the overall process stability (Rincón et al. 2008). Bicarbonate/carbonate and VFAs have a significant role for the digestion process because, along with ammoniacal N, they are the main buffer systems for the pH in digestates (Georgacakis et al. 1982). Bicarbonate/carbonate concentration in digestates is typically in the range 500–1500 mg L^{-1}. For the typical pH range of the digestates inorganic C is mainly (>95%) found as bicarbonate species (HCO_3^-).

Among the various VFAs contained in the digestates, the most abundant species is acetate (CHO_2^-). However, due to the inhibitory effect of VFAs on methanogenesis, their concentration should kept relative low (<500 mg/L) for a stable AD process. Recently, there is an increased interest in performing AD at low pH in order to favor the hydrolytic only phase and inhibiting methanogenesis to convert organic matter into VFAs, which are a potential C source for the production of different commodities such as biosurfactants, bioflocculants, and bioplastics (Wang et al. 2014). Regarding microalgae cultivation, these accumulated VFAs may serve as energy and/or C source for the mixotrophic or heterotrophic production of microalgal biomass (Venkata Mohan and Prathima Devi 2012; Chiranjeevi and Venkata Mohan 2017).

Nitrogen

Total N, which is the sum of organic and inorganic N, of the digestate can range between 1.2 and 9.1 g Kg^{-1} FM, while inorganic N can be 44–81% of total N (Table 1). N is mostly contained in the ammoniacal form (NH_4^+/NH_3), while NO_3^- and NO_2^- are in trace concentrations. A fraction, however, of N remains in organic form, and its concentration depends mostly on the degree of biodegradation and mineralization of the organic matter. Ammoniacal N mainly derives from the mineralization of proteins and amino acids (contained in plant-derived substrates or from undigested feed proteins/amino acids excreted in manures), urea, or uric acid (main nitrogen form in animal and poultry manures, respectively), with a lower proportion originating from other nitrogenous compounds. A small fraction of ammoniacal N is utilized by anaerobes for their metabolic needs and their cell multiplication (Rajagopal et al. 2013). The ratio of NH_4^+ to NH_3 (free ammonia) is

Table 1 Summary of nutrient content in digestates

Parameter	Digestates	Liquid fraction
pH	7.3–9.0	7.9
Total solids (%)	1.5–13.2	4.5–6.6
COD	210–6900	
Total inorganic carbon (mg L^{-1})	940–1350	
Volatile solids (% DM)	63.8–75.0	–
Total N (g Kg^{-1} FM)	1.20–9.10	4.0–5.1
Total NH_4^+ (g Kg^{-1} FM)	1.5–6.8	1.8–3.0
NH_4^+ share on total N (%)	44–81	40–80
Total C content (% DM)	36.0–45.0	48
C/N ratio	3.0–8.5	3.7–4.8
Total P (g Kg^{-1} FM)	0.4–2.6	0.7–1.0
Water-soluble P (% of total P)	25–45	–
Total K (% DM)	1.9–4.3	3.5–5.2
Total K (g Kg^{-1} FM)	1.2–11.5	–
Total Mg (g Kg^{-1} FM)	0.3–0.7	7.9
Total Ca (g Kg^{-1} FM)	1.0–2.3	–
Total S (g Kg^{-1} FM)	0.2–0.4	–

Adapted by Möller and Müller (2012).

mainly dictated by the pH and temperature. NH_3 can be calculated from the total ammoniacal N by the following equation (Hansen et al. 1998):

$$NH_3 = \frac{\text{Total ammoniacal } N}{1 + \frac{10^{\text{pH}}}{10^{-\left(0.09018 + \frac{2729.92}{T}\right)}}}$$

where pH is the actual pH value of the solution and T is the temperature (in K). For the typical pH range of digestates, ammoniacal N is mainly (>95%) in the form of ammonium (NH_4^+).

The ionic status of ammonium allows it to interact with other ions in the digestate to form various complexes, with the most significant one to be the struvite ($NH_4MgPO_4 \cdot 6H_2O$) which precipitates in the solid state. Struvite formation occurs in two stages, (i) nucleation and (ii) crystal growth, and arises when the concentrations of Mg^{2+}, NH_4^+, and PO_4^{3-} surpass the struvite solubility product, which is also a function of pH. As pH increases, struvite solubility decreases and its formation is favored (Marti et al. 2008; Pastor et al. 2010).

Phosphorus

Total inorganic and organic P content in the digestates can range between 0.4 and 2.6 g kg^{-1} FM of which around 25–45% is in water-soluble forms (Table 1). After mineralization P is contained mainly in orthophosphates with HPO_4^{2-} and PO_4^{3-} (pK_{a2} ≈ 7.21) dominating speciation at the pH range typical for digestates. At higher pH the chemical equilibrium shifts toward favoring the formation of phosphate (PO_4^{3-}), which tends to complex with cations (Ca^{2+}, Mg^{2+}, etc.) and subsequently precipitate as phosphate salts, such as $Ca_3(PO_4)_2$ or $MgHPO_4$. As mentioned before, at adequate concentration of NH_4^+ and Mg^{2+} struvite is formed; this is a very interesting form of fertilizer P. Marti et al. (2008) have shown that most P, 58% of the fixed P, precipitated as struvite, 15% as calcium phosphates in the form of hydroxyapatite ($Ca_5(PO_4)_3(OH)$), and the other 27% was adsorbed on surfaces of the solids.

Digestates from the agro-industrial sector that contain animal manures are rich in P because typically the feeds of monogastric (nonruminants) animals such as swine and poultry are excessively supplemented with inorganic phosphate salts to provide the required P for their growth. This is because monogastric animals and poultry lack the enzyme phytase and cannot metabolize phytic acid, the principal P form in plant matter that comprises animal and poultry feed, typically a mixture of various crops such as corn, wheat, oat, and soya. Moreover, monogastric animals and poultry have a low uptake efficiency for P from phosphate forms, which results that almost 70% of the P contained in the feed is excreted unmetabolized in manures (Gupta et al. 2015; Jorquera et al. 2008). Consequently, cattle manure contains in general lower P amounts compared to swine and poultry (Kleinman et al. 2005).

Other Nutrients

Agro-industrial digestates, especially derived by the AD of animal manures, besides N and P, are also rich in other nutrients including K, S, Mg, Cu, Zn, and Fe. Some of these nutrients originate in the feed, while other nutrients (especially trace metals, such as Cu, Fe, Zn) originate mostly from the various feed supplements used to formulate feed rations. These elements are usually added in excess, as highly soluble metal salts, and therefore excess amounts are excreted in manures (Zhang et al. 2012). Regarding energy crops or crop residues, they contain very low amounts of trace metals, and therefore, when digestates as sole substrate (without manures), they have to be supplemented in the digesters (Brulé et al. 2013). In any case, regardless of the substrate type, it is very probable that digestate will contain all essential nutrients to support microalgal growth. However, due to the slight alkaline pH of the digestates, most of the cation nutrients might form several complexes and precipitate as carbonates and phosphates or attach to solid surface (Möller and Müller 2012). In this case their bioavailability for microalgal growth could be limited and might be necessary to be externally added to the cultivation medium.

2.2 Inhibitory Compounds and Contaminants Present in Digestates

Digestates, due to their complex nature, besides the valuable and reusable nutrients, contain also various organic, inorganic, or biological compounds that could potentially inhibit microalgal growth or affect the overall quality and safety of microalgal biomass. Besides the suspended solids and the colored dissolved compounds of the digestates that limits light penetration reducing growth rates, the most significant potential inhibitory compounds in digestates that might affect microalgal growth include ammonia, various organic acids, and heavy metals. On the other hand, the contamination of biomass with pathogens or chemical contaminants originated in digestates could restrict biomass utility for the production of various commodities.

Ammonia

One of the most significant inhibitory compound of digestates is ammoniacal N. In particular the free ammonia (NH_3) form is highly toxic to microalgae. While ammonium is actively taken up by cells and therefore its intracellular concentration is metabolically regulated, NH_3 diffuses passively and uncontrolled into the cells and at elevated concentrations act toxic. Ammonia acts by damaging the photosynthetic machinery and other cellular components, resulting in reduced photosynthesis activity and lower growth rates or even in cell death (Markou et al. 2016; Azov and Goldman 1982; Drath et al. 2008). Free ammonia has inhibitory effects on microalgae in relatively low concentrations (>25 mg-N/L) (Abeliovich and Azov 1976;

Azov and Goldman 1982; Markou and Muylaert 2016); however, ammonia toxicity is microalgal species dependent, i.e., some species, such as the cyanobacterium *Arthrospira platensis*, display higher ammonia tolerance than other species (Markou et al. 2016; Markou and Muylaert 2016).

As mentioned before, in the NH_4^+/NH_3 ionization system, the formation of NH_3 is favored by alkaline pH ($pK_a \approx 9.25$). Microalgae are therefore more susceptible to ammonia toxicity when pH of the cultivation medium is high. This should be taken into consideration especially for microalgal cultures without a pH control, where pH may reach values higher than 10 due to the liberation of hydroxides (OH^-) during CO_2 uptake. At such pH values, ammoniacal N will be mostly found as free ammonia (>50% of the total ammoniacal N at pH > 9.25) increasing the digestate toxicity potential.

Organic Acids

Even as some microalgal species have the ability to utilize, mixotrophically or heterotrophically, organic acids such as fermentative acetate or butyrate as C and/or energy source (Turon et al. 2015), these could be inhibitory when their concentration is high. For example, butyrate exhibited inhibitory effects on *Chlorella sorokiniana* and *Auxenochlorella protothecoides* at concentrations higher than 0.1 gC L^{-1} and 0.25 gC L^{-1}, respectively (Turon et al. 2015), or acetate on *Chlamydomonas reinhardtii* at concentrations higher than 0.4 g L^{-1} (Chen and Johns 1996b). However, significant higher acetate concentrations (> 3 g L^{-1}) have been used for the cultivation of some microalgae without showing any inhibition, reflecting that the inhibition of organic acids is microalgal species dependent (Perez-Garcia et al. 2011; Chen and Johns 1996a), while cultivation mode (fed-batch, perfusion, chemostat) could improve the overall cultivation efficiency (Chen and Johns 1995, 1996a).

Heavy Metals

Heavy metals are generally defined as the metals or metalloids with a specific density >5 g mL^{-1}, including Cu, Co, Cr, Cd, Fe, Zn, Pb, Hg, Mn, Ni, As, Mo, and V. Some of them, such as Cu, Co, Fe, Mn, and Mo, are essential for microalgal growth and are supplemented in various synthetic cultivation media as trace metals. These elements are contained in various enzymes or biomolecules and play a significant biological role. Depletion of these essential elements could have a negative effect on microalgal growth.

Digestates frequently contain heavy metals. These can originate in agro-industrial wastes and wastewaters that are contaminated by fertilizers, plant protection chemicals, or processing agents that contain heavy metals. Most manures contain relative high amounts of heavy metals especially Zn, Cu, and As as these are supplemented in animal feed as growth promoters or for the treatment of various diseases. They are usually added as soluble metal salts, commonly in excess amounts of the physi-

Table 2 Example of heavy metal content of digestates

Heavy metal	mg L^{-1}
Cr	<1.2
Co	0.02–0.04
Cu	0.09–21.4
Fe	0.9–65
Pb	0.03–2.8
Mn	0.1–17
Mo	<1.8
Ni	<1.4
Zn	0.9–13

Adapted from Xia and Murphy (2016)

ological requirements and therefore are excreted along with feces and urine (Zhang et al. 2012). Heavy metal species and concentrations in digestates are generally depended on the feedstock and in particular the ratio of the different wastes/wastewater used (Table 2). Toxicity of heavy on photosynthetic organisms, including microalgae, has been widely studied, and it is well known that at high concentrations, they damage the photosynthetic machinery affecting negatively cell growth. However, the level of toxicity depends on the heavy metal species, its concentration, and cultivation parameters, such as light intensity, pH, and vary with microalgal species (Švec et al. 2016; Napan et al. 2015; Torres et al. 2017). However, since digestates usually require dilution before, it is used for microalgal cultivation, their toxicity potential is rather low. No inhibition of microalgae cultivated in digestates is reported to be attributed to heavy metal toxicity. Contamination of biomass with heavy metals would though limit the potential of biomass to be used to produce several commodities (e.g., food, feed).

Biological Contamination

Digestates contain a plethora of microbes, the majority of which are strict anaerobes, but also include some facultative anaerobic acidogenic bacteria (Gerardi 2003). During microalgal cultivation facultative anaerobes as well microbes that externally contaminate the cultures could grow in a synergistic or competitive relationship with microalgae. The synergistic relationships are based on the fact that microbes degrade organic matter into CO_2 which is then taken up by microalgae, while microalgae produce oxygen that is used by the microbes. Antagonism between microalgal and bacteria may occur when they compete for nutrients (Munoz and Guieysse 2006). On the other hand, some bacteria present in digestates produce phytohormones that are growth-promoting agents (Qi et al. 2017). Phytohormones play a regulatory role in microalgae cell division and elongation and in chlorophyll and protein metabolism and enhance tolerance to several stresses such as heavy metal toxicity, osmotic, and salt stresses (Pei et al. 2017; Salama et al. 2014). The addition of phytohormones to cultures promotes growth and increases biomass density (Pei et al. 2017). Presence of pathogens creates a significant disadvantage for the use of digestates for the cultivation of microalgae. Contamination of microalgal biomass with pathogens would definitely limit biomass utility in various applications (food, feed, high-value products). The term pathogens include all those agents, such as bacteria (e.g., *Campylobacter* spp., *Clostridium* sp., *Escherichia coli*, *Listeria monocytogene*s, *Salmonella* sp., or *Yersinia enterocolitica*), fungi (e.g., *Aspergillus* sp., *Penicillium* sp., *Rhizomucor*), protozoa, worms, viruses (e.g. enteroviruses, rotaviruses, adenoviruses, hepatitis E viruses, caliciviruses, reoviruses, parvoviruses), and prions that can cause diseases (Bicudo and Goyal 2003; Ray et al. 2013). The main source of pathogens in the digestates originates from animal and poultry manure used in the mixtures. During AD several pathogens are fully inactivated; however some are resistant and can survive. The major AD parameters that play a role in the inactivation of pathogens are time and temperature of treatment, the latter being the most significant one. Thermophilic (>50 °C) AD is generally more effective than mesophilic (>30–38 °C) for pathogen reduction; however some pathogens such as some spore-forming *Clostridium* or *Bacillus* can survive thermophilic AD (Sahlström 2003; Bagge et al. 2010). In several countries, a pasteurization stage (70 °C for 1 h) either before or after the AD digestion is integrated in the process to warrant the hygiene of the digestates; however spore-forming pathogens are not always fully inactivated (Schnürer and Schnürer 2006; Sahlström 2003; Bagge et al. 2010).

Chemical Contaminants

Agro-industrial wastes/wastewater and hence their digestates can contain several other xenobiotic compounds that could pose a risk of contamination of microalgal biomass. The most important xenobiotics include pharmaceuticals (e.g., steroidal hormones, antibiotics, and parasiticides), mycotoxins, and dioxins (Ray et al. 2013; Van Boeckel et al. 2017) (Khan et al. 2008; Bártíková et al. 2016). Even as most of these xenobiotics are degraded during AD, there are some categories, such as steroidal hormones, or some antibiotics that are not extensively degraded. The efficiency of AD for degradation of xenobiotics varies widely and is a function of the physicochemical characteristics of the compound in question and some AD parameters, such as retention time and temperature (Stasinakis 2012).

3 Cultivation of Microalgae Applying Digestates

3.1 Removal of Nutrients from Digestates

Microalgae require a range of nutrients to synthesize the biomolecules that consist their biomass; C, N, P, K, Mg, S, Cl, Fe, Ca, Mn, Co, Cu, B, and Zn are essential for an unhindered cell growth. Lack of one or more of these essential nutrients will cause a cessation of cell growth resulting in biomass production reduction.

Moreover, lack of nutrients will lead to alteration of the biochemical composition of biomass, typically triggering the accumulation of carbonaceous compounds (lipids or carbohydrates) and downregulation of protein production (Pancha et al. 2014; Kamalanathan et al. 2015). However, this biochemical composition alteration due to nutrient starvation/limitation in favor of carbonaceous compounds has been suggested as a strategy for the accumulation of lipids or carbohydrates as feedstock for various applications (see Sect. 4). Due to the fact that microalgae can remove nutrients from their surroundings, they have been proposed as a biological mean for the treatment of waste/wastewater for the removal of inorganic pollutants, such as N and P (Olguín 2012) which are among the main targets in the conventional wastewater treatment plants.

During microalgal cultivation on digestates, nitrogen is removed through three main mechanisms, i.e., biomass uptake, volatilization, and denitrification. Biomass uptake refers to the active or passive transport of the nutrients into the interior of the cells. N can be taken up being in various forms, such as ammoniacal, nitrite, nitrate, or organic form (N_2 can be utilized only by a limited number of N-fixing cyanobacterial species). In general, ammoniacal form is the most preferable N form because it is already in a reduced status and can be utilized immediately by the metabolic pathway for protein synthesis. In contrast, nitrogen oxides have first to be reduced intracellularly, therefore consuming energy (Perez-Garcia et al. 2011). If different N forms are present in the cultivation medium, ammoniacal N is preferentially taken up, and only after it is exhausted other N forms are taken up (Fernandez and Galvan 2007; Vílchez and Vega 1994). Besides inorganic N uptake, microalgae can also grow on organic N, such as urea or amino acids (glycine, glutamate, glutamine). Urea is taken up indirectly, because it is first hydrolyzed to ammonia and carbonic acid which can then be taken up by cells. However, amino acid uptake is species dependent, and growth rates can vary between microalgal species (Neilson and Larsson 1980; Flores and Herrero 2005). Removal of N through volatilization occurs only when N is in the free ammonia form. As was mentioned before (Sect. 2.2), free ammonia dominates as pH increases, and therefore the potential of ammonia removal through volatilization increases at high pH. Depending on the cultivation conditions, ammonia volatilization losses can be significant (González-Fernández et al. 2011; Markou et al. 2014a). The pH is the most important factor for the volatilization of ammonia, whereas other various physicochemical characteristics (e.g., solid content, electrical conductivity) of the digestates do not have any significant influence on the volatilization potential (Markou et al. 2017). Removal of N through denitrification refers to the transformation of ammoniacal N into nitrite/nitrate and finally into molecular nitrogen (N_2). Denitrification is a process that could take place when dissolved oxygen is not easily available and nitrifying microbes are present in an aqueous body, such as is case for microalgal-bacterial cultivation systems. Denitrification could account for 20–25% of the N removal (González-Fernández et al. 2011).

P is removed mainly through two mechanisms: biomass uptake and precipitation. P is taken up by cells mainly in the phosphate form (PO_4^{3-}) by metabolically driven processes. However, microalgae can take up P from other inorganic or organic forms as well (Huang and Hong 1999; Whitton 1991). Organic P can be taken up after mineralization of the organic matter to produce as PO_4^{3-}, or hydrolysis of other inorganic forms than PO_4^{3-}, by extracellular phosphatase enzymes, the main mechanism of organic P uptake (Hua-sheng et al. 1995; Dyhrman and Ruttenberg 2006). The ability of microalgae to take up organic P depends on the chemical composition of the organic molecules (Dyhrman and Ruttenberg 2006). Precipitation of P complexes with polyvalent cations (Ca, Mg, etc.) might occur, especially in cultures where pH is increased due to photosynthesis. The presence of organic substances, such as humic acids, could favor the formation of phosphate-metal-humic complexes that are also of low bioavailability for microalgal uptake (Hartley et al. 1997; Hoffmann 1998; Li and Brett 2013). P removal from the solution is frequently reported to reach very high rates (>95%), attributed either to biomass uptake or precipitation (Yang et al. 2017; Franchino et al. 2013).

Although inorganic carbon (IC) is not a target for conventional wastewater treatment, microalgae require adequate amounts of CO_2. Most microalgae can take up IC only from CO_2 and/or HCO_3^-, while CO_3^{2-} is extreme rarely taken up (Camiro-Vargas et al. 2005). Bicarbonate concentration in digestates, especially when they are used in a diluted form, seems not to be adequate for a satisfactory biomass production, and therefore it has to be externally provided to the cultures (Bjornsson et al. 2013; Park et al. 2010). As a source of CO_2, either biogas (that contains about 35–45% CO_2) or flue gases obtained after biogas combustion could be used (Kao et al. 2012; Salafudin et al. 2015).

For an unhindered cell growth and biomass production, digestates should contain the following nutrients in adequate amounts to cover metabolic needs: C, N, P, K, Mg, S, Cl, Fe, Ca, Mn, Co, Cu, B, and Zn. All these nutrients will be taken up during microalgal growth, and the degree of their removal from the medium depends on the microalgal species and the cultivation conditions (Markou 2015). As was mentioned before, digestates are a multipart medium, and the various ions interact with each other forming complexes that lead to some nutrients (e.g., P, Mg) to become unavailable to microalgae (Möller and Müller 2012). The unavailability of nutrients or an unbalanced C/N/P nutrient ratio could result in nutrient limitation with a negative effect on cell growth and biomass productivity (Beuckels et al. 2015). However, the level of nutrients availability will have an effect on the differential accumulation of target compounds like carbohydrates, lipids, and proteins. As nutrient availability decreases the accumulation of carbohydrates or lipids is triggered, while protein productivity increases as long bioavailability of nutrients increases (Dickinson et al. 2015).

3.2 *Removal of Organic Matter*

Organic matter removal during microalgae cultivation may occur through two main mechanisms: biomass uptake and degradation. As was mentioned before, microalgae can utilize some organic molecules as source of C and/or energy. Under mixotrophic conditions, i.e., in the presence of light, microalgae can take up organic molecules and utilize them as C and/or energy source, while under heterotrophic conditions where no light energy is available, microalgae take up organic molecules as C and energy source (Chojnacka and Marquez-Rocha 2004). Organic molecules that can be directly taken up by microalgae include several sugars (e.g., glucose, fructose), amino acids, organic acids (e.g., acetate, butyrate), and glycerol (Perez-Garcia et al. 2011). Removal of organic matter through degradation occurs either through their degradation by microalgae themselves or by various bacteria that are typically present in microalgal cultures. In both cases the degradation is carried out by various lytic enzymes that break organic matter down either into simpler molecules or into CO_2 that is then taken up by microalgae. Both mechanisms contribute in a significant reduction of organic matter (measured as chemical oxygen demand – COD or biological chemical demand – BOD), reaching values as high as >90%.

However, organic matter cannot be removed completely because during microalgal growth, cells excrete extracellular organic matter (EOM) comprising of various compounds such as proteins, nucleic acids, lipids, and polysaccharides, with the latter a main portion of EOM (Myklestad 1995). However, depending on the cultivation mode (mixotrophic or heterotrophic), the EOM can be dominated by other compounds such as proteins (Wang et al. 2015). Among the main digestate constituents, it was reported that in cultures of *Arthrospira platensis* and *Chlorella vulgaris*, volatile fatty acids were removed at rates >90%, while proteins were removed only in cultures of *C. vulgaris* and not of *A. platensis*, whereas carbohydrates were accumulated in the medium of both cultures (Markou 2015). Hence, the total removal of organic matter from the cultivation medium supplemented with digestates is the difference between the organic matter removed and organic matter excreted by microalgae.

3.3 Cultivation Operational Parameters

Light penetration, mixing, and hydraulic retention time (HRT) are significant cultivation parameters that influence microalgal growth, especially when using digestates as the nutrient source, which are rich in suspended solids and dissolved colored compounds. The suspended solids contribute to turbidity, which along with the dissolved colored compounds absorb the incident light and reduce its penetration into the cultures, resulting in general in lower photosynthesis and biomass production (Wang et al. 2012; Depraetere et al. 2013; Curtis et al. 1994). Therefore, adequate mixing is important because it generates turbulence moving cells from dark zones to the light zones of the culture subjecting them to more light to conduct photosynthesis. The fluctuation in light intensity caused by mixing should be short enough (10 ms) for best light harvest (Eriksen 2008); however, strong mixing could cause shear stress reducing biomass production (Eriksen 2008; Marshall and Huang 2010). Turbulence caused by mixing is important because it also avoids cell sinking and the formation of nutrient or thermal gradients and increases the mass transfer between the liquid medium and the atmospheric CO_2 and removes excess dissolved oxygen (Grobbelaar 2000).

HRT is a significant parameter because, for a given digestate type, it defines the load of the influents, the degree of nutrient removal, and the biomass concentration in the effluents. HRT depends on various parameters, such as light intensity, temperature, nutrient availability, microalgal strain(s), photobioreactor configuration, etc. (Whitton et al. 2015; Munoz and Guieysse 2006) and should be as high as it is needed for best nutrient removal and higher biomass concentrations and productivity. Depending on the cultivation conditions, for sufficient removal of inorganic and organic loads, HRT needs to be 2–10 days (Whitton et al. 2015; Munoz and Guieysse 2006). HRT length should be set to allow cells to reach their logarithmic growth phase to favor biomass productivity and wastewater treatment efficiency (Kim et al. 2014a; Medina and Neis 2007).

3.4 Pretreatment of Digestates to Facilitate Microalgal Growth

A pretreatment of digestates might be required before using them as a nutrient source for the cultivation of microalgae. The pretreatment targets the removal of growth-limiting or growth-inhibitory compounds, such as suspended solids, dissolved colored compounds, and ammonia, in order to render digestates more appropriate for microalgal growth. The simplest pretreatment is dilution by which the concentration of limiting/inhibitory compounds will be decreased at appropriate levels. Depending on the physicochemical characteristics of the digestates, they need a significant dilution, probably more than five times dilution (Xia and Murphy 2016). To address the often limited availability of operational water for the dilution, brackish water, seawater, or low-strength wastewater may be used, including the recycling of the medium after cell harvest (Farooq et al. 2015; Delrue et al. 2015; González-López et al. 2013; Deng et al. 2018).

A common pretreatment method of digestates is solid/liquid separation which generates a solid fraction rich in fibers and compounds attached to them and a liquid fraction rich in soluble compounds (Möller and Müller 2012; Hjorth et al. 2010) (Drosg et al. 2015). Solid/liquid separation, especially of digestates with high solid content, followed by filtration should be considered as a necessary step, as any suspended solid would decrease light penetration or may lead to cells clumping (Uggetti et al. 2014; Xia and Murphy 2016). Solid/liquid pretreatment will have a positive effect on HMs by reducing their concentration, since a great fraction of HMs will be attached onto the solids and removed from the liquid fraction. However, a fraction of P will as well be removed with the solid faction (Table 1)(Möller and Müller 2012), decreasing the available P for microalgal growth. Solid-liquid separation efficiency can be improved by the addition of precipitating agents; however, it should be taken into consideration that various essential nutrients could be removed as well. Laboratory studies have investigated other pretreatment methods, such as ammonia removal through stripping and decolorization by activated carbon (Marazzi et al. 2017), or decolorization using precipitation agents (Depraetere et al. 2013) or by nutrient recovery through adsorption onto geo-minerals (Markou et al. 2015; Markou et al. 2014b) prior microalgal cultivation, showing that generally the pretreatment improves microalgal growth and biomass production. Other pretreatment methods, such as coagulation and flocculation, flotation, and electrochemical treatment (Fu and Wang 2011; Gupta et al. 2012; Marazzi et al. 2017; Kim et al. 2014b; Depraetere et al. 2013), might also be employed; however, there is lack of studies for evaluating their impact on microalgal growth.

4 Potentials for Microalgal Biomass Uses

4.1 Biofuels

Microalgal biomass has been recently considered as a highly potential feedstock for the production of biofuels, such as biodiesel, bioalcohols, biogas, or bio-oil. Microalgal biomass could be used by any biomass-to-energy conversion technology available. Microalgae typically contain about 20–30% lipids; therefore they are of strong interest for biodiesel production (Lam and Lee 2012; Chisti 2007). For the production of biodiesel, lipids are transesterified, i.e., the triglycerides react with short-chain alcohol (i.e., methanol or ethanol) in the presence of catalyst converting them to fatty acid esters. However, one of the major challenges for an economically feasible and sustainable microalgal biodiesel production is to avoid drying of biomass, which after harvesting contains high moisture contents (90–95%) before biofuel production. Presence of water will inhibit several downstream processes, such as lipid extraction and transesterification (Lam and Lee 2012; Chisti 2007; Taher et al. 2014; Macías-Sánchez et al. 2015).

To avoid biomass drying, which is an energy consuming process, for the production of microalgal biofuels other biomass-to-energy conversion technologies that utilize wet biomass could be used; the ones with the greatest potential are AD, alcoholic fermentation, and hydrothermal liquefaction. The most challenging issue with AD is the recalcitrant nature of cell wall that hinders digestion. Most microalgal cell wall is composed of organic compounds with slow biodegradability, and therefore a disruption step is required to facilitate the release of the intracellular biomass compounds in order to be available for digestion (Gonzalez-Fernandez et al. 2015). Bioalcohol production is also a promising route for the production of microalgal biofuels (de Farias Silva and Bertucco 2016). Bioalcohol fermentation is commonly performed in two steps, hydrolysis and fermentation, which steps can be carried out separately or simultaneously. The main drawback of bioalcohol production is the complex, multistep, energy consuming and costly processes required (de Farias Silva and Bertucco 2016).

During hydrothermal liquefaction (HTL), i.e., under relative high temperature (200–350 °C) and relative high pressures (15–20 MPa), biomass is converted to a crude oily liquid (Guo et al. 2015). Compared to other technologies, such as biodiesel and bioalcohols, HTL is considered to be more efficient because lipids, proteins, and carbohydrates are converted into bio-oil with a final high energy density (Tian et al. 2014; Guo et al. 2015). However, since microalgae are relative rich in proteins, the content of N in the bio-oil is high, which lowers its quality and restricts its use (Biller et al. 2012).

4.2 Animal Feed

Because microalgae are rich in proteins, carbohydrates, lipids, and micronutrients, recent research on the use of microalgae for animal feed supplements received widespread attention (Chew et al. 2017). Adding microalgae biomass to animal feed provides vitamins, essential amino acids, polysaccharides, and n-3, n-6 polyunsaturated fatty acids, as well as minerals and pigments, such as carotenoids and chlorophyll (Priyadarshani and Rath 2012). Feeding livestock with microalgae at a replacement rate of 5–10% of the conventional feed can improve body weight gain, feed intake and feed conversion ratio, immune response, weight control, antioxidant status, fertility, and external appearance, such as healthy skin and fur (Certik and Shimizu 1999; Holman et al. 2012; Tsiplakou et al. 2017; Kulpys et al. 2009; Milledge 2011). Microalgae are able to synthesize all amino acids and become a source of essential amino acids. Allegedly, the average mass of most microalgal proteins is higher than traditional plant proteins and is similar to that of yeast, soy flour, and milk, with a well-balanced amino acids profile (Becker 2007). Adding microalgae to dietary supplements can improve the nutritional quality of meat, increase the ratio of PUFA/SFA (polyunsaturated fatty acids/total saturated fatty acids), and increase the DHA (docosahexaenoic acid) and total amount of n-3 fatty acids (Díaz et al. 2017). Microalgal carbohydrates are also an important nutrient component. Studies have found that *Arthrospira* carbohydrate is beneficial to animal internal organs (Kovač et al. 2013). Microalgae are rich sources of almost all important minerals and are therefore suitable to be used as animal feedstuffs for mineral supplementation (Christaki et al. 2011). The presence of copper, iodine, iron, potassium, zinc, and other elements in microalgae is abundant. At the same time, vitamins such as A, B1, B2, B6, B12, C, and E and nicotinic acid, anonicotin, biotin, and folic acid are also present in microalgae (Christaki et al. 2011; Priyadarshani and Rath 2012). However, long-term high-concentration microalgae in feed can lead to a decrease in the palatability of the feed and thus reduce the feed intake (Spolaore et al. 2006; Lamminen et al. 2017).

4.3 High-Value Products

A very interesting property of microalgae is their potential to produce a wide range of high-value products for various applications, such as pharmaceuticals, nutraceuticals, cosmeceuticals, and food additives. Microalgae could be an excellent source of carotenoids, such as astaxanthin (Aki et al. 2003), lutein (Fernández-Sevilla et al. 2010), phycocyanin, and phycoerythrin (Khajepour et al. 2015), or polyphenols, a source of mycosporine-like amino acids, or various secondary metabolites that have antioxidant, antibacterial, antitumor, or antidiabetic activities, improve immune system, etc. (Chew et al. 2017). Several of these high-value microalgal products are already commercialized, while there is a clear trend and high opportunities for new products to be placed in the market (Borowitzka 2013). However, these high-value products are very expensive to be produced, mainly due to their very low concentration in the microalgal biomass or due to the complex and costly extraction processes used. Therefore, the use of digestates for the production of high-value products is unlikely to lead to any significant improvement in the economics of the production of such commodities. However, the use of digestates could be an approach to support the sustainability of such production systems and especially when digestates could be derived by the leftover of microalgal biomass after the extraction of the target compounds. Such a scheme will be based on a closed-loop production system, where the nutrients contained in the biomass will be mineralized during AD and recycled back to the cultures for further biomass production (Prajapati et al. 2014).

4.4 Contamination Potentials

A great concern about using digestates for the production of microalgal feed or high-value products is the content of various hazardous pollutants, such as HMs, pathogens, and xenobiotics, which have the potential to contaminate the produced biomass, lowering its quality and rising safety issues upon its use and consumption. Contamination of microalgal biomass with hazardous pollutants can occur by cell surface sorption and/or intracellular accumulation. Surface sorption denotes the adhesion of the compounds in question onto the cell surface, while intracellular accumulation denotes the passive diffusion or active transportation of the compounds across cellular membranes into the interior of cells (Perales-Vela et al. 2006; Suresh Kumar et al. 2015; Basile et al. 2012). Microalgae display a very high sorption capacity for HMs (up to 100 mg g^{-1}) and therefore have been considered as means for HMs removal from wastewater (Anastopoulos and Kyzas 2015; Suresh Kumar et al. 2015). However, this capability to take up high amounts of HMs could restrict the application potential of the production of microalgal commodities for human and animal consumption, since some HMs, such as Ni, As, Hg, and Cd, which are frequently contained in digestates, are highly toxic and their content limits on feed/food and products is strictly regulated worldwide.

It has been frequently reported that microalgae generate appropriate conditions, e.g., high pH and high concentration of dissolved oxygen, that result in a significant reduction (3–4 log) of the population of some indicator bacteria (Posadas et al. 2015; Heubeck et al. 2007; Schumacher et al. 2003; Al-Gheethi et al. 2017). However, the mechanisms underlying in the pathogen reduction during microalgal growth are still unclear, and there is lack of knowledge whether pathogens can be hosted by microalgal cells resulting in the contamination of the produced biomass. It is very probable that pathogens will be adsorbed in the microalgal cells because microalgal cell walls consist of several compounds, such as polysaccharides and proteins (e.g., adhesins), which have charged functional groups and favor the attachment of pathogens, such as viruses and bacteria (Verbyla and Mihelcic 2015; Marshall 1985).

Microalgal cultivation systems are able to remove xenobiotics by the following mechanisms: adsorption onto cell wall, cell uptake, volatilization, photodegradation and biological degradation, and transformation (Zhang et al. 2014; Wang et al. 2017). The extent of xenobiotics removal depends on the chemical compound and its physicochemical characteristics and the cultivation environmental conditions (light penetration, pH, retention time) (Zhang et al. 2014; Wang et al. 2017; Matamoros et al. 2015). Studies on the contamination of microalgal biomass with xenobiotics are scarce, but the available data show that there is a potential of biomass contamination with xenobiotics; for example *Desmodesmus subspicatus* was found to adsorb around 30% of the estrogens contained in the cultivation medium (Maes et al. 2014), while *Phaeodactylum tricornutum* displayed a sorption capacity for oxytetracycline of about 29 mg g^{-1} (Santaeufemia et al. 2016).

Given that contaminants have a variety of physicochemical characteristics (i.e., hydrophilic or hydrophobic properties, etc.), there is a high potential that using one of the available extraction methods (Cuellar-Bermudez et al. 2015; Günerken et al. 2015; Gerardo et al. 2014), they could be extracted as well along with the target compound. However, there is lack of related studies, and more research is needed to identify and develop methods for avoiding transferring contaminants along with the extracted target compounds.

5 Conclusions

Digestates contain all necessary macro- and micronutrients and can be utilized as cultivation medium (or supplement) for microalgal biomass production. However, some of the main physicochemical characteristics of the digestates, such as high content of inhibitory compounds, turbidity, and colored dissolved compounds might negatively influence microalgal growth, and therefore they need to be adjusted by using one or a combination of pretreating methods (e.g., dilution, solid/liquid separation, filtration, etc.) in order to render them appropriate for cell growth. So far, the majority of the published work regards laboratory-scale investigations, in which however it is demonstrated that microalgae can be successfully cultivated on media consisted from digestates. Low-value microalgal products, such as biofuels, seem not to be economical feasible yet, and therefore the best route of valorizing digestates with microalgae is the production of high-value products (feed, food supplemented, pigments, etc.). However, there are some concerns about the potential contamination of microalgal biomass with unwanted hazardous pollutants, such as heavy metals and pathogens originating in digestates. More research is needed toward this route in order to optimize the production of a safe and valuable microalgal biomass.

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Industrial Wastewater-Based Microalgal Biorefinery: A Dual Strategy to Remediate Waste and Produce Microalgal Bioproducts

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1 Introduction

Fossil-based fuels play a significant role in the modern energy market. These fuels are nonrenewable and getting diminished gradually. Their continuous utilization has resulted in many environmental problems like increasing emissions of greenhouse gases, global warming, climate change, etc. (Chisti 2007, 2008). Therefore, governments, industries, and R&D institutes have gained their interest toward developing an alternative energy source that is renewable, economically competitive, and environmental friendly (Mussgnug et al. 2010).

Various types of biomass can be utilized as a source of biofuel, which can be of a different variety. Biomass can be converted into bioethanol, bio-butanol, biohydrogen, biogas, or biodiesel through biological or thermo-chemical methods. The two most common biofuels, which are the likely candidates to replace diesel and petrol in the near future, are biodiesel and bioethanol, respectively (John et al. 2011). The choice of biomass feedstock for biofuel conversion depends upon many factors like availability, cost of raw materials, etc. there are resources like sugar/food crops and lignocellulosic biomass that can be used as feedstocks for biofuel generation and are known as first- and second-generation biofuels, respectively. The

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problem in utilizing these substrates as a biofuel feedstock is the use of arable land and freshwater for their cultivation (thus giving rise to the critical food versus fuel debate), seasonal and geographical variations in productivity, as well as their uses as medicinal sources (Sun and Cheng 2002). A similar situation can be envisaged for biodiesel produced from animal fats and vegetable oils through transesterification by chemical or enzymatic catalysis (Leung et al. 2010). Therefore, there is an urgent need for the development of renewable and sustainable fuels.

1.1 Microalgae as a Feedstock for Biofuels and Valuable Bioproducts

Microalgae are a diverse group of organisms that include both prokaryotic and eukaryotic, O_2-evolving photosynthetic organisms, excluding higher plants. Microalgae can be subdivided into green algae (Chlorophyceae), red algae (Rhodophyceae), diatoms (Bacillariophyceae), brown algae (Phaeophyceae), and cyanobacteria (Cyanophyceae) (Brodie and Lewis 2007). Microalgae have gained importance as a biomass feedstock for biofuel production (Schenk et al. 2008). Microalgal biomass has several advantages over terrestrial energy crops such as high solar energy utilization efficiency and high growth rates. Additionally, microalgae can also be used for agro-industrial and domestic wastewater treatments. Organic matter in the wastewater can be converted into algal biomass, which can serve as a source for biofuel and other valuable products with subsequent economic and environmental benefits (Markou and Georgakakis 2011; Rawat et al. 2011). A microalga having the ability to grow mixotrophically (i.e., ability to grow both photoautotrophically as well as photoheterotrophically) is also used to treat wastewater. In mixotrophic microalgal growth, production of both biomass and lipid is higher than the photoautotrophic condition (Pancha et al. 2015).

Nowadays, research is also focused on improving the lipid productivity of microalgae for biodiesel production. Microalgal lipids, mainly TAGs, are important for biodiesel. Microalgal lipid composition can be modified or increased by changing the cultivation conditions, e.g. cultural and environmental factors like media ingredients, light, temperature, etc. (George et al. 2014). Microalgal lipids are a group of compounds, which include neutral lipids, polar lipids, wax esters, sterols, and hydrocarbons as well as prenyl and pyrrole derivatives. Lipids produced by microalgae can be divided into two classes, structural and storage lipids. Microalgae can produce substantial amounts of lipids as a storage lipid under various stress conditions. Lipids in microalgae have various physiological roles like structural support as a membrane and as signaling molecules. Storage lipids are different from both structural and signaling lipids, as they are mainly made up of glycerol esters of fatty acids, more commonly known as TAGs (Yu et al. 2011). TAG biosynthetic pathway is divided into three major steps: (1) formation of acetyl co-a, (2) acyl chain elongation, and (3) triacylglycerols (TAGs) formation (Hu et al. 2008). These TAGs can be easily converted into biodiesel by transesterification. Structural lipids contain a high amount of polyunsaturated fatty acids (PUFAs), which are nutritionally important for aquatic animals and humans.

Utilization of lipid-rich microalgae for biofuel production has following advantages over land-based crop plants:

1. High growth rate and volumetric productivity.
2. Efficient CO_2 utilizing ability thereby reducing GHGs emission.
3. Ability to grow in seawater, brackish water, and wastewater.
4. Ability to grow in land unsuitable for agriculture thus avoiding the competition for arable land for food production.
5. Very short harvest cycle compared with other feedstock (which harvest once or twice in a year).
6. Compatibility of integrated biofuel and coproducts within biorefineries, which makes microalgae clean, renewable, and efficient feedstock for biodiesel production.
7. Microalgae can accumulate 20–50% of the dry weights of their biomass as lipids.
8. Oil-extracted biomass can serve as a good source of feed and fertilizers, which can be fermented to produce bioethanol and biogas (Chen et al. 2013).

To enhance the economic feasibility of algal biodiesel, biomass and lipid content are the key parameters to be improved. Unfortunately, higher accumulation of microalgal lipids occurs in environmental stress conditions (mainly nutrient limitations), which often results in low biomass production. Therefore, the enhancement of oil content without affecting the biomass productivity is crucial for the development of microalgal biofuel.

Microalgal biomass is also a good source of other valuable coproducts like nutritionally important PUFAs and pigments, which can be used in medical and cosmetic industries (Spolaore et al. 2006). Cyanobacteria, a group of microalgae, are considered as a very good source of phycobiliproteins, pigments with high antioxidant, anti-inflammatory, and other nutraceutical properties, which can be explored for the industrial applications (Patel et al. 2005; Sonani et al. 2017). Apart from these, cyanobacteria also contain a high amount of proteins and therefore can be used as food and feed supplements (Gantar and Svirčev 2008). Microalgae also contain a group of pigments known as carotenoids, which are excellent single oxygen scavengers and possess high antioxidant and other nutraceutical properties (Guedes et al. 2011).

2 Wastewater Characteristics and Phycoremediation Potential of Various Microalgae

Various types of wastewater, viz., industrial, agricultural, and municipal, contain different toxic compounds and must be treated before their disposal to the natural water system; otherwise it may cause serious problem to the aquatic life or human health. Different chemical and biological treatments are generally used to remove the toxic substrates from wastewaters (Zeng et al. 2015). Utilization of these treatment methods increases the expenditure cost for the industries or local municipality. Apart from the toxic substrates, the wastewater also contains very high amount of nitrogen, phosphorous, and organic carbon compounds, which can be used for the growth media for various types of microorganisms as carbon, nitrogen, and phosphorous are the basic requirement for the cultivation of any microorganisms.

As discussed earlier, at present, microalgae are considered to be the potential resource for the production of biofuels and various valuable compounds. One of the major challenges in the commercialization of microalgal-based bioproducts is its high production cost, mainly due to its high cultivation cost (Pancha et al. 2014; Pate et al. 2011). One of the alternatives to lower down the production cost associated with microalgal biomass production is the cultivation of microalgae in wastewaters which will reduce the water and nutrient cost for the large-scale microalgal cultivation (Chokshi et al. 2016). Utilization of microalgae for the treatment of wastewater is not new; it has been reported by Oswald et al. in 1957 that microalgae have the ability to utilize the nutrients from wastewater. It has also been reported in USDOE aquatic species program that microalgae-based biofuel coupled with wastewater treatment is a promising way to cut down the production cost of algal biodiesel (Sheehan et al. 1998). Apart from water and inorganic nutrients like nitrate and phosphates, wastewaters are generally rich in various organic compounds that increase the microalgal biomass productivity since it has been reported that mixotrophic growth of microalgae is more supportive for higher biomass as well as lipid and other valuable products compared to the normal photoautotrophic cultivation (Pancha et al. 2015; Wu et al. 2014). It has also been proposed that apart from the biodiesel potential, the spectrum of bioproducts can be extracted from the wastewater-cultivated microalgae (Hu et al. 2011).

The composition of wastewaters differs based on their origin, but it mainly contains inorganic nutrients like nitrate, phosphate, and ammonia, organic compounds like glucose and other amino acids, and various types of heavy metals like Pb, As, etc. (Chiu et al. 2015; Sutherland et al. 2014). Total nitrogen (TN) and total phosphorous (TP) are the major components of any wastewater used for the cultivation of microalgae. Nitrogen is one of the important nutrients and limiting factor for the growth and lipid production in microalgae (Pancha et al. 2014). Microalgae can utilize nitrogen in the form of nitrate, ammonia, or organic nitrogen source. In wastewater, most of the nitrogen is in the form of ammoniacal nitrogen, one of the most preferred nitrogen sources for microalgal absorption; however, at higher concentration, it inhibits the growth of microalgae. Therefore, dilution of wastewater or controlling the desired pH is preferred for the maximum utilization of dissolved nitrogen in wastewater-based cultivation medium (Razzak et al. 2013). The TN content is reported to be in the range of 15–90 mg/L in normal sewage wastewater, while the highest TN of 4165 mg/L is reported in animal wastewater (Burks and Minnis 1994; Barker et al. 2001). Phosphorous is another important nutrient for microalgal cultivation since it is important for the synthesis of ATP and the nucleic acids in microalgae. In wastewater, phosphorous is generally available as $H_2PO_4^-$ and HPO_4^-. Typical TP concentration ranges from 5 mg/L in municipal sewage wastewater to 988 mg/L in animal wastewater (Burks and Minnis 1994; Barker et al. 2001). Apart from inorganic nutrients, wastewaters from agricultural and dairy industries are rich in organic compounds like glucose and acetic acid, which can be good resources for the cultivation of microalgae under mixotrophic and heterotrophic mode (Lowrey et al. 2015).

Phycoremediation is a process for remediating the toxic wastes using microalgae or macroalgae (Chokshi et al. 2016; Rawat et al. 2011). It is a green and sustainable approach to not only remove or treat the toxic wastewater but also produce various valuable compounds which can be used for diverse applications. Phycoremediation includes (1) removal of nutrients from various types of wastewater, (2) biosorbents of toxic compound and nutrients through algae, (3) CO_2 sequestration, (4) transformation or degradation of xenobiotics, and (5) detection of the toxic compound by microalgal-based biosensors (Lavoie and De la Noüe 1985). Table 1 shows micro-

Table 1 Microalgae used for the treatment of different wastewaters

Name of microalgae	Wastewater	Ref
Chlorella pyrenoidosa	Anaerobic-digested starch wastewater	Yang et al. (2015)
Chlorella ellipsoidea YJ1	Domestic secondary effluents	Yang et al. (2011a)
Chlorella zofingiensis	Piggery wastewater	Zhu et al. (2013a)
Chlorella saccharophila	Industrial wastewater	Chinnasamy et al. (2010b)
Chlorella sorokiniana	Raw sewage	Gupta et al. (2016)
Chlorella vulgaris	Saline wastewater	Shen et al. (2015)
Scenedesmus obliquus	Secondarily pretreated wastewater	Álvarez-Díaz et al. (2015)
Scenedesmus quadricauda SDEC-13	Campus sewage	Han et al. (2015)
Scenedesmus obliquus	Brewery effluent	Mata et al. (2012)
Desmodesmus sp.	Anaerobically digested wastewater	Ji et al. (2014)
Micractinium inermum NLP-F014	Domestic wastewater	Park et al. (2015)
Leptolyngbya sp. *ISTCY101*	Municipal wastewater	Singh and Thakur (2015)
Neochloris sp.	Dyeing industry effluent	Gopalakrishnan and Ramamurthy (2014)
Mix microalgae	Domestic sewage	Subhash et al. (2014)
Micractinium reisseri	Municipal wastewater	Abou-Shanab et al. (2014)
Tetraselmis sp.	Digestate effluent	Erkelens et al. (2014)
Galdieria sulphuraria	Urban wastewaters	Selvaratnam et al. (2014)
Chlorococcum sp. *RAP-13*	Dairy wastewater	Ummalyma and Sukumaran (2014)
Botryococcus braunii UTEX 572	Piggery wastewater	An et al. (2003)
Botryococcus braunii	Urban wastewater	Órpez et al. (2009)
Chlamydomonas reinhardtii	Different stage wastewater	Kong et al. (2010)

algae used for the treatment of different wastewaters. Selection of microalgae for the cultivation in wastewater is one of the important criteria for the production of bioproducts linked with microalgal-based cultivation. Generally, microalgae having ability of high growth rate and biomass production, to obtain nutrients from various types of wastewater, to tolerate toxicity by various xenobiotics, to sequester high amount of atmospheric CO_2, to accumulate high amount of desired end products, and to withstand wide variety of physicochemical parameters like high temperature and high light intensity, are preferred for wastewater remediation (Chen et al. 2015; Cai et al. 2013; Zhou et al. 2014).

Researchers have shown that native microalgae isolated from natural environments have more potential to remediate toxic waste as well as produce bioproducts compared to microalgae obtained from the culture collection centers. This is mainly due to their better acclimatization in native environments (Zhou et al. 2012; Bhatnagar et al. 2011). Among various microalgae used for wastewater treatment, microalgae genus from *Chlorella* and *Scenedesmus* is the most dominant species mainly due to their high nutrient removal ability as well as capability to withstand wide variety of environmental parameters (Chiu et al. 2015; Ji et al. 2014; Kim et al. 2016). Apart from these two green algae, various other strains like *Galdieria sulphuraria*, *Micractinium minimum*, *Chlorococcum* sp. RAP-13, etc. have also been reported for their ability to remediate wastewater as well as produce various bioproducts. Cyanobacteria like *Arthrospira* sp., *Synechococcus nidulans*, etc. have the ability to obtain nutrients from wastewater as well as to produce various nutraceutical compounds (Markou et al. 2012; Sydney et al. 2011). Ruiz-Marin et al. (2010) reported that immobilized *Scenedesmus obliquus* and *Chlorella vulgaris* have higher ability to remove nutrients from wastewater compared to the suspended algal culture. This indicated that cultivation strategy is also important for the phycoremediation of wastewater.

Many researchers also reported that consortia of microalgae or algae-bacteria are more promising to remove nutrients from the wastewater compared to the monoculture and also accumulate high amount of various bioproducts during the cultivation (Olguín 2012; de-Bashan et al. 2004). Compared to only microalgal consortia, microalgal-bacterial consortia are more efficient because both the species utilize their ability to realistically remediate the waste. Therefore, both the organisms have higher growth rate as well as they also easily withstand environmental extreme compared to the monocultures (Brenner et al. 2008). One of the most studied microalgal-bacterial interaction is *Azospirillum* sp. – *Chlorella sorokiniana* system. The bacterium is known to produce various growth-promoting substances, which influence the growth and nutrient removal ability of microalgae compared to the monoculture microalgal wastewater remediation. de-Bashan et al. (2004) reported that co-immobilization of algal-bacteria in alginate beads removes almost 100% ammonium compared to only algal cultivation, which removes about 75% of ammonium from the wastewater.

3 Bioapplications Linked with Microalgae-Based Phycoremediation

Microalgal biomass is mainly rich in lipid, carbohydrates, and various types of pigments, which are a potential resource for the production of biofuels and other health products. Dual-purpose system involving microalgae-based wastewater treatment along with the production of biodiesel and other valuable compounds has recently become popular due to its vast advantage compared to only single-product production. In this section, we will briefly discuss the integrated microalgal-based wastewater treatment along with the spectrum of bioproduct development in a biorefinery manner.

3.1 Biodiesel Production

Lipid is one of the major biochemical components of microalgae. It has been reported that microalgae can accumulate almost 60% of the TAG under various stress conditions which is a good source for its conversion to biodiesel (Pancha et al. 2014). Generally, cultivation conditions like nutrient starvation or salinity stress (Pancha et al. 2015, Chokshi et al. 2017a, 2017b), change in cultivation temperature (Chokshi et al. 2015), or light intensity (George et al. 2014) increase the accumulation of lipid in microalgae. Utilization of various types of wastewater as an organic and inorganic nutrient source is a promising approach for the sustainable production of microalgal biomass. A study carried out by Chinnasamy et al. (2010a, 2010b)) shows that wastewater-linked microalgal biodiesel production can reach up to 0.40–0.78 t/ha/year. The lower yield of biodiesel is mainly due to lower lipid content of microalgae which can be further improved by cultivating microalgae in wastewater having a higher amount of organic nutrients. Table 2 shows lipid content of various microalgae grown in various wastewater.

A study carried out by Ledda et al. (2015) shows that microalgae *Nannochloropsis gaditana* grown in centrate from wastewater treatment can produce the biomass of 0.1 g/L in tubular raceway ponds. However, a higher concentration of centrate (beyond 30%) is inhibitory for microalgal growth and reduces their biomass production and chlorophyll content. This suggests that selection as well as dilution or pretreatment of wastewater before the cultivation of microalgae is also important for the higher biomass production. Another study carried out by Gupta et al. (2016) indicated that compared to microalgae *Scenedesmus obliquus*, *Chlorella sorokiniana* have high ability to remove nutrients and produce lipid content of about 22% w/w of biomass when used to treat the raw sewage for treatment. However, the study carried out by Ji et al. (2015) indicated that *Scenedesmus obliquus* have an ability to utilize the nutrients from food wastewater and remove 38.9 mg/L TN and 12.1 mg/L TP from 1% of food wastewater along with higher accumulation of lipid and carbohydrate in the cells, which may further be used for biodiesel and bioetha-

Table 2 Lipid content of various microalgae grown in various wastewaters

Microalgae	Type of wastewater	Lipid content	Ref
Selenastrum minutum	Mix wastewater	37%	Gentili (2014)
Consortia of microalgae	Municipal wastewater	28.5%	Mahapatra et al. (2014)
Scenedesmus sp.	Domestic wastewater	23.1%	Nayak et al. (2016a)
Mix microalgae	Domestic wastewater	26.2%	Soydemir et al. (2016)
Coelastrella sp. QY01	Swine wastewater	24.8%	Luo et al. (2016)
Consortia of microalgae	Dairy farm wastewaters	16.89%	Hena et al. (2015)
Scenedesmus bijuga	Food wastewater effluent	35.06%	Shin et al. (2015)
Chlorella sp. GD	Piggery wastewater	29.3%	Kuo et al. (2015)
Chlorella vulgaris	Domestic wastewater	32.7%	Lam et al. (2017)
Chlorella vulgaris	Brewery wastewater	18%	Farooq et al. (2013)
Chlorella zofingiensis	Piggery wastewater	45.81%	Zhu et al. (2013a)
Rhodosporidium toruloides + Chlorella pyrenoidosa	Distillery and local municipal wastewater	63. 45%	Ling et al. (2014)
Micractinium reisseri	Municipal wastewater	40%	Abou-Shanab et al. (2014)
Chlamydomonas mexicana	Piggery wastewater	33%	Abou-Shanab et al. (2013)
Chlamydomonas polypyrenoideum	Dairy wastewaters	42%	Kothari et al. (2013)
Ourococcus multisporus	Municipal wastewater	31%	Ji et al. (2013)
Euglena sp.	Municipal wastewater	25.6%	Mahapatra et al. (2013)
Monoraphidium sp.	Municipal wastewater	26%	Holbrook et al. (2014)
Chlorella sp. ZTY4	Domestic wastewater	79.2%	Zhang et al. (2013)
Scenedesmus sp. Z-4	Molasses wastewater	28.9%	Ma et al. (2017)

nol production. This suggests that same type of microalgae might behave differently or the origin of microalgae may have a significant effect on wastewater remediation as well as co-production of biofuels.

Prior to utilization as a growth medium of microalgae, sometimes pretreatment or dilution of wastewater is needed since the high amount of organic or ammonia load may inhibit the microalgal growth. This was clearly shown by Zhu et al. (2013b) who indicated that microalgae *Chlorella zofingiensis* utilizes the piggery wastewater and produces 30/mg/l/day biodiesel; but this strain shows good growth and nutrient removal efficiency when the COD of the wastewater was 1900 mg/L. As reported earlier, generally microalgae accumulate high amount of lipid under the nutrient starvation phase. A novel two-stage wastewater treatment was reported by Hemalatha and Mohan (2016) who first grew microalgae in mixotrophic condition using pharmaceutical wastewater for the higher production of biomass and subsequently transferred the culture in the tap water for the higher accumulation of lipid. In summary, we can say that dual role of phycoremediation along with microalgal biodiesel production is one of the promising ways, but the selection of microalgae, mode of cultivation, as well as pretreatment of wastewater are key characteristics, which should be considered before the full-scale operation.

3.2 Bioethanol Production

Apart from lipids, another most abundant carbon reserve in microalgae is carbohydrate that can be used for the production of bioethanol. Microalgal carbohydrates have additional advantages compared to plant carbohydrate since microalgae do not contain lignin in their cellular composition (Pancha et al. 2016). Many studies demonstrate that microalgae accumulate high amount of carbohydrates when grown in wastewaters. A study by Wang et al. (2015) demonstrates that microalga *Chlorella vulgaris* JSC-6 have an ability to remove 60–70% of COD and 40–90% NH_3-N from swine wastewater along with the production of biomass containing 58% carbohydrate that can be converted into bioethanol. Another study carried out by Jiang et al. (2015) with *Spirulina subsalsa* shows that the cyanobacteria have an ability to utilize the nutrients from the wastewater from glutamate factory along with the production of biomass containing 41% protein and 18% carbohydrate. In another study by Nayak et al. (2016b), microalgae *Scenedesmus* sp. cultivated in a closed and open reactor with domestic wastewater and coal-fired flue gas accumulated about 35.6% lipid and 10.4% carbohydrate.

Apart from freshwater microalgae and cyanobacteria, certain marine microalgae also have the ability to obtain nutrients from wastewater and simultaneously accumulate carbon reserve in their cells. A recent study by Reyimu and Özçimen (2017) shows that microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* can be used to treat the municipal wastewater. They reported that both microalgae have the different ability for their growth in wastewater; *Nannochloropsis oculata* can grow in a media with up to 75% of wastewater, while *Tetraselmis suecica* can grow best with only 25% of wastewater. However, the bioethanol production capacity of *Tetraselmis suecica* was much higher compared to that of *Nannochloropsis oculata*. Apart from only bioethanol, Ellis et al. (2012) showed that 9.74 g/L ABE (acetone-butanol-ethanol) was produced by *Clostridium* spp. using wastewater-grown microalgae as a fermentation source. This indicates that not only ethanol but another type of fermentation-based fuels can also be produced using wastewater-grown microalgae. After cultivation, wastewater-grown microalgae generally require pretreatment to hydrolyze the complex sugars into simple and readily metabolizable carbon source by fermentative microorganisms. A study carried out by Choi et al. (2011) showed that sonication of wastewater-grown microalgae *Scenedesmus obliquus* YSW15 for 60 min is seven times more effective for the production of bioethanol. In another study, Castro et al. (2015) showed that treatment of 10%

wastewater-grown microalgae with 1.0 M sulfuric acid for 120 min at 80–90 °C produced 5.23 g/L of total ABE and 3.74 g/L of butanol.

3.3 Biohydrogen and Biogas Production

It is not necessary that wastewater-grown microalgae have high lipid accumulation. One of the alternatives to utilize such type of wastewater-grown microalgae is the conversion of the whole microalgal biomass into biohydrogen or biogas through anaerobic digestion. Generally, anaerobic digestion is conducted by two processes: in the first process, the simple sugar is converted into the alcohols by fermentative bacteria in the anaerobic digest, and in the next stage, metanogenic microorganisms utilize these compounds and produce biomethane (Danquah and Harun 2011). Various studies showed that wastewater-grown microalgae have high potential to be used as a substrate for anaerobic digestion and subsequent utilization for biogas or biohydrogen production (Prajapati et al. 2014; Passos et al. 2016; Prandini et al. 2016). One of the major advantages is one can directly utilize harvested wet microalgal slurry for biogas production, since moisture content is very important in the process. By using such approach, one can also reduce the drying cost, thereby reducing the overall production cost (McKendry 2002).

Various factors affecting the final biomethane production yield are the type of microalgal biomass, temperature for digestion, pH, C/N ratio in the biomass, etc. When algal biomass has low C/N ratio, co-digestion with other biomass is also carried out to enhance the final biomethane yield (Cheah et al. 2016). A study conducted by Caporgno et al. (2015) indicated that freshwater microalgae *C. kessleri* and *C. vulgaris* cultivated in urban wastewater produced 346 ml CH_4/g_{vs} and 415 ml CH_4/g_{vs}, respectively. Similar to bioethanol production, pretreatment of microalgal biomass also enhances the anaerobic digestion process and further increases the yield of biogas. A study conducted by Passos et al. (2013) indicated that microwave pretreatment increases the solubilization of microalgal biomass; this pretreatment increased the biogas production rate (27–75%) and final biogas yield (12–78%) in BMP (biomethane potential) tests. Apart from single microalgae, a consortium of wastewater-grown microalgae can also be used for biomethane production. Choudhary et al. (2016) showed that native consortia PA6 has high nutrient removal ability with theoretical methane potential of up to 0.79 m3kg/VS. One of the major advantages of utilization of wastewater-grown microalgae for biogas production is that one can also utilize the residual biomass after extraction of lipid for the biodiesel production. With such biorefinery approach, one can remediate the wastewater along with producing biodiesel and biogas in a sustainable way.

Another gaseous fuel one can produce using the wastewater-grown microalgal biomass is biohydrogen. Hydrogen gas is also recently considered as a major fuel option. Biohydrogen can be produced through the metabolic activity of microorganisms using various types of biomass as the carbon source; microalgal biomass is among one of them. A study carried out by Batista et al. (2015) indicated that microalgae *Scenedesmus* could obtain the nutrients from urban wastewater and the biomass obtained through the cultivation can produce 56.8 ml H_2/g_{vs} which is similar to that when cultivated in the synthetic growth medium. Table 3 shows bioethanol, biogas, and biohydrogen potential of various microalgae grown in wastewater. A two-stage biohydrogen and biodiesel production system was demonstrated by Ren et al. (2014), which reduces 28.3% of COD. It is also possible to produce biohydrogen by the metabolic pathway of microalgae without using fermentative microorganisms under certain environmental stress conditions like sulfur starvation. Using this approach, Faraloni et al. (2011) reported that microalgae *Chlamydomonas reinhardtii* produce 150 ml H_2/L of culture when cultivated with olive oil wastewater along with TAP, which was significantly higher than the normal control cultivation process. Another microalga *Micractinium reisseri* YSW05 can grow photoheterotrophically on acetate- and butyrate-rich wastewater and produce a high amount of biohydrogen during their cultivation process (Hwang et al. 2014). Apart from the production of this type of biofuels, it is also possible to directly utilize the wastewater-grown microalgae to produce bio-crude by hydrothermal pyrolysis or to generate electricity using the gasification process.

Table 3 Bioethanol, biogas, and biohydrogen potential of various microalgae grown in wastewater

Microalgae	Type of wastewater	Lipid content	Ref
Mix microalgae	Lagoon wastewater	0.311 g/g ABE	Ellis et al. (2012)
Tetraselmis suecica	Municipal wastewater	7.26% ethanol	Reyimu and Ozçimen, (2017)
Chlorella vulgaris JSC-6	Swine wastewater	58% carbohydrate	Wang et al. (2015)
*Scenedesmus obliquus*YSW15	Swine wastewater	0.316 g/g ethanol	Choi et al. (2011)
Scenedesmus obliquus	Urban wastewater	56.8 ml H_2/g_{vs}	Batista et al. (2015)
Chlorella vulgaris	Urban wastewater	415 ml CH_4/g_{vs}	Caporgno et al. (2015)
Mix microalgae	Urban wastewater	309 ml CH_4/g_{vs}	Passos et al. (2013)
Native microalgae consortia	Rural wastewaters	0.79 m^3kg CH_4/vs	Choudhary et al. (2016)
Mix microalgae	Piggery wastewater	171 ml CH_4/g COD	Molinuevo-Salces et al. (2016)
Scenedesmus sp.	Starch wastewaters	1466 ml H_2/L	Ren et al. (2015)
Algae-bacteria	Activated sludge	0.21 L/g_{vs}	Bahr et al. (2013)
Micractinium reisseri YSW05	Acetate- and butyrate-rich wastewater	191.2 ml/ml of effluent	Hwang et al. (2014)
Scenedesmus sp.	Synthetic wastewater	252 ml CH_4/kg_{vs}	Kinnunen and Rintala (2016)

3.4 Pigments and Other Valuable Compounds

Apart from biofuel production, microalgae are also considered as a rich source of pigments like phycobiliproteins, carotenoids, and various fatty acids having nutraceutical applications. Wastewater-grown microalgae can also be utilized for such purpose, but one of the problems is the possibility of the presence of certain toxic metals and other pollutants in the biomass. However, an application like natural colorants from algal biomass is a safe and good alternative (Dufossé et al. 2005). Generally, nutraceutical and health applications from algal biomass generate more revenue compared to its biofuel applications. Therefore, utilization of wastewater-grown microalgae is a promising alternative to generate high-value products from microalgae. Few studies reported the use of wastewater-grown microalgae for such applications. A study carried out by Rodrigues et al. (2014) showed that *Phormidium autumnale* produce the high amount of carotenoids, namely, all-trans-β-carotene (70.22 μg/g), all-trans-zeaxanthin (26.25 μg/g), and all-trans-lutein (21.92 μg/g) using agro-industrial waste as a cultivation medium. In another study, Kim et al. (2007) showed that microalgae *Scenedesmus* sp. can obtain the nutrients form fermented swine wastewater as well as produce about 2.8- and 2.7-folds higher astaxanthin and lutein, compared to the control culture.

Microalgae with an ability to withstand a wide range of environmental conditions are the best candidate for the bioremediation-linked bioproducts production. A study conducted by de-Bashan et al. (2008) indicated that microalgae *Chlorella sorokiniana* UTEX 2805 is a good candidate for bioremediation of ammonia-containing wastewater since this microalga has high ammonium removal ability as well as an ability to grow in the temperature range from 40 to 42 °C along with high-light tolerance. When immobilized with the bacterium *Azospirillum brasilense*, its ability to remediate the waste along with the production of photosynthetic pigments increased significantly. All these studies indicate that phycoremediation-based microalgal nutraceutical production is the best way to produce high-value products with minimum investment.

4 Biorefinery with De-oiled Microalgal Biomass

After biodiesel preparation, a large amount of de-oiled biomass, mainly rich in carbohydrates and protein, remains as a major by-product. Utilization of this biomass for valuable products like bioethanol, biomethane, or as a biosorbent for removal of heavy metal and dyes from the wastewater, for nanoparticles preparation, etc. reduces the cost of microalgal biodiesel production (Maurya et al. 2016a, Chokshi et al. 2016). Carbohydrates in microalgae are mainly cellulose in the cell wall and starch in the plastids without lignin and low hemicelluloses contents that can be easily converted into reducing sugars (Chen et al. 2013). A study by Lee et al. (2013) shows that enzymatic hydrolysis of lipid-extracted residual biomass of the microalga *Dunaliella tertiolecta* with AMG 300 L produced 21.0 mg/mL of reducing sugar with a yield of 42.0% (w/w) based on the residual biomass at pH 5.5 and temperature of 55 °C. Another study by Goo et al. (2013) shows almost 90% recovery of extracellular polysaccharide from defatted microalgae *D. tertiolecta* by sulfuric acid (2 M) hydrolysis or by one-step enzymatic saccharification. Lam et al. (2014) reported the highest maltodextrin yield (90%) using 3% sulfuric acid at operating temperature of 90 °C after 1 h of hydrolysis time from de-oiled microalgae *Chlorella vulgaris*. Recently, Pancha et al. (2016) observed almost 97% saccharification of the mixotrophically grown de-oiled biomass of *Scenedesmus* sp. by enzymatic hydrolysis; further 78% ethanol conversion efficiency was also achieved. Apart from the energy production, de-oiled microalgal biomass can also be used for the cultivation of microalgae and as a fertilizer substitute (Maurya et al. 2016b, 2016c). Chokshi et al. (2016) synthesized silver nanoparticles using the de-oiled biomass of *Acutodesmus dimorphus* grown in dairy wastewater.

5 Economic Analysis of Microalgae-Based Wastewater Treatment

Apart from its advantages, microalgal-based biofuels are not yet commercialized mainly due to their high operating cost of production. Various studies conducted so far indicated that the price for microalgal-based biodiesel is between \$ 2.6 and \$ 20.53/gal, which is comparatively higher than the fossil-based diesel (Davis et al. 2011; Abayomi et al. 2009). The main cost involved in the biodiesel production is the cost of water, nutrients like N and P, as well as harvesting and dewatering. One of the solutions to reduce the production cost is the cultivation of microalgae in wastewater, which might result in 50% reduction in cultivation or final biodiesel cost (Zhou et al. 2014; Pittman et al. 2011). Wastewater cultivation of microalgae produce a high amount of biomass compared to normal photoautotrophic cultivation since most of the wastewater contains some amount of organic carbon compounds that improve the microalgal productivity (Lowrey et al. 2015). Utilization of microalgae also reduces the environmental risk. Many LCA analyses indicate that utilization of microalgal biomass for biofuel production is environmentally sustainable approach compared to other energy biofuel production crops (Clarens et al. 2010; Mu et al. 2014). Yang et al. (2011b) reported that 3.726 kg of water is required to produce 1 kg of microalgal biomass using wastewater-based cultivation. Further, this type of cultivation does not require any additional nutrients, since wastewater itself contains almost all the essential elements required for microalgal growth. However, various cost estimation and LCA analysis show different results mainly due to the utilization of different type of wastewater, utilization of different end products, as well as utilization of different methods to produce biofuels, viz., transesterification, pyrolysis, fermentation, etc., which significantly alter production cost (Chiaramonti et al. 2015).

6 Conclusion

Microalgal biomass is considered as a rich bioresource for the production of valuable compounds and biofuel precursor, but due to its high cost, mainly associated with its cultivation and product purification, it has not been used commercially. However, a biorefinery approach to extract high-value product along with biofuel precursor makes a good model and an attractive approach to make economical and sustainable microalgal products. In addition to biorefinery approach, cultivation of potential microalgae in various types of wastewater further reduces the cultivation cost along with freshwater and nutrient inputs. This chapter mainly describes major microalgae being utilized for treatment and co-production of various economically important products along with biorefinery of de-oiled microalgal biomass for different applications which in turn make the overall process environmentally sustainable. Finally, the cost analysis and LCA study of wastewater-integrated microalgal system suggest that this cultivation strategy not only reduces the cost of microalgal-based bioproducts but also reduces the water and carbon footprint of the overall system, which is a major achievement of wastewater-integrated microalgal biorefinery.

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Phyco-Remediation of Dairy Effluents and Biomass Valorization: A Sustainable Approach

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1 Introduction

Dairy industries will continue to expand in its size and capacity due to the increasing world's dairy product demands (Kushwaha et al. 2011) and, hence, will create terrific volumes of dairy effluent (Briã and Tavares 2007). Wastewater from dairy industry has been mostly originated from the washing and cleaning operations during the processing of milk. Dairy industry produces approximately 0.2–10 L of wastewater in the processing of one litre of milk (Ummalyma and Sukumaran et al. 2014; Vourch et al. 2008). According to Munavalli and Saler (2009), approximately 2% of the total milk is wasted during its processing. In general, dairy industry wastewater has high organic (dissolved sugars and protein) and inorganic (nitrogen and phosphorus) contents along with a high level of cleaning agent (detergents and sanitizing compounds) (USDA-SCS 1992). Presence of organic wastes in the dairy effluent has been considered as a severe environmental threat attributable to elevated chemical oxygen demand (COD), biological oxygen demand (BOD) level and difficulties associated with its putrefaction. This organic nutrient load may lead to eutrophication of water bodies, and presence of detergents and sanitizers could negatively have an effect on aquatic life (Su et al. 2012). Due to high pollution load, old effluent treatment plant setup and limited wastewater treatment capacity, industries

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Table 1 Minimal standard parameter values for discharge of wastewater effluents from the dairy industries into the environment

Parameters	Maximum value for discharge (mgL^{-1})	
	Report of World Bank[a]	CPCB New Delhi, India[b]
pH	6–9	6.5–8.5
Total dissolve solids (TDS)	–	450
Suspended solids (SS)	50	150
Total nitrogen	10	10
Total phosphorous	2	–
Oil and grease	10	10
BOD_5	50	100 (BOD_3/27 °C)
COD	250	–
Coliform bacteria	400 MPN/100 ml	

[a]Source: Pollution Prevention and Abatement, Fruit and Vegetable processing. "Pollution Prevention and Abatement: Dairy Industry," January 31, 1996 P621. World Bank, environmental Department 1996

[b]Source: http://www.cpcb.nic.in/Industry-Specific Standards/Effluent/DairyIndustry.pdf

discharging wastewater with treatment or after partial treatment into the natural habitat can cause serious ecological imbalance (Montuelle et al. 1992). Indian government and World Bank have put forth very strict set of laws for the discharge of effluent into water reservoir to save the environment (Table 1).

Generally, dairy effluent treatment processes involve physico-chemical methods (coagulation-flocculation, membrane filtration), which are often very costly. Biological methods (anaerobic digestion and aerobic digestion) have also been employed in some cases but have its own limitations. Most commonly fungi and bacteria have been used for reducing organic pollutant from dairy industry wastewater (Kothari et al. 2012; Tastan et al. 2010; McMullan et al. 2001). Since the last few decades, the industrial and municipal wastewaters have been used by various groups of scientists for algal cultivation to generate third-generation biofuel feedstock as well as for waste remediation (Pacheco et al. 2015; Kothari et al. 2012; Su et al. 2012). Microalgae have remarkable capacity to reduce atmospheric CO_2 and produce more oil as compared to terrestrial oil-producing crops (Chisti 2007). Usually, the cultivation of microalgae can be performed by four different methods (mixotrophic, photoheterotrophic, phototrophic and heterotrophic), and it mainly depends on algal strain and adaptation with their corresponding cultivation conditions. Phototrophic cultivation of microalgae needs light and atmospheric CO_2 for growth, but it shows restricted biomass and hence limited oil yield/productivity. Heterotrophic algal cultivation needs external carbon source such as glucose, glycerol, sodium acetate, etc. and offers the possibility of higher biomass yield under dark condition. The most often phototrophic method of cultivation is commercially used for large-scale microalgae biomass production that can be used for biofuel production (Danquah et al. 2009). The cost associated with nutrients, harvesting and suitable water used for algal cultivation has been the most important hurdles for the economical production of algal biomass. Freshwater has also been anticipated as one of

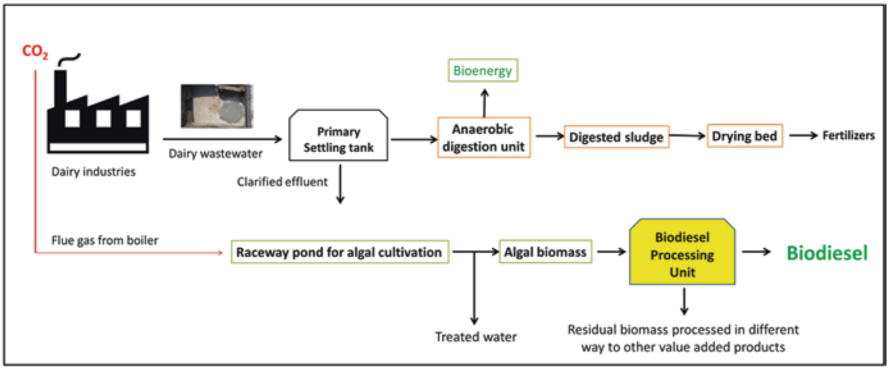

Fig. 1 Schematic diagram of the integrated dairy effluent treatment biofuel production: sustainable technologies

the depleting resources, and hence utilization of freshwater for algal cultivation may be considered as unethical. Using wastewater for algal cultivation could be a strategy to produce low-cost biomass for liquid biofuel production (Su et al. 2012). Developing countries like India have great potential for generating industrial effluents. As per an assessment, Indian dairy industries produce around 72.65 billion gallons of dairy effluent annually (Kushwaha et al. 2011; Chokshi et al. 2016). Therefore, a combined sustainable approach (Fig. 1) could be established for algal biofuel production and waste remediation (Htet et al. 2013).

This chapter discusses the importance of dairy industry wastewater effluent as alternative nutrients for algal cultivation. Besides this, the chapter also explores how efficiently microalgae remediate organic nutrients from dairy industry wastewater. We have attempted to summarize the study that integrates dairy waste remediation with unicellular algal strain cultivation and its potential application for biofuel production. Brief account of *mass balance analysis and scale-up potential* for algal biomass production using dairy industry waste has also been discussed.

2 Dairy Wastewater for Microalgal Culture

2.1 Physiochemical Characteristics of Dairy Wastewater

Milk processing practices result in disposable liquid- or water-carried waste known as dairy wastewater (DWW). Typically DWW contains large quantities of oxygen-demanding waste, pathogenic organisms, milk solids, oil and grease, carbohydrates (lactose), nitrogen, phosphorus, surfactants and sanitizers. It has very high-chemical oxygen demand (COD) (80–95,000) and biological oxygen demand (BOD) (40–48,000) which depend on the types and source of DWW. Table 2 presents typical

Table 2 Physico-chemical characteristics of dairy industry wastewaters (composition in mgL^{-1} except for pH)

Dairy waste water type	*pH*	*TSS*	*VSS*	*COD*	*BOD*	*TKN/TN*	NO_3-*N*	NO_2-*N*	NH_3-*N*	Phosphorous (PO_4^{-3}/*TP*)	Fats	References
Dairy wastewater	7.5	–	–	2470	1840	–/160	94.3	48.3	–	120.6/183.4	–	Khemka and Saraf (2017)
Dairy industry	7.80	–	–	2593.33	–	–	–	0.95	277.40	5.96/–		Chokshi et al. (2016)
Dairy industry	8.18	–	–	2593.00	–	283.00/–	–	–	181.50	–/115.90		Lu et al. (2015)
Dairy industry	9.90	–	–	1448.00	672.5	–	–	–	–	–		Shivsharan et al. (2013)
Dairy industry	6.58	–	–	13054.00	11,000	69.32/–	6.62	–	–	13.12/–	4203.8	Amini et al. (2013)
Dairy industry	7.30	–	–	–	–	–	66.4	0.95	–	21.00	–	Kothari et al. (2012)
Dairy industry	4.5–9.4	24–4500	–	80–95,000	40–48,000	–/15–180	–	–	–	12–132	–	Gutierrez et al. (2007)
Dairy industry	8.0–11.0	–	635	4000	2600	–/55	–	–	–	35	400	Kasapgil et al. (1994)
Dairy industry	–	800	–	4500	2300	–/60	–	–	–	50	350	Koyuncu et al. (2000)
Yoghurt and buttermilk	7.2	191	–	1500	1000	–/63	–	–	–	–	–	Koyuncu et al. (2000)
Cheese industry	7.32	1100	–	4430	3000	–/18	–	–	–	14	–	Monroy et al. (1995)
Dairy industry	–	7175	–	18,045	8239	–/329	–	–	–	–	4890	Arbeli et al. (2006)
Dairy industry	8.0–11.0	350–1000	330–940	2000–6000	1200–4000	–/50–60	–	–	–	–	–	Ince (1998)
Dairy industry	4.7–11.0	250–2750	210–1890	430–15,200	650–6240	–/14–90	–	–	–	–	160–1760	Passeggi et al. (2009)

pH Initial pH, *TSS* Total suspended solids, *VSS* Volatile suspended solids, *COD* Chemical oxygen demand, *BOD* Biological oxygen demand, *TKN* Total Kjeldahl nitrogen, *TN* Total nitogen, NO_3-*N* Nitrate, NO_2-*N* Nitrite, NH_3-*N* Ammoniacal nitrogen, *TP* Total phosphorous.

composition of DWW in terms of nitrogen, phosphorous and total organic and inorganic contents.

2.2 Growth Supplement from Dairy Wastewater for Microalgal Culture

DWW contains enormous amount of organic form of nutrients (protein, fats, lactose and inorganic salts) as discussed in previous section. Besides these they also contain different forms of nitrogen (organic-N, ammonium, nitrates and nitrite) and phosphorous (phosphate) which makes them suitable for microalgal cultivation. Reports are available which have shown that DWW also contains some metals (Chokshi et al. 2016; Cai et al. 2013; Kothari et al. 2012). Hence, DWW offers ideal conditions for microbial (i.e. bacteria) growth and putrefaction of organic matter by oxygenation. However, microbes are less efficient in the removal of inorganic nutrients such as phosphorus, which is usually the main cause of eutrophication of freshwater ecosystems. Thus, an additional step followed by bacterial decomposition must be applied before the release of DWW in natural waterways, which tends to increase the process cost (Guzzon et al. 2008).

The microalgae grown in different fractions of anaerobically digested dairy manure, raw DWW and pretreated effluent collected at different stages of effluent treatment plant showed satisfactory results for biomass and lipid productivity plus waste remediation efficiency (Table 3). Microalgae cultivation using DWW is influenced by a number of factors. The efficiency of microalgae growth depends upon the control of critical nutrients and environmental factors, such as initial nutrient concentrations (nitrogen, phosphorous and carbon), pH, temperature, availability of light, mixing velocity, CO_2 and O_2. The presence of heavy metals, organic compounds and contaminants such as bacterial pathogens and predators (zooplankton) results in inhibitory effects on microalgae growth. The presence of suspended solids and high turbidity affects algal growth as it reduces the light brightness and may become a limiting factor in algal growth.

The concentration of abovesaid components probably depends on the nature and origin of DWW. The COD is indirect measurement of the organic and inorganic form of nutrients present in wastewater. The organic carbon from dairy industry effluents supports the heterotrophic cultivation of microalgae. Heterotrophic mode of cultivation also improved microalgal growth rate, lipid productivity and nutrient remediation rate (Lu et al. 2015). Sufficient amount of carbon source has been present in cheese whey wastewater, but it requires pretreatment such as dilution, pH adjustment and others before algal inoculation for growth in cheese whey wastewater. Industrial dairy effluents have limited amount of carbon and hence required supplementation of external carbon source (Lu et al. 2015).

Table 3 Biomass, lipid productivity and nutrient remediation of the microalgae cultures using dairy wastewater

Microalgae sp.	Dairy waste water type	Biomass		Lipid		% of nutrient removal			Reference
		Cell conc. (gL^{-1})	BP ($gL^{-1}d^{-1}$)	Lipid content (% dcw)	LP ($gL^{-1}d^{-1}$)	COD	Nitrate	Phosphate	
Chlorella sp.	DI	2.25	0.260	–	–	81.21	79.29	100	Lu et al. (2015)
Chlorella pyrenoidosa	DI	6.8	0.450	–	–	–	60.0	87.0	Kothari et al. (2012)
Axenic algal strain	DFW	0.86	0.140	–	–	90	–	92	Ding et al. (2015)
Acutodesmus dimorphus	DI	0.840	0.210	–	–	90.1	100.0	100.0	Chokshi et al. (2016)
Algal consortium	DFW	2.76	0.276	23.62	0.066	98.8	99.4	98.8	Henaa et al. (2015)
Chlorella sp.	DM	–	0.082	11.35	0.009	–	–	–	Wang et al. (2010)
Chlorococcum sp.	DE	0.8	0.053	31	0.017	93	–	–	Ummalyma and Sukumaran (2014)
Chlorella vulgaris	DE	1.23	0.123	–	–	80.62	85.47	65.96	Choi (2016)
Chlorella pyrenoidosa	DE	–	–	–	–	84.55	98.29	97.1	Yadavalli and Heggers (2013)
Microalga consortium	DE		0.229	13.4	0.031	91	82	99.7	Tsolcha et al. (2016)
Chlamydomonas polypyrenoideum	DI	–	–	42	–	50	90	70	Kothari et al. (2013)
Chlorella vulgaris	UV treated DI	1.218	0.287	14.38	0.044	>73	–	>94	Qin et al. (2014)
Chlorella vulgaris	DI	1.870	0.450	10.30	0.048	>51	–	>91	Qin et al. (2014)
Desertifilum tharense MSAK01	DWW	0.8–1.11	0.05–0.07	–	–	94	98	56	Khemka and Saraf (2017)
Algal consortium (*Chlorella* sp./*C. zofingiensis*/*Scenedesmus* sp.)	DWW	4.72–5.41	0.657–0.773	19.03–21.09	0.127–0.150	57.01–62.86	87.04–91.02	91.16–95.96	Qin et al. (2016)

DI dairy industry, *DE* dairy effluents, *DM* digested dairy manure, *WP* whey permeates, *DFW* dairy farm treated wastewater, *BP* biomass productivity, *LP* lipid productivity

3 Effectiveness of Microalgal Cultivation in Dairy Wastewater

3.1 Biomass Valorization of Algal Culture in Dairy Wastewater

Numerous studies have been performed by different research groups to evaluate the mass culture of microalgae for biomass production using DWW. Wang et al. (2010) evaluated the effectiveness of anaerobically digested dairy manure as a nutrient add-on for the growth of oleaginous green alga *Chlorella* sp. and observed that they survived in all the dilutions (10 × to 25 ×) of dairy manure. The maximum growth rate 0.409 d^{-1} was achieved by *Chlorella* sp. grown in 25 × diluted dairy manure. Mulbry et al. (2008) have carried out the mass culture of microalgae consortium dominated by the green algae *Rhizoclonium hieroglyphicum* using raw as well as anaerobically digested dairy manure effluents. The maximum algal biomass productivity was 21.3 ± 2.4 g $DWm^{-2}d^{-1}$ and 20.4 ± 2.5 g $DWm^{-2}d^{-1}$ at 1.60 ± 0.01 gTN $m^{-2}d^{-1}$ loading rate from indoor laboratory scale ATS without and with CO_2 addition, respectively, in the raw dairy effluent. The maximum biomass productivity was 17.3 ± 2.7 $DWm^{-2}d^{-1}$ and 21.0 ± 3.4 $DWm^{-2}d^{-1}$ at 1.56 ± 0.01 gTN $m^{-2}d^{-1}$ loading rate used in digested dairy effluent without and with added CO_2, respectively. Kothari et al. (2012) in an experimental study observed that *Chlorella pyrenoidosa* NCIM 2738 has better growth and adaptation in 75% concentration of the dairy wastewater in comparison with control. Chokshi et al. (2016) demonstrated that *Acutodesmus dimorphus* biomass yield was 0.84 gL^{-1} using unsterilized raw dairy effluent after fourth day of batch cultivation, which was five- to sixfold more as compared to standard modified BG-11 grown culture (0.149 gL^{-1}). Similarly, the 7.4-fold increase in the cell number of *Acutodesmus dimorphus* (from 0.47×10^7 to 3.48×10^7 cells mL^{-1}) was also observed after 4 days of batch cultivation in DWW (Chokshi et al. 2016). Ding et al. (2015) observed that microalgae efficiently grown in unsterilized dairy wastewater generated maximum biomass yield (0.86 gL^{-1}) in 20% DWW in 8 days indoor lab-scale batch cultivation. Huo et al. (2012) observed fourfold enhancements in the cell number of *Chlorella zofingiensis* (from 3.0×10^6 to 11.0×10^6 cells mL^{-1}) after 5 days of batch cultivation in DWW. *Chlorella* sp. was efficiently grown in raw DWW in outdoor conditions and achieved biomass productivity of 110.0 $mgL^{-1}d^{-1}$ (Lu et al. 2015). *Chlorella* sp. quickly altered to raw DWW in indoor bench-scale conditions, and maximum biomass obtained was 2.25 gL^{-1} and 3.05 gL^{-1} for 0.17 gL^{-1} and 0.34 gL^{-1}, respectively, in only 8 days of batch culture. It was also concluded that initial inoculum concentration has greater impact on algal growth (Lu et al. 2015). Biomass yield for *C. vulgaris* grew in untreated, UV pretreated and NClO pretreated DWW which ranged from 0.861 to 1.870 gL^{-1} (Qin et al. 2014).

Cai et al. (2013) investigated the effluent loading influence on marine alga *Nannochloropsis salina* biomass productivity and observed to be maximum, i.e. 92 $mgL^{-1}d^{-1}$ (biomass yield 0.92 gL^{-1}) at an effluent loading of 6% which was higher than *Nannochloropsis salina* grown in municipal wastewater (0.212 gL^{-1})

(Jiang et al. 2011). Biomass productivity dropped as an effluent loading increased from 6% to 24% and was found to be 68 $mgL^{-1}d^{-1}$ at 24% effluent loading. The above findings suggested the inhibition of microalgal growth at high effluent concentration. Similarly, inhibition in the growth of *Nannochloropsis* sp. in municipal wastewater was observed as volumetric loading rate increased from 50% to 80% (Cai et al. 2013; Jiang et al. 2011).

3.2 Lipid Production of Algal Culture in Dairy Wastewater

Reports suggest that microalgae grown in various forms of DWW have profoundly better lipid content as compared to normal growth media. *Acutodesmus dimorphus* stored 25.05% dry cell weight (dcw) lipid content with 50 $mgL^{-1}d^{-1}$ lipid productivity grown in DWW and was observed to be 1.2-fold higher as compared to that grown in BG11 as a media (21.08% dcw) after 8 days of batch cultivation (Chokshi et al. 2016). Algal consortium grown in raw DWW accumulated 19–21% (dcw) lipid after 1 week of batch cultivation (Qin et al. 2016). Similarly, Woertz et al. (2009) reported 28% (dcw) of lipid accumulation by algal consortium cultivated in 25% DWW. Henaa et al. (2015) obtained 11–52 $mgL^{-1}d^{-1}$ lipid productivity by *Chlorella sorokiniana* grown in dairy farm effluent. Tsolcha et al. (2016) observed maximum oil content 13.4 ± 2.0% dcw after cultivation of green microalgae in DWW. *Choricystis* sp. dominated microalgal-bacteria mixture in 17% secondary cheese whey wastewater. *Chloroccum* sp. accumulated 31% lipid when grown in the dairy effluents (DE) under mixotrophic mode, while under heterotrophic cultivation, they accumulated 39% and 42% lipid when grown in DE supplied with 4% and 6% external carbon source (biodiesel industry waste glycerol), respectively (Ummalyma and Sukumaran 2014). Availability of carbon in DE was insufficient to support enhanced growth of microalgal culture under heterotrophic cultivation; hence from economic point of view, it was suggested to use alternate cheap carbon source (Ummalyma and Sukumaran 2014).

A green freshwater microalgae *Chlamydomonas polypyrenoideum* grown in DWW was shown to accumulate 42% lipid content (Kothari et al. 2013). *Nannochloropsis salina* stored lipid content 35% of the dcw when grown in 3% effluent loading rate, and it was also observed that the increase (from 3% to 24%) in rate of effluent loading resulted in the decline of lipid productivity from 29.2 to 14 $mgL^{-1}d^{-1}$ (Cai et al. 2013).

3.3 Nutrient Remediation Efficiency of Algal Culture from Dairy Wastewater

For microalgae growth NH_3-N has been the favourite among all forms of nitrogen source present in wastewater, as it easily metabolizes and requires reasonable amount of energy for its consumption from liquid medium (Kumar et al. 2010).

Acutodesmus dimorphus grown in unsterilized DWW completely (100%) removed nitrite (0.95 to 0.00 mg L^{-1}) and NH_3-N (277.40 to 0.00 mg L^{-1}) within culture from 4 to 6 days, respectively (Chokshi et al. 2016). Ding et al. (2015) observed 83.20% removal (73.8 to 12.4 mg L^{-1}) of NH_3-N from 8-day-old culture of microalgae in the 20% DWW. Tsolcha et al. (2016) reported inclusive removal of total Kjeldahl nitrogen (TKN) after 7-day-old culture of microalgal and bacterial mixed population dominated by *Choricystis* sp. in 35% DWW. Lu et al. (2015) reported total remediation of NH_3-N at the end of 6 days batch culture of *Chlorella* sp. in 75% diluted DWW, whereas total nitrogen (TN) reduction was observed from 83.38% to 85.17% after 8 days cultivation of *Chlorella* sp. in differently diluted (5% to 25%) raw DWW. *Desertifilum tharense* grown in DWW removed 66.25% of TN (from 160 ± 2.06 to 54 ± 1.4 mgL^{-1}) efficiently after 15 days of batch cultivation. The TN removal rate was found to be the maximum (6.58 $mgL^{-1}d^{-1}$) in 25% raw DWW and was 3.21 and 2.51 fold higher than that for 5% and 10% raw DWW, respectively. Cai et al. (2013) found inclusive removal of total nitrogen by *N. salina* after 10 days of culture at 3% and 6% effluents loading. *Chlorella* sp. grown in anaerobically digested dairy manure consumed 82.5% TN (from initial TN 250 mgL^{-1}) after 22 days of culture (Wang et al. 2010). Total removal of NH_3-N from DWW was observed using immobilized *Chlorella pyrenoidosa* cells (Yadavalli and Heggers 2013).

Phosphate (P-PO_4^{-3}) is the key component for microalgal cell cultivation as it is responsible for energy transfer, cell membrane formation and nucleic acid synthesis and has been present in sufficient amounts in DWW. The consumption of phosphate has been found to be slower as compared to nitrate; however, it has been completely consumed (from 5.96 to 0.00 mgL^{-1}) by microalgae *Acutodesmus dimorphus* after 8 days of cultivation in unsterilized DWW (Chokshi et al. 2016). Similarly, Tsolcha et al. (2016) observed 88.20% to 100% removal of phosphate by microalgal population dominated by green microalga *Choricystis* sp. after 7 days of cultivation in differently diluted secondary cheese whey wastewater (SCWW). *Chlorella pyrenoidosa* NCIM 2738 removed 87% of phosphate after 10 days cultivation in DWW (Kothari et al. 2012). Cai et al. (2013) observed 99% total phosphate (TP) removal after 10-day batch cultivation of *N. salina* in 3% and 6% effluent loadings. Total phosphorus removal has been more than anticipated based on N/P ratio (i.e. 7) which could be due to the ability of *N. salina* to take up excess phosphorous. Immobilized cells of *Chlorella pyrenoidosa* used for DWW treatment removed 98% of phosphate within 96 h (Yadavalli and Heggers 2013). A cyanobacteria *Desertifilum tharense* MSAK01 grown in DWW efficiently removed 56.5% of TP (from 183.4 ± 1.14 to 79.7 ± 0.3 mgL^{-1}) in 15-day batch cultivation (Khemka and Saraf 2017).

Besides being consumption of different forms of nitrogen and phosphorous from DWW, microalgae have also been capable of removing different forms of organic and inorganic compounds from DWW. COD has been used for indirect measurement of total organic and inorganic compounds present in wastewater. The microalgae *Acutodesmus dimorphus* grown in unsterilized DWW consumed 91.71% of COD (from 2593.33 to 215.00 mgL^{-1}) within 4 days with removal rate of

579.5 $mgL^{-1}d^{-1.}$ After this, there has been no further reduction in COD observed (Chokshi et al. 2016). The reason hypothesized that the microalgal growth depends on other nutrients present in DWW (such as nitrite, ammoniacal nitrogen) that has been utilized or would be utilized making it a growth-limiting factor for microalgae. Similarly, Ummalyma and Sukumaran (2014) reported 93% (from 984 to 60 mgL^{-1}) of COD and 82% (from 37 to 7 mgL^{-1}) of BOD reduction after 12 days and 15 days cultivation of *Chloroccum* sp. in DE, respectively. They also observed 73% of COD reduction within first 3 days of cultivation. The slow reduction of COD in later days could be accredited to the presence of residual carbon as a slowly biodegradable material. In another study, about 90.0% reductions in COD has been observed at the end of 8th day of microalgae cultivation in unsterilized DWW (Ding et al. 2015). Tsolcha et al. (2016) observed 92.3 ± 2.0% of COD reduction after 7 days cultivation of algal population dominated by green microalgae *Choricystis* sp. Lu et al. (2015) reported a higher percentage of COD consumption (54.82%) than L. Wang's group which showed COD percentage consumption at 27.4–38.4% (Wang et al. 2010). However Lu et al. (2015) also reported consumption rate of 41.31 $mgL^{-1}d^{-1}$ in the same study for *Chlorella* sp. grown in 25% raw DWW in outdoor conditions. The better results obtained by Lu et al. (2015) may be due to the presence of indigenous bacteria in raw DWW which probably affect the COD removal. Besides this, the COD reduction was comparatively less efficient than indoor controlled conditions. Lu et al. (2015) reported 83.33% and 91.25 $mgL^{-1}d^{-1}$ COD reduction percentage and COD removal rate, respectively, for *Chlorella* sp. grown in 25% raw DWW under controlled indoor conditions.

4 Limitations of Algal Growth/Lipid Productivity by Dairy Wastewater

The average lipid content within microalgae cells and the biomass productivity in gm L^{-1} d^{-1} is collectively known as lipid productivity. For an algal species, the lipid productivity could be considered as a more useful indicator of the prospective liquid biofuel production cost. A microalga accumulates more lipids under stressed and nutrient depletion conditions. Microalgae shift their metabolic pathways to produce and accumulate more lipids in the cytoplasm as a reserve energy source under stressed cultivation condition. However, the cultivation of microalgae in stressed condition can inhibit cell division, leading to decline in overall lipid productivity. Factors such as selection of suitable algal species, bioreactor used for algal cultivation, light, temperature, pH, CO_2, available nutrients, level of contamination and also some others affect the productivity and success of wastewater remediation of dairy-based industries. The choice of algal strains totally depends upon the physicochemical composition and source of wastewater. Besides these the geographical location of the plant, environmental conditions where it grows and the fate of bioprocess could also be considered.

It has always been preferred to use robust indigenous algal strains that have desired characteristics as per process needs. Wide range of algae strains, for example, *Actinastrum* sp., *Spirogyra* sp., *Nitzschia* sp., *Micractinium* sp., *Golenkinia* sp., *Chlorococcum* sp., *Closterium* sp., *Acutodesmus dimorphus*, *Chlorella* sp., *Botryococcus braunii*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*, cultivated well particularly in unsterilized and sterilized raw industrial effluents (Woertz et al. 2009; Chinnasamy et al. 2010; Kothari et al. 2012). In case of DWW, there have been several algal strains found comparably efficient for biomass as well as nutrients remediation as shown in Table 3. To make process economically achievable, it has always been advisable to choose algal strains which have higher growth rate (may reduce the culture area required), large cell size, colonial and filamentous morphology, self-flocculation capacity (easy gravity-based harvesting and reduces harvesting and downstream processing cost), adaptive to seasonal and diurnal variation, high product content and no excretion of auto-inhibitors.

Microalgae sp. predominantly found in wastewater treatment with high rate algal ponds (HRAPs) include *Coelastrum* sp., *Dictyosphaerium* sp., *Actinastrum* sp., *Pediastrum* sp. and *Micractinium* sp. and habitually form large colonies (50–200 µm) making it easier to harvest and reduce the cost involved in downstream processing (Benemann et al. 1978; Borowitzka 1992; Park and Craggs 2010). Algal cultivation in open raceway pond has been more economically viable than mass production in photobioreactor, even though they have low volumetric productivity (~0.5 gL^{-1}) in the open raceway pond (Jorquera et al. 2009; Chisti 2007). This is because the net energy ratio (NER) for algal biomass has been more in the raceway ponds as compared to flat panel photobioreactors and also the installation, capital and operation cost of photobioreactor have been higher than that of raceway pond reactor (Jorquera et al. 2009). The raceway pond was most commonly contaminated by grazers and parasites that reduce the overall productivity. Zooplanktons (such as rotifers and cladocerans) at elevated concentration reduce the algal cell yield up to 90% within 48 h (Oswald 1980). Approximately, 99% reduction in algal pigment was observed as result of *Daphnia* grazing (Cauchie et al. 1995). Besides these fungal and viral contaminations significantly affect and reduce algal population (Kagami et al. 2007). Algae growth has also been affected by light intensity, and its growth increases consistently as light intensity increases in the absence of nutrients' limitation. Study suggests that neutral lipid accumulation has been higher at high light intensity (Yantao et al. 2011). In general, algae grown in high rate algal ponds (HRAPs) get sporadically exposed to the light by eddies and paddle wheel mixing. These systems not only agitate the water but increase the productivity as compared to other conventional techniques. The optimum temperature for the maximum algal growth rate has been observed between 25 °C and 35 °C, above which overall productivity goes down (Pulz 2001). The ionic equilibrium, pH and O_2/CO_2 solubility have also been affected by the temperature of cultivation medium (Bouterfas et al. 2002). In most of the cases, the optimum pH for algal growth in wastewater has been 8.0. Above this pH the algal growth reduced and thus decreased the overall productivity of the algal pond (Park et al. 2011; Park and Craggs 2010). Consumption of CO_2 and HCO_3^- during the photosynthetic activity increases the pH (Park and Craggs 2010) and hence enhances the algal productivity.

The significant nutrients affecting the algal growth in wastewater have been nitrogen and phosphorous. Many researchers have found that a number of microalgae having high lipid content adapt well in DWW and produce higher biomass as compared to normal commercialized growth medium used for autotrophic cultivation of microalgae. Microalga has now been used to cultivate in DWW for dual purposes, one for biomass which can be used as animal or biofuel feedstocks and another for nutrient remediation present in DWW (Pittman et al. 2011; Woertz et al. 2009). The problem associated with algal cultivation using DWW is its low biomass yield. It has been stated that the maximum cell density of microalgae grown in DWW was less than 0.7 gL^{-1}. As shown in Table 1, the DWW has high COD content ⟨ 7500 mgO_2 L^{-1}) and BOD content ⟨ 4500 mgO_2 L^{-1}) (Christenson and Sims 2011). These ranges have been increased in case of dairy cheese whey wastewater and reached up to 70,000 mgO_2 L^{-1} (COD) (Öztürk et al. 1993). These high organics in DWW may cause substrate inhibition. To prevent such problems, the researcher proposed different approaches such as dilution of DWW before algal inoculation (Kothari et al. 2012; Woertz et al. 2009). The microalgae growth has been basically influenced by the nitrogen concentration (nitrate, ammonia), phosphorus (phosphate) and carbon source present in the medium.

Hypothetically, low C:N:P ratio was not favourable for algal growth in DWW and yields low biomass and low nutrient remediation rate but may yield high lipid content due to nutrient limitation. Hence, it has been supposed to optimize the nutrient supplementation (Zhang et al. 2014) in DWW, not only to enhance the algal biomass but also for waste remediation improvement. The average ammonia nitrogen (NH_3-N) content in DWW ranges from 48 to 500 gL^{-1} (Longhurst et al. 2000). The microalgae grown in DWW consumed total NH_3-N within 72 h and that may be the reason that leads to low microalgal biomass yield (Lincoln et al. 1996). To eliminate such nutrient limitations during algal cultivation in DWW, some researchers proposed mixing of DWW with other effluents. Gentili (2014) mixed the dairy final effluents with pulp and paper influents, hence, improved nutrient profile of DWW and obtained higher biomass yield (1.12 gL^{-1}).

5 Life Cycle Assessment: Mass Balance Analysis and Scale-Up Potential of Dairy Wastewater-Derived Microalgae Biofuel a Case Study

The life cycle assessment (LCA) of a process has been used to measure the environmental sustainability before it is fully implemented. Importantly, LCA is not restricted to the study of technologies that are functioning at optimized conditions. LCA can also be used to recognize environmental effect in the systems which have not yet been operational at full scale. Pittman et al. (2011) discussed the LCA of wastewater-derived algal biofuel and considered that the process with positive energy output has been economically viable. Higgins and Kendall (2012) developed a life cycle inventory cost model to simulate algal turf scrubber (ATS) treatment system, where the produced biomass has been used to generate heat and electricity, the data shows by means of an ATS and eutrophication gets reduced significantly with high lipid productivity. The estimated cost of wastewater treatment system has been around $ 1.42 per cubic meter by using ATS technology. This chapter has not been going to discuss the life cycle cost assessment of integrated process for algal biofuel production and dairy waste remediation because till date debates exist regarding LCA of large-scale algae-based biofuel production integrated with waste remediation. This chapter summarizes the mass balance analysis for an integrated system and its commercial-scale feasibility. Rothermell et al. (2013) have analysed LCA of a coupled process for biofuel production and treatment of wastewater which demonstrated that the energy production and nutrient removal capacities of an algal photobioreactor (PBR) were being fed with wastewater as well as calculated associated energy demand and environmental influences in this coupled system. The LCA showed that maximum energy demand came up during harvesting of the algal mixtures by centrifugation or filtration process. It has been concluded that it may be a feasible process to remediate DE while producing renewable biofuels, but this process has to be optimized to decrease life cycle environmental effects and leads to a net energy gain before implementation in large-scale operation.

Chokshi et al. (2016) carried out a theoretical mass balance analysis for integrated DWW treatment and a unicellular microalgae *A. dimorphus* cultivation and found that 100 kg of dried algal biomass produced 27.3 kg biofuels. Similarly, to evaluate the feasibility of an integrated model system for algal biofuel production and DWW treatment, *Chlorell*a sp. has been considered and used as a model algal strain for the cultivation in DWW (Fig. 2). *Chlorella* sp. adapted well to grow and uptake nutrients in raw DE under both indoor and outdoor conditions and accumulates 30% of dcw lipid content (Lu et al. 2015). As per literature review, the average carbohydrate accumulated in *Chlorella* sp. was ~23% dcw (Lu et al. 2015). The biomass productivity of *Chlorella* sp. was found 160 $mgL^{-1}d^{-1}$ (1.28 gL^{-1}) after 8 days of batch cultivation in outdoor cultivation conditions. Chokshi et al. (2016) reported that various dairy industries generated 2–3 L DE per litre of milk processed. Based on the data available on web blog of one of India dairy industry (Shyam Dairy Products, Prayagraj, U.P., India), 1460 million litres of milk are processed annually (http://shyamdairy.com/about_us.htm) and approximately 2920 million litres of DE are produced, which means that eight million litres DE generated daily. To tackle this huge amount of wastewater by algal cultivation means from a dairy industry required 10.6 ha land for raceway ponds. The raceway pond of working volume of 600,000 L with 100 m × 10 m × 0.6 m dimensions is needed to prepare considering 8-day batch cultivation of microalgae. The total 107 open raceway ponds will be required for continuous cultivation of *Chlorella* sp. in DWW (Higgins and Kendall 2012). Considering biomass production of 1.28 gL^{-1} (outdoor culture) (Lu et al. 2015), 37,376 quintals of dry biomass is produced annually by cultivating *Chlorella* sp. in DWW. This biomass might produce 11,213 quintal oil

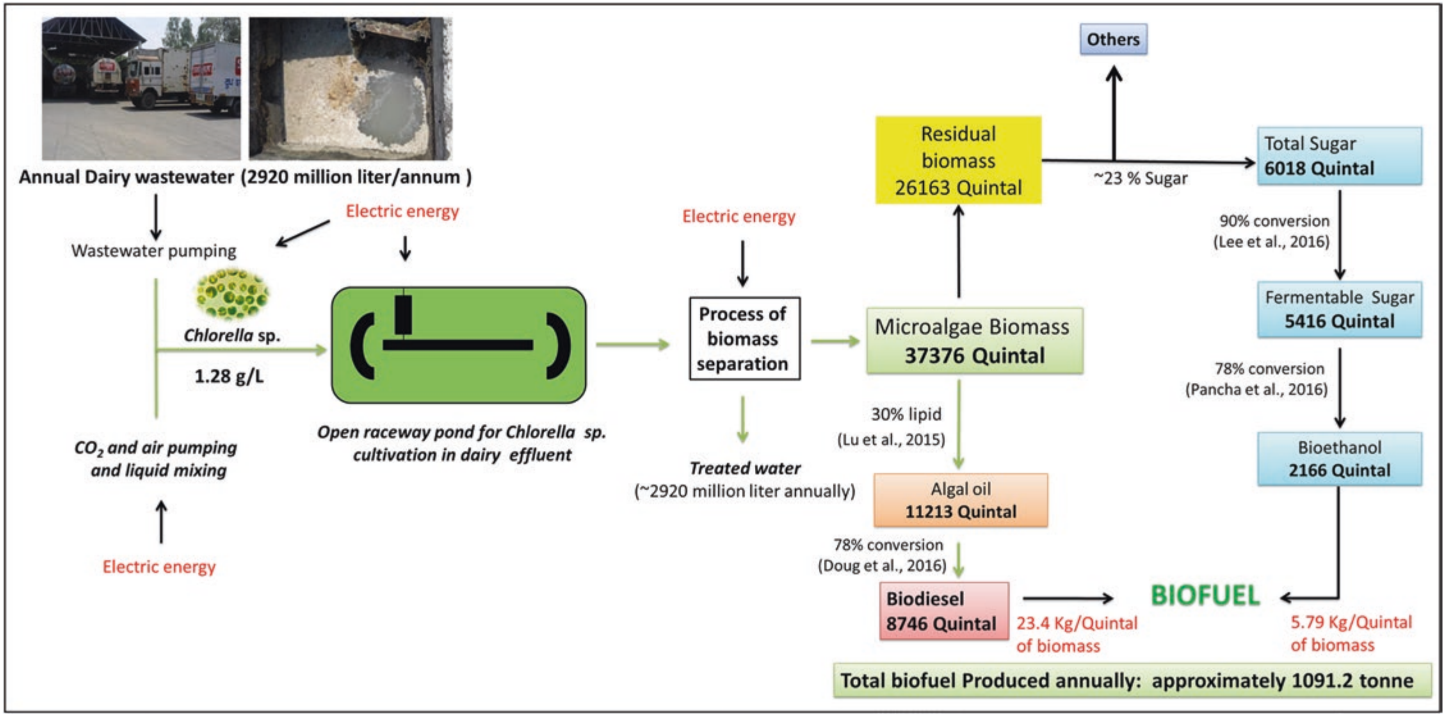

Fig. 2 Theoretical mass balance for biofuel production from algae grown in dairy wastewater (DWW)

(8746 quintal biodiesel) (12.2 L oil $m^{-2}annum^{-1}$/ 9.53 L biodiesel $m^{-2}annum^{-1}$) and 2166 quintal bioethanol, annually (Lu et al. 2015; Dong et al. 2016; Lee et al. 2015; Pancha et al. 2016). This reveals that 100 kg dry biomass of *Chlorella* sp. may produce approximately 23.4 Kg biodiesel and 5.79 Kg bioethanol, which sum up to 29.2 Kg biofuel from per 100 kg dried biomass of *Chlorella* sp. Further calculations, on CO_2 consumption, based on previous experimental work by Chisti (2007), for 1 kg of algae biomass production, 1.8 kg of CO_2 (this is on assumption that algal biomass consists of approximately 50% carbon) has been used since carbon is 27.3% of CO_2(w/w), hence, 67276.8 quintals of CO_2 consumed annually. 1 kg of microalgae-based biodiesel consumes approximately 0.33 kg nitrogen and 0.71 kg phosphorus (Yang et al. 2011). From these results of the lab-scale studies, it might be assumed that such integrated approach definitely helps to reduce not only the production cost of algal biofuel but also wastewater treatment cost. Besides these benefits, it will also reduce the burden on the freshwater reservoir and save our carbon credit.

The lipid-extracted biomass further may be utilized in several ways such as bioethanol production, hydrogen gas production, removal of heavy metals and textile dyes, animal feeds and fertilizers. Such value-added applications improve the economics of the process; however space available to develop sustainable microalgae biorefineries is one of the constraints. Research opportunities are also available in algal research in the area of scale-up of cultivation process; development and designing of high-efficient bioreactors; development in downstream processing especially in harvesting, oil extraction and transesterification of algal oil; and process development of value-added products as pigment, PUFA, cosmetics, proteins and fine chemicals.

6 Conclusion

The present chapter reveals the prospective of algal culture in DWW in connection with nutrient removal and biomass production. DWW treatment by using microalgae has been recognized as an effective, efficient and eco-friendly as compared to existing conventional methods. Microalgae cultivation can remove more than 90% of nutrients from the raw DWW, and it can be more enhanced/improved with supplement such as growth inducers and developing growth models with optimized key process factors. The mode of cultivation and physical factors such as temperature, photoperiod, light intensity, aeration and scale of operation have also influenced the algal biomass, lipid yield and nutrient remediation. As a whole, microalgae cultivation has the potential for the treatment of DWW and in addition can be used to produce third-generation biofuel feedstocks.

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Commercial Potential of Phycoremediation of Wastewater: A Way Forward

Sudheer Kumar Shukla, Prerana Pandey, and Keunje Yoo

Abbreviations

ATS	Algal Turf Scrubber
BGA	Blue-green algae
EU	European Union
GoI	Govt. of India
HM	Heavy metals
HRAP	High-rate algal ponds
HTL	Hydrothermal liquefaction
LED	Light-emitting diode
MFA	Monounsaturated fatty acids
NPs	Nanoparticles
SFA	Saturated fatty acids
SIO	Scripps Institution of Oceanography

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1 Introduction

Phycoremediation is the use of various algal species to remove various pollutants from wastewater including carbon dioxide from the waste air. It comprises several applications: nutrient removal (i.e. nitrogen and phosphorus) from organic-nutrient, and xenobiotic-rich wastewater, carbon dioxide sequestration, transformation and degradation of xenobiotics, and a biosensor to detect toxic compounds in water (John 2000; Olguin 2003). The use of research on the application of microalgae for the wastewater treatment has been one of the primary areas in the wastewater research (Wang et al. 2013; Wang et al. 2010), which is very favorable to other conventional technologies (de la Noie et al. 1992). Concentrations of several heavy metals have also been shown to be reduced by the cultivation of microalgae (Arunakumara and Xuecheng 2008). The advantage of algal-based treatment is to produce much-valued biomass with the treatment of wastewater (Bolan et al. 2004; Munoz and Guieyssea 2006). The conventional biological treatment systems like activated sludge process, trickling filter, and anaerobic treatment are though efficient; however, the sensitivity of these systems toward pH is inevitable, and even small variation of pH could create problem to the process. However, microalgae are strong enough to withstand extreme pH. Moreover, most microalgal species stabilize the pH of the wastewater in less than 9, and pH 9 is the upper limit of discharge standard for treated industrial wastewaters. Thus the use of microalgae technology eliminates the dependency on external pH balancer.

2 Phycoremediation of Wastewater and Biomass Generation

2.1 Composition and Structure of Algae

In general, algal cell walls are made up of two components:

1. The *fibrillar component*, which forms the skeleton of the wall, and
2. The *amorphous component,* which forms a matrix within which the fibrillar component is embedded. The most common type of *fibrillar* component is cellulose, a polymer of 1,4-linked D-glucose. The amorphous mucilaginous components occur in the great amounts in the *Phaeophyceae* and *Rhodophyta,* the polysaccharides of which are commercially exploited (Fig. 1).

2.2 Photosynthesis and Photorespiration in Microalgae

It's well known that algae are C_3 plants, but there has always been a debate regarding the existence or nature of photorespiration in algal species (Cheng and Colman 1974; Chollet and Ogren 1975; Tolbert 1974). In higher plants, both evolution and

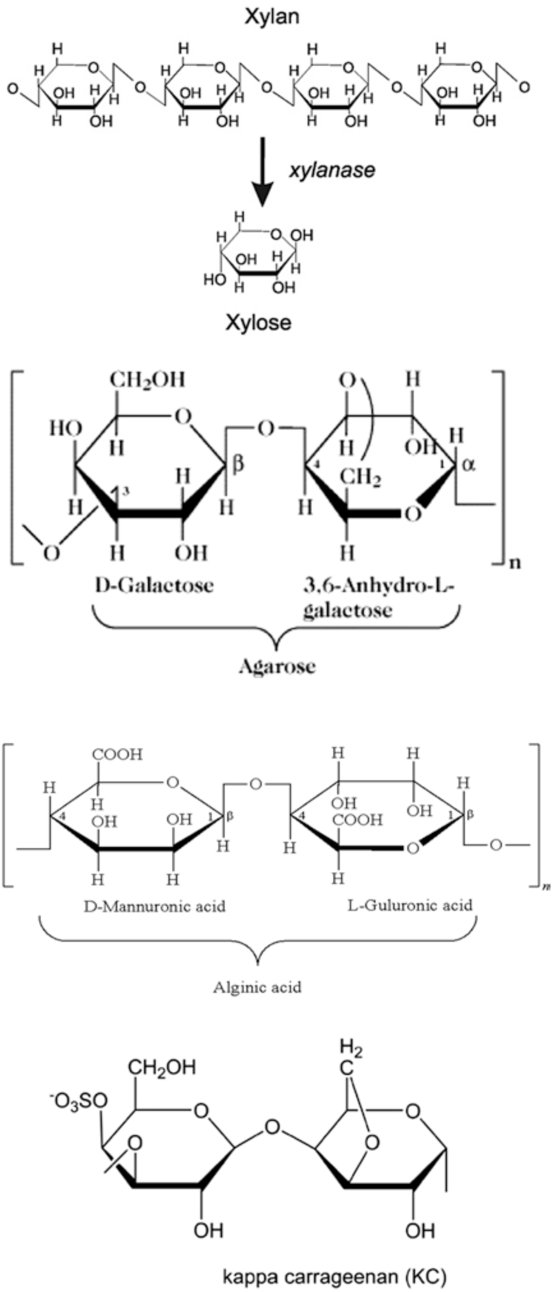
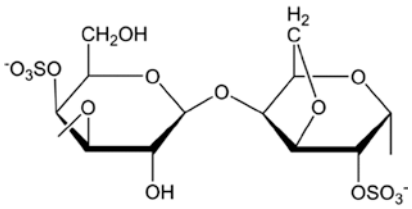
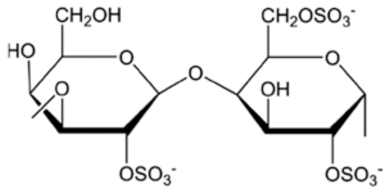


Fig. 1 Composition and structure of algae



iota carrageenan (IC)



lambda carrageenan (LC)

Fig. 1 (continued)

exchange of CO_2 are the most widely studied aspects of photorespiration (Zelitch 1971). Although in algal species CO_2 evolution during photosynthesis has just been suggested (Tolbert 1974), little has been studied regarding CO_2 exchange because of the difficulties imposed by the aqueous medium.

2.3 Removal of Heavy Metals and Nutrients from Wastewater

Microalgae have an extensive spectrum of mechanisms both extracellular and intracellular that make them able to cope with heavy metal toxicity and nutrient removal. Their considerable presence and, also, ability to grow and absorb heavy metals make them a perfect choice for wastewater treatment processes. Heavy metal removal by microalgae is assumed to be far better to the presently used physico-chemical processes. The heavy metals like Cd, Cr, Cu, Hg, Pb, and Zn are known to cause severe environmental and health problem (Hong et al. 2011). Heavy metal removal capacities of various marine and freshwater algae are reported (Doshi et al. 2006) to be much higher than the chemical absorbents like activated carbon, natural zeolite, etc. Khan et al. (2008) report that the *Sargassum* biomass has superior Cd(II) metal binding capacity as compared to organic and inorganic sorbents. Various researchers reported heavy metal (Zn, Cu, Mn, Ni, Cd) removal using different algal species like *Chlorella*, *Scenedesmus*, *Cladophora*, *Spirulina*, *Oscillatoria*, *Anabaena*, *Chlorella vulgaris*, *Sphagnum*, *Tetraselmis suecica*, and *Kappaphycus alvarezii* (Perales-Vela et al. 2006; Khan et al. 2008; Pérez-Rama et al. 2002) and through different treatment processes like the high-rate algal ponds

(HRAP) and Algal Turf Scrubber (ATS) (Perales-Vela et al. 2006; Khan et al. 2008; Pérez-Rama et al. 2002; Kumar et al. 2007, 2008). Becker (1983, 1994) concludes that planktonic algae could be used for the removal of residual metals from wastewaters because of its high uptake of heavy metals, and at the same time, value-added biomass could be produced which could be further utilized in production of biogas, fertilizer, fodder, etc. Wilke et al. (2006) reported simultaneous sorption processes, with 30 different algal species, and suggested the following order of selective sorption: $Pb_4Ni_4Cd_4Zn$. Algal affinity and selection criteria for metal sorption (Tüzün et al. 2005) has also been discussed, wherein *Chlamydomonas reinhardtii* was reported to have maximum Hg(II), Cd(II), and Pb(II) ion biosorption capacities (72.270.67, 42.670.54, and 96.370.86 mg g1 dry biomass, respectively).

2.4 Cultivation and Harvesting

Microalgae cultivation processes, which are in use, are mostly based either on open ponds or closed photobioreactors (Davis et al. 2011). For harvesting algal biomass, sedimentation, flocculation, and flotation are used to recover around 1% (dry basis) biomass, and then centrifugation is used for further concentration to 20% (Dassey and Theegala 2013; Barros et al. 2015). These multiple operations are time-consuming and quite costly. A recent techno-economic study conducted by Davis et al. (2011) reveals that the biomass harvesting costs alone account for 21% of the total capital cost of an open pond cultivation system.

2.5 Significance of Phycoremediation

Phycoremediation is the use of microalgae for the removal or biotransformation of pollutants and nutrients from wastewater and capture of CO_2 from the waste air. This process could tackle simultaneously more than one problem which is not possible by conventional chemical processes. The major significance of this process is that it is very much case specific which can be either operated in batch, semicontinuous, or continuous way. The process is highly compatible and cost-effective with existing operations. CO_2 sequestration, which is need of an hour, is very possible alongside. With this process, co-production of biofuels and biofertilizers can be done simultaneously with wastewater treatment. Algal treatment is very selective to remove contaminants (heavy metals) from the wastewater which is being treated over. Above all the significance of phytoremediation that has been mentioned here the most important one is its sustainable and eco-friendly nature from an ecological perspective.

3 Commercial Application of Microalgae

3.1 Biofuel

The microalgae have future prospects for biofuel generation because of its ability of CO_2 sequestration and a high percentage of oil content (Chen et al. 2011). Microalgae are considered as one of the prospectives for clean and economical energy resources (Metting 1996; Spolaore et al. 2006; Thajuddin and Subramanian 2005; Tan 2007). Energy can be extracted from the biomass produced from microalgae using different energy conversion processes (Amin 2009; Brennan and Owende 2010; Mata et al. 2010). The process to convert algal biomass to energy is the process which is generally used for oil extraction and depends on the types and sources of biomass (Lee 2001).

Major steps in algal biofuel generation are the cultivation of biomass and conversion of the biomass (Fig. 2). Photobioreactors and open-air systems are used for this purpose. Photobioreactors allow more precise parameter control for cultivation process which improves the production of biomass; however, the open-air systems are cheaper and simpler in operation but are less efficient. Demirbas and Demirbas (2011) investigated the importance of algae oil as a source of biodiesel. The result shows that different biofuels can be generated through different varieties of algae. Schenk et al. (2008) conclude that algal biofuels appear to be the only current renewable source that could meet the global demand for transport fuels. When *Cladophora* sp. is compared with (Hossain et al. 2008) *Oedogonium* sp. and *Spirogyra* sp., it showed that *Cladophora* sp. produced a higher quantity of bio-

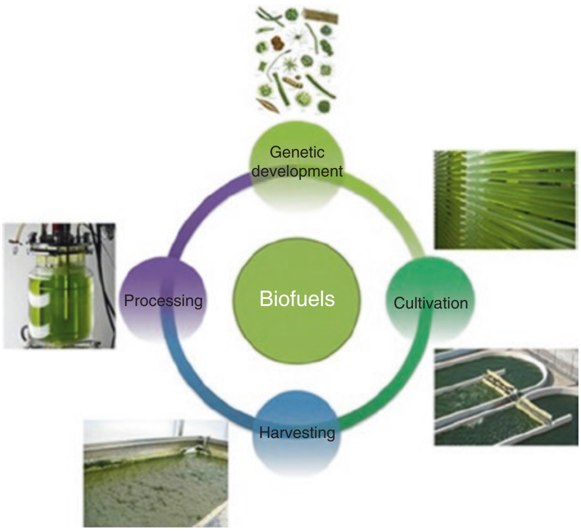


Fig. 2 Process of biofuel production from microalgae

diesel than Spirogyra sp., and extracted oil in the former one was also higher than the latter. In a recent study, Piligaev et al. (2015) observed that strains *Chlorella vulgaris* A1123 and *S. abundans* A1175 have a high total content of saturated fatty acids (SFA) and monounsaturated fatty acids (MFA) (67.0% and 72.8%, respectively) that would allow its use as a source of high-quality biofuels.

3.2 Microalgae in Human and Animal Nutrition

Microalgae, which are rich in biological wealth, are one of the most promising resources which can provide new products with plenty of applications (Pulz and Gross 2004). They can be utilized to increase the nutritional value of human and animal food. Microalgae have been considered as a good diet supplement for malnourished people since 1950; however, nowadays it is popular among health-conscious people as a protein supplement (Spolaore et al. 2006). Becker (1988) suggests that 20 g daily consumption of algae has no harmful effects on the human body. Gross et al. (1978) in their study with *Scenedesmus obliquus* algae 5 g/daily dose for children and 10 g/daily dose for adults, along with their normal diet, found that in the 4-week test period, all the parameters are normal and a slight weight gain was also observed which is good for children. However, adults except health enthusiasts are quite resistant in welcoming their foods with microalgae (Feldheim 1972), maybe because of their conservative ethical and religious values (Gross and Gross 1978; Becker 1994).

3.3 Aquaculture Feed

Microalgae are also used to feed aquaculture for better production. There are two types of cultures used as an aquaculture feed, monoculture and extensive culture. Most recommended monoculture genera for the larval stages of bivalves, shrimp, and certain fish species feed include *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis*, *Nannochloropsis*, *Pavlova*, and *Skeletonema* (Brown et al. 1997; Enright et al. 1986; Thompson et al. 1993). A shrimp larva actively takes up microalgae as a feed, which plays a very important role in nutrition at that stage of its life cycle (Marínez-Córdova and Peña-Messina 2005; Kent et al. 2011).

3.4 Chemicals and Pharmaceuticals

Marine algae have gained a lot of awareness as sources of bioactive metabolites and been appraised by the pharmaceutical industry in drug development. Algae have various medicinal characteristics which make them stand out from synthetic drugs.

Wide ranges of products like antimicrobials, antivirals, therapeutic proteins, drugs, and antifungals can be derived from algae (Budiyono and Kusworo 2012; Sulaymon et al. 2013; Silva et al. 2013; Tomar 2012; Ariyant et al. 2012; El-Sheekh et al. 2012). The strongest water-soluble antioxidants found in algae are polyphenols, phycobiliproteins, and vitamins. This is a widely known fact that antioxidants are very useful in the inhibition of cancer growth by causing regression of premalignant lesions (Ichihara et al. 2016; Bisen 2016; Pastorino et al. 2016; Nguyen et al. 2016; Brandão and Longhi 2016; Liu et al. 2016). Antioxidants fight many diseases including chronic disorders, cardiovascular diseases, and inflammations. Microalgae have a great ability of coiling or protein folding. By folding into specific three-dimensional shape, proteins are able to perform their biological function. Algae can be used to produce human antibodies and therapeutic drugs to treat patients suffering from pulmonary emphysema (Vanadate 2014; Suryanarayanan and Johnson 2015; Singh et al. 2014; Silva et al. 2013; Visconti et al. 2015; Basystiuk and Kostiv 2016; Montoya-Gonzalez et al. 2016). As scientists are looking for cheaper biological drugs, green algae have been the recent area of research shift; with so many applications, it's becoming popular. With its ability to produce the bioactive compound, it is a boon to the pharmaceutical research.

3.5 Biofertilizers

Biofertilizers can not only increase crop yield but also can improve soil health at the same time. Algal biofertilizers like the blue-green algae (BGA) such as *Nostoc* sp., *Anabaena* sp., *Tolypothrix* sp., *Aulosira* sp., etc. have the potential to fix atmospheric nitrogen and are used in paddy fields. Some other types include mycorrhizae, organic fertilizers, and phosphate-solubilizing bacteria. The most important group of biofertilizers are *Azolla-Anabaena* and *Rhizobium*.

3.6 Phyconanotechnology

Algal nanoparticle synthesis can be done in three major steps, "(1) preparation of algal extract in water or in an organic solvent, (2) preparation of molar solutions of ionic metallic compounds and (3) incubation of algal solutions and molar solutions of ionic metallic compounds under controlled conditions" (Thakkar et al. 2010; Rauwel et al. 2015). So far, several seaweeds, namely, *Chaetomorpha linum* (Kannan et al. 2013), *Enteromorpha flexuosa* (Yousefzadi et al. 2014), *Fucus vesiculosus* (Mata et al. 2009), *Turbinaria conoides* (Rajeshkumar et al. 2012), *Sargassum wightii* (Singaravelu et al. 2007), *Stoechospermum marginatum* (Rajathi et al. 2012), *Ulva fasciata* (El-Rafie et al. 2013), and *Ulva reticulata* (Sudha et al. 2013), have been used for synthesizing AgNPs of different sizes and shapes. The polysaccharides are excellent substance for stabilizing and controlling the size of nanoparticles (NPs) when compared to conventional extracts and gum.

4 Challenges in the Commercialization of Algal-Based Technologies

4.1 Availability of Carbon Dioxide

It is well known that the most important factor for the growth of algae is carbon dioxide. At least 1.83 tons of carbon dioxide is used up in growing 1 ton of algal biomass (Chisti 2007). It is reported that nearly every commercial-scale algal culture depends on acquired Co_2 that contributes (~50%) to the algal production cost. The production of biofuel from microalgae is not possible unless free carbon dioxide is available (Chisti 2007). CO_2 produced from different industrial activities can be used to produce algal biomass; also our atmosphere contains around 0.039% of carbon dioxide by volume (Kumar et al. 2010). By anyhow, if this source can be efficiently utilized, the need for carbon dioxide exploited through fossil would entirely fade. Also, utilizing atmospheric carbon dioxide would greatly reduce the carbon footprint of algal biofuels. Unfortunately, till now there is no method for growing algae at a high productivity using only atmospheric carbon dioxide.

4.2 Supply of N and P Nutrients

Along with carbon dioxide, nitrogen (N) and phosphorous (P) are principal nutrients required for algal growth. Phosphorus grant is fixed (Cordell et al. 2009; Gilbert 2009). If we talk about nitrogen, almost as much N fertilizer can be generated, but that will surely demand fossil energy. Haber–Bosch process (Travis 1993), which is presently used for trapping atmospheric nitrogen, needs an enormous amount of energy. As studied by Metz et al., almost 1.2% of global energy consumption is used for producing N fertilizers for agriculture.

4.3 Anaerobic Digestion

Some studies do show that biogas produced by anaerobic digestion can be burnt to fulfill the need of electricity which is quite essential for of algal biomass production and its separation from the water (Chisti 2008; Harun et al. 2011). The extraction of oil through biomass drying is not feasible, or the net energy recovery would be quiet low (Chisti 2012). As mentioned in many studies, cultivating algal species in marine water is the only possible option for biomass production for biofuel generation at commercial scale. Algal biomass derived from the saline source may have to be washed with fresh water to reduce the salt content. This is how N and P fertilizer material redeemed from the anaerobic digester are not too saline to be used in agriculture. Therefore it can be stated that commercializing algal fuels requires vast on-field research on anaerobic digestion for biogas generation and nutrient recovery. A substantial amount of protein is present in algal biomass (González López et al. 2010) and other useful products. Consequently, we can very well interpret that the residual biomass could be regarded as a budding source of animal feed and other products.

4.4 Wastewater as a Source of Nutrients

These days due to the raising of urban population, huge amount of wastewater is generated from cities. The domestic wastewater is a very good source of nitrogen and phosphorous; therefore domestic wastewater provides a very good substrate for algal biomass production (Kosaric et al. 1974; Woertz et al. 2009; Kumar et al. 2010; Christenson and Sims 2011; Craggs et al. 2011). Unfortunately, algal fuels from wastewater can make only a minuscule contribution to the fuel supply. The wastewater produced by a city of ten million people, producing wastewater, could annually provide approximately 425,000 metric tons of algal oil. Approximately 1.25 L of algal oil is energetically equivalent to a liter of petroleum (Chisti 2012). At present not many industrial-scale wastewater-based algal biofuel production plants are present in the world. However, it is envisaged that more plants could be seen in the near future as people are realizing the significance of algal biomass and its useful by-products.

4.5 Carbon Footprint

Any biofuel could be accepted only when its carbon footprints are smaller than the footprint of petroleum on an equal energy basis. Several studies show that the greenhouse emissions from both algal-based biodiesel and soy- or corn-based bioethanol would be equal (Zamboni et al. 2011; Liu et al. 2012). Greenhouse gas emissions from soybean biodiesel were calculated around 49 g MJ-1, which is 82.3 g MJ-1 for petroleum diesel (Hill et al. 2006). This has been estimated that the greenhouse gas emissions for algal biofuel range from 78 to 351 g MJ-1 depending on the production and consumption approaches (Shirvani et al. 2011). Contrary to above, greenhouse gas emissions from the life cycle of algal-based biodiesel have been estimated to be at most about 50% of the emissions from biodiesel produced from canola (Campbell et al. 2011). A life-cycle analysis of marine algae production shows algae are much better than corn, canola, and switchgrass when compared to a raw biomass energy basis. Algal-based biodiesel emits a similar amount of greenhouse gas as switchgrass but much less than canola biodiesel, if emission per vehicle/kilometers traveled are to be compared (Clarens et al. 2011).

5 Future Prospects

5.1 Genetic and Metabolic Engineering for Improving Light Supply to Algae Culture

Research on the application of light on cultivation can be traced back to 1962 when Pipes and Koutsoyannis (1962) established a *Chlorella* family cultivation model and proved that cell density is proportional to irradiation time. From this study, the irradiated *Chlorella* appeared with the amount of growth of 0.275 mL, confirming theoretical predictions. LED lamps (Tamulaitis et al. 2005) were used with four wavelengths for comparison with traditional experiments using high-pressure sodium lamps for cultivating radish and lettuce seedlings: (1) 455 nm, which is closely related to phototropism, (2) 660 nm for photosynthesis, (3) 735 nm (far infrared) for changing plant growth type, and (4) newly produced 640 nm LED lamps. Their experiments revealed that LED light sources outperformed high-pressure sodium lamps at photosynthesis and growth. Advantageously, the new LEDs cost much less than early LED lamps. The experiment proved the critical effects of wavelengths 640 nm and 660 nm on photosynthesis, and plant shape showed obvious changes under far infrared irradiation. An LED photobioreactor can be utilized to enhance the biological production and cell density of *Chlorella* (Fu et al. 2012). Such research proved the feasibility of using LED lighting and carbon dioxide in microalgae biotechnology to further improve biological productivity and other benefits.

5.2 Policy Solutions

Research and development on microalgae-based energy started in the 1950s. There was a huge oil crisis in 1970 in the American subcontinent. The US government has lots of emphasis on the scientific research in the area of marine algae. It was estimated that the USA have invested over 450 million dollars in seaweed energy scientific research since 2008. By the end of the year 2010, this research has been shifted toward refinement of algal biomass for the commercialization of military aviation fuel. The only laboratory called “Pacific Northwest National Laboratory” developed a hydrothermal liquefaction (HTL) method, to achieve a high yield of algae bio-oil production with less time and cost (Sukenik and Shelef 1984). Some institutes and companies like “Scripps Institution of Oceanography (SIO)” and

"Algenol Biotech, USA," achieved a significant breakthrough in algal research. The company produced 9000 gallons of ethanol per acre per year and 1100 gallons of hydrocarbon fuel per acre per year (Lane 2013). European Union established the "Carbon Trust" in 2008 to fund microalgae biofuel-based projects. And it is planning to invest around £20–30 million in order to reduce the production cost of microalgae-based biofuel by developing new methods and processes. The Indian government has also formulated a National Policy on Biofuels, which was adopted on December 24th, 2009. The Policy aims to produce biofuels from indigenous biomass feedstocks (Aradhey 2016). Four challenges have been identified for the commercialization of algae: culture stability and management, scalable system designs, nutrient sources, and water sustainability.

5.3 Prospects of Algae Commercialization

There has been a continuous progress of the algae biofuel's research and commercialization of the same (Singh and Cu 2010). It has been proven that ethanol and diesel production from algae is practicable. The only barrier in the commercialization of the algal biofuels, which has been much reported, is its economic aspects (Ziolkowska and Simon 2014). Algae remain a potential biofuel material for future. It can grow in marginal lands and seawaters; they neither compete with crops or with other biofuel feedstocks for land and fresh water and provide both bioenergy and other coproducts. The basic research is necessary to understand the biological mechanisms of algae's metabolic pathway, which helps in increasing biomass and lipid content production. Research must continue in the area of fundamental genetic principles, growth physiology, metabolite production, and strain robustness (Energy 2016). Algal genetic modification tools remain a technical obstruction. Advanced research in phycology could contribute to the algae genetic engineering. Remarkable production of biofuels should be the long-term goal of the industries to minimize the depletion of our natural recourses. At the same time, the industries need to survive by producing the higher coproducts to offset the costs in short term. Coproducts could help the algal-based companies to make profits and keep the scale-up development of algae products in short and long term as well. Government support places a pivotal role in the commercialization of algal biofuel.

6 Conclusion

The potential use of microalgae for biofuel production with the wastewater treatment has received the great attention of researchers in recent years. Algal biofuel production using wastewaters research has achieved great technological advancement. However, commercialization has still not been taken place at the magnitude which can show visible effects in the energy sector. There are many obstructions to the commercialization of algal biofuels like high cost, the technological bottleneck in cultivation and harvesting, and policy issues. Governments of many countries have invested a large amount of money and resources in order to promote technologies to reduce production cost. Although widespread availability of algal fuels is not likely to happen in near future in a long time, it could provide a sustainable alternative to conventional energy resource. Interest in commercial production of algal fuels particularly by the different governments shows the possibility of an economically viable production. Many algae bioenergy companies are coming to the picture with the multi-supports of investment from governments. However, there is still much work to do to achieve commercialization. Algal biofuel cost can be reduced by giving emphasis on production of high value-added products such as nourishment, medicines, and cosmetics with the oil fuel.

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Potential Biotechnological Applications of Microalgae Grown in Wastewater: A Holistic Approach

Amit Kumar Singh and Abhay K. Pandey

1 Introduction

Surface water pollution, rise in the world's population and industrialisation have emerged as serious threats to the environment as they release millions of litres of wastewater to the nearby waterbodies in the mistaken belief that "solution of pollution is dilution" (Umamaheswari and Shanthakumar 2016). The primary catalyst for industrial revolution was coal, the unsustainable source of energy, and to fulfil the demand of the industrialised world, people started exploiting petroleum fuels and natural gas, and in the very less time, these unsustainable sources of energy became the lifeline of current society. Drawback associated with the use and dependence on fossil fuels is their limited resource. In a study it has been reported that with the current rate of consumption, petroleum reserves will deplete in less than 50 years. Moreover, their utilisation causes adverse effect on our health and environment (Rawat et al. 2011). During burning process of fossil fuel, various aromatic compounds, toxic heavy metals and particulates are discharged into the environment that are responsible for various health ailments including cancer, breathing problem, lung injuries, nerve damage, etc. Scientists around the world are working on innovative ideas to move from petroeconomy to bioeconomy (Lim et al. 2010). So, the need of the hour is to search for a system which provides a strategy to efficiently and economically depollute the wastewater and also help in production of renewable source of energy. Keeping this objective in mind, algae seem to be the appropriate choice to function as an eco-friendly tool for treatment of wastewater with biomass production coupled to biofuel generation (Singh and Pandey 2018). Phycoremediation is a promising option for treatment of wastewater as it lessens the requirement of chemicals and energy in conventional wastewater treatment methods, i.e. centrifugation, filtration, floatation, gravity settling, etc. (Wu et al. 2012).

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Microalgae utilise solar energy and CO_2 to make their food and are found in both freshwater and marine habitats. Microalgae are important resources of bioactive secondary metabolites that have various industrial uses. Algal biomass contains significant amount of proteins, essential amino acid, fatty acids, carbohydrates, chlorophylls, carotenoids, vitamins, etc. that can be utilised as health supplements for humans and animal feed, cosmetics and pharmaceuticals industry, CO_2 sequestration, biofuel production and nutrient removal from wastewater (Sarkar et al. 2006). Algal metabolites have been investigated for their medicinal properties and reported to have antibacterial, antioxidant, antifungal, anticancer, anti-inflammatory and antidiabetic activities (Dominguez 2013). Algae must be grown with appropriate environmental management control to produce product with superior quality and purity. This chapter describes the utility of freshwater and marine microalgae in wastewater treatment, in biofuel production as well as in pharmaceutical and other industrial applications (Singh et al. 2017).

2 Microalgae Industry: Necessity of Wastewater

Microalga are potential sources of next-generation sustainable fuel in comparison to other oil crops, because not only their rate of accumulation of triacylglycerol is higher than other high-oil-yielding crops but also their biomass productivity. Other positive points associated with microalga-based industries are that they do not affect agricultural production and water and land availability because they have the potential to grow on nonarable lands fed with brackish or wastewater (Mishra et al. 2016). Studies have reported that wastewater acts as a growth medium for microalgae-based biofuel production (Chaput et al. 2012). Nowadays algal biomass production is not economically feasible, and the actual cost is about 100€/kg biomass production. Acien et al. (2012) after 2 years of continuous operation calculated that the production cost of a 30 m^3 photobioreactor microalgae plant is about 69€/kg of dry biomass. The reason for this higher production cost is lack of optimised processes and small-scale operations. Based on this small-scale operation and by using techno-economic extrapolation, large-scale production cost is calculated. The average cost of algal biodiesel production is approximately € 2.5 per litre (Quinn and Davis 2016). Still, this is not cost-effective as the production cost of petroleum/diesel is 0.6€/L, and petrochemical industries are sceptical about the use of alga for biofuel generation. Hence reduction in production cost of algae-based biofuel is a major challenge. Economic and environmental drawbacks such as higher nutrient price, water scarcity, phosphorus, the non-renewable source of nutrients can be surpassed by utilising agricultural, municipal and industrial wastewater as nutrient medium. So the need of nutrients and freshwater could be minimised, thereby lowering the production cost and reducing adverse environmental effects of the process and simultaneously depolluting the wastewater, so it is a win-win paradigm (Guo et al. 2015).

3 Microalgae: An Eco-Friendly Approach for Wastewater Treatment

The term phycoremediation is used to address remediation carried out by alga. Oswald reported the applicability of microalgae in wastewater treatment for the first time in the early 1950s (Oswald and Gotaas 1957). Since then it has been in trend, and research have been carried out for applicability of microalgae for wastewater treatment. Usually, this process requires the use of algal species such as *Chlorella* spp., *Scenedesmus* spp., *Chlamydomonas reinharditi*, *C. pyrenoidosa*, etc. for removal or transformation of hazardous toxic compounds to nonhazardous forms, and this process also results into the algal biomass production (Fig. 1), which might be applicable for production of biofuel or other bioactive compounds (Mulbry et al. 2008; Olgum 2003).

3.1 Composition of Typical Wastewater

Mostly, the wastewater contains agricultural and industrial effluents and household discharges which are enriched with nutrients. Various pollutant contaminates the quality of water channel and interrupt the quality and quantity both. The daily life style and technologies being used in the particular society define the wastewater composition (Rai et al. 2011). The pollutant present in wastewater (organic and inorganic both) adversely affects the human and other organisms' health. There are two kinds of wastes that are found in water; one is organic and the other is inorganic. Organic waste is composed of carbon-containing biodegradable substances such as carbohydrate, fats, and proteins, while inorganic waste includes sodium, potassium, nitrate, phosphate, toxic heavy metal, etc. (Gochfeld 2003). Table 1 summarises the

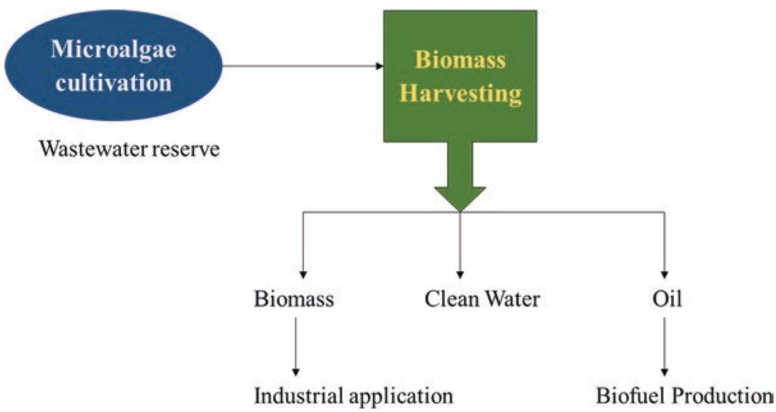

Fig. 1 Integrated wastewater treatment method and biofuel and other bio-based chemical production from microalgae

Table 1 Major classes of municipal wastewater contaminants, their significance and sources

S. N.	Contaminant	Source	Significance
1.	Nutrients (N and P)	Domestic, rural run-off, industrial	High levels of nitrogen and phosphorus in surface water will create excessive algal growth (eutrophication). Dying algae contribute to organic matter
2.	Micro-pollutants (heavy metals, organic compounds)	Industrial, rural run-off (pesticides)	Non-biodegradable compounds may be toxic, carcinogenic or mutagenic at very low concentrations (to plants, animals, humans). Some may bioaccumulate in food chains, e.g. chromium (VI), cadmium, lead, most pesticides and herbicides and PCBs
3.	Pathogenic microorganisms	Domestic	Severe public health risks through transmission of communicable waterborne diseases such as cholera
4	Settled solids (sand, grit)	Domestic, run-off	Settled solids may create sludge deposits and anaerobic conditions in sewers, treatment facilities or open water
5.	Total dissolved solids (salts)	Industrial, (salt water intrusion)	High levels may restrict wastewater use for agricultural irrigation or aquaculture

contaminants present in municipal wastewater. There is no standard compositional analysis of industrial wastewater because it varies with the chemical ingredients present in the manufacturing/processing material used; the components may be the fragmented or modified products of the input material used in the industry. Generally, industrial wastewater is deficient in carbon content but rich in nitrogen and phosphorus contents that support the growth of microalgae in wastewater (Ahluwalia and Goyal 2007).

3.2 Removal of Nitrogenous and Phosphorogenous Compounds

Alga in biotreatment of wastewater for removal of nutrients such as phosphorous and nitrogen are used since several decades ago (Abdel-Raouf et al. 2012; Chan et al. 2014). Over the years, several investigations and laboratory works were carried out to improve the productive efficiency of the treatment. Metabolic interconversions of extra-derived compounds are the primary source of nitrogen accumulation in discharged sewage (Costanzo et al. 2001; Ferreira et al. 2007. And synthetic detergents contribute around 50% or more in total phosphorous content. Nitrogen generally occurs in three physical forms, i.e. NH_4^+, NO_2^- and NO_3^-. $\mathbf{PO_4^{3-}}$ is the principal form of phosphorous in effluents (Duenas et al. 2003; Meybeck 1982;

Ruiz et al. 2003). Together these elements are considered as nutrients, and their extraction forms an effluent termed as nutrient shipping. After treatment of wastewater, where it is aerobic or anaerobic, the inorganic compounds such as nitrate, phosphate and ammonium ions are still present and lead to eutrophication and stimulate the growth of harmful algal bloom in rivers (Marti et al. 2001; Graneli et al. 2008; Davidson et al. 2012; O'neil et al. 2012). Researchers reported that P and N contribute to eutrophication; thus, further treatment of wastewater is necessary to supress the eutrophication of rivers. The use of microalgae in treatment of wastewater is cost-effective. Microalgae's high capacity for mineral intake and ability to grow in mass culture support their use in tertiary phase of water treatment. Also, the use of these biological approaches to treat wastewater produces less or no secondary pollution in comparison to chemical treatment procedures. Several studies carried out on the action of microalgae in the treatment of wastewater provided encouraging results (Kelly and Whitton 1995; Smith and Schindler 2009). It has been shown that *Chlorella vulgaris* removed the inorganic P and N with the removal efficacy of 78% and 86%, respectively (Lau et al. 1995). Other workers have also revealed the nutrient removal efficiency of microalgae from nitrogen- and phosphorus-enriched wastewater (Przytocka-Jusiak et al. 1984).

3.3 Removal of Heavy Metals

Microalgae are great absorbers of heavy metals. Release of toxic contaminants in wastewater has increased with developing industrialisation (Bhargava et al. 2012; Carolin et al. 2017). Heavy metal concentration is significantly high in wastewater, and due to this a better option for sewage treatment is needed for complete removal of these contaminants. Microalgae-mediated metal bioaccumulation may provide a reasonable method for treating heavy metal-contaminated wastewater (Horikoshi et al. 1981). Moreover, algae can be easily cultivated in reservoirs with minimum input of nutrients. It is well reported that the heavy metals are accumulated by several marine and freshwater algae inside the cells. Researchers also concluded that microalgae-mediated heavy metal removal from wastewater effectively produces more reusable effluent water (Pandi et al. 2009).

Different mechanisms are involved in metal sequestration processes by microalgae. It depends on the metal ions and the algal species (whether living/nonliving) and the solution conditions. Trace metals (Co, Ca, Cu, Cr, Mg, Mo, Pb, Se and Zn) in living cells accumulate intracellularly through active transport (Singh et al. 2012). Experiment performed in rice puddles with algae showed Cd^{2+} accumulation by a factor of about 1000 times (Liu et al. 2009). Algae also accumulate tributyltin and organochlorides. Some of these compounds are also broken down by them (Wu and Kosaric 1991).

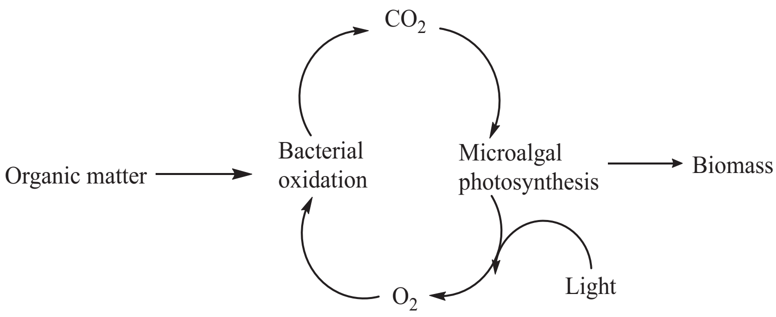

Fig. 2 Mechanism of photosynthetic aeration in BOD removal process

3.4 Reduction in BOD and COD

BOD is used as an abbreviation for biological/biochemical oxygen demand which is described as the quantity of oxygen needed by aerobic microorganism to break the compounds present in a given sample (water). So, it measures the respiratory demand of a microorganism. Chemical oxygen demand (COD) demand, is similar to BOD in that it measures the compound that can be chemically oxidised (Cai et al. 2013).

Photosynthetic microorganisms during their autotrophic growth release O_2 in the waterbodies; this is known as photosynthetic aeration. This photosynthetic aeration results into the reduction of the energy requirements during BOD removal in wastewater by providing oxygen to aerobic microorganism for their growth and breakdown of organic pollutants, and this process releases carbon dioxide utilised by microalgae as depicted in Fig. 2. (Colak and Kaya 1988; Munoz and Guieysse 2008).

4 Common Sources of Biofuel

Biodiesel production has received significant attention worldwide because of increasing demand and limited supply of fossil fuel. Researchers all around the world have started to take series of measures to overcome this issue. Countries that do not have energy resources and completely depend on import of petroleum fuel, their aim is to search for alternative energy sources. Biodiesel is a biomass-derived oil mainly from vegetable oils. Utilising it, has several advantages and seems to be an attractive energy source, i.e.:

1. First, biodiesel is a renewable source of energy and can be supplied sustainably. With the current rate of consumption, petroleum reserves will be exhausted in the next 50 years.

2. Second, it is eco-friendly as it does not result in net increase in CO_2 release and has miniscule Sulphur content.
3. Third, biofuel is environmental friendly as it does not have any aromatic compounds or other complex chemical ingredients.
4. Fourth, microalgae sequester carbon dioxide from the environment through photosynthesis and do not require fertile land as oil crops do (Huang et al. 2010).

Conventional sources of biodiesel are oil crops such as palm, soybean, sunflower, rapeseed, etc. Recently, microalgae have emerged as an oil reserve for biofuel production. In comparison to other oil crops, it has several advantages, viz. much land is not required for their cultivation as required by oil crops and they can be cultivated also on nonarable lands. Also they grow very rapidly and are a rich source of oil. Recent year's technological advancements allowed algae to grow on industrial wastewater, as an alternative strategy for wastewater treatment coupled to biomass production for biofuel production (Chisti 2007).

5 Biofuel Production from Wastewater-Derived Algal Biomass

Large-scale production of microalgae has been used as food supplement or for treating wastewater (Chisti 2007). Based on the studies, it has been found that microalgae have the potential to grow well in wastewater, an appropriate medium for sustainable biomass production. They grow in wastewater because of the presence of nutrient elements, i.e. nitrogen, carbon and phosphorus. Some microalgal species under certain growth medium composition produce lipid at higher concentration (about 80% of dry weight), and the accumulated lipid, whether saturated fatty acid, unsaturated fatty acid, phospholipid or TAG, will depend on species and growth condition. Algal species grown in a photobioreactor in laboratory condition are shown to have higher concentration of cellular lipid (Chisti 2007; Griffiths and Harrison 2009). Scientists also reported another way to increase lipid content in algal species by giving nutrient stress (limitation of N/P) (Dean et al. 2010). Higher lipid production is coupled with lower biomass production, so studies suggested that algal growth must focus on biomass productivity instead of lipid productivity, the basic need of algal biofuel production. Wastewater treatment coupled to biomass production gives an effective method for permanent removal of nutrients from waterbodies (Rodolfi et al. 2009).

Industrial wastewater comprises of very less amount of nutrients (nitrogen and phosphorus) and significant amount of toxic heavy metal pollutants (Cd, Cr, Pb, As, etc.) and other organic toxicants (surfactants, hydrocarbons), responsible for increased generation time of microalgae. However, in municipal and agricultural wastewater, water microalgae show rapid growth rate. Based on the recent findings by Chinnasamy et al. (2010), it has been proposed that carpet mill industry wastewater can be utilised as a medium for generation of considerable amount of algal biomass and subsequently biodiesel, and this is responsible for lowering the cost of algal biofuel production and processing.

6 Microalgae-Derived Bioactive Compounds

Nowadays algae are emerging as a promising source of sustainable crops having various therapeutic application benefits including protein, antioxidants and ω-3 fatty acids. Bioactive compounds present in algal species having various pharmaceutical potential need to be explored. Scientists around the globe are working with an aim to enhance the production and stability of particular bioactive pigments and compounds within certain algal species reported to have nutraceutical or pharmaceutical value. Algae possess significant commercial importance because of their considerable impact on food, pharmaceutical and health sectors. Besides macromolecules, microalgae possess various compounds which are biologically active inside the human body. Researchers around the world have identified several microalgal species for their therapeutic applicability on mammals, and these therapeutically important bioactive compounds are accumulated either inside the cell or released extracellularly into the medium by the algal species (Bhagavathy et al. 2011). Bioactive compounds present in these microorganisms are proteins, fatty acids, polysaccharides, enzymes, vitamins, sterols and several other high-value compounds having pharmaceutical and nutritional applicability that can be explored for commercial use (Priyadarshani and Rath 2012). Various microalgae have shown to accumulate these bioactive compounds in their biomass; however, some algal species excrete these metabolites into the extracellular medium, therefore called as exo-metabolites. Based on the recent findings, it has been proposed that many of these metabolites such as linolenic acid, cyanovirin, palmitoleic acid, oleic acid, lutein, vitamin E, B12, β-carotene, plastocyanin, zeaxanthin, etc. have anticancer, antioxidant, antimicrobial, antiviral and anti-inflammatory properties, with the potential to prevent initiation and progression of disease (Smee et al. 2008; Ibanez and Cifuentes 2013; Markou and Nerantzis 2013; Harun et al. 2010).

Chlorella, a freshwater green alga, has significant amount of proteins, chlorophyll, vitamins, polysaccharides and minerals with essential amino acids that constitute about 53% protein, 23% carbohydrate, 9% lipids and 5% other minerals (Costa and Morais 2013). One of the most prominent bioactive compounds present in *Chlorella* is β-1,3-glucan that reduces free radical formation, blood cholesterol level and heart diseases and also has immunomodulatory properties. And this compound has been examined for its efficacy against gastric ulcers, sores, constipation, atherosclerosis, hypercholesterolaemia and tumour (Spolaore et al. 2006). *Dunaliella* is a unicellular microalga reported to have a huge amount of bioactive natural products with many commercial applications. *Dunaliella* biomass has various therapeutic applications such as antihypertensive, antioxidant, muscle relaxant, bronchodilatory, hepatoprotective, analgesic, and antiedemal properties (Madkour and Abdel-Daim 2013). It is a good source of bioactive compounds like natural β-carotene and produces up to 14% glycerol, lipids, enzymes and vitamins (Francavilla et al. 2010).

Cynobacterial species such as *Anabaena*, *Spirulina*, *Nostoc* and *Oscillatoria*, have higher concentration of biochemically active compounds, viz. lipopeptides, fatty acids, amino acids, macrolides and amides, and they act as reversers of multidrug resistance, antimalarial, antifungal, antifeedant, herbicides and immune modulators. Out of them *Spirulina* is one of the most comprehensively studied blue-green algae, for its use in the treatment of cancer, hyperlipidaemia, obesity, HIV, diabetes and hypertension (Ambrosi et al. 2008).

6.1 Industrial Application

Microalgae synthesise various biologically active compounds that can be harnessed for industrial application. Table 1 summarises the bioactive compounds of microalgal origin having biotechnological applications. Microalgal species such as *Chlorella*, *Chaetoceros*, *Isochrysis*, *Arthrospira* (*Spirulina*), *Dunaliella*, *Nannochloropsis*, etc. are most commonly used for commercial production of proteins, carbohydrates, lipids, carotenoids, vitamins, cosmetics, food colourants and feed additives. Algae such as *Spirulina* and *Chlorella* are now being consumed by humans as food supplements and also used as animal feed. In addition to this, microalgae are now being explored as an eco-friendly tool for treatment of wastewater and assessment of environmental toxicants. As the fossil fuel reserves are decreasing day by day, another potential applicability of microalgae is production of biofuel from their biomass. It is advantageous to include microalgal biomass for biofuel production because there is no such food vs. fuel dilemma (Singh et al. 2017) (Table 2).

7 Microalgae and Human Food

Food industry is one of the major commercial markets for microalgae. These tiny photosynthetic microorganisms contain higher concentration of nutraceuticals such as protein, fibre, carbohydrates and fatty acids; vitamins like A, C, thiamine, riboflavin, pyridoxine and niacin; and minerals like Mg, Fe, K, I and Ca. Therefore, they are major food sources for Asian natives such as Chinese, Japanese, Malaysian, Korean, etc. *Nostoc* has been used by Chinese people for survival during famine about 2000 years back which represented the first use of microalgae by mankind (Ashton et al. 1984). *Spirulina maxima*, *Chlorella vulgaris*, *Dunaliella salina* and *Haematococcus pluvialis* are some of the commercially relevant algal species. *Chlorella* and blue-green algae *Spirulina* are gaining considerable attention

Table 2 Microalgae-derived bioactive compounds, their producers and application

S.N.	Compounds	Microalgal species	Application
1.	*Eicosapentaenoic acid (EPA)*	*Pavlova*, *Nannochloropsis*, *Monodus*, *Phaeodactylum*	Nutritional supplements, aquaculture feed
	Docosahexaenoic acid (DHA)	*Schizochytrium*	Nutraceuticals, infant formula, aquaculture feed
	γ-Linoleic acid	*Cryptocodium*	Nutritional supplements
	Arachidonic acid	*Arthrospira* spp. *Porphyridium*	Nutritional supplements
2.	Phycocyanin	*Spirulina platensis*	Natural dye for food and cosmetics, antioxidants property
	Phycoerythrin	*Porphyridium cruentum*	Used in biomedical research, in diagnostic lab as fluorescent agent
3.	Mycosporine-like amino acid	*Aphanizomenon flos-aquae*	Sunscreen protecting agent
	Sporopollenin	*Characium terrestre*	
	Scytonemin	*Scytonema* spp.	
4.	Single-cell protein	*Spirulina*, *Chlorella*	Food supplements
5.	*ß-carotene*	*Dunaliella salina*	Food colourant, antioxidant, anticancer, provitamin A
	Astaxanthin	*Haematococcus pluvialis*	Anti-inflammatory, antioxidant, pigment for salmon
6.	Lipids and TAG	*Chlorella* spp., *Scenedesmus* spp., *Nannochloropsis* spp.	Biofuel production from biomass
7.	*Biotin*, *α-tocopherol*, *Ascorbic acid*	*Dunaliella salina* *Chlorella* spp.	Nutraceuticals, antioxidant antioxidant
8.	Halogenated compounds	Red algae	Antibacterial, antifungal, antiviral and anti-inflammatory

worldwide as nutritional supplements which are available in markets as single-cell protein in tablet form (Colla et al. 2007; Sajilata et al. 2008). Hills and Nakamura (1978) reported that by-product of *Chlorella* is also economically important as preservative for fruits and vegetables. They are also reported to have probiotic compounds that maintain the balance of intestinal flora. β-Carotene and photosynthetic pigments are obtained from *D. salina*. This species is also used as food colourant for orange juice and as a vitamin C supplement (Becker 2004; Pulz and Gross 2004). Hydrocolloids such as agar, alginates and carrageenan are polysaccharides in nature and available in the market. They have potential uses in foods and industrial products. Food processing industries use these hydrocolloids for providing desired texture and as food preservatives because of their water-holding capacity (Venugopal 2009; Bixler and Porse 2011).

8 Application of Microalgae in Cosmetic Industry

Algae are the undisputed treasures of the sea and are of great importance in cosmetic industry. Microalgal species such as *Chlorella* and *Arthrospira* are well established in the cosmetic industry. Algal extracts are being utilised as skin care products such as anti-irritant in peelers, dirt removal hair care products, anti-ageing cream and emollient. In addition, they are also used in hair care products and sunscreen industries. Based on the finding, it has been reported that crude extract of edible seaweed, called *Alaria esculenta*, induces a significant decrease in the expression of gene that encodes a protein, progerin, which accumulates in ageing tissues and is expressed more in older fibroblasts (Singh et al. 2017; Verdy et al. 2011).

Bioactive compounds present in algal extract promote blood circulation and maintain moisture and functioning of sebaceous glands of the skin. Among the bioactive components isolated from microalgae which have significant use in cosmetic industry, polysaccharides are used as thickeners and gelling agents in various cosmetic formulations. They also provide moisturisation because of their water retention property (Jain et al. 2005). *Fucus vesiculosus* and *Turbinaria conoides*, the brown algae, produce polysaccharides such as laminaran, fucoidan and alginates which show antioxidant activities and are applied topically as anti-ageing cream to prevent skin disorders (Jea et al. 2009). Some of the algal species that are being studied and utilised in cosmetic industries are *Botryococcus*, *Chlorella*, *Dunaliella*, *Haematococcus*, *Phaeodactylum, Porphyridium*, *Spirulina*, etc. (Rosenberg et al. 2008; Raja et al. 2008; Borowitzka 2013).

Biologically active ingredients extracted from microalgae shown to increase the renewable capacity of cell, metabolism, provides resistance towards bacteria and fungus, antioxidant and anti-inflammatory property. *Porphyra umbilicalis* synthesises a large amount of substances, mycosporine-like amino acids, that have the tendency to not only absorb ultraviolet radiation but also decrease melanin synthesis (Shick and Dunlap 2002). Pigments isolated from red algae species are being used in cosmetic industries as colourants for cosmetic formulations such as eyeliners, face make-up and lipstick (Kim et al. 2008). Compounds isolated from seaweeds have antibacterial and antifungal property, and when used in skin care product, they maintain the skin flora by preventing the growth of such organisms. Red algae *Rhodomela confervoides* and brown algae *Padina pavonica* extracts are reported to be effective against *Candida albicans* and *Mucor ramaniannus*, respectively (Saidani et al. 2012).

Microalgal species are reported to produce compounds like mycosporine-like amino acid, sporopollenin and scytonemin to protect themselves from sun damage, and nowadays cosmetic industries are using these components to produce sunscreen (Shick and Dunlap 2002). Proteasomal activity of skin cells protects them from adverse effects of UV rays and improves skin elasticity and firmness. Extract from *Phaeodactylum tricornutum* promotes proteasome activity in skin cells (particularly melanocytes, fibroblasts and keratinocytes) and delays the appearance of wrinkles and/or reduces their depth (Nizard et al. 2007).

9 Conclusion

Microalgae are being used as an eco-friendly tool for wastewater treatment. They utilise the carbon and nitrogen sources present in the wastewater for their growth and development. And the use of microalgal biomass produced for commercial application is significantly an increasing and promising area of research. The dual use of microalgae for wastewater treatment as well as for industrial application of biomass is an attractive choice to overcome operational cost. Various bioactive compounds isolated from microalgae have significant uses in healthcare sectors and industries. Major challenges associated with algal biomass production utilising wastewater are designing optimum cultivation system production and downstreaming of algal metabolites of commercial uses.

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Microalgal Biofuels Production from Industrial and Municipal Wastewaters

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1 Introduction

World energy demand is continuously increasing due to increasing world population growth and industrialization, and it is expected to reach 9.2 billion by 2050 and 11.2 billion by 2100 (Lage et al. 2018; United Nations WPP 2017). Continued use of fossil fuels will increase energy prices and diminish crude oil reserves. Therefore, in the future, energy based on fossil fuels may be economically unsustainable (Hill et al. 2006). The main challenge of the global economy is energy security and environmental conservation. At present, the global economy mainly depends on nonrenewable and fossil fuels. Excessive use of fossil fuels poses challenges relating to energy insecurity and environmental pollution. Thus, industry needs to look for alternative strategies to overcome such challenges by developing economic and environment friendly biofuels (Chen et al. 2011; Kassim and Meng 2017).

Microalgae biomasses have attracted considerable attention as a potential feedstock for producing sustainable biofuels, including biodiesel, bioethanol, biomethane, biosyngas, and biohydrogen. Microalgal biofuel is one of the best potential alternatives to fossil fuel. However, during algal biofuel production, cultivation costs exceed approximately 70% of total costs, which is the major constraint for the algal biofuel industry. To reduce the cost of cultivation, various strategies are adopted. Among others, the use of various types of treated and untreated wastewater as nutrient media for the cultivation of algae seems to be best option for the production of biomass for biofuels at relatively lower cost. The use of wastewater to cultivate microalgae substantially reduces the need for chemical fertilizers and their

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related economic burden on the life cycle of wastewater-based algal biofuels (Clarens et al. 2010; Rawat et al. 2013; Soratana et al. 2013).

Green microalgae, including diatoms, are sunlight-driven cell factories that can convert atmospheric carbon into valuable carbohydrates, lipids, proteins, pigments, and vitamins. Hence, they can be used as potential sources for biofuel, food, feed, and high-value bioactive compound production. In addition, arable land is not required for microalgae growth and they can be grown in different types of wastewater. Microalgae are used for wastewater treatment because they can exhibit fast growth rates all year round, efficiently remove nutrients (carbon and nitrogen sources) and heavy metals from wastewater, while simultaneously utilizing atmospheric carbon dioxide (CO_2) in large amounts (Singh and Gu 2010; Markou and Nerantzis 2013; Koller et al. 2014; Fu et al. 2015; Simas-Rodrigues et al. 2015).

Microalgae are a highly diverse group of photoautotrophic organisms that occur in various natural water habitats, while some species are capable of growing under heterotrophic/mixotrophic conditions. In recent years, much attention has been paid to microalgae cultivation in different wastewater sources due to their relatively high tolerance to varying nutrient sources and loads. Various physicochemical parameters of wastewater, such as pH, irradiance, temperature, carbon, nitrogen, phosphorus, and micronutrient (e.g., Cu, Fe, Mn, Zn) influence microalgal growth and its biochemical composition. Thus, microalgae biomass productivity and its biochemical composition are strongly dependent on the mode of cultivation and the nutrient profile of the growth medium. Therefore, species selection and cultivation conditions may improve the growth, biomass yield and productivity of value-added products produced by microalgae growing in wastewater (Guedes et al. 2011; Abdel-Raouf et al. 2012; Nur and Buma 2018). Using microalgae for various wastewater treatments is an energy efficient way to reduce challenges associated with wastewater treatment costs (Tam and Wong 2000; Wu et al. 2015). Wastewater can be used as media for microalgae cultivation to produce biomass that can be used for the production of various biofuels. By photosynthesis, microalgae convert wastewater nutrients into useful carbohydrates (sugars) and triacylglycerols (fats) that can be used as raw materials for the production of third-generation biofuels (Chisti 2007; Singh et al. 2016).

Microalgae utilize the available nutrients of wastewater, so the wastewater gets treated over the course of cultivation. However, nutrient removal depends on cultivation conditions, type of species, geographical conditions, and other factors. Integrated phycoremediation (wastewater treatment) and biofuel technology appear to be the best way to sustainably produce biofuels. The potential of microalgal growth in various wastewaters on land that thus far has been considered nonarable with high biomass productivity makes phycoremediation an interesting subset of bioremediation (Gupta et al. 2015). All kinds of phycoremediation techniques have been proposed because of the potential using such techniques to treat different types of wastewater and biomass production. It has already been proved that microalgal species can accumulate lipid contents of up to 70% in their cells; however, lipid yields differ from one species to another as well as culture conditions. Microalgal biomass produced from wastewater can be utilized for the production of various biofuels, including crude oil, biodiesel, biomethane, bioethanol, and biohydrogen, for example (Li et al. 2011; Gupta et al. 2016a, b, c). Based on substantial lipid yields, microalgae possess more advantages over other oil-yielding crops because arable lands are not required for microalgae cultivation purposes (Gupta et al. 2017). In addition, microalgal biomass can be used as a main feedstock for the production of various biofuels, so it has attracted much attention from researchers in recent years, but large-scale biomass production is still not economically feasible because commercial media are expensive. Microalgal biomasses are considered the most promising sustainable biofuel feedstock. However, the high water use and fertilizer demand for microalgal cultivation still make large-scale commercial application difficult (Pires et al. 2013). The growth efficiency of microalgae in various industrial wastewaters for biomass production depends on the pH, temperature, concentration of heavy metals, essential nutrients, including nitrogen, phosphorus, and organic carbon, CO_2 levels, and light availability. Currently, the treatment of industrial wastewaters using microalgae is being explored by academic researchers and researchers in various industries. The cyanobacterium *Arthrospira platensis*, when grown in olive-oil mill wastewater, removed the maximum 73.18% of chemical oxygen demand (COD), while phenols, nitrates, and phosphorus were completely removed (Markou and Nerantzis 2013). Similarly, *Chlorella pyrenoidosa* removed about 60–80% of total nitrogen and 80–85% total phosphorus from dairy wastewater. The freshwater microalgae *Chlorella zofingiensis*, grown in piggery industry wastewater (PWW), at an optimum COD concentration of 1900 mg L^{-1}, showed the highest biomass, lipid, and biodiesel productivity of 296.16 mgL^{-1} day^{-1}, 110.56 mg L^{-1} day^{-1}, and 30.14 mg L^{-1} day^{-1}, respectively (Zhu et al. 2013).The potential of microalgae to grow in different wastewaters represent the best way to overcome this hurdle, in combination with wastewater treatment (Chinnasamy et al. 2010). This is beneficial for reducing the use of freshwater, reducing the cost of nutrients, removing total nitrogen and phosphorus, which are responsible for eutrophication, and producing microalgal biomass as a bioresource for biofuels or high-value byproduct production (Kothari et al. 2012; Kumar et al. 2015; Lu et al. 2015). However, before microalgae is produced on a large scale using wastewater, some obstacles, such as unsuitable microalgae species, bacterial contamination, ammonia inhibition, and turbidity, should be overcome (Hu et al. 2012).

2 Integrated Industrial Wastewater Treatment and Microalgal Biomass Production

2.1 Utilization of Piggery and Brewery Wastewater

Nutrient pollution through piggery wastewaters (PWWs) is becoming a severe problem when disposed of without proper treatment due to the presence of high levels of organic materials. However, PWW is extremely rich in ammonium nitrogen and phosphorus, which can be used as a nutrient source for the cultivation of microalgae, which could be one of the best sustainable measures to control pollution as well as production of biofuel feedstocks (Bai et al. 2012). This could be a promising approach to producing sustainable energy to avoid future energy crises and mitigate global climate change. Various studies have demonstrated that PWW could serve as an excellent nutrient medium for the production of different oleaginic microalgae species such as *Chlorella vulgaris*, *C. zofingiensis*, *Scenedesmus quadricauda,* S. *dimorphus,* and *Arthrospira platensis* (Bai et al. 2012; Zhu et al. 2013).

The microalgae *C. sorokiniana* and *Euglena viridis* are stress-tolerant specific strains capable of growing in four fold-diluted swine slurries with bacteria-activated sludge (de Godos et al. 2010). In such cultivation systems, microalgae can supply oxygen for aerobic bacteria to biodegrade organic pollutants and in turn take up the CO_2 that is released by bacteria via respiration (Munoz and Guieysse 2006). However, microalgae may detrimentally affect bacterial growth by increasing the culture pH/temperature or by releasing inhibitory metabolites (Gonzalez and Bashan 2000), while some bacteria may release extracellular enzymes like cellulose that can damage microalgae cells (Zhang et al. 2012). Due to very high ammonium concentrations and bacterial loads, PWWs may inhibit microalgal growth if used directly. Therefore, the pretreatment of PWW represents a major challenge for its use as a culture medium requirung some eco-friendly techniques instead of chemically based pretreatment methods. The highest specific growth rate of 0.033 day^{-1} of *Chlorella* was observed in undiluted digested piggery wastewater pretreated with fungi (Liu et al. 2017). The green microalga *C. zofingiensis* efficiently utilized PWW at a level of 1900 mg L^{-1} COD, resulting in high biomass, lipid, and biodiesel productivity of 296.16 mg L^{-1} day^{-1}, 110.56 mg L^{-1} day^{-1}, and 30.14 mg L^{-1} day^{-1}, respectively (Zhu et al. 2013).

The brewing industry utilizes large quantities of water and generates huge amounts of wastewater. The wastewater derived from brewing is rich in organic compounds, which includes proteins, phosphates, nitrate, or ammonia. In addition, brewery wastewater (BWW) contains easily biodegradable sugars, soluble starch, ethanol, and volatile fatty acids (Raposo et al. 2010). However, BWW can pose a serious problem to human beings and aquatic life if released untreated into the environment. Therefore, the treatment and safe disposal of BWW have become important aspects in the brewing industry (Farooq et al. 2013).

Due to the potential of microalgae to utilize wastewater for growth, they can be used in BWW treatment. Using BWW for the cultivation of microalgae is beneficial for minimizing the usage of freshwater, reducing nutrient costs, removing nutrients, and producing biomass as feedstock for biofuel production and value-added products (Schneider et al. 2013). However, microalgae cultivation in BWW requires optimum temperatures, pH, air, and light intensity. The pH range from 6.1 to 8.5 and temperature from 28 to 32 °C in BWW are suitable for microalgae cultivation. Few studies have demonstrated the potential use of microalgae for BWW treatment (Choi 2016).

2.2 Utilization of Aquaculture Wastewater

Wastewater generated from aquaculture contains high amounts of nitrogen, phosphorus, total suspended solids (TSSs), volatile suspended solids, biochemical oxygen demand (BOD), and COD. Untreated aquaculture wastewater discharged into natural water bodies results in the eutrophication of aquatic systems (Khatoona et al. 2016; Lananan et al. 2014; Mook et al. 2012). Microalgae can be used for simultaneous aquaculture wastewater (AWW) nutrient removal. High rate microalgal ponds (HRMP) and algal settling ponds are commonly used for aquaculture wastewater treatment. HRMPs are systems with a large surface area and shallow profile (0.2–1 m deep) that increase the exposure of microalgal cells to sunlight for enhanced biomass production (Park et al. 2011).

Microalgae such as *Tetraselmis suecica*, *Isochrysis galbana*, and *Dunaliella tertiolecta* have been found to be suitable species for the treatment of unsterilized gray mullet *Mugil cephalus* wastewater with high biomass production (Andreotti et al. 2017). In addition, many microalgal genera such as *Chlamydomonas, Chlorella, Nannochloropsis*, and *Scenedesmus* have been found to be suitable candidates for simultaneous nutrient removal from wastewater and biomass production for biofuel production (Sirakov et al. 2015). These genera could be applied to various AWW treatments without any secondary pollution, and the biomass produced is reused. Using AWW for microalgae cultivation is seen as a low-cost and eco-friendly technique and an alternative wastewater treatment technique for conventional methods of wastewater treatment (Ahmad et al. 2013; Hii et al. 2011).

2.3 Utilization of Dairy Industry Wastewater

In recent years, the consumption of milk and milk products has increased, and thus the number of dairy-related companies has also increased along with dairy wastewater (DWW). DWW is easily biodegradable due to the presence of high organic matter, high protein content, and lower levels of heavy metals (Sarkar et al. 2006). However, despite its low levels, DWW should be properly treated prior to disposal; otherwise, it poses a serious risk to the environment and aquatic life (Karadag et al. 2015). Therefore, DWW treatment and safe disposal has become an important consideration for the dairy industry. Generally, DWW is treated in anaerobic digesters to remove its high organic content. The use of DWW for microalgal cultivation has the potential to significantly improve the economics of biofuel production. Microalgae consume these organic carbons largely for their metabolic activity (Wang et al. 2010). Microalgae can perform the dual role of phycoremediation of DWW and high biomass and lipid production (Farooq et al. 2013).

Currently, various microalgal genera are being used for DWW treatment in order to achieve both the treatment of DWW and biomass production. Due to the potential of microalgae to use DWW for their growth, microalgae are particularly useful for the removal of inorganic nitrogen and phosphorus from DWW (Hena et al. 2015; Kothari et al. 2013). Kothari et al. (2012) reported a removal rate of 80%–85% phosphorus and 60–80% nitrogen by *Chlorella pyrenoidosa* from DWW. Raw DWW provides better conditions than the synthetic medium BG11 for the cultivation of *Chlorella* sp., *C. zofingiensis*, and *Scenedesmus* sp. All three of these strains removed 100% ammonia from raw DWW in less than 6 days. A study by Shu et al. 2018 revealed that microalgal biomass produced using DWW would be suitable feedstock for biodiesel production.

2.4 Utilization of Sewage Wastewater

Sewage wastewater (SWW) is mostly discharged directly into waterways without any pretreatment in most developing countries, and this is increasing day by day due to run-down state and poor management of conventional water treatment plants. However, SWW is an ideal medium for various microorganisms, including bacteria, viruses, protozoa, and cyanobateria, for example. It contains high amounts of organic and inorganic materials. Three quarters of the organic carbon in SWW is amino acids, carbohydrates, proteins, fats, and volatile acids. Similarly, the inorganic constituents comprise ammonium salts, bicarbonate, calcium, chlorine, magnesium, phosphate, potassium, sodium, sulfur, and heavy metals (Lim et al. 2010; Abdel-Raouf et al. 2012).

Ammonia, nitrate, and phosphate removal from SWW by a tertiary process is about four times more expensive than the primary treatment. In addition, this conventional approach is not sustainable. The cultivation of microalgae in SWW offers an environmentally sustainable platform for tertiary and quaternary treatments due to the inherent ability of green algae to consume nitrogen and phosphors for their growth. Around 60 years ago, the fascinating idea of the biotreatment of SWW using microalgae was launched by Oswald and Gotaas (1957) in the USA, and since then it has been intensively studied in several countries (Abdel-Raouf et al. 2012). Microalgae serve the dual purpose of bioremediation and potentially valuable biomass production, which can be used for various applications (Gupta et al. 2016a, b, c). A study by Gupta et al. (2016a) showed that the biomass production of a green microalga, Chlorella vulgaris was 1.39 gL^{-1} dry cell weight in glucose (5 g/L)-supplemented municipal wastewater (MWW) and the biomass productivity of 0.13 gL^{-1} day^{-1}. This biomass accumulated 19.29 ± 1.83% of total lipid that contained 61.94% of saturated fatty acid methyl esters. *C. vulgaris* utilized the MWW as a nutrient medium for the potential production of renewable biomass and high-value microalgal oil. Microalgae are known to remove excess nutrients from MWW by nutrient uptake and biomass production. Nutrient removal efficiency depends on the microalgal species (Wang et al. 2010; Zhang et al. 2017). Microalgal biomass can be used for the production of biofuels (bioethanol, biodiesel, biomethane, and biohydrogen), animal feed, poultry feed, and other high-value products such as astaxanthin and carotenoid (Ding et al. 2016). The biomass productivity of three green microalgae, such as *Chlorella vulgaris*, *C. kessleri*, and *Scenedesmus obliquus* grown in SWW, was higher than the control of those species cultivated in a synthetic medium (Alvarez-Diaz et al. 2017). These species are promising sources of bioenergy and reducers of wastewater pollution. Utilization of SWW as a growth medium for the cultivation of oleaginous microalgae for lipid production could also be an efficient means of reducing biofuel production costs.

2.5 *Utilization of Textile Wastewater*

Textile wastewater (TWW) contains comparably higher color, salinity, temperature, BOD, TSS, total dissolved solids (TDS), COD, and varying pH (Kaushik and Malik 2009). The high to very high concentration of such parameters makes it tougher to clean TWW by biological methods. However, biological treatments using microalgae could be a cost-effective and an efficient alternative method for the treatment of TWW. Microalgae can decolorize TWW through several mechanisms like biosorption, bioconversion, and bicoagulation (Khalaf 2008). Therefore, microalgae are considered a better choice for TWW treatment compared to other microorganisms owing to their photosynthetic capabilities, which can be useful in biofuel production and removal of nutrients and other pollutants (Kumar et al. 2014; Fazal et al. 2018). TWW is an inexpensive source of water for microalgae cultivation because it contains various nutrients such as phosphate, nitrates, micronutrients, and organic dyes. Microalgae can be used in TWW treatment to remove color and nutrients and to produce microalgal biomass that can be used for the production of various types of biofuels. This integration process could potentially improve economic biodiesel production as well as wastewater treatment (Fazal et al. 2018). Some microalgae species, for example *Chlorella vulgaris, C. pyrenoidosa*, *Spirogyra* sp., *Oscillatoria tenuisin*, and *Scenedesmus* sp., have shown their capability to grow in TWW (Khalaf 2008; David et al. 2014). However, these microalgae are not only used to treat TWW but also synthesize large amounts of lipids that can be used for biodiesel production (Wu et al. 2012; Khandare and Govindwar 2015; Roberts et al. 2013).

3 Optimization of Biomass Yield

Microalgal biomass can be used for the production of long-chain polyunsaturated fatty acids, vitamins, pigments, and biofuels including photobiological hydrogen, biodiesel, biomethane, and bioethanol (Chisti 2007). Algae can be used for wastewater treatment and mitigation of greenhouse gases (GHGs) by CO_2 fixation. However, in recent decades, microalgae have attracted significant attention for their potential in biofuel production. High actual photosynthetic yield compared to terrestrial plants may lead to the large-scale production of microalgal biomass in photobioreactors, which could be several tens of tons per hectare per year, depending upon the species and cultivation conditions. Several growth enhancement techniques can be used to improve the yield of microalgal biomass. Large-scale cultivation will decisively contribute to sustainable industrial-scale production of biomass and cost-effective value-added products. Many species of microalgae exhibit potential for large-scale cultivation, but there is scanty information about commercial-scale trials on biomass production and wastewater treatment. A large volume of microalgal biomass is required to compete with other feedstocks for sustainable biofuel production. Successful microalgae cultivation techniques will need to generate large quantities of biomass if they are to be used for biofuel production (Khan et al. 2018).

Microalgae can be cultivated by different methods and under different physicochemical conditions. Mainly they need a light source to convert water and CO_2 into biomass through photosynthesis (Ozkurt 2009). Carbon (C) is a most essential nutrient for the growth of any living organism. More specifically, the form of available carbon induces the metabolic pathway by which the microalgae fix carbon. In general, there are two ways of carbon fixation in microalgae: (1) autotrophic, in which the inorganic carbon (CO_2) fixation take place by the Calvin-Bensen cycle (photosynthetic growth); and (2) heterotrophic, where organic carbon assimilation take place in the absence of light (Lee et al. 1996). However, some microalgae can exist in either photoheterotrophic or mixotrophic conditions (Ogawa and Aiba 1981; Marquez 1995; Lee et al. 1996). The importance of metabolic pathways to microalgal biomass production relates to the effect on growth rates, biomass yields, and their biomolecules such as carbohydrates, proteins, and lipids. In addition, there will be corresponding infrastructural and optimum process condition input requirements (e.g., light and carbon source) associated with each metabolic condition.

Natural metabolic mechanisms are promising avenues for biomass and lipid production in microalgae. Ideally, cultivation of microalgae using wastewater by heterotrophic or mixotrophic will acquire improved flexibility in production of algal biomass as well as it's comparably economic while generating a more concentrated biomass and valuable product. These metabolic pathways for culturing microalgae in wastewater can yield greater biomass and biomolecule accumulation. However, because insufficient information exists on major commercially attractive microalgae species, they must be explored for specific target products like lipids, carbohydrates, proteins, pigments, and wastewater to ensure that increases in biomass yields are not offset by challenges or costs. Huge quantities of wastewater are produced by several industries through various processes that contain various pollutants in different quantities (Hussain and Khan 2003). However, the majority of industries discharge heavy metals in wastewater. The physicochemical characteristics of industrial wastewater vary from one industry to the next. Optimization of culture conditions for the cultivation of oligogenic microalgae in industrial wastewater is needed.

To achieve the maximum growth of microalgae, the cultivation temperature should be maintained between 20 and 30 °C. In addition, several nutrients are also required in huge quantities for microalgal growth. Thus, nutrient supplementation can be done through industrial wastewater (Abdel-Raouf et al. 2012; Dalrymple et al. 2013; Tan et al. 2017). Usually, microalgal growth increases with increasing temperature up to a certain level. Direct cultivation of microalgae in industrial wastewater is quite difficult due to varying pH levels. Nevertheless, the majority of microalgal species are able to tolerate varying levels of pH tolerance abilities (Zhu 2015).

4 Optimization of Lipid Yield

Microalgae can accumulate large amounts of neutral lipids in the form of hydrocarbons or triacylglycerol (TAG). This lipid is considered a promising renewable resource in the production of biodiesel and omega-3-rich oil (Naghdi et al. 2016). For pilot-scale microalgae-based biodiesel production, improvements in lipid productivity is very important to make algal-based biofuels economically viable. So far, significant advancements have been made at the laboratory scale and in field testing only (Hu et al. 2008). Large-scale production of microalgae-based biodiesel still lacks economic viability due to the higher production cost with lower lipid yield. Therefore, the optimization of lipid yield of microalgae is most important for microalgae-based biodiesel production (Yang et al. 2014). Some oleaginous species when grown under optimal conditions can allocate 70% of their biomass. As the lipid synthesis metabolism is species-specific, however, it is heavily influenced by environmental conditions. Extensive researches have revealed that both nutritional and environmental conditions can efficiently alter lipid synthesis metabolism in microalgae (Sharma et al. 2012).Therefore, to meet specific production goals, various strategies are applied to improve the yield of lipids and modify the fatty acid composition of algal biomass. The optimization of physiochemical parameters such as mode of nutrition, pH, temperature, and nutrient concentration are well-known engineering processes and is a simple approach that can make changes in biochemical composition including lipid quantity and quality of microalgal biomass (Lari et al. 2016). Most microalgae can accumulate lipids under specific stress conditions (e.g., N depletion or high salinity). Nutritional and environmental factors, mainly the concentration of CO_2/light intensity, and growth phase are the major cultivation parameters that affect lipid content and quality in microalgae (Van Wagenen et al. 2014). For instance, the lipid yield of *Nannochloris* sp. UTEX LB1999 had an 83.08% increase following a decrease in the concentration of nitrogen to 0.9 mM (Takagi et al. 2002)

Nitrogen sources in organic and inorganic forms are considered a very important nutrient factor for the growth and lipid synthesis of microalgae (Yeh and Chang 2011). However, nitrogen starvation stress results in comparatively slower growth but in significantly higher lipid accumulation in microalgae. Nevertheless, prolonged nitrogen starvation in culture medium can eventually lead to a high mortality of microalgae cells. Therefore, it is very important to establish appropriate cultivation conditions specific to either biomass rich in protein and carbohydrate or biomass with maximum lipid content (Wan et al. 2013). Saline wastewater is produced from various industrial processes, including food processing, aquaculture, oil production, tanneries, textiles, and wine production (Lefebvre and Moletta 2006). The treatment of saline wastewater offers more benefits compared to other bioremediation technologies as large quantities of biomass can be produced during such treatment processes that can be used as biofuel or fertilizer (Church et al. 2017). In addition, salinity stress can also improve lipid synthesis in oleaginous microalgae species (BenMoussa-Dahmen et al. 2016; Church et al. 2017). In mixotrophic cultivation, *Chlorella vulgaris* produced 15.4–25.6% lipids when grown in 35 g L^{-1} NaCl medium rather than no NaCl (Heredia-Arroyo et al. 2011). The new cultivation techniques that involve manipulating culture conditions for the potential enhancement of biomass and lipid production are essential nowadays.

Intensity of light is also a major limiting factor in pilot-scale cultivation of microalgae. Light duration and intensity can directly affect microalgal photosynthesis and affect biomass and lipid yield. However, the light intensity requirements for microalgae species vary from one species to another. The optimum light intensities for most microalgae species range from 200 to 400 μM photons/m^2/s (Krzeminska et al. 2014). Kitaya et al. (2005) experimentally demonstrated that a light intensity of 100 μmol/m^2/s is optimum for some microalgal species. Similarly, temperature is another important physical factor that also directly influences the growth of microalgae and the synthesis of macro biomolecules such as carbohydrates, lipids, and proteins. The optimal temperature of different species for growth is entirely different. When the temperature increases, exponential increases in the microalgal growth can be observed up to the optimum range and vice versa. However, an increase or decrease in temperature beyond the optimal level retards or even stops microalgal growth and activity (Bechet et al. 2017). A temperature range of 20–30 °C is the optimum for most microalgal species (Singh and Singh 2015). The pH of the growth medium is another very important factor directly affecting microalgal growth and lipid synthesis. Generally, microalgal species have different pH requirements. However, a pH range of 6–8.76 is optimal for most species. Most microalgae species are sensitive to pH, but few can endure a broad range of pH (Lam and Lee 2012).

5 Scope of Pilot-Scale Biomass Production Using Industrial Wastewater

The current method of biofuel production from crops depends entirely on limited arable lands, so large-scale production is impossible to meet global biofuel demand without disrupting food production. However, large-scale microalgae biomass production using industrial wastewater could offer new insights for the biofuel and wastewater treatment industries. To reduce the cost of biomass production, various industrial wastewaters could be used as growth media for cultivating microalgae. This is a prerequisite for economical microalgae biomass production to sustain the present energy market.

The pilot-scale production of microalgae biomass using industrial wastewater will help to reduce demands/requirements of chemical fertilizer and mitigate the environmental burdens associated with CO_2 and GHGs. Growing microalgae in industrial wastewater has a number of benefits, including providing a supply of nonlignocellulosic biomasses, which will minimize the hydrolysis costs of the downstream process for lignin removal and maximize the generation of biogas yield in a shorter amount of time. Also, microalgae biomass can be made available throughout the year.

Integrating the approaches of industrial wastewater treatment and biomass production using microalgae is a strategy for economical large-scale microalgal biomass production. This coupling process can reduce the costs of culture media, promote onsite local industries, and eliminate the large negative environmental footprint. Microalgae have the ability to effectively remove a broad category of chemicals from industrial wastewater by various modes of nutrition such as phototrophy, heterotrophy, and mixotrophy.

The most promising use of microalgae biomass is in the energy market as biodiesel, bioethanol, biomethane, syngas, biohydrogen, and biochar, as well as in the production of aquaculture feed and biofertilizer, for example. Microalgae biomass–derived biochar could be used as a commercial substrate for plant growth in hydroponic systems as it can meet the requirements for high capacity of nutrient solution maintenance.

The scale-up of biomass production using industrial wastewater will be indispensable in the future because of rapid urbanization and industrialization. At this juncture, the integrated approach of industrial wastewater treatment, as well as biomass production using microalgae, is one of the most promising strategies for economical and large-scale production of biofuels. However, the expansion of advanced appropriate cultivation methodologies is necessary to improve biomass yield up to the desirable quality. The commercialization of biomass production using industrial wastewater is unavoidable in the future.

6 Use of Algal Biomass for Biofuels

6.1 Biodiesel

Third-generation biodiesel produced from lipids of microalgae has attracted serious attention globally because of its significant advantages over first- and second-generation biodiesels, which are synthesized from edible and nonedible oils. Microalgae biomasses are the most feasible feedstock for third-generation biodiesel. Microalgae can produce and store TAGs within their cells in a stressed environment. In such circumstances, the microalgae cells stop cell division and store TAGs to withstand these adverse conditions (Narala et al. 2016).

For successful cultivation of microalgae for industrial-scale biodiesel production, huge amount of nutrients are needed, especially nitrogen and phosphorus. Generally, inorganic fertilizers are used as the main nutrient sources. However,

Table 1 Microalgae producing >25% lipid content using MWW as growth medium

Microalgae	Lipid (%)	Reference
Chlorococcum sp.	~28	Chinnasamy et al. (2010)
Nannochloropsis sp.	33.8–59.9	Jiang et al. (2011)
Chlorella ellipsoidea YJ1	43	Yang et al. (2011)
Scenedesmus acutus	28.3	Sacristan de Alva et al. (2013)
Scenedesmus sp. ZTY1	32.3	Zhang and Hong (2014)
Scenedesmus obliquus	51	Han et al. (2016)
Scenedesmus obliquus	30.5	Han et al. (2018)

extensive use of chemical fertilizers results in water pollution, such as eutrophication (de Oliveria and Crispim 2013; Abdel-Raouf et al. 2012; Lam and Lee 2012). Various studies have demonstrated that industrial wastewater can be used as the nutrient source for microalgae cultivation as it contains high concentrations of nitrogen and phosphorus. Some indigenous microalgae species, such as *Botryococcus braunii, Chlorella protothecoides, C. saccharophila, C. vulgaris, Spirulina maxima, S. platensis, Dunaliella tertiolecta, Nannochloris oculata, Tetraselmis suecica, T. chuii, Phaeodactylum tricornutum*, and *Pleurochrysis carterae* are being grown in carpet industry wastewater with decent biomass production of 9.2–17.8 t ha^{-1} a^{-1} with 6.82% lipid content (Chinnasamy et al. 2010; Pittman et al. 2011; Zhou et al. 2014).

The utilization of MWW instead of commercial artificial media for microalgae biomass production on a large scale is a cost-effective option for microalgae cultivation for various value-added products. MWW is a suitable and sustainable growth medium for various microalgal communities. As shown in Table 1, some microalgae can be grown quite easily in MWW and yield moderate levels of lipids (>25%). Due to the presence of different types of key nutrients, wastewater is considered a potential medium for the growth of several types of oleaginic microorganisms including microalgae, bacteria, fungi, and yeast (Abdel-Raouf et al. 2012). From an energetic point of view, microalgae consuming 0.52 MJ m^{-3} for nutrient removal, which is much less energy than the 3.6 MJ m^{-3} utilized for conventional systems (Sepulveda et al. 2015). Presently, different microalgal strains are being used for MWW treatment to produce biodiesel (Schulze et al. 2017).

6.2 Bioethanol

The biorefining of microalgae biomass presents opportunities to develop a sustainable and economical means of bioethanol production (Nobre et al. 2013). Bioethanol production by fermentation is a simple process that requires less energy compared to biodiesel production systems. Bioethanol production from microalgae biomass is still under investigation and has not yet been commercialized Harun and Danguah (2011). Defatted microalgal biomass can also be used for bioethanol production

Table 2 Carbohydrate concentration and bioethanol yield of selected microalgal biomass

Microalgal species	Total carbohydrate concentration	Bioethanol yield	Reference
Chlamydomonas reinhardtii UTEX 90	59.7% (w/w)	235 mg ethanol g–1 algae	Choi et al. (2010)
Chlorococcum humicola	32.52% (w/w	52% (g ethanol g–1 algae)	Harun and Danguah (2011)
Chlorella vulgaris FSP-E strain	50.39% (w/w)	92.3%	Ho et al. (2013a)
Scenedesmus obliquus CNW-N	51.8%	99.8%	Ho et al. (2013b)
Chlorella vulgaris	22.4%	89%	Kim et al. (2016)
Chlorella sp. KR-1	49.7%	79.3%	Lee et al. (2015)
Nannochloropsis limnetica	24.14%	68.41 gL-1	Sivasankar and Kumar (2017)
Microcystis aeruginosa	16 mM/mL	60 mM/mL	Khan et al. (2018)

because it contains large amounts of fermentable carbohydrates in its cell walls (Ansari et al. 2015). As shown in Table 2, microalgal species have the highest carbohydrate content and thus can be used as raw materials for bioethanol production by fermentation. However, the carbohydrate content of microalgae is insufficient for large-scale bioethanol production (Khan et al. 2018). Therefore, the concentration of carbohydrates in microalgae should be enhanced by the optimization of nutritional or environmental factors (Chen et al. 2011).

6.3 Biohydrogen

Biohydrogen is a highly advantageous, clean, and environmentally safe fuel and a potential candidate to replace fossil fuels with the highest energy density. In addition, it has many other benefits, including technical, socioeconomic, and environmental, which make it superior among all other known fuels (143 GJ per tonne). This is the only known fuel that contains lower emissions than carbon-based fuels because the combustion of hydrogen does not release CO_2 as a byproduct (Azwar et al. 2014; Sharma and Arya 2017). Both the fertilizer and petroleum industries are the most prominent hydrogen users and account for approximately 50% and 37% of its use, respectively (Chang and Lin 2004). However, microalgae-based hydrogen production is still at the laboratory scale. Therefore, design and process parameters must be optimized for scaling up the hydrogen production (Sharma and Arya 2017).

The classical methods of hydrogen production via steam reforming of natural gases, coal gasification, and electrolysis of water are energy-intensive processes requiring temperatures exceeding 840 °C and are not environmental friendly. Next, biofuel feedstocks, such as sucrose, starch crops, corn, and sugarcane, as well as lignocellulosic materials including rice straw and switchgrass, are also used for hydrogen production by microbial fermentation. However, the cost of hydrolysis of lignocellulosic materials is very high. Biohydrogen production through renewable carbon sources can be considered a CO_2 offset. Different carbon sources including wastewater can be utilized for biohydrogen production, but glucose and sucrose are preferred as model substrates for hydrogen production due to their readily degradable capacity. Otherwise, carbon need to be obtained from the complex composition polymeric carbon sources. Complex polymers contain tightly bound cellulose, hemicelluloses, and lignin. The first two can be degraded under the same conditions and increase costs, which is a matter of concern (Azwar et al. 2014; Behera et al. 2015).

Biohydrogen production by the fermentation of microalgae grown in wastewater and flue gases is more beneficial due to the reduced environmental footprint. Microalgal biomass fermentation for biohydrogen production is a rather unexplored field. More research is needed considering both organic and inorganic contaminants present in microalgal biomass produced by cultivation in wastewater and flue gases, which could inhibit fermentation (Lage et al. 2018). Batista et al. (2015) integrated the process of urban wastewater treatment using microalgae such as *Chlorella vulgaris, Scenedesmus obliquus*, and a group of wild algal species. The biomass was used for the production of biohydrogen. The highest H_2 yield obtained from *Scenedesmus obliquus* was similar to that obtained from the biomass of the same algae cultivated in synthetic media. The results were promising in demonstrating the application of wastewater-based biomass production and its successful use for biohydrogen production.

6.4 Syngas

Synthesis gas (syngas) is an eco-friendly fuel with high potential for commercial applications including transportation, heat, and electricity generation via fuel cells in the near future. It can also be utilized as an intermediate resource for the production of hydrogen, industrial chemicals, ammonia, and biological processes (syngas fermentation) (Chen et al. 2016). Currently, large-scale syngas is supplied from fossil fuels, mostly from the gasification of coal and natural reforming processes. The high reliance on fossil fuels releases large amounts of CO_2 and hence increases the concentration of GHGs in the atmosphere (Raheem et al. 2017; Yoon et al. 2010). Biomass-based syngas appears to be a promising fuel for various applications. Although different biomasses have extensively been utilized in syngas production, the utilization of lignocellulosic feedstock may have a series of issues such as arable land usage particularly for food production and other high-value-added products and technical obstacles for product conversion; these make the production of syngas economically less appealing. Moreover, lignocellulose containing biomass affects downstream processes in the removal of remaining lignin and maximizes gas synthesis conversion and production competences (Maddi et al. 2011; McKendry 2002; Nigam and Singh 2011).

Microalgae are the best renewable feedstock as algal cells has high calorific value but no lignine component. These benefits provide microalgae with a potent capacity to replace syngas production commodities. A possibility for improving the energy recovery from microalgal biomass is the combination of different conversion processes with anaerobic digestion or fermentation. After extracting lipids, the remaining biomass can be used as feedstock for biogas production by anaerobic digestion. Gasification is an emerging thermochemical conversion technology for syngas production from biomass. The quality and quantity of syngas vary with the biomass type and its composition. Through thermochemical gasification technology, biomass is converted into gaseous products and categorized depending on the gasifying agent such as air, steam, and oxygen enriched air, for example. In thermochemical gasification techniques, biomass residues convert into a syngas under partial oxidation, mainly H_2, CO, carbon dioxide, and methane. However, both the quality and quantity of syngas are highly dependent on the feedstock composition and process parameters including biomass loading, process temperature, gasifying agent, and equivalence ratio (Raheem et al. 2017).

6.5 Biochar

Biochar (BC) is a carbon-rich byproduct of wood biomass and crop residues obtained by pyrolysis in a closed container with trace amounts of or no oxygen (Lehmann and Joseph 2009). BC contains a stable form of carbon that cannot be oxidized by soil microorganisms. Due to the presence of the stable form of carbon in BC, it has unique properties, can be used for sequestering atmospheric CO_2, increases plant growth by retaining soil fertility, remediation of AWW, eutrophic natural waterways, saline wastewater (Awad et al. 2017; Bird et al. 2011), mitigation of climate change, and energy generation (Ahmad et al. 2014; Amin et al. 2016). Using microalgal biomass for BC preparation is a promising biorefinery approach, which is associated with the production of high-value byproducts due to its renewable and sustainable nature (Yu et al. 2017). Microalgae can be easily cultivated in closed photobioreactors or membrane photobioreactors with wastewater can be used as feedstock for BC production (Fig. 1), thereby significantly reducing the costs of BC feedstock (Yu et al. 2018).

The chemical composition of microalgal biomass is very different from lignocellulosic biomass because it contains substantial amounts of specific functional groups such as carbohydrates, lipids, and proteins, which are primarily located on microalgal cell walls and may endow microalgal-based biochar (BC) with special physicochemical properties. In recent years, extracting biofuels and producing BC simultaneously from microalgae has attracted much attention in the biorefinery field (Tripathi et al. 2016). Microalgal BC can be utilized for the removal of various types of micropollutants from wastewater. The BC prepared from *Chlorella* sp. Cha-01 can easily be used as an adsorbent for the removal of p-nitrophenols (PNP) micropollutant as BC exhibits a superior adsorption capacity of 204.8 mg $g^{-}1$ PNP versus raw microalgae powder and powder-activated carbon (PAC). The algal BC contains high N/C and O/C ratios and increased numbers of polarizable O-containing func-

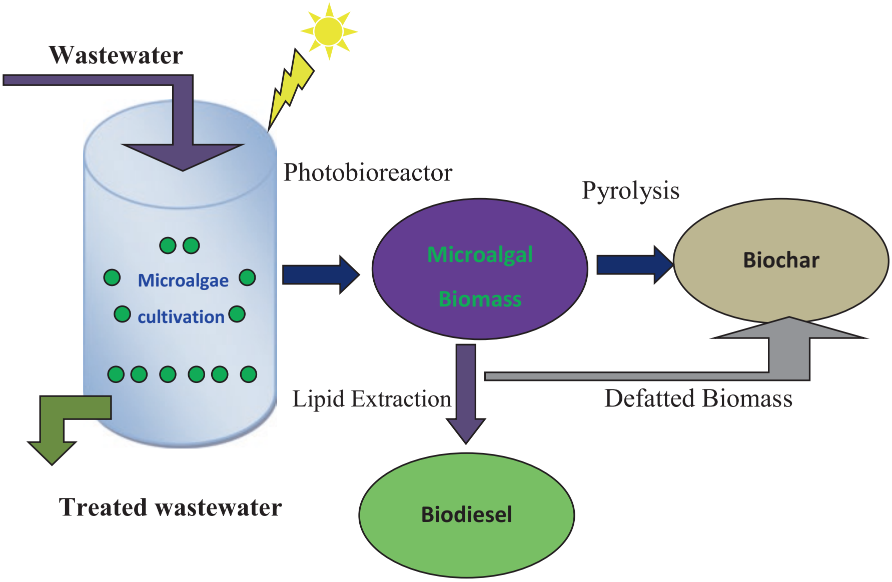

Fig. 1 Biochar preparation from raw and defatted microalgae biomass grown in wastewater

tional groups. These could be the reason for the high adsorption capability of microalgal BC for both ionic and nonionic PNP. Due to its highly polarized capacity, microalgal-based BC could be applied as an adsorbent for organic pollutant removal from wastewater without further activation (Zheng et al. 2017).

Using microalgal BC in large-scale wastewater treatment has obvious advantages like high adsorption capability, excellent storage stability, and greater efficiency. It is a more convenient technique for emergency wastewater treatment than using microalgal live cells. However, BC preparation is an intensive thermal process that requires high energy. Therefore, the energy efficiency and economic issues have become a main obstacle and limiting factor for the application of BC. Some research is being conducted on how to minimize the energy consumption of microalgae BC preparation by adding chemical agents or using appropriate pretreatment to lower the temperature for carbonization and shorten the carbonization time and high adsorption capability (Zheng et al. 2017). A recent study utilized temperatures ranging from 500 °C to 700 °C for the pyrolysis of microalgae biomass for preparing BC with good chemical stability (Chang et al. 2015). Similarly, a high-quality BC produced from microalgal biomass by carbonization of biomass at 600 °C (Dong et al. 2015; Maddi et al. 2011).

6.6 Hydrothermal Liquefaction

Hydrothermal liquefaction (HTL) is an attractive type of thermochemical conversion process by which biomass can be converted to fuel energy with hot compressed waste. HTL consumes less energy, making it a promising method for biomass conversion. Compared to pyrolysis, HTL can perform at lower temperatures ranging from 200 to 375 °C, but it requires higher pressures in the range 4–40 MPa, resulting in higher capital investment. In HTL, factors such as temperature, ash content, total solids, algal lipid content, and retention time affect yields and quality of microalgae bio-oil or biocrude (Biller and Ross 2012; Chen et al. 2017; Gollakota et al. 2018; Lage et al. 2018; Yoo et al. 2015). The elemental composition of microalgal biomass bio-crude obtained by HTL is given in Table 3.

7 Application of Defatted Microalgal Biomass for Biofuels

7.1 Biomethane from Defatted Microalgal Biomass

Anaerobic fermentation of biomass for biomethane production represents an alternative means of gaseous fuel generation. Different types of biomasses have been identified as possible potential feedstock sources for the production of biomethane. Among the various biomasses, microalgae biomass is considered a very suitable source for biomethane production by fermentation. Anaerobic digestion (AD) is a highly efficient technique, by which 88% conversion efficiency is possible with the appropriate biomass. The AD performs the dual process of nutrient recycling and energy cogeneration by methane production (Klassen et al. 2017; Lum et al. 2013). By AD, methane can be produced from raw microalgae biomass or residues (defatted). Defatted biomass is economically more viable for methane production than whole biomass because, after lipid extraction, the remaining residual biomass is rich in proteins and polysaccharides making it a better substrate for AD. Therefore, defatted microalgal biomass can easily be used for biomethane production by AD. In addition, using wastewater for microalgae cultivation would minimize water and nutrient media requirements, thereby improving the economics of biomethane production (Sarat Chandra et al. 2014). However, defatted microalgae biomass is not a suitable substrate for the AD process because microalgae have rigid cell walls that are highly resistant to decomposing microbes and a low carbon-to-nitrogen (C/N) ratio (Klassen et al. 2016). These can be overcome by using physical or enzyme-based pretreatment techniques (Mahdy et al. 2015; Marsolek et al. 2014; Mendez et al. 2014).

In anaerobic digestion, all the biomolecultes of the algal cells get utilized, which is why microalgal biomass is considered a potential feedstock for biomethane production (Sialve et al. 2009). To enhance the digestibility of microalgae biomass,

Table 3 Elemental composition of bio-crude obtained from microalgal biomass by hydrothermal liquefaction

Microalgal biomass	Elemental composition of biomass (%)								Reference
	C	H	N	O	S	Ash	Moisture	HHV*	
Spirulina sp	68.9	8.9	6.5	14.9	0.86	–	–	32.6	Vardon et al. (2011)
Nannochloropsis gaditana	76.1	10.3	4.5	8.8	0.4	–	–	38.0	Barreiro et al. (2015)
Scenedesmus almeriensis	74.9	9.1	5.9	9.6	0.7	–	–	36.20	Barreiro et al. (2015)
Nannochloropsis sp	77.2	9.9	4.7	8.2	0.5	–	–	39.0	Barreiro et al. (2015)
Nannochloropsis Oceana	77.6	4.9	3.4	–	0.3	–	–	37.70	Caporgno et al. (2016)
Scenedesmus sp	72.6	9.0	6.5	10.5	1.35	–	–	35.5	Vardon et al. (2012)
Spirulina sp	72.2	9.1	8.1	9.2	1.41	–	–	35.8	Vardon et al. (2012)
Nannochloropsis sp	51	7	9	0.6	28.5	–	3	–	Valdez et al. (2012)
Cyanobacteria sp	76.02	9.10	6.29	7.44	1.15	–	–	36.51	Huang et al. (2016)
Bacillariophyta sp	76.09	9.11	5.60	8.28	0.92	–	–	36.45	Huang et al. (2016)
Spirulina	48.10	6.97	10.14	34.13	0.66	–	–	–	Nautiyal et al. (2014)
Chlorella	51.33	7.90	9.80	30.38	0.59	–	–	–	Nautiyal et al. (2014)
Nannochloropsis gaditana	40.3	5.97	6.30	14.49	0.37	28.3	4.1	18.53	Nautiyal et al. (2014)
Microcystis	42.26	6.27	7.88	43.07	0.52	6.14	9.59	16.2	Hu et al. (2013)
Dunaliella tertiolecta	53.3	5.2	9.8	31.7	–	–	–	19.8	Minowa et al. (1995)
Chlorella vulgaris	52.6	7.1	8.2	32.2	0.5	–	–	23.2	Biller and Ross (2011)
Nannochloropsis oculata	57.8	8.0	8.6	25.7	–	–	–	17.9	Biller and Ross (2011)
Botryococcus braunii	77.04	12.40	1.23	9.86	0.18	–	–	35.6	Liu et al. (2012)
Nannochloropsis Salina	55.16	6.87	27.3	33.97	1.27	2.48	4.95	25.40	Toor et al. (2013)
Chlorella sp	47.54	7.1	6.73	38.63	–	5.93	6.8	18.59	Phukan et al. (2011)
Anabaena	42.78	7.74	7.91	–	–	38.1	7.4	9.61	Wagner et al. (2016)

**HHV* higher heating value

pretreatments such as microwave-assisted alkaline/acid hydrolysis autoclaving and ultrasonication can be applied (Gonzalez-Fernandez et al. 2012).

In bioenergy production, the microalgal defatted biomasses of some microalgal species such as *Nannochloropsis salina*, *Nannochloropsis* sp., *Auxenochlorella protothecoides*, *Chlorella variabilis*, *Microspora* sp., and *C. sorokiniana* have been identified as potential feedstock for biomethane production via anaerobic fermentation (Bohutskyi et al. 2015a, b; Cheng et al. 2016; Kinnunen et al. 2014; Quinn et al. 2014; Zhao et al. 2014). Thermally pretreated defatted biomass of *Scenedesmus dimorphus* yielded 137–162 mL g^{-1} more methane than untreated biomass. Up to 60% methane yield increased after pretreatment of the biomass as about 6.2% of solubility was increased in the pretreated biomass (Sarat Chandra et al. 2014). However, the low C/N contained defatted biomass is not suitable for methane production because lipid-extracted biomass has high protein and excessive which inhibits fermentation (Sialve et al. 2009). This difficulty could be overcome by the co-digestion of defatted microalgal biomass with some other waste that contains high amounts of carbon. Some studies have suggested that the digestion of defatted microalgal biomass with grease waste or glycerol increased the yield of methane by 4–7% than individual digestion (Ehimen et al. 2009; Neumann et al. 2015; Park and Li 2012). The application of defatted microalgae biomass for methane production is a simple and promising alternative technology.

7.2 *Bioethanol from Defatted Microalgae Biomass*

Bioethanol is one of the best biofuels and an alternative source for petroleum oils that can be used as a blend with gasoline to improve the octane of fuel and reduce GHG emissions (Wyman 1996). Due to this positive impact, research has been directed toward economical ethanol production. The bioethanol can be prepared by fermentation using a wide range of carbohydrate feedstocks (Dinus 2001; Bai et al. 2008). In the biorefinery concept, every component of the biomass could be used to produce commercially important products (Nobre et al. 2013).

Defatted microalgae are also a valuable alternative protein ingredient for aquaculture and poultry (Ju et al. 2012; Leng et al. 2014). The single biomass can be used for lipid-based biofuels and ethanol biofuels (Chaudhary et al. 2014). Whole-cell algal biomass or lipid-extracted algal biomass requires proper pretreatment for lysis of the complex carbohydrates to simple fermentable sugars that can be readily metabolized and fermented for ethanol production by fermenting microorganisms. To facilitate this, different single and combined pretreatment methods such as thermal, thermochemical, ultrasonic, enzymatic, and thermo-chemo-sonic digestion have been applied. Application of these technologies leads to the dissolving of many organic compounds during the process (Uma et al. 2012; Kavitha et al. 2013; Selvakumar and Sivashanmugam 2017). Defatted biomass of *Scenedesmus* sp. was saccharified by chemo-enzymatic hydrolysis with yields of 37.87% (w/w) and

43.44% for pretreatment of defatted biomass using 0.5 M HCl and Viscozyme L (20FBGU/g), respectively (Pancha et al. 2016).

8 Economic Sustainability of Microalgae Biofuel Production Using Industrial Wastewater

The continued use of fossil fuels has led to the rapid depletion of fossil fuel reserves, rising crude oil prices, global climate change, and environmental degradation. This has forced the scientific community to explore other alternative energy sources. The potential of microalgae has gained considerable attention as an alternative source for renewable energy. Unfortunately, large-scale microalgae cultivation for biofuel production is not economically viable. However, coupling microalgae cultivation with industrial wastewater treatment is considered one of the most promising means of producing biofuels in an economically viable and eco-friendly manner since huge amounts of synthetic culture media required for microalgal growth can be saved (Zhou et al. 2014).

In addition, in raceway pond systems, the freshwater should be supplied to compensate for water evaporation. One suggestion is that microalgae could be cultivated in wastewater. However, pretreatment of wastewater is required to remove microalgal-growth-inhibiting components, and this process could raise the energy demand. Freshwater resources are a costly option for biofuel production from microalgae. Therefore, supplying adequate amounts of low-cost water is critical to the success of biofuel production from microalgae. With microalgal cultivation in open ponds, the water demand is as high as 11–13 million L ha^{-1} $year^{-1}$. Therefore, depleting freshwater resources has brought into question the feasibility of microalgal biofuel production. Most of the water used for industrial and domestic purposes turns into wastewater, pollutes the environment, and creates health hazards. If the available 95 billion m^3 (50%) of this utilized water for microalgae cultivation, approximately 247 million tons of microalgal biomass and 37 million tons of oil could be generated. For example, in the Dalton area in north-central Georgia, the carpet industry generated 40–55 million m^3 $year^{-1}$ of wastewater along with sewage. This wastewater has the potential to produce up to approximately 15,000 tons of microalgal biomass, which could produce approximately 2.5–4 million L biodiesel and also remove approximately 1500 tons of N and 150 tons of P from carpet industry wastewater per year (Chinnasamy et al. 2010).

The microalgal-based treatment (MBT) technology of MWW treatment is highly efficient nutrient and energy recoveries while absorbing CO_2. Finally, producing a well oxygenated and treated effluent. During the MBT of MWW, microalgae exhibit high productivity and fast growth rates and do not compete with land-based food crops (Chalivendra 2014). The resulting wastewater-grown microalgal biomass can be used for a broad range of third-generation biofuels including biodiesel, bioethanol, and biomethane (Smith et al. 2010; Ge et al. 2018). However, biomass harvesting and dewatering increase costs and energy use. Therefore, economic harvesting and dewatering are also a need of the day for algal industry. Higher microalgal biomass yield pave ways an economic route of biofuels production through different biochemical processes. Meeting requirements for larger volumes of freshwater is the basic challenge for the production of microalgal biomass. When cultivating microalgae in closed photobioreactors, the water use may be much larger for cooling. Similarly, open pond systems also consume large amounts of freshwater for microalgae biomass production, for example, annual water consumption in raceway ponds is 11–13 million L ha^{-1} (Wijffels and Barbosa 2010; Chinnasamy et al. 2010). Therefore, the reuse of MWW that enables nutrient recycling would subsequently reduce the cost of microalgal biomass production (Santiago et al. 2013).

9 Future Prospects

- Identification of suitable wastewater triggers for lipid synthesis in microalgae will lead to better understanding of the productivity of biofuels.
- Novel microalgal species need to be isolated, cultivated, and developed by simple mutation techniques for the utilization of highly toxic wastewater.
- Process parameters for the integration of wastewater nutrient removal and biomass production need to be optimized.
- Besides the microalgae can be efficiently remove wastewater nutrients while
- There is a need to assess the prospect of simultaneous nutrient removal from wastewater utilizing large quantities of atmosphere CO_2.
- Polyculture of microalgal strains in wastewater and assessment of their feasibility as biofuel feedstock need to be initiated.
- There is a need to assess the prospect of biodiesel and ethanol/methanol production from single microalgal strains.
- Process parameters for biogas production from defatted microalgae biomass need to be optimized.

10 Conclusions

Microalgae are tiny cell factories since they function as renewable, sustainable, and economical sources of biofuel. Microalgae have great potential to utilize wastewater for their growth through which they produce enormous amounts of biomass. Microalgal biomass is considered a bioresource for the production of various biofuels and high-value byproducts. Culturing microalgae in wastewater can significantly improve water management by providing an inexpensive and eco-friendly wastewater treatment. Wastewater of different origins, such as aquaculture, municipal wastewater treatment, textile industry, dairy industry, agriculture, piggeries, and brewing wastewater, offers a great nutritional source for microalgal growth. Wastewater reduces the cost of microalgal cultivation compared to synthetic media containing nutrients or fertilizers, and at the same time microalgae remove nutrients from wastewater and energy is saved during wastewater treatment. Wastewater-grown microalgal biomass and its biomolecules, such as carbohydrates and lipids, are more suitable for use as feedstock of biofuels than as food or feed. Upgrading of microalgal biofuel technology from the lab scale to the pilot scale or commercial level will be possible when overcoming various bottlenecks associated with potentially costly steps in biofuel production.

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Potential of Microalgae for Wastewater Treatment and Its Valorization into Added Value Products

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1 Introduction

Activated sludge processes have dominated both domestic and industrial wastewater treatment during the past twentieth century as a result of their effective carbon and nutrient removal and tolerance to low-moderate temperatures. On the other hand, upflow anaerobic sludge blanket reactors (UASB) have been extensively used in tropical countries based on their ability to support a cost-effective carbon removal concomitantly with bioenergy generation. In this context, the extensive design and operational experience accumulated over the past decades still supports the widespread implementation of activated sludge processes and UASBs despite their high operating costs and poor nutrient removal, respectively (Tchobanoglous and Stensel 2003). Most recent research and development efforts have focused on process intensification, which has resulted in compact membrane, floating carriers, or granular bioreactors. These technologies provide a superior wastewater treatment performance in bioreactor configurations with lower footprints but at the expenses of higher energy consumptions and environmental impacts (Lema and Suarez 2017). In addition, energy efficiency enhancement has been also a driving force in the optimization and scale-up of Anammox processes applied to centrate treatment and in

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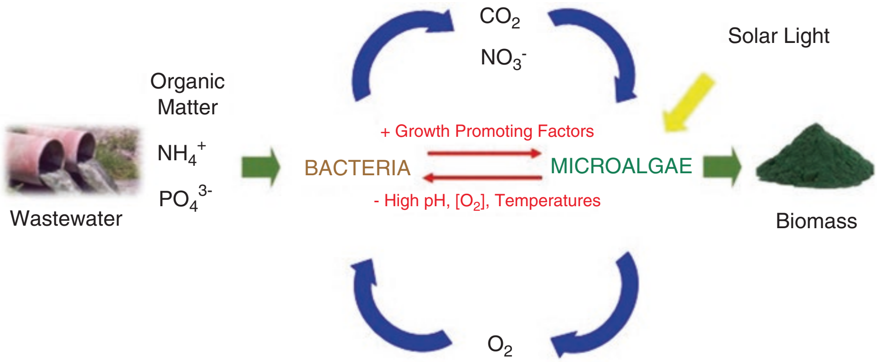

Fig. 1 Symbiotic and antagonistic interactions between microalgae and bacteria supporting an enhanced wastewater treatment

the emerging research on microalgae-based wastewater treatment. The latter provides an effective carbon/nutrient recovery at lower energy demands and environmental impacts but at the expenses of a significantly larger footprint compared to activated sludge, membrane, or granular processes (Muñoz and Guieysse 2006).

Microalgae-based wastewater treatment relies on the bacterial oxidation of organic matter and ammonium present in wastewater using the O_2 produced by microalgae. In return, bacteria release the CO_2 required by algal photosynthesis to sustain the process (Fig. 1). Apart from this symbiotic exchange of CO_2 and O_2, bacteria can release agents that promote algal growth (i.e., vitamin B12, phytohormones, etc.). On the other hand, microalgae are capable of generating in the mixed liquor of the photobioreactor high concentrations of dissolved oxygen, pH, and high temperatures, which favor the elimination of pathogens and emerging contaminants.

Traditionally, this symbiosis between microalgae and bacteria has supported the treatment of domestic, industrial, and livestock wastewater, although the photoautotrophic metabolism of microalgae renders them the best candidates for the recovery of nitrogen and phosphorus from anaerobic digestates. In addition, recent studies in the field of algal microbiology have revealed the enormous metabolic versatility of microalgae, which are able to heterotrophically degrade from aromatic compounds such as cresol, phenol, and phenanthrene to azo dyes (Papazi et al. 2012). Despite research in the field of microalgae-based wastewater treatment originated in the 1960s at the Berkeley University laboratories of Prof. William J. Oswald, it only experienced an exponential increase from 2009 onward (Fig. 2) (Oswald 1988). Most research and development projects carried out in the past decade have focused on decreasing the hydraulic retention time (to decrease the inherently high footprint of this process) and on upscaling high-rate algae ponds (to obtain precise data on energy consumption and validate the technology). The

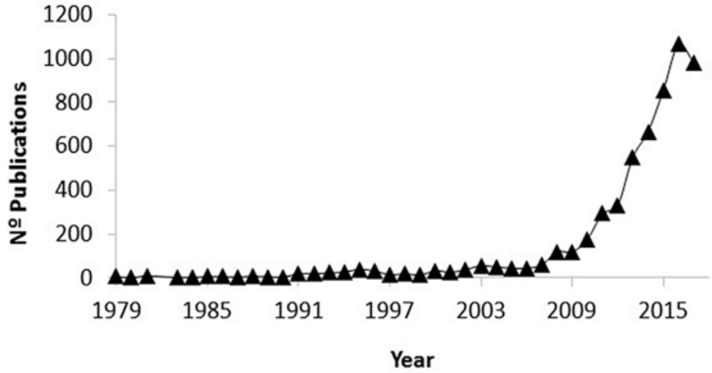

Fig. 2 Time course of the number of publications indexed in ISI Web of Knowledge using the search term "microalgae" and "wastewater"

operation of high-rate algae ponds (HRAPs) at hydraulic retention times of 3–4 days allows to achieve eliminations of organic matter of 70–80%, of total nitrogen of 60–70%, of ammonium of 98–100%, and of phosphate of 40–60% (Posadas et al. 2015). Recent studies carried out within the FP7 ALL-GAS project have estimated that this technology can reduce wastewater treatment costs from 0.22 \$ m^{-3} (in activated sludge processes) to 0.17 \$ m^{-3} and even to 0.15 \$ m^{-3} if the commercialization of microalgae as biofertilizer is considered. This technology supports also a reduction in electricity consumption of 400% compared to conventional active sludge processes (Acién et al. 2017a). This reduction in cost and energy consumption has been achieved with an R&D effort of less than 10 years, compared to the 100 years of development of its counterpart, the activated sludge process.

Despite the progress made in such a short period of time, algal-bacterial photobioreactors still suffer from severe limitations that restricts their widespread implementation as an alternative platform for wastewater treatment: (i) poor process performance in carbon-limited wastewaters (i.e., digestates), (ii) poor biomass settling, (iii) limited number of process configurations and metabolic functions investigated, and (iv) limited understanding of the microalgae-based bioconversion processes into added-value bioproducts in the context of wastewater treatment.

This book chapter represents a state-of-the-art review of two innovative microalgae-based processes devoted to produce biopolymers (polyhydroxyalkanoates) and biofuels (biomethane) in the context of wastewater treatment, which will boost the economic viability of microalgae biorefineries. The fundamentals of microalgae-based cultivation and wastewater treatment will be initially reviewed in order to lay the foundations of the two target processes further discussed.

2 Application of Microalgae for Wastewater Treatment

2.1 Main Characteristics of Wastewater

Domestic and agro-industrial wastewaters and centrate are characterized by their high content in carbon and nutrients (mainly nitrogen and phosphorous) (García et al. 2017a). In addition, other pollutants such as heavy metals are commonly found in these waste streams (Table 1). The concentration of these pollutants must be reduced before being discharged into natural water bodies in order to avoid eutrophication, oxygen depletion, and toxicity issues (Razzak et al. 2017). In this context, microalgae, which present a high tolerance level against adverse environmental conditions, are able to grow in different types of wastewater supporting a high

Table 1 Characteristics of domestic wastewaters, centrate, and piggery effluents

Parameter	Domestic wastewater	Centrate	Piggery wastewater
pH	7.1–7.8	6.8–9.2	7.3–7.6
COD (mg L^{-1})	395–1179	134–1043	987–11,241
TOC (mg L^{-1})	112–292	16–891	3935–10,340
IC (mg L^{-1})	68–186	450–974	1450–1750
TN (mg L^{-1})	49–166	316–1570	475–3680
N-NH_4^+ (mg L^{-1})	41–102	316–1143	>367
N-NO_3^-(mg L^{-1})	0–0.5	0.2–8	<5
N-NO_2^-(mg L^{-1})	0–0.5	0	<5
TP (mg L^{-1})	10–52	45–297	44–85
P-PO_4^{3-} (mg L^{-1})	4–41	26–135	–
S-SO_4^{2-} (mg L^{-1})	27–51	0–25	–
Cu (μg L^{-1})	1–250	60–10,400	1210–1290
Pb (μg L^{-1})	0–53	<2800	40
Cd (μg L^{-1})	0–4	<1000	<1
Cr (μg L^{-1})	5–33	<1100	–
Zn (μg L^{-1})	15–365	30–6340	2740–2900
Mn (μg L^{-1})	43–180	30–4570	1660–1840
Mo (μg L^{-1})	2	<1800	20
Fe (μg L^{-1})	65–922	250–22,400	1590–1690
B (μg L^{-1})	–	790–2870	250–290
References	Posadas et al. (2013), Krustok et al. (2016), García et al. (2017a), Norvill et al. (2017), Saddoud et al. (2006), Andreo-Martínez et al. (2016), Kumwimba et al. (2017), Gani et al. (2017)	Morales-Amaral et al. (2015), Marín et al. (2018), Singh et al. (2011), Zhao et al. (2015), Ji et al. (2014)	García et al. (2017b), Kim et al. (2016), Zheng et al. (2018)

nutrient removal and a cost-effective oxygenation potential (Posadas et al. 2013). Moreover, these effluents typically present a pH of 7–9, which matches the optimal range for microalgae growth (Posadas et al. 2017a).

In domestic wastewaters, most of the nitrogen is present as ammonium (NH_4^+), with low concentrations of nitrite and nitrate. This feature favors nitrogen consumption by microalgae since NH_4^+ assimilation requires less energy than NO_3^- and NO_2^- conversion into structural nitrogen (Cai et al. 2013). However, domestic wastewaters present a C:N ratio (3.5:1) and a C:P ratio (20:1) too low in comparison with the optimum ratios for microalgae growth (C:N:P of 100:18:2) (Woertz et al. 2009; Posadas et al. 2014). In this context, carbon limitation often occurs during microalgae-based domestic wastewater treatment, which decreases microalgae growth rate and therefore nutrient removal by microalgae (Arbib et al. 2013). Recent studies have demonstrated that CO_2 supplementation (i.e., from biogas or flue gas) can improve carbon availability during wastewater treatment, increasing biomass productivity (and nutrient assimilation) while simultaneously mitigating the emission of a potential greenhouse gas (de Godos et al. 2010).

Agro-industrial wastewaters such as piggery wastewater contain higher concentrations of organic matter, nitrogen, and phosphorus in comparison with domestic wastewaters (Table 1). However, its composition depends on farming practices and animal nutrition (de Godos et al. 2009). Similarly, nitrogen and phosphorous concentrations in centrate (the liquid fraction of the digestate produced as by-product of the anaerobic digestion) are considerably higher than in typical domestic wastewaters. In this particular case, centrate composition depends on the type of sludge digested, digestion temperature, supplementation of trace elements, organic loading rates, and the digester configuration (Xia and Murphy 2016). Similar to urban wastewater, the C:N:P ratios in agro-industrial wastewaters and centrates are lower than those required for microalgal growth and nutrient removal by assimilation; thus additional carbon supply is required for the photosynthetic treatment of these wastewaters. On the other hand, despite ammonium is the preferred form of nitrogen for microalgae growth, NH_4^+ concentrations >100 mg L^{-1} at pH > 8 decreases microalgae growth in some species due to free ammonia toxicity (Posadas et al. 2014). As a result, agro-industrial wastewaters and centrate must be diluted or fed at low loading rates to microalgae-based treatment technologies (Posadas et al. 2017b; Serejo et al. 2015). Moreover, dilution strategies or pretreatment steps contribute to reducing or removing the dark color of these effluents, thus avoiding problems of light limitation in the cultivation broth of the photobioreactors (Depraetere et al. 2013).

Heavy metals such as Cu, Pb, Cd, Cr, or Zn are commonly found in wastewaters (Table 1). Heavy metals can inhibit photosynthetic activity and bacterial growth at low concentrations. For instance, Hamed et al. (2017) reported the inhibition of *Chlorella sorokiniana* and *Scenedesmus acuminatus* growth when exposed to Cu concentrations of 1.6 mg L^{-1} and 3.2 mg L^{-1}, respectively. In contrast, some metals at trace level concentrations may improve microalgae growth (Cheng et al. 2015). Indeed, Zhang et al. (2013a) observed an increase in *Ostreococcus tauri* growth at arsenic concentrations of 0.75–2.25 mg L^{-1}, while Huang et al. (2009) reported that Cd concentrations of ~4.5 mg L^{-1} stimulated *Chlorella vulgaris* growth.

2.2 Environmental Parameters

Wastewater composition is one of the main factors governing microalgae productivity. CO_2 and nutrient limitation, as well as the presence of toxic compounds in wastewaters, may inhibit microalgae growth. Nevertheless, under excess of CO_2 and nutrients, environmental parameters such as temperature, light intensity, pH, dissolved oxygen, and the presence of biological predators also impact microalgae growth.

- *Light*

Light availability is a relevant factor affecting the rate and efficiency of the photosynthetic process and, consequently, microalgae growth (Grobbelaar 2009). Light provides the energy required to convert dissolved inorganic carbon into organic biomass via photosynthesis (Sutherland et al. 2014). In the absence of nutrient limitation, microalgae growth increases at higher light intensities until a maximum value where the culture becomes light saturated (Carvalho et al. 2011). Higher intensities above the light saturation point can lead to photoinhibition or photodamage. Most microalgae reach this saturation point at light intensities of ~200 μmol m^{-2} s^{-1}, which is approximately 8% of the summer and 17% of the winter maximum light irradiances (2500 and 1200 μmol m^{-2} s^{-1}, respectively) (Torzillo et al. 2013). However, due to the fact that about 10–20% of the total solar radiation is lost by reflection in the photobioreactor and only 48% of the solar irradiance is photosynthetically active radiation (PAR), the maximum solar energy potentially fixed by microalgae ranges from 12.8 to 14.4% depending on the climate, photobioreactor configuration, and algal strains (Park et al. 2011). Furthermore, temperature fluctuations and photoinhibition typically decrease the photosynthetic efficiency of microalgae to 1–5% in conventional photobioreactors. Microalgae growth is also affected by the length of the light/dark cycle (Zhou et al. 2017). For instance, Jacob-Lopes et al. (2009) studied the effect of the photoperiod algal productivity in a bubble column photobioreactor using *Aphanothece microscopica Nägeli*. The results showed a decrease in the biomass concentration and CO_2 fixation potential when the duration of the light period was reduced. A 12:12 h light/dark photoperiod resulted in higher biomass productivities and cell densities compared to 14:10 and 16:8 h light/dark photoperiods. Krzemińska et al. (2014) observed higher growth rates in *Botryococcus braunii* and *Scenedesmus obliquus* cultures under continuous light regime, while 12:12 light/dark photoperiods promoted the growth of the three species of *Neochloris* tested.

- *Temperature*

Temperature governs most metabolic processes, which ultimately impacts on microalgae growth (Chinnasamy et al. 2009). Other properties such as the solubility of gases (O_2 and CO_2), the ionic equilibria of the cultivation broth and the pH also depend on the temperature (Bouterfas et al. 2002). The optimal temperature for microalgae growth often ranges between 15 and 30 °C, but it is highly species-specific, some strains being able to tolerate or even prefer lower or higher temperatures (Zhou et al. 2017). For instance, *Chlorella* sp. exhibits an optimal activity between 30 and 35 °C (Muñoz et al. 2015). De Jesus et al. (2018) cultivated *Spirulina* sp. *LEB-18* in two different locations and found higher growth rates between 27.1 and 37.3 °C at average PARs of 476–1784 μmol m^{-2} s^{-1} than those obtained at 19.1–25.2 °C and 22 to 559 μmol m^{-2} s^{-1}. De Oliveira et al. (1999) observed a significant decrease in the metabolic activity of *Spirulina maxima* and *S. platensis* at temperatures below 17 °C, while growth was not inhibited at 40 °C. On the other hand, Butterwick et al. (2005) found that low temperatures (2 °C) positively impacted *Asterionella formosa* growth, being unable to survive at 27 °C.

- *pH of the Cultivation Broth*

The pH modifies the enzymatic activity and the energetics of the cells associated with microalgal growth and mass transfer phenomena associated to the absorption of acidic or basic gas pollutants (Perez-Garcia et al. 2011). Indeed, pH influences the NH_3/NH_4^+ and $CO_2/HCO_3^-/CO_3^{-2}$ equilibria and also phosphorus and heavy metal availability. This directly impacts on nutrient removal through NH_3 volatilization and orthophosphate precipitation at pH between 9 and 11 (Muñoz and Guieysse 2006). The pH tolerance and the optimal pH value for microalgal growth differ among strains (Zhou et al. 2017). For instance, acidophilic microalgae such as *Chlamydomonas acidophila* present an optimal growth at pH below 6 (Cuaresma et al. 2006). However, most microalgae show a maximum activity at pH 7–8 (Muñoz et al. 2015).

During wastewater treatment, the pH of the cultivation broth depends on the rates of algal/bacterial respiration, nitrification, photosynthetic activity of microalgae, and on the alkalinity and ionic composition of the wastewaters (Park et al. 2011). Typically, the increase in pH resulting from CO_2 consumption via photosynthesis, which could inhibit microalgae and bacteria growth at values >11, can be controlled by CO_2 addition (Arbib et al. 2013). On the contrary, photobioreactors with a high nitrification activity may undergo a severe pH decrease due to H^+ release from NH_4^+ oxidation. In this context, a high alkalinity in the cultivation broth (high concentration of inorganic carbon) results in a high buffer capacity which can maintain a constant pH, while low alkalinity systems might need alkali addition in order to compensate the pH drop caused by nitrification (Posadas et al. 2017b).

- *Dissolved Oxygen Concentration*

The large amounts of oxygen produced during the photosynthetic process might result in dissolved oxygen (DO) concentrations in the cultivation broth of 10–40 mg L^{-1} (Peng et al. 2013). High concentrations of DO can inhibit the activity of enzymes involved in the photosynthesis (e.g., RuBisCO), induce light energy dissipation by photorespiration, or cause photochemical damages to membrane structures and the photosynthetic apparatus, among others, which in turn results in a decrease in microalgal growth (Pawlowski et al. 2015). For instance, Jiménez et al. (2003) observed a decrease in biomass concentration at DO concentrations >25 mg L^{-1}. Therefore, a proper control of the DO levels is a key issue in the design of photobioreactors, particularly in closed systems (Rubio et al. 1999). In this sense, CO_2 supplementation using flue gas and the implementation of degassing units in photobioreactors devoted to photoautotrophic microalgae growth are common strategies to promote dissolved oxygen stripping, although the inherent O_2 demand caused by bacterial activity during wastewater treatment typically maintain DO below inhibitory levels (Mendoza et al. 2013).

- *Herbivorous Predators and Pathogens*

Proliferation of zooplankton grazers, especially cladocerans and rotifers, in open photobioreactors such as HRAPs, can severely reduce the algal concentration within a few days (Wang et al. 2013). Grazing can change the microalgal dominance, increase the colony size, and trigger structural modifications like protective spines, which affects the biomass settleability and consequently the capacity of predators to consume microalgae (Montemezzani et al. 2016; Schlüter et al. 1987; Verschoor et al. 2009). These predators reach high population densities at higher cultivation broth temperatures, longer hydraulic retention times (HRTs), and at pH of ~ 8. Grazer populations can be controlled by setting low DO concentrations during the night, high nutrients concentration, temperature fluctuations, high pH (~ 10 can promote free ammonia toxicity), and low HRTs (Montemezzani et al. 2016). Phytoplankton-lytic bacteria can also reduce microalgae population by excreting toxic extracellular substances or through direct contact with microalgae cells (Shunyu et al. 2006; Zhou et al. 2011). In addition, fungal parasitism and viral infection can induce changes in algal cell structure and diversity, negatively affecting algal growth (Park et al. 2011).

2.3 Operating Parameters

Hydraulic retention time, mixing and gas-liquid mass transfer represent the main operating parameters influencing wastewater treatment in microalgae-based processes.

- *Hydraulic Retention Time*

The average period of time that the wastewater remains in the photobioreactor is given by the hydraulic retention time (HRT). Therefore, both carbon and nutrient load supplied to the photobioreactor and consequently the biomass productivity are determined by the HRT (Arbib et al. 2013; Metcalf and Eddy 2003). The HRT can be calculated according to Eq. 1:

$$HRT = \frac{V}{Q} \tag{1}$$

where V is the photobioreactor volume (m^3) and Q the influent wastewater flow rate ($m^3\ d^{-1}$).

HRTs between 2 and 10 days are commonly required for an efficient removal of both organic pollutants and nutrients, this value depending on the characteristics of the wastewater, the photobioreactor configuration, and environmental conditions. In this sense, closed systems usually require lower HRTs (2–5 days) compared with open photobioreactors (i.e., 4–9 days for HRAPs) (Muñoz and Guieysse 2006; Luo et al. 2017).

Biomass productivity is expected to decrease due to biomass washout when the HRT is lower than the specific growth rate (μ). Ruiz et al. (2013) observed maximum biomass production and carbon dioxide biofixation at a HRT of 2 μ^{-1}, whereas a HRT close to μ^{-1} allowed for an optimum nutrient removal.

- *Gas-Liquid Mass Transfers*

Carbon, the major substrate for photoautotrophic microalgal growth, is mainly provided to the microalgae culture in the form of CO_2 via bacterial respiration of organic matter during wastewater treatment. Carbon fixation from the atmospheric CO_2 is not efficient due its low concentration in the atmosphere (~0.03%) and reduced solubility, while the alkalinity present in the wastewater is limited. In this context, sparging a CO_2-laden stream (such as flue gas or biogas) in the cultivation broth would improve biomass productivity and therefore boost nutrient assimilation (Pires et al. 2017). In this sense, increasing the CO_2 mass transfer from the gas to the cultivation medium is necessary to increase the total inorganic carbon available for microalgae growth (Chang et al. 2017). At this point, it is worth noting that the mass transfer coefficient (k_La) can be affected by numerous factors such as the agitation of the cultivation broth, airflow, air pressure, temperature, reactor geometry, properties of the fluid (density, viscosity), or presence of antifoaming agents, among others (Barbosa et al. 2003; Janssen et al. 2003). Different strategies such as improving culture mixing or implementing gas recirculation have been proposed for overcoming mass transfer limitations. Similarly, according to Fick's Law, a decrease in the bubble size improves the mass transfer, resulting in faster CO_2 dissolution, slow rising, and high surface-to-volume ratio (Zimmerman et al. 2011; AL-Mashhadani et al. 2015).

- *Mixing*

Mixing provides turbulence and homogeneity to the cultivation broth, preventing anaerobic conditions, light saturation and inhibition, and the formation of nutrient, gas, or thermal gradients (Eriksen 2008; Ugwu et al. 2008). When algal growth is not limited by other parameters, efficient mixing is the key parameter determining the biomass yield as a result of its direct influence on light availability. In photobioreactors, agitation can be divided into mechanical (such as stirring, mechanical pumps, or the paddle wheels used in open ponds, where the shear rate is a function of the diameter of the impeller and the spinning rate) and nonmechanical through gas sparging in closed photobioreactors (Ugwu and Aoyagi 2012; Acién et al. 2017b). Mixing optimization is crucial since strong agitation could result in excessive shear stress, impairing metabolism and ultimately resulting in cell death, while low mixing could lead to insufficient CO_2 mass transfer to the culture broth, limited oxygen removal, and poor access of microalgae to the photic zone in the photobioreactor, thus limiting cell growth. Moreover, mixing is an important contributor to the energy consumption and therefore, to the operating costs of the process. In this sense, some authors have studied the potential reduction of energy consumption during mixing by modifying the reactor configuration. For instance, Zeng et al. (2016) assessed the performance of 15° inclined blades in a raceway pond, observing a better mixing for the same power consumption and an increase in biomass areal productivity of 15%, while Zhang et al. (2013b) assessed the performance of a tubular PBR with helical static mixers, obtaining a biomass productivity of 37.3% higher than in conventional tubular PBRs.

Whereas linear velocities of 10–30 cm s^{-1} are commonly reported in HRAPs, this value depends on several factors such as the microalgae strain or the photobioreactor scale and configuration (Sullivan et al. 2003; Ugwu and Aoyagi 2012). On the other hand, linear velocities of 1 m s^{-1} are highly recommended to prevent biofouling in tubular photobioreactors treating wastewater.

2.4 Photobioreactor Configuration

Microalgae cultivation has been traditionally performed in open (circular or HRAPs) and closed (tubular, column, or flat paneled) photobioreactors (PBRs) with either artificial or natural light (Fig. 3). Key factors in the design of PBR for microalgae-based wastewater treatment are a large surface area, an efficient light supply with short internal light paths, an enhanced gas exchange, and an efficient mixing (Carvalho et al. 2006; Eriksen 2008). The photobioreactor footprint must be also taken into account in land-restricted sites. Additionally, contamination control

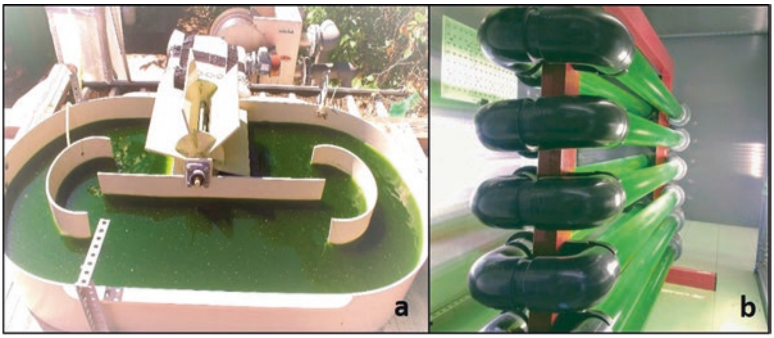

Fig. 3 HRAP (**a**) and tubular (**b**) photobioreactors located in the facilities of Department of Chemical Engineering and Environmental Technology, Valladolid University (Spain)

becomes a crucial parameter in photobioreactors devoted to biomass revalorization into high-added value products.

Open Ponds

Open ponds are the most common configuration for large-scale microalgae cultivation. The widespread use of open ponds is mainly attributed to their simple design, construction, and operation, their low energy requirements, and, thus, lower costs. On the contrary, open ponds present large space requirement, a low photosynthetic efficiency mediating a low biomass density, a poor gas-liquid mass transfer (due to the limited contact time gas – culture), and a limited control over environmental parameters. Moreover, the exposure of the culture to the open atmosphere facilitates microbial contamination and water evaporation (Chisti 2007; Posten 2009; Chang et al. 2017).

Circular ponds (Fig. 4b) are one of the oldest pond configuration used for commercial algae cultivation, particularly suited for easily settleable biomass. They are commonly constructed in concrete and lined with materials (i.e., plastic sheets, inert membranes). A rotating arm is mounted in the center of the pond to provide better mixing of the cultivation broth (Borowitzka and Moheimani 2013).

However, more than 95% of the algae production worldwide is performed in HRAPs (also named raceway ponds), the most frequent open photobioreactor used at commercial scale due to their flexibility and easy scale-up (Fig. 4a). In HRAPs, the culture broth containing the microalgae, wastewater, and nutrients is continuously recirculated around a racetrack consisting of two or four parallel channels (Posten 2009; Acién et al. 2017b; Benemann 2013). Water channel depth is limited to improve light penetration: the lower the water layer, the higher the light penetration, biomass concentration, and culture stability (Acién et al. 2017b; Singh and Sharma 2012). They are equipped with a paddle wheel that provides a continuous, slow, and nonturbulent mixing, preventing sedimentation and reducing cell damage induced by shear stress (Fig. 4a). However, this mild mixing might also result in flocculation of the cells, impeding proper light penetration and leading to lower

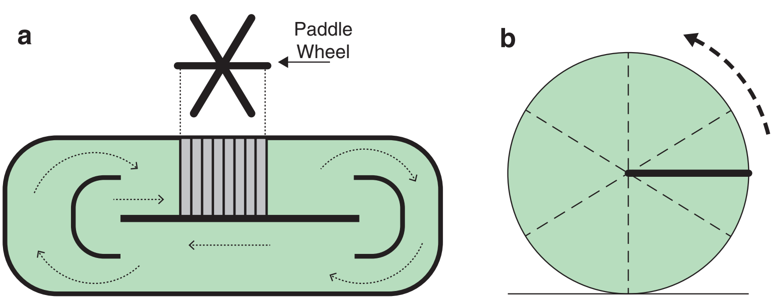

Fig. 4 Schematic representation of most typical open pond configurations: (**a**) raceway ponds and (**b**) circular pond

biomass productivities (Ugwu and Aoyagi 2012). In order to increase the gas-liquid contact time and overcoming poor CO_2 mass transfer efficiency, raceway ponds are usually endowed with sumps or interconnected to external absorption columns (Park et al. 2011; Posadas et al. 2017b). Typical design parameters are HRT of 2–8 days, total surface area ranging from 100 up to 10,000 m^2, water depths from 20 to 40 cm, and length-to-width (L/W) and surface-to-volume (S/V) ratios of 10–20 and 5–10 m^{-1}, respectively. Further information on the design criteria and power consumption calculations can be found elsewhere (Acién et al. 2017b; Posadas et al. 2017a; b). Microalgae species such as *Chlorella* sp., *Dunaliella* sp., or *Spirulina* sp. are commonly cultivated in raceway ponds due to their tolerance to high pH and salinities that might inhibit the growth of other weed algae or pathogenic bacteria (Ugwu and Aoyagi 2012). When HRAPs are used to treat wastewater, microalgae from the genera *Chlorella* or *Scenedesmus* tend to dominate the cultivation broth based on their tolerance to organic pollution.

Closed Photobioreactors

Closed PBRs overcome the main disadvantages encountered in open systems, allowing for a better control of environmental parameters such as temperature, pH, light, and CO_2 concentration, minimizing CO_2 losses and water evaporation and preventing culture contamination. This last advantage is of key importance when targeting the production of high-quality, complex bioproducts since they facilitate the cultivation of single, pure algal strains. They also provide large S/V ratios, shortening internal light paths and improving photosynthetic efficiency (Janssen et al. 2003; Carvalho et al. 2006; Chang et al. 2017). The high internal recycling rates of the cultivation broth offer an efficient mixing and boost gas-liquid mass transfer but restrain their applicability to high-shear-sensitive microalgal strains. On the contrary, closed systems are limited by the poor settleability of the biomass, possible biomass washout, and harvesting limitation. Moreover, the dissolved oxygen concentration in the photobioreactor might reach inhibitory levels for microalgae if the system is operated at long HRT or low organic loading rates (Molina Grima et al. 2001; Costache et al. 2013).

Tubular PBRs are the most commonly implemented closed photobioreactor configuration at industrial scale for microalgae cultivation (i.e., Mera Pharmaceuticals in Hawaii operating 25 m^3 reactors or the 700 m^3 plant in Klötze, Germany (Eriksen 2008)) (Fig. 3b). In tubular PBRs, microalgal culture flows through long, transparent tubing of <0.1 m of inner diameter arranged either horizontally, vertically, inclined, or helically. The cultivation broth is recirculated by mechanical pumping or aeration to provide turbulence and prevent biomass settling in the tubes (Carvalho et al. 2006; Fernández et al. 2013). Tubular photobioreactors present S/V ratios of ~80 m^{-1}, the length and diameter of the tubes being key design parameters that determine both the dissolved O_2 accumulation and head loss. Power requirement in tubular photobioreactors ranges from 10 to 100 W m^{-2}, and the liquid velocity through the tubes is usually set between 0.1 and 0.8 m s^{-1} in order to minimize power consumption and avoid cell damage. If aeration is used, aeration rates of 0.01–0.10 v v^{-1} min^{-1} are selected, the mass transfer coefficient depending on the type of diffuser and gas flow rate (Acién et al. 2017b). Additional information on the design parameters of tubular PBRs can be found elsewhere (Carvalho et al. 2006; Acién et al. 2017b).

Vertical tubular photobioreactors are compact, easy to operate reactors with a high S/V ratio, low contamination risk, and high biomass productivity, thus suitable for large-scale cultivation of microalgae (Chang et al. 2017). Depending on the type of mixing, they can be classified into stirred tank PBRs, bubbling columns, or air-lift PBRs (Acién et al. 2017b) (Fig. 5). Stirred tank reactors use mechanical agitation through one or several impellers, the CO_2 being supplied by CO_2-enriched air bubbling from the bottom of the reactor (Fig. 5a). Although this configuration has been applied for the production of high-value products because of the superior control over processing parameters and microbial contamination, insufficient illumination for the photosynthetic microalgal activity usually hinders their implementation at large scale (Carvalho et al. 2006; Singh and Sharma 2012; Fernández et al. 2013). Bubble column PBRs are vertical cylindrical aerated columns which provide a high homogeneity of the culture broth conditions, an improved mass and heat transfer, and an efficient oxygen abatement (Fig. 5b). However, photosynthetic efficiency greatly depends on the gas flow rate, since the erratic turbulence created by gas sparging might result in uneven exposure of microalgal cells to light intensity, and sedimentation is more likely to occur (Singh and Sharma 2012; Chang et al. 2017). Finally, air-lift PBRs consist of two interconnected zones: the riser, where the gas is sparged, and the internal downcomer, the region that does not receive gas. Most common configurations used for air-lift PBRs are internal loop (Fig. 5c), internal loop concentric (Fig. 5d), and external loop vessels (Fig. 5d), the latter offering an enhanced mixing because of the distance between the riser and the downcomer. The presence of the two zones generates a circular and homogeneous mixing pattern, where the liquid culture moves continuously through dark and light zones. The residence time of the culture broth in each zone will affect heat transfer, mass transfer, mixing, and turbulence (Chisti and Moo-Young 1993; Degen et al. 2001; Xu et al. 2009; Monkonsit et al. 2011; Chang et al. 2017). Interestingly, due to mixing pattern of air-lift PBRs, higher growth rates are achieved in this configuration compared with those obtained in bubble column PBRs (Xu et al. 2009).

2.5 Carbon and Nutrients Removal

Microalgae can fix CO_2 in order to obtain the inorganic carbon needed for growth, being also able to uptake soluble carbonates as a source of carbon. The most common form of inorganic carbon in wastewaters is bicarbonate (HCO_3^-); it is actively transported into microalgal cells and subsequently converted into CO_2 before being fixed by the enzyme RuBisCo (ribulose bisphosphate carboxylase oxygenase), producing two molecules of 3-phosphoglycerate through the Calvin cycle (Picardo

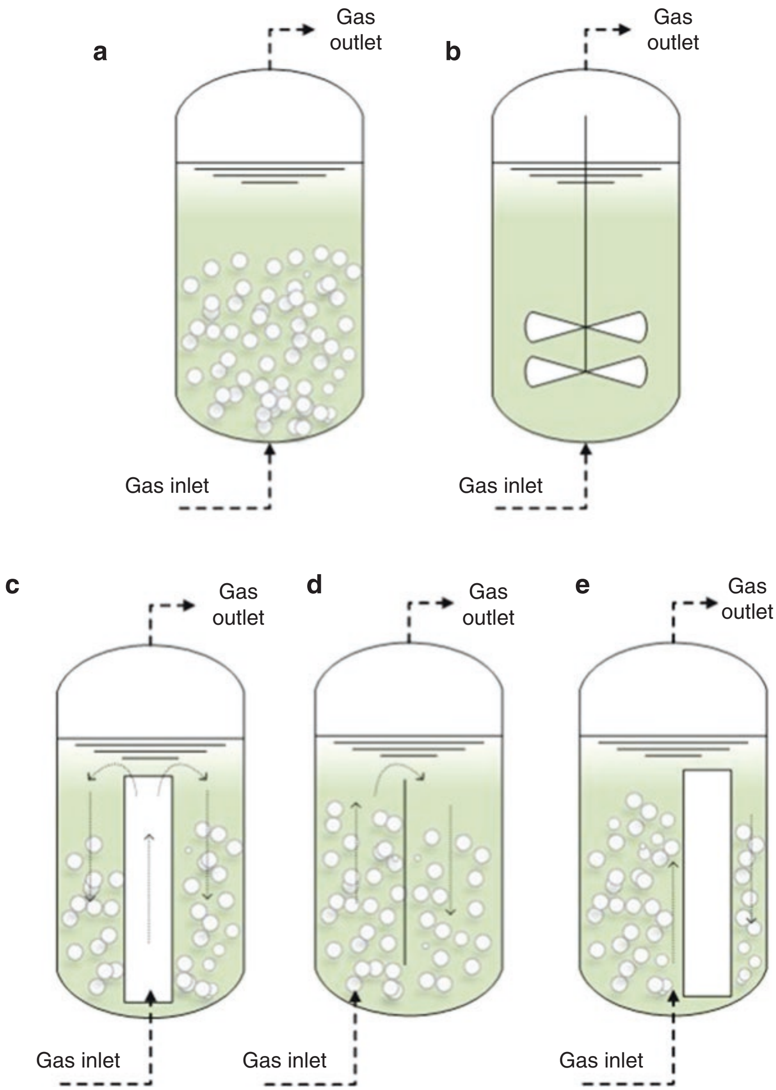

Fig. 5 Schematic representation of typical vertical tubular photobioreactor configurations: (**a**) bubble column, (**b**) stirred tank, (**c**) internal loop split airlift, (**d**) internal loop concentric tube airlift, (**e**) external loop vessels airlift. (Adapted from Carvalho et al. 2006; Massart et al. 2014)

et al. 2013; Sydney et al. 2014). According to Alcántara et al. (2013), microalgae consume ~1.8 kg of CO_2 per kg of photosynthetic biomass. High CO_2 concentrations might result in photosynthesis inhibition, besides hindering algae growth due to low pH values. For instance, a complete growth inhibition was observed in *Anabaena variabilis* when exposed to CO_2 concentrations of 18%, with no lag phase observed on cell growth at CO_2 concentrations between 4% and 13% (Yoon et al. 2002). On other hand, some microalgae species have shown extremely high tolerance to CO_2. For instance, *Chlorella* ZY-1 isolated from soil exhibited a high growth rate and cell density at CO_2 concentrations between 30% and 50% (Yun et al. 1997).

Likewise, some microalgae can use organic carbon as the sole carbon and energy source. This heterotrophic growth of microalgae is able support the degradation of acetate, glucose, glycerol, and ethanol, concomitantly with the photoautotrophic fixation of CO_2 (Neilson and Lewin 1974; Brennan and Owende 2010).

Nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), ammonia (NH_3), and molecular nitrogen (N_2) are the most common forms of inorganic nitrogen that cyanobacteria (prokaryotic microalgae) can use as a nitrogen source. Atmospheric N_2 is converted into N-NH_3 at low O_2 concentrations, which can be either incorporated into amino acids and proteins or excreted to the environment (Eq. 2) (Cai et al. 2013):

$$N_2 + 8H^+ + 8E^- + 16\,ATP \xrightarrow{\text{Nitrogenase}} 2NH_3 + H_2 + 16\,ADP + 16P_i \tag{2}$$

On other hand, eukaryotic microalgae can assimilate NH_4^+, NO_3^-, and NO_2^-. These nitrogen forms enter microalgal cells through active transport at the plasma membrane. First, the reduction of NO_3^- into NO_2^- is catalyzed by the enzyme nitrate reductase, using NADPH as reducing agent (Eq. 3). Afterward, nitrite reductase catalyzes the reduction of NO_2^- into NH_4^+ (Eq. 4), which is actively incorporated into microalgal cells and converted into amino acids via the glutamine synthase enzyme (Eq. 5). At this point it is important to highlight that large amounts of the NH_4^+ present in the wastewater can be volatilized before algal assimilation at high pH and temperature (Grobbelaar 2004; Barsanti and Gualtieri 2006; Crofcheck et al. 2012):

$$NO_3^- + 2H^+ + 2e^- \xrightarrow{\text{Nitrate reductase}} NO_2^- + H_2O \tag{3}$$

$$NO_2^- + 8H^+ + 6e^- \xrightarrow{\text{Nitrite reductase}} NH_4^- + 2H_2O \tag{4}$$

$$\text{Glutamate} + NH_4^+ + ATP^- \xrightarrow{\text{Glutamine synthase}} \text{Glutamine} + ADP + 16P_i \tag{5}$$

Phosphorus is mainly present in wastewater as PO_4^{3-} and can be removed by microalgae assimilation or by precipitation (at pH > 9). Phosphorous enters microalgal cells either as $H_2PO_4^-$ or HPO_4^{2-} through active transport at the plasma membrane. The assimilation into organic compounds follows a three stage process where ATP is produced from adenosine diphosphate (ADP) and energy: phosphorylation at a substrate level, oxidative phosphorylation, and photophosphorylation (Eq. 6). Energy can be obtained from the oxidation of organic substrate via the electron transport system of the mitochondria and from light energy transformation by energy transfer and nucleic acid synthesis (Eq. 6) (Martinez et al. 1999; Su and Mennerich 2012; Cai et al. 2013; Wang et al. 2014).

$$ADP + P_i \xrightarrow{\text{Energy}} ATP \tag{6}$$

The ability of microalgae and cyanobacteria to simultaneously remove nitrogen and phosphorus from different wastewater streams (e.g., agricultural, industrial, and municipal) has been widely demonstrated. This process has been traditionally implemented as tertiary treatment, where nutrients are uptaken into their cells reaching removal efficiencies of 60–99 and 54–95% for nitrogen and phosphorus, respectively (Chavan and Mukherji 2008; Posadas et al. 2013; Mustafa et al. 2012; Gonçalves et al. 2017). For instance, Di Termini et al. (2011) obtained a 99% nitrogen and phosphorous removal from a real wastewater in a tubular PBR inoculated with *Scenedesmus* and operated at a biomass productivity of 0.25 g L^{-1} d^{-1}. However, in the past decade, both secondary and tertiary treatment have been conducted in the algal-bacterial photobioreactor. For instance, Novoveská et al. (2016) successfully coupled biomass production and wastewater treatment in an outdoor PBR, with removals of 75% of total nitrogen, 93% of phosphorous, and 92% BOD from municipal wastewater. Efficient organic matter (>78%) and nitrogen (>70%) removals are typically found in single stage raceway ponds operated at 5–10 days of HRT, while these removal efficiencies increase up to 90 and 80%, respectively, when implementing anoxic-aerobic photobioreactor configurations (Gutzeit et al. 2005; Alcántara et al. 2015; Arcila and Buitrón 2016; Gutiérrez et al. 2016).

3 Potential Revalorization of Wastewater by Microalgae

Microalgae are mainly constituted by proteins (40–60%), lipids (5–20%), carbohydrates (20–30%), and ashes (5–15%) (Alcántara et al. 2015; Chisti 2007). Several authors have assessed the potential of microalgae cultivation in a wide range of wastewaters (such as urban wastewater, swine waste, dairy manure, cattle residues, and poultry waste) combined with the simultaneous utilization of the resulting biomass for the production of different high-value products such as biofuels, lipids, carbohydrates, proteins, vitamins, pigments, and biopolymers (Fig. 6, Table 2) (Olguín et al. 2003; Hu et al. 2008; Samantaray et al. 2011; Borowitzka 2013; Bhati and Mallick 2016).

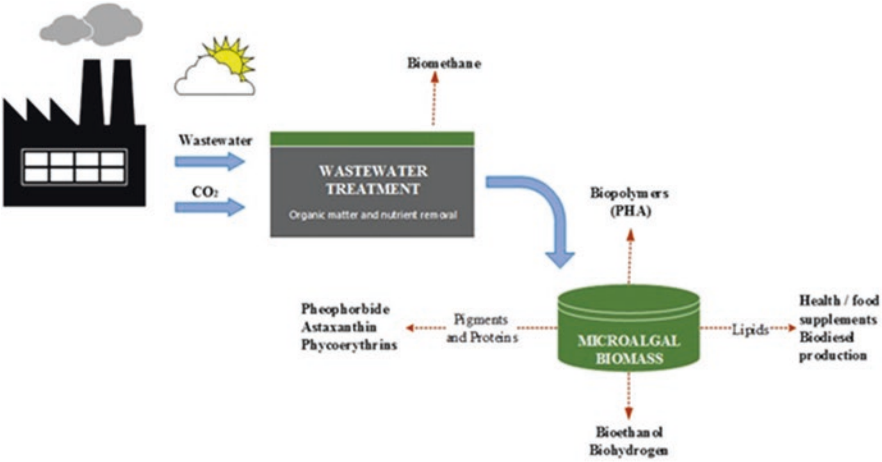

Fig. 6 Schematic representation of the valorization process of microalgae biomass into biofuels and added-value products. (Adapted from Haddadi et al. 2018)

Table 2 Examples of microalgae biomass revalorization into biofuels and added-value bioproducts

Microalgae species	Product	Reference
Nostoc	Biofertilizer	Roger and Kulasooriya (1980)
Chlorella emersonii	Biodiesel	Illman et al. (2000)
Dunaliella salina	β-carotene	Borowitzka (2005)
Schizochytrium sp.	Biodiesel	Chisti (2007)
Cyanobacterial phycobiliproteins	Pigments with different pharmaceutical application	Larsson et al. (2007)
Chlorella protothecoides	Biodiesel	Xiong et al. (2008)
Chlorella sp.	Photosensitizer effects	Busch et al. (2009)
Scenedesmus obliquus CNW-N	Bioethanol	Ho et al. (2013)
Oscillatoria sp.	Antioxidant	Ali et al. (2014)
Chlorella sp.	Phenolic	
Scenedesmus obliquus	Carotenoids	
Synechococcus sp. PCC	Bioethanol	Mollers et al. (2014)
Chlorella zofingiensis	Biodiesel	Zhu et al. (2014)
Scenedesmus obliquus	Biohydrogen	Batista et al. (2015)
Microalgae-bacterial	Biomethane	Posadas et al. (2015)
Chlorella sorokiniana	Biomethane	Meier et al. (2017)

3.1 Biofuel Production

The production of third-generation biofuels such as biodiesel, bioethanol, biogas, or biohydrogen usually raises potential conflicts with food production. In this context, the use of microalgae as feedstocks and their cultivation coupled with wastewater treatment has been widely explored as a cost-efficient and sustainable alternative (Milano et al. 2016; Collottaa et al. 2018).

- Biodiesel

Microalgae are able to accumulate from 20 up to 70% of lipids (dry matter basis) within their cells depending on the microalgae species and cultivation conditions. This makes them attractive for biodiesel production as their fatty acid composition is similar to that of vegetable oil and animal fat. Lipid productivity and composition depend on several factors such as the feed composition (especially the nitrogen forms and content), temperature, pH, CO_2 supplementation, or the cultivation mode (Koutra et al. 2018). For instance, Chisti (2007) obtained a 77% lipid content in *Schizochytrium* sp. and Illman et al. (2000) achieved a 63% lipid content in *Chlorella emersonii* cultivated in low nitrogen medium. Zhu et al. (2014) cultivated *Chlorella zofingiensis* in pilot scale PBRs with artificial wastewater in winter conditions, recording a lipid content >50% dcw suitable for good quality biodiesel production. Unfortunately, scarce research has been performed so far on tailoring the fatty acid profile, which is of key interest since it determines the biodiesel quality and properties (Koutra et al. 2018). Moreover, the cost competitiveness of algal-biofuel production over petroleum-based fuels is strongly dependent on yield and process improvements (Sun et al. 2011; Borowitzka and Moheimani 2013).

- Biogas and Biohydrogen

Green gaseous biofuels such as biogas can be obtained from the anaerobic digestion of algal biomass, which presents a competitive advantage over other biofuels since it does not require oil or lipids extraction and all of the macromolecules of the microalgae are utilized during the methanogenic fermentation. Besides the typical factors affecting anaerobic digestion (organic loading and retention time, temperature, pH, C/N ratio, inoculum to substrate ratio), biogas production from algal biomass digestion must also consider other parameters such as the microalgal species, the biomass pretreatment, and the cultivation methods in order to ensure an acceptable biogas yield and composition (Jankowska et al. 2017; Koutra et al. 2018). For instance, low C/N ratio in the cultivation media of microalgae devoted to biogas production via anaerobic digestion may negatively impact the digestion process due to ammonia accumulation and pH increase (Koutra et al. 2018).

Biohydrogen production by cyanobacteria and green algae has emerged as a promising alternative to the present carbon-based fuels. In this sense, microalgae and cyanobacteria can produce biohydrogen by bio-photolysis (using light energy and converting water to H_2), or they can be used as a substrate for dark fermentation by anaerobic bacteria (Argun et al. 2016; Ferreira et al. 2012). Moreover, dark fermentation might be also combined with urban wastewater treatment using microalgae and the subsequent energetic valorization of the obtained biomass. For instance, Batista et al. (2015) cultivated *Scenedesmus obliquus* in an outdoor photobioreactor using urban wastewater under nutritional stress with the production of hydrogen gas via dark fermentation, recording a specific hydrogen production of 56.8 mLH_2/g_{vs} (volatile solid). Nevertheless, whereas the potential of biohydrogen production from microalgal biomass has been widely demonstrated, its production must be combined with other types of biofuels to ensure economic feasibility.

- Bioethanol

The cost-effective production of bioethanol from microalgal biomass relies on the adequate selection of the species and the cultivation substrate, since their content in fermentable carbohydrates is usually relatively low for large-scale production (5–23%). On the contrary, microalgae present highly fermentable carbohydrates and lack of lignin in comparison with terrestrial feedstocks (Singh and Dhar 2014; De Farias and Bertucco 2016; Koutra et al. 2018). Despite the potential of ethanol production from microalgal biomass, there are still important constraints for its industrial implementation which makes it not economically favorable compared with current fossil fuel production. In this sense, research must focus on improving both the carbohydrate content and the biomass productivity, together with the optimization of hydrolysis and fermentation technologies (De Farias and Bertucco 2016).

3.2 Synthesis of High-Added Value Products: PHAs

The economic feasibility of large-scale production of biofuels from microalgae is usually constrained by the high capital and investment costs. Thus, the co-production of high-added value products such as pigments, proteins, antioxidants, or biopolymers has been recently explored in order to increase the profitability of the process. In this sense, the high content in proteins found in microalgae cultivated in wastewaters boosts their application as biofertilizers (Mulbry et al. 2005; Sepúlveda et al. 2015). Microalgae are also capable of storing high concentrations of proteins such as astaxanthin, used as antioxidant agent, and phycobilins, employed as fluorescent labeling reagents. In addition, pigments such as pheophorbide A (utilized as photosensitizer in photodynamic therapy) are obtained from the breakdown of the chlorophyll synthesized by microalgae as *Chlorella* sp. and *Spirulina* (Busch et al. 2009; Yen et al. 2013). Similarly, *Dunaliella salina* was the first microalgae used for the commercial production of β-carotene, frequently used in the pharmaceutical industry as a powerful antioxidant for human health. Previous studies demonstrated that *Dunaliella salina* was able to accumulate up to 14% of dcw (Aasen et al. 1969; Borowitzka 2005).

- Polyhydroxyalkanoates (PHAs)

Biopolymers, biologically originated polymers produced by living organisms, have recently emerged as a sustainable alternative to conventional plastics obtained from nonrenewable fossil fuels. They are produced from natural substrates under unbalanced nutrient conditions (i.e., N limitation) and are completely biodegradable and biocompatible, with thermal and mechanical properties similar to those of petroleum-derived plastics (i.e., polyethylene or polypropylene). By varying the producing strains, substrates, and co-substrates, a number of polyesters can be synthesized with different monomer composition. In particular, polyhydroxyalkanoates (PHAs), such as poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and their copolymer (PHBV), are polyesters with structural properties comparable to polypropylene which are synthesized and stored intracellularly by bacteria, serving as energy and carbon storage compounds (Shrivastav et al. 2010) (Table 3). PHAs have numerous applications in several industrial sectors such as pharmaceutical, agriculture, biofuels, medical use, disposables, and chromatography (López et al. 2018; Singh et al. 2016). Depending on the monomer composition, the carbon source, the microbial strain used, and the purity of the final biocomposite, the market price varies from 4 up to 20 € $kgPHA^{-1}$. This price is still not competitive compared to that of fossil-based polyesters, the high costs of carbon source acquisition accounting for 30–40% of the final PHA price (López et al. 2018). In this sense, the accumulation of PHAs by microalgae and cyanobacteria cultivated in wastewater might improve the economic balance, boosting the competitiveness of biologically based polymers.

Several studies have evidenced the capacity of cyanobacteria to accumulate intracellular PHAs under nutrient deprivation conditions (Table 4). For instance, *Arthrospira subsalsa* was able to accumulate 14.7% dcw of PHB under N limitation (Shrivastav et al. 2010); *Synechococcus* sp. MA19 and *Nostoc muscorum* exhibited intracellular PHB contents of 55 and 21.5% dcw, respectively, when cultivated under P limitation and CO_2 addition (Nishioka et al. 2001; Haase et al. 2012), while a 13% dcw of intracellular PHB was achieved in *Synechocystis* sp. PCC 6714 under N and P limitation (Kamravamanesh et al. 2017). Some strains also require the supplementation with an organic carbon substrate to promote biopolymer production. In this context, *Aulosira fertilissima* accumulated up to 77.2% dcw and 58.6% dcw under P and N deficiency, respectively, with 0.5% acetate supplementation (Samantaray and Mallick 2015).

All these experiments were performed using a synthetic cultivation medium, while low-cost substrates for microalgae growth and PHB accumulation are preferred from an economic point of view. In this sense, the combination of wastewater or digestate treatment with biopolymers synthesis has been also successfully

Table 3 Physical properties of PHB and the conventional polymer polypropylene (Balaji et al. 2013)

Properties	PHB	Polypropylene
Melting temperature (ºC)	177	176
Glass transition temperature (ºC)	2	–10
Crystallinity (%)	60	50–70
Tensile strength (MPa)	43	38
Extension to break (%)	5	400

Table 4 Accumulation of PHAs in cyanobacteria under different culture conditions

Cyanobacteria species	Culture condition: Cultivated	Culture condition: Nutrient deficiency (N or P)	Culture condition: Culture conditions	Type of polymer	mg L^{-1}	(% dcw)	References
Synechococcus sp MA19	BG-11	P	50 °C	PHB	–	62	Nishioka et al. (2001)
Nostoc muscorum	BG-11	–	0.2% Acetate Dark incubation	PHB	–	35	Sharma and Mallick (2005)
Chroococcus sp. M1	BG-11 modified Allen's	–	–	PHB	140	63.57	Beyatli et al. (2006)
Oscillatoria limosa 1966/1380		–	–		55	85.45	
Chroococcus sp. M1	BG-11	–	–	PHB	145	18.62	
Oscillatoria limosa 1966/1380		–	–		705	10.64	
Synechocystis sp. PCC6803	BG-11	P	0.4% (w/v) Acetate Pre-grown in BG-11 +0.1% glucose	PHB	–	28.8	Panda et al. (2006)
		N			–	14.4	
Nostoc muscorum	BG-11	N	0.2% Acetate 0.4% Propionate	[P(3HB-co-)3HV]	–	28.2	Mallick et al. (2007)
Arthrospira subsalsa	Modified ASNIII	N	5% NaCl	PHA	147.75[a]	7.45	Shrivastav et al. (2010)
Aulosira fertilissima	BG-11	P	0.5% (w/v) Acetate	PHB	160.1	77.2	Samantaray and Mallick (2012)
		N			143.5	58.6	
Nostoc muscorum	ES medium	P	Aeration 2.4L h^{-1} air +1.8 mL day $^{-1}$ CO_2	PHB	–	21.5	Haase et al. (2012)
Nostoc muscorum	BG-11	N	10g/L Poultry litter 10% CO_2 air flow 0.28% Acetate 0.38% Glucose 0.30% Valerate	PHA	773.5	65	Bhati and Mallick (2016)

(continued)

Table 4 (continued)

Cyanobacteria species	Culture condition			Type of polymer	mg L^{-1}	(% dcw)	References
	Cultivated	Nutrient deficiency (N or P)	Culture conditions				
Synechocystis salina	Digestate supernatant	–	–	PHB	88.7	5.5	Meixner et al. (2016)
Synechocystis sp. PCC 6714	BG-11	N and P	2% CO_2 at a flow rate of 0.02 vvm (20 mL min^{-1})	PHB	342	14.6	Kamravamanesh et al. (2017)
Mixed cyanobacterial culture	Wastewater	N	12:12 Cycles light: dark	PHB	61.61	6.5	Arias et al. (2018)
		P	24:0 Cycles light: dark		104	5.7	

[a]PHA content mg/g (cell dry weight)

achieved for some cyanobacteria species. As an example, Bhati and Mallick (2016) demonstrated the capacity of *Nostoc muscorum* to accumulate up to 65% of [P(3HB-co-)3 HV] when cultivated in poultry waste and supplemented with a 10% CO_2-laden air stream. Arias et al. (2018) optimized the light/dark cycle and nutritional regime to enhance the intracellular accumulation of PHBs and carbohydrates by a mixed wastewater-borne cyanobacterial culture, reaching 5.7% dcw accumulation under P limitation and constant illumination. Moreover, natural digestate was evaluated as a nutrient solution for producing PHB by *Synechocystis salina* in a 200 L tubular photobioreactor. While a maximum PHB content of ~6% was obtained, the quality of the produced PHB in terms of thermal and rheological properties was similar to commercial PHB (Meixner et al. 2016; Kovalcik et al. 2017).

Preliminary studies have demonstrated that cyanobacteria are able to achieve comparable PHB yields to bacterial strains, thus representing a potential alternative for PHA production. Besides, they allow for a reduction of the costs associated to biopolymer production using wastewaters as a low-cost nutrient source. However, downstream processing is more difficult due to the lower cell densities of cyanobacterial cultures, which constitutes the main drawback for full-scale implementation.

4 Simultaneous Wastewater Treatment and Biogas Upgrading

In addition to the microalgae applications presented in previous sections (including wastewater treatment, Sect. 2, and valorization through the production of highly valuable compounds, Sect. 3), the symbiotic activity of a microalgae-bacteria consortium has been also applied for the simultaneous treatment of wastewater and biogas.

Biogas is a by-product of the anaerobic digestion process, mainly composed of methane (CH_4) (55–70%) and CO_2 (30–45%). The biogas quality depends on the type of anaerobic digestion system and on the characteristics of the digested organic matter (i.e., sewage sludge, industrial wastewater, animal manure, crops residues, etc.). Besides CH_4 and CO_2, the biogas may contain other impurities, such as N_2 (<15% v/v), O_2 (<3% v/v), H_2O (<5% v/v), H_2S (<10,000 ppm$_v$), ammonia (<100 ppm$_v$), and siloxanes (<40 mg/m^3) (Bauer et al. 2013).

The produced biogas can be used in many applications such as heat or steam generation, electricity production through combustion, or as a substitute of natural gas, either by its direct injection into the natural gas grid or as transport fuel. However, biogas upgrading and purification is recommended or even mandatory before its utilization. The main objectives of the upgrading process are to increase the biogas calorific value by CO_2 removal, simultaneously reducing the transportation costs, and to prevent corrosion in the equipment (i.e., piping, compressors, etc.) by removing harmful compounds such as H_2S, which might also result in human toxicity. There are several physical-chemical technologies for biogas purification;

however, they are very costly due to their chemical and energy consumption (Awe et al. 2017). In contrast, biogas upgrading based on photosynthetic process appears as a novel, sustainable, and cost-effective alternative, which uses the removed pollutants (i.e., CO_2 and H_2S) as feedstocks for the simultaneous treatment of wastewater and the production of valuable products (biomethane and algal biomass) (Bahr et al. 2014; Muñoz et al. 2015).

The biogas upgrading process carried out by algal-bacteria consortia is based on the simultaneous CO_2 fixation by the microalgae and H_2S oxidation to sulfate by sulfur-oxidizing bacteria, which uses the dissolved oxygen produced during photosynthesis. For this process both gases must be transferred from the biogas to the liquid broth by absorption (Bahr et al. 2014). In addition, the broth alkalinity controls the removal of CO_2, an acidic gas. Thus, the use of centrate from anaerobic digestion process as nutrient source also provides the additional inorganic carbon (IC) required, supporting both an enhanced biogas upgrading and wastewater treatment (Posadas et al. 2017b; Toledo-Cervantes et al. 2016). The optimal IC concentration in the centrate that should be supplied has been recently quantified at ~1500 mg/L, guaranteeing a CO_2 removal >99% (del Rodero et al. 2017).

The process setup should provide good mass transfer of the target gases from the biogas to the medium broth, safe operation conditions by reducing the risk of an explosive atmosphere due to CH_4 and O_2 mixing, reduced CO_2 emissions, and simple construction and operation. From this point of view, the implementation of HRAPs interconnected with an absorption column (AC) has been consistently proven as one of the optimum operational systems allowing algal biomass production and efficient biogas upgrading at low costs (Muñoz et al. 2015).

Several studies have been performed with the objective of optimizing the HRAP-AC system, always achieving a high biomethane quality (>97% CH_4) regardless of the gas-liquid flow configuration in the absorption column. However, the optimum biomethane composition was obtained at a liquid to gas flowrate ratio of 0.5 (L/G) and cocurrent operation (Fig. 7). On the contrary, the countercurrent flow

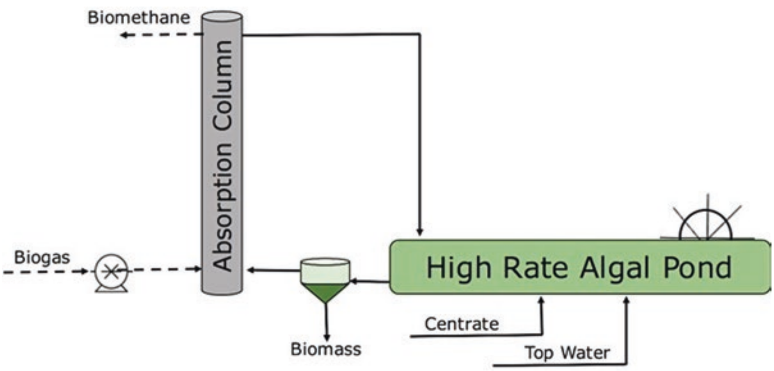

Fig. 7 Schematic representation of a photosynthetic biogas upgrading system consisting of a HRAP interconnected to an absorption column

in the absorption column resulted in a decrease in the biomass productivity and in the pH of the cultivation broth. Furthermore, exceptional carbon, nitrogen, and phosphorous recoveries from the influent wastewater have been observed in this configuration due to the decoupling of the hydraulic retention time from the solids retention time (SRT) through algal biomass settling (Toledo-Cervantes et al. 2016; Toledo-Cervantes et al. 2017).

When using a mass balance approach analysis to the process, it is possible to elucidate the compound's fate. The carbon fixation into biomass is mainly associated with the light intensity, while CO_2 striping is associated to the pond turbulence, both operational parameters affecting directly the HRAP performance. Therefore, a proper combination of light intensity and mixing in the photobioreactor increases biomass productivity and reduces CO_2 emission. In addition, if nitrogen is supplemented as NH_4^+ (e.g., from anaerobic centrates), this nitrogen form is assimilated at a rate governed by biomass production, stripped out as a result of the high pH of the cultivation broth (in open systems) or nitrified into NO_3^-/NO_2^- if sufficient O_2, SRT, and CO_2 is available. Finally, the phosphorus is almost completely assimilated by the microalgae biomass (Alcántara et al. 2015). In addition, the specific evaluation of the illumination regime (light/dark cycles) showed the ability of the system to regenerate the alkalinity consumed in the absence of light (Franco-Morgado et al. 2017). In summary, the application of the photosynthetic biogas upgrading by a microalgae-bacteria consortia provide a high-quality biomethane, with CO_2 removals >90% and final CH_4 concentrations >96% in the upgraded biogas, associated to biomass productivities of up to 15 g/(m^{2}d) (Toledo-Cervantes et al. 2017).

Regarding the microbial communities involved in the process, the selection of a high growth rate microalgae might increase the biomass productivity and the performance of the system. However, the population into the photobioreactors changes significantly during long-term operation, adapting to the operational conditions (Serejo et al. 2015). In addition, molecular characterization of the community has revealed high bacterial richness and high microalgae diversity based on the Shannon-Wienner diversity index (Posadas et al. 2015). Therefore, the microbiology developed in the HRAP is directly affected by the operational conditions of the system; however, the resilience of the evolving community allows for a stable biogas upgrading and wastewater treatment performance.

This technology was developed and studied indoors under artificial illumination, exhibiting excellent performances on CO_2 and H_2S removal; and it has been also recently validated outdoors under continental climate conditions (Posadas et al. 2017b). This study demonstrated the capacity of the technology to achieve complete H_2S degradation regardless of the environmental conditions and operation parameters. However, lower CO_2 removals were observed during winter as a result of the lower biological activity, in spite of the increase in gas solubility associated to the lower temperature of the cultivation broth. In addition, the IC accumulation during spring and summer triggered an increase in alkalinity and pH, resulting in an enhanced performance. Finally, optimization of the operation parameters for minimizing O_2 concentration in the upgraded biogas still requires further research (Marín et al. 2018).

5 Conclusions and Future Prospects

Current trends toward sustainability in the energetic and the industrial field demand renewable, unrestricted resources; cost-effective, environmentally friendly, and easily scalable techniques; and cheap feedstocks for the production of valuable products. Microalgae-based techniques meet all these requirements, as they can be used as a renewable carbon source, present rapid growth rates per unit area, and show high adaptability to harsh environmental conditions (i.e., wastewater composition and characteristics). However, in order to enhance the environmental and economic benefits of the process, algal biomass production using waste streams such as wastewater or CO_2 from waste gases (flue gas or biogas) and the subsequent valorization of the produced biomass via the simultaneous production of high-added value products is crucial. This general approach might boost their commercial application, resulting in one of the most sustainable ways to produce bioenergy and bioproducts through a biorefinery approach.

Nevertheless, in spite of the numerous studies devoted to explore the potential of microalgae for wastewater treatment in combination with the production of marketable by-products, further research at laboratory and full scale is mandatory to determine the optimal conditions for both biomass production and accumulation of the target products. Moreover, improving the photobioreactor design is also of key importance since the application of open systems (commonly implemented for algal biomass cultivation at industrial scale) for the production of profitable biomass derivatives is limited and they have little margin for technological improvement.

Finally, it is important to highlight that the growth of the algal biomass is only part of the whole process and downstream processing including biomass harvesting, dewatering, extraction, and final processing of the target product must be taken into account. In this sense, the large cultivation broth volumes, the low biomass densities (between 0.2 and 2 g/L), and the small size of algae cells arise as the main challenges for harvesting and dewatering stages. Therefore, the development of a sustainable and cost-efficient process for the production of high-added value products from microalgae requires a holistic approach.

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Microbial Carbon Capture Cell: Advanced Bio-electrochemical System for Wastewater Treatment, Electricity Generation and Algal Biomass Production

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1 Introduction

Water quality has been degraded due to receiving partially treated or untreated wastewater from domestic and industrial sources as well as from non-point sources. In recent years, exponential population growth and accelerated industrial development has resulted in increased quantity of generation of wastewater with high concentrations of diverse nature of pollutants. Hence, maintaining water quality in the water bodies has become an utmost important issue that needs immediate attention.

On the other hand, depletion of fossil fuel reserves has prompted the need of developing sustainable energy generation technologies. One such innovative and renewable technology is microbial fuel cell (MFC), which can simultaneously treat organic matter from wastewater as well as generate energy in the form of electricity for onsite applications. These bio-electrochemical cells convert chemical energy from wastewater used as fuel into electricity. Several researches are being carried out with a goal to facilitate and accelerate the development of MFC, which uses microorganisms to transform the chemical energy present in organic compound into electricity (Logan et al. 2006).

Generally, MFC is composed of an anodic and cathodic chamber, which is separated by a proton exchange membrane (separator). In the anodic chamber, the exoelectrogenic microorganisms, also known as electrogens, act as biocatalysts and oxidize organic matter into electrons, protons and carbon dioxide. The electrons are transferred to the cathode via external electrical circuit, and protons are transferred

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into cathodic chamber through membrane. Oxygen, or any another chemical oxidant, combines with electrons and protons on cathode to favour oxygen reduction reaction to form water, or other reduced compounds if alternate chemical oxidants are used. By this process, the energy stored in the chemical bonds of organic matter present in wastewater can be converted to electricity, which could be used directly. Thus, an efficient but easily available electron acceptor and PEM are prerequisite for good performance of MFC (Pant et al. 2010). The cost of external mechanical aeration in cathodic chamber for supplying oxygen and the high cost of ion exchange membrane need to be minimized for field scale application of MFC for wastewater treatment and electricity generation.

High oxidation potential and clean reduction product (water) make oxygen as the most common electron acceptor to be used in MFC. On the other hand, supply of oxygen in the cathodic side is, however, energy intensive mainly due to energy cost associated with external mechanical aeration. Microbial carbon capture cells (MCCs) nullify this challenge by using the ability of algae to produce oxygen with an added advantage of using harvested algae as a feed stock for production of emerging biodiesel as a fuel source. MCCs with different configurations (Elmekawy et al. 2014), different electrodes (Wang et al. 2010), various substrates (Wu et al. 2014; Ganesh and Jambeck 2013) and numerous algal species (Cui et al. 2014; Saba et al. 2017) have been widely studied. Microalgae employed in cathodic chamber should have high photosynthetic productivity and higher lipid content, which can qualify it as a good feedstock for biodiesel production (Morita et al. 2000).

MCC is a mix of employing different processes in two different compartments and hence, the performance of MCC rely upon individual efficiency of these two distinct processes occurring in anodic and cathodic compartments, depending on operating conditions and design parameters. The interrelationship of these dependable variables basically influences the overall performance of MCC. The present chapter provides a detailed knowledge on MCC along with an idea about the prominent and relevant parameters that influence its performance and efficiency.

2 Microbial Carbon Capture Cell

Carbon capture can be achieved in MFC by modifying it to MCC, for providing an encouraging solution of utilizing the CO_2 to synthesize the algal biomass in cathodic chamber, upon harvesting which can act as feedstock for biodiesel production, with simultaneous removal of organic matter from wastewater in anodic chamber and energy recovery in the form of electricity. The MCC consist of integration of MFC and algal species cultured in cathodic chamber for CO_2 sequestration and O_2 production to support cathodic reduction reaction (Fig. 1). The use of algal species in cathodic chamber generates oxygen during photosynthesis, making it available for cathodic reduction, thus reducing the cost of external aeration as required in aqueous cathode MFC.

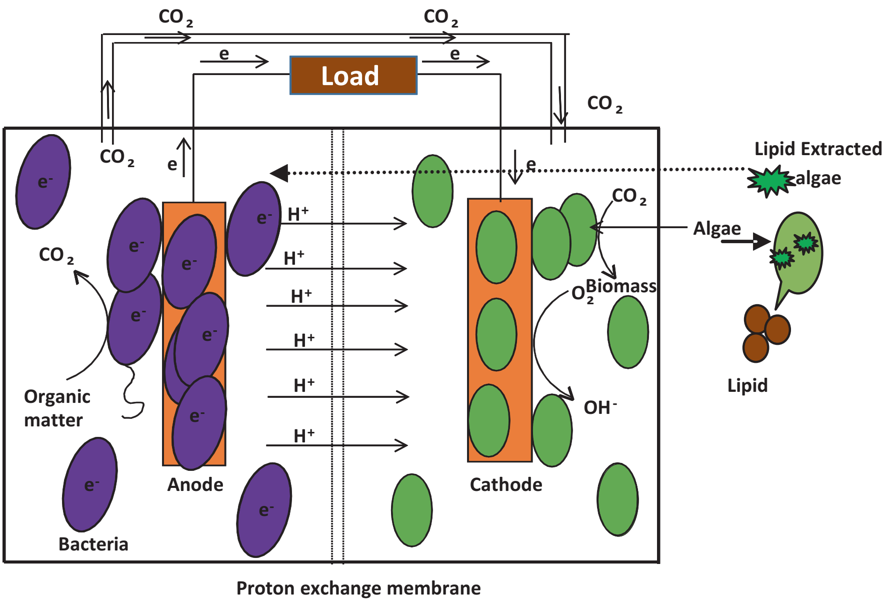

Fig. 1 Schematic representation of CO_2 capture and cathodic reactions involved in MCC

In MCC, the CO_2 generated during oxidative degradation of organic matter in the anodic chamber can be used for synthesis of the useful algal biomass with photosynthetic activity, attaining simultaneous electricity generation, CO_2 sequestration, wastewater treatment and biomass production. Thus, this addition of anodic off gases, produced during oxidation of organic matter, into cathodic chamber for favouring algae growth in cathodic chamber demonstrates an effective way for reducing CO_2 emission and in addition provides opportunity for simultaneous electricity generation without need of external aeration (Wang et al. 2010).

The overall biochemical reactions that occur at the anode and at the cathode of the MCC are as illustrated in Eq. (1) and Eqs. (2 and 3), respectively.

Anodic chamber:

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^- \tag{1}$$

Cathodic chamber (during exposure to light):

$$nCO_2 + nH_2O \rightarrow \left(CH_2O\right)_n + nO_2 \tag{2}$$

$$2O_2 + 8e^- + 8H^+ \rightarrow 4H_2O \tag{3}$$

During light phase, algae carry out photosynthesis to produce oxygen and algal biomass, whereas during the dark phase, respiration occurs where the oxygen is consumed as per reaction explained in Eq. (4) (González et al. 2013).

$$C_2H_4O_2 + 2O_2 \rightarrow 2CO_2 + 4H_2O \quad (4)$$

Hence, there is a need to maintain the optimum light: dark period to get best possible performance from MCC.

3 Advancement in MCC

Research efforts on MCC mainly focused on the enhancement of performance and byproduct synthesis by optimizing the cathodic configuration and operating conditions, making favourable environment to support algal growth and enhancing cathodic reaction kinetics. Several aspects of MCC research have been discussed here.

3.1 Application of MCC

Wastewater Treatment

The organic matter present in the wastewater can be utilized as a carbon source for microbial communities in anodic chamber during oxidation, and, thus, wastewater can be effectively treated in MCC (Fig. 2). Pant et al. (2010) reviewed utilization of various substrates ranging from simple sugars (e.g. glucose, acetate) to complex wastewaters (e.g. starch processing wastewater, kitchen wastewater). The agricultural wastewater, domestic wastewater, effluent from food-processing industries,

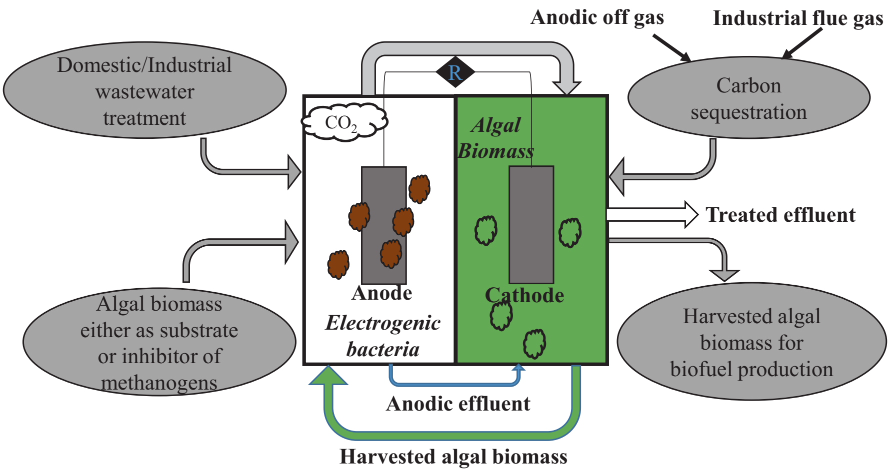

Fig. 2 Flow chart representing applicability of MCC

ligno-cellulosic waste, animal waste, waste activated sludge, etc. can be effectively treated in MFC, hence also in MCC, along with generation of bioelectricity. Hou et al. (2016) used *Golenkinia* sp. in cathodic chamber of MCC and achieved a power density of 6.3 W/m^3 along with chemical oxygen demand (COD) removal of 44%. Some of the researchers have even used algal biomass as a substrate for anodic oxidation and produced higher power output (Rajesh et al. 2015). Further, integrating microalgae with MFC (i.e. MCC) will provide an added advantage of removing nutrients from wastewater streams, if provided at cathodic side, along with carbon capture (Neethu and Ghangrekar 2017).

Electricity Generation

Pandit et al. (2012) supplied CO_2–air mixture to *Anabaena* sp. grown in cathodic chamber of MCC and generated a power density of 57.8 mW/m^2. The electrical output from MCC varies with fluctuations in oxygen concentration synthesized by algal species. Jadhav et al. (2017) reported 1.5 fold higher power generation with use of *Chlorella pyrenoidosa* algal species over *Anabaena ambigua* sp., due to superiority in oxygen production rate of *Chlorella*. However, the electricity generated in MCC depends upon many physiological conditions that favour bacteria in the anodic chamber, which include pH of anolyte, electrode materials, design parameters as well as cathodic conditions such as algal concentration, CO_2 concentration, etc.

Carbon Capture and Algal Biomass Production

Large quantity of $CO_{2,}$ emitted from various conventional wastewater treatment methods for organic pollutant degradation, is added to the environment annually (Campos et al. 2016). Capturing and supplying this extent of CO_2 to the cathodic chamber of MCC will result in high algal biomass production by making the process sustainable with additional benefits (Kokabian et al. 2013). Additionally, microalgae have been recognized as one of the most productive biological systems for generating biomass and capturing carbon while treating the wastewater (Fig. 2). Considering properties of each algal species, the particular class of *Chlorella* sp. have high photosynthetic rate that favours high growth kinetics and results in higher biomass production. During MCC operation of 25 days, an algal biomass production of 66.4 mg/(L.day) was reported for *Chlorella* sp. and over 50.4 mg/(L.day) for *Anabaena* sp. grown in cathodic chamber due to variations in cell structure and growth kinetics (Jadhav et al. 2017). However, the carbon capture rate and biomass generation yield for individual algae depends upon the growth kinetics, nutrient availability for growth, operating conditions, light intensity and other environmental parameters.

Other Applications

Along with above discussed applications, the electricity generated from MCC can be capable to power the different electronic biosensors and electronic appliances. Additionally, algae in cathodic chamber are capable to remove nutrients from the anodic effluent and thus provide the polishing treatment before disposal of treated effluent (Fig. 2). The algal biomass harvested during photosynthesis can be further used as feedstock for synthesizing byproducts such as biodiesel, bioethanol, biohydrogen, methane, etc. (Wang et al. 2015). Also, while treating sewage, first it can be treated in anodic chamber for organic matter removal and then in cathodic chamber for removal of nutrient, and in this way higher pathogen destruction in cathodic chamber is also expected, producing superior quality treated water for desired onsite reuse.

3.2 Photosynthetic Algal Microbial Fuel Cell

Algae have been used commonly in MFCs to produce oxygen in the cathodic chamber so as to have oxygen reduction at cathode and harvested algal biomass, with or without oil extraction, can be utilized as a substrate for bacteria. However, sufficient electric current can also be generated at anode, where cytochromes help indirect shuttling of electrons generated in photosystem II of the algal cells and can be called as photosynthetic algal microbial fuel cell (PAMFC) (Shukla and Kumar 2018). He et al. (2014) reported a COD removal up to 92% with power density of 2.5 W/m^3 in PAMFC having *C. vulgaris* in cathodic chamber. The immobilization of microalgal cells on polymeric or biopolymeric matrices can help in separation of algal cells together with enhancement of performance of MCC. Immobilization of microalgae provides additional advantage of increased cell density, resistance to toxic matter and stable operation with high metabolic activities over time.

3.3 Prospective of Algae

Algae as Substrate

Biomass is a good choice of feed stock to convert it to electrical energy, and algae are the most easily available source of biomass with high yield per unit area of land. The algal dry mass, considered as major pollutant vector in the streams, can be used as a potential substrate for electricity generation in anodic chamber of MCC (Cui et al. 2014). The algal biomass harvested during cathodic photosynthesis can be pretreated with heat treatment, enzymatic treatment or chemical treatment so that treated (or even untreated or live) algal mass can be utilized as a carbon source for anodic oxidation to produce the electrons (Shukla and Kumar 2018).

Algae as Biocathode

Since algae are photosynthetic microorganisms, the availability of light as well as the electron-donating anodic process may have significant effects on the performance of the biocathode. The phototrophic microorganisms can serve as biocatholytes in MFCs because the oxygen produced is an electron acceptor for the harvested electrons from the anodic chamber. Researchers used photosynthetic biocathode in sediment type MCC and achieved effective wastewater treatment (Commault et al. 2014; Neethu and Ghangrekar 2017). Previously, researchers also confirmed the advantageous use of algae as a viable biocathode in microbial desalination cells to supply electron acceptors in an sustainable manner (Kokabian and Gude 2015).

Algae as Inhibitor of Methanogens

Considering the varieties of microbial populations present in the mixed anaerobic sludge inoculum used in anodic chamber, major substrate is consumed by methanogenic consortia for methane production and other non-electrogenic reactions. To reduce the substrate consumption by non-electrogenic bacteria and recover maximum coulombs from the substrate, researchers have used algal powder to suppress the growth of methanogens and also to serve as a substrate for anodic oxidation (Rajesh et al. 2015, 2014). Hexadecatrienoic acid, a long-chain saturated fatty acid, present in the marine algae *Chaetoceros* was found to inhibit the growth of methanogenic *archaea* by the process of adsorption as well as disruption of cell membranes and achieved the Coulombic efficiency (CE) of 45.18% with a power density of 21.43 W/m^3 (Rajesh et al. 2015).

Algae for Wastewater Treatment

Along with carbon capture, microalgae can assist in effective removal of nutrients present in wastewater by utilizing these elements during their cell metabolism (Elmekawy et al. 2014). Electro-migration along with diffusion of ions from anodic to cathodic chamber concurred for recovery of nutrients from the wastewater (Colombo et al. 2017). Recently, Huang et al. (2017b) reported maximum PO_4^{3-}-P removal up to 37.2% using *C. vulgaris* biocathode in MFC. Nitrogen is utilized as nutrient source for algal cultivation and can be effectively treated in MCC at loading of 2 g/L of nitrate (Neethu et al. 2018). However, additional photo-bioreactor coupled with MFC is reported to be capable of removing about 92% phosphorous and 99% NH_4^+-N. Moreover, Kokabian and Gude (2015) proposed coupling of algae in cathodic chamber of microbial desalination cell and achieved complete salt removal in such system. Single-chambered air cathode MFC was capable of removing COD, colour and heavy metals (Zn-98%; Cr-80%) from wastewater (Logroño et al. 2017).

Algae as a Carbon Source for Electrode Material

Harmful algal blooms, including blue-green algae, can act as a promising electrode material for sodium-ion batteries and can be used for fuel cell applications. The algae carbonization helps to develop low-cost green electrode material for high-capacity batteries and also contribute to solve the issue of harmful algal blooms (Meng et al. 2015). Also, nanoporous carbon having large specific surface area can be synthesized from microalgae as a promising composite electrode material (Zhou et al. 2012).

Microalgal Biorefinery

Microalgae contains high concentration of proteins, lipids and carbohydrates, and its biomass after harvesting can be utilized as a potential feedstock for biodiesel production, lipid extraction and other applications. In microalgae biorefinery applications, CO_2-neutral MFC was developed for producing feedstock for bioethanol production, algae oil extraction and bioelectricity generation simultaneously during treatment of fermented beer yeast in the anodic chamber (Powell and Hill 2009). However, some algal species requires pretreatment to release carbohydrates stored in the cells. In addition, photobiological and fermentative ways of biohydrogen production from algal biomass employing *S. obliquus* and *Chlamydomonas reinhardtii* in cathodic chamber of MCC are well established.

4 Factors Governing the Performance of MCC

4.1 Algal Biocathode

Oxygen is one of the most widely recognized electron acceptors utilized within the cathodic chamber of MFC owing to its high oxidation potential (0.8 V vs. SHE), and certainty it produces a clean end product, i.e. water, after reduction (Ucar et al. 2017). In any case, most investigations demonstrated that supply of oxygen to the cathodic chamber consumes energy. Microalgae may provide a viable alternative to cathodic oxygen supply; however, its efficiency as an eminent biocathode depends on various factors which are discussed briefly here. The oxygen that is given away during photosynthesis originates from water and not from the part of CO_2. The light reaction responsible for oxygen production occurs at the thylakoid membranes of the cell chloroplast, thus rate of oxygen evolution is believed to be dependent mainly on cell type and concentration, light intensity and operating conditions (Perrine et al. 2012).

Algal Species, Type and its Concentration

Performance of MCC is reliant on algal species as the photosynthesis rate and cell multiplying time are diverse for different types of microalgae communities (Sun et al. 2016). An investigation by Jadhav et al. (Jadhav et al. 2017) showed the dominance of *Chlorella* over *Anabaena* for capturing CO_2 and generation of photosynthetic oxygen that encouraged the cathodic reduction. The *Chlorella sp*. is the most preferred biocathode on account of their resistance for high level of CO_2, higher tolerance to municipal wastewater and reasonable lipid content. A study utilizing diverse types of *Chlorella* in cathodic chamber showed that a superior performing MCC was acquired with *C. vulgaris* as biocathode to demonstrate higher biomass yield and CO_2 fixation rate (Hu et al. 2015).

Gautam (2016) reported that biocathode with mixed algae collected from natural pond performed better over pure culture of *C. pyrenoidosa* biocathode in MCC under natural sunlight conditions and it can be suitable option for practical application of MCC at large scale. In this way the power yield is, by implication, dependent on the biomass concentration, which in turn relies upon algal species used and cell doubling time. Equally important is the cell concentration; lower cell concentration infers less oxygen (electron acceptor) availability, thus resulting in reduction in performance of MCC. Power can be enhanced with increment in concentration of algal biomass up to a certain concentration; however, beyond certain concentration of algae, the efficiency decreases due to self-shading of algae where cells close to the surface utilize a portion of the light and shade those more deep in the water (Ugwu and Aoyagi 2008). Another critical unfavourable impact of higher cell concentration is the metabolic loss because of formation of excess metabolites. Algae used in MCC serve as feedstock for biodiesel production as well as sequesters the anodic off gas introduced into the cathodic chamber. Hence, while choosing the algal species, it is crucial to choose species that have high photosynthetic efficiency to capture CO_2 and also that can yield lipid having proper unsaturated fats for biodiesel synthesis.

Light, CO_2 Supply and Oxygen Concentration

Different environmental parameters influence algal growth kinetics either directly or in a roundabout way, these include light/irradiance/temperature, CO_2, pH, blending/aeration, salinity, etc. Keeping in mind one of the end goals, i.e. to improve the microalgal growth in cathodic chamber of MCC, the light prerequisite is a standout among the most vital parameters; hence, proper intensity, duration and wavelength of light should be provided with extreme care. Extreme intensity may prompt photo oxidation leading to growth prevention, while reduced light intensity will lead to growth restrictions. There have been a few advancement in the utilization of light by using changed light sources, such as texturized optical filaments and LEDs with particular wavelengths and proper lens to collimate light beam (Carvalho et al. 2011). The part of incident light that isn't utilized gets changed over into heat energy; consequently, utilization of red and blue light is ideal to keep up the survivable temperature for algal cultivation (Michael et al. 2015). Thus, the utilization of internally illuminated LED can bring out different favourable circumstances of less heat energy scattering along with avoidance of self-shading. Low temperatures increase the solubility of CO_2, and this indeed promotes the high growth rate and yield of microalgae.

Equally important is the presence of carbon source, especially CO_2. Some green algae are accounted for to effortlessly grown at high CO_2 concentration, and *Chlorella* are one of the known species that are used for carbon sequestration having high photosynthetic efficiency to convert CO_2 to O_2 (Singh and Singh 2014). Enhancement in power generation, quantity of biomass and lipids with increase in CO_2 concentration were reported earlier by many authors (Wang et al. 2010; Sato et al. 2003; Andersen and Andersen 2006; Tang et al. 2011). Optimum concentration of CO_2 varies with species as evident in the case of *C. vulgaris* with a carbon fixation rate of 6.17 mg/L.h (Bhola et al. 2011) unlike *Scenedesmus* species having the optimal CO_2 consumption rate of 59.19 mg/L.h (Ho et al. 2012). When CO_2 is consumed in the presence of light due to cell metabolic reactions, O_2 is liberated, which acts as electron acceptor in MCC. One of the significant limitations of algal growth in a photo-bioreactor is that high oxygen concentration suppresses the growth, which is not an issue in MCC as oxygen released constantly get reduced by means of oxygen reduction reaction (ORR) for cathodic reduction. In MCC, a voltage of 706 mV was observed with a DO concentration of 6.6 mg/L in cathodic side, hence making algae as a suitable candidate for production of O_2 that is necessary for stable performance of MCC (Kang et al. 2003).

Nitrate Concentration

Different microalgal species will respond differently to concentration of nutrients provided depending on their quota flexibility. Microalgae expel nutrients from wastewater essentially by utilizing it for algal metabolism (Aslan and Kapdan 2006). Nitrogen has a key impact in deciding the productivity of microalgae as far as biomass and lipid production is concerned. Neethu et al. (2018) reported that power generation was increased with increase in nitrate concentration from 0.5 to 2.0 g/L and further increase in nitrogen resulted into decrease in power output of MCC. Also, Converti et al. showed that decrease in concentration of nitrogen in the medium can expand the lipid portions of biomass dry weight (Converti et al. 2009). Hence, it is important to have an optimum concentration of nitrogen at which biomass and lipid content can be boosted. For this situation, the domestic wastewater, which contains moderately less inorganic nutrients, can permit appropriate development of microalgae alongside the accumulation of lipid content in algal cells.

4.2 Operating Conditions and Anodic Constraints

Performance of MCC is highly determined by the operating condition as discussed earlier. Thus, if the condition is unfavourable to the exoelectrogenic bacteria (on anode) and algal community (in cathodic chamber), the overall performance of MCC will be adversely affected. For optimum COD removal and power production, operating conditions such as organic loading rate (OLR), hydraulic retention time (HRT), inoculums, substrate, anodic environment and anolyte pH ought to be rightly chosen. The OLR mainly depends on the substrate concentration (i.e. COD loading) and flow rate, which is again dependent on HRT. Substrate fed in MFC can range from complex molecules of starch to simple acetate (Pant et al. 2010). Complex substrates have to be broken down to simple organic molecules before being used as carbon source in MFC for easy metabolism by bacteria (Pant et al. 2010). In a study, when acetate was compared with butyrate, propionate and glucose as substrate in MFC, the acetate-fed MFC gave the highest CE (Chae et al. 2009). Similar results proving the higher efficiency of MFC by usage of simple compounds were reported by Liu et al. (2009), which compared the efficiency of acetate-induced consortia and protein-rich wastewater as substrate. Operation mode can be either batch mode or continuous mode, where the later one is preferred in practical application for continuous generation of electricity. Also, the HRT to be provided depends mainly on the complexity or degradability of the substrate, the influence of which on performance of MFC being reported in several literature (Sharma and Li 2010; Akman et al. 2013; Rahimnejad et al. 2011). Hence an optimum HRT should be fixed, which gives the best result in the prevailing environmental, bacterial as well as other operating conditions.

In addition to the previously mentioned parameters, electrolyte pH is likewise as vital as it governs the performance of MFC. Majority of researches arrived at a conclusion that the consortia best performs in alkaline condition (Zhuang et al. 2010; Puig et al. 2010; Behera and Ghangrekar 2009). The reason for this conclusion is differently addressed in various studies. Certain work demonstrated the higher internal resistance at lower pH (Behera and Ghangrekar 2009), whereas Yuan et al. focused on the effect of anolyte pH on electrocatalytic activity of anodic biofilms (Yuan et al. 2011); alongside Zhuang et al. explained this by more negative anodic potential as a result of alkaline pH (Zhuang et al. 2010).

4.3 Design Parameters

Proper design of MCC can lower the overall internal resistance and improve efficiency of organic matter oxidation and TDS removal. Design parameters that affect the performance include the electrode spacing, electrode material, membrane thickness and area, mixing, size of chambers and reactor layout. The reactor configuration is a critical parameter that influences the power generation and algal production in MCC (Table 1). Algae are generally cultivated in photo-bioreactor (PBR), and subsequently while incorporating algae in MFC system, it can take two configurations – the PBR externally connected to the MFC and the PBR incorporated inside the MFC components (Fig. 3). The first configuration can be a photo-reactor bottle connected using peristaltic pump to the MFCs, where complete recirculation occurs in a closed-loop system (Gajda et al. 2015; Jiang et al. 2013) or PBR kept separately without PEM wherein CO_2 generated in anodic chamber is directly released into the photo-bioreactor (Powell et al. 2009). In case of later, among the diverse reactor designs proposed by researchers, the generally adopted design is dual-chambered MCC.

In a two-chambered MCC, anolyte (anodic chamber) and catholyte (cathodic chamber) are separated by a membrane (proton exchange membrane), and electrodes are linked by an external circuit. In this type of MCC, the CO_2 produced in anodic chamber is generally transferred to the cathodic chamber through a vent made at top of each chamber (Khandelwal et al. 2018). Apart from dual-chambered system, certain studies were carried out using single-chambered MCC, where the electrodes were placed in a single chamber without separators and algal bacterial symbiosis was observed in such MCC (Fu et al. 2010). Aside from this, an airlift MCC system, established by Hu et al. (2015), simultaneously achieved the high level of carbon sequestration along with wastewater remediation.

Design of algal chamber of MCC is one of the major technical aspects that should be given importance for the production of microalgal biomass in cathodic chamber. In contrast to the usual MFCs, there must be a transparent surface keeping in mind the end goal to guarantee the light is received by the algal cells in cathodic chamber of MCC. For the most effective utilization of incident light, several studies have been carried out in terms of reactor design and its architecture. While designing the cathodic part of MCC, no light should be lost, and no dark area ought to happen in which algae don't grow. So light capturing, channelling and scattering play an important role while considering the design of MCC. Scientists have explored the utilization of Fresnel focal points and light guides to focus, carry and deliver direct light into the algae suspension (Zijffers et al. 2008). Also, few studies were conducted to enhance the horizontal dispersion to an expansive stretch out by roughening the surface of the illuminating surface of the distributor (Csögör et al. 1999). Hence, while planning the lighting arrangement to MCC, importance should be given to the following: (i) enough light is available as required for the growth of species used, (ii) light intensity that can be adjusted accordingly, (iii) light wavelength (blue, red) that can be shifted to support the algal growth, (iv) light frequency should be variable, which can match the prevailing condition of region, (v) uniform distribution of light throughout the media and (vi) proper mixing can help in uniform distribution of incident light.

Biocompatibility of carbon-based material such as graphite rod, carbon felt, carbon cloth, etc. makes it the most suitable material to be used as electrodes in MCC (Table 1). Surface area and its roughness are two major factors that determine the adhesion of bacterial or algal biofilm on electrode surface. Compared to other electrode materials, graphite felt provide the desired surface area and texture required

Table 1 Effect of various design parameters on performance on MCC

Anode material	Cathode material	Reactor configuration	Substrate	Algal biocathode/ catholyte	Power/current density	References
Carbon felt	Carbon felt	Double chamber	Synthetic wastewater (SWW)	*C. Pyrenoidosa*	6.4 W/m^3	(Jadhav et al. 2017)
Graphite felt	Graphite felt	Dual chamber	SWW	*C. vulgaris*	2.7 W/ m^3	(Khandelwal et al. 2018)
Carbon felt	Carbon felt	Dual chamber	Sediment (inoculums) + SWW	*Chlorella*	202.9 ± 18.1 mW/m^2	(Zheng et al. 2017)
Carbon felt	Carbon felt	Dual chamber	UASB (inoculums) + SWW	*Chlorella*	158.2 ± 15.1 mW/m^2	(Zheng et al. 2017)
–	Stainless steel	MBR-MFC	SWW	*C. pyrenoidosa*	1.2 W/m^3	(Yang et al. 2017)
Carbon cloth	Carbon cloth	Dual chamber	SWW	*Cyanobacteria*	1.48 mA	(Rago et al. 2017)
Carbon cloth	Carbon cloth	–	–	*Scenedesmus sp*	–	(Angelaalincy et al. 2017)
Carbon brush	Stainless steel	Tubular	SWW	*Chlorella*	200 mA/m^2	(Ma et al. 2017)
Carbon cloth	Carbon cloth	Two chamber	SWW	*Spirulina*	0.85 W/m^2	(Colombo et al. 2017)
Graphite rod	graphite rod	Two chamber	*C. pyrenoidosa*	$K_3[Fe(CN)_6]$	6030 mW/m^2	(Xu et al. 2015)
Carbon paper	carbon paper	Two chamber	*C. pyrenoidosa*	*C. pyrenoidosa*	2.5 mW/m^2	(Xu et al. 2015)
Graphite rod	Graphite rod	Two chamber	SWW	*C. vulgaris*	0.6 mW/m^2	(Mitra and Hill 2012)
Carbon brush	Carbon felt	Two chamber	SWW	*C. vulgaris*	187 mW/m^2	(Liu et al. 2015)
Graphite felt	Graphite felt	Two chamber	SWW	*Desmodesmus* sp.	64.2 mW/m^2	(Wu et al. 2014)
Carbon fibre	carbon paper	Two chamber	SWW	*Microcystis aeruginosa*	58.4 mW/m^3	(Cai et al. 2013)

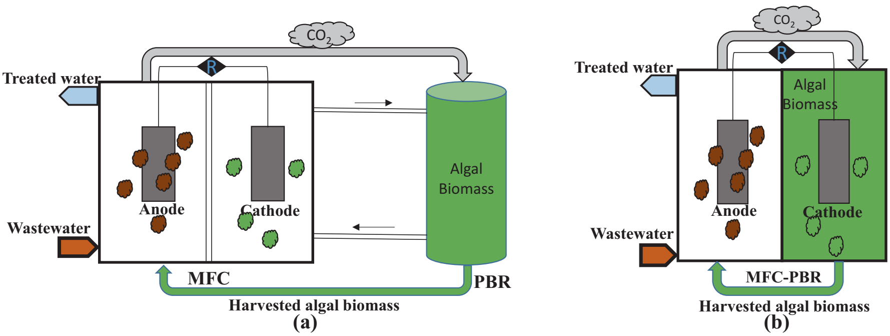

Fig. 3 Configurations of microbial carbon capture cell (a) PBR connected to MFC and (b) PBR within MFC

for uniform biofilm formation and colonization of bacteria. An enriched biofilm formation on anode surface will help in easy transfer of electrons to anode. Another major design factor that determines the performance of an MCC is the spacing between anode and cathode. Though utilization of appropriate material can diminish the activation losses (Mustakeem 2015; Zhou et al. 2011), bringing the anode and cathode closer can bring down the ohmic losses (Doherty et al. 2015). The power generation decreases with an increasing electrode spacing; whereas, keeping the electrodes too close can quicken substrate and oxygen diffusion, bringing about fast biofouling of the cathodes (Tartakovsky and Guiot 2006). To overcome these unfavourable impacts on performance of MCC, there should be an ideal separation between the electrodes. Electrode spacing could be related with different factors in controlling efficiency of MFC. Previous studies showed the effect of external resistance (Ghangrekar and Shinde 2007), where a maximum power density was observed at lower spacing of 20 cm between the electrodes, apart from this certain studies proved that the control over electrode spacing for improving power generation is dependent on substrate concentration (Lee and Huang 2013). Ahn et al. have tried different electrode configurations to optimize the performance of a multi-electrode MFC; a better power and coulombic efficiency was attained by separator electrode assembly configuration, whereas better wastewater treatment efficiency was achieved in configuration with closely spaced electrodes (Ahn et al. 2014).

Maintaining the anaerobicity of anodic chamber is a prerequisite for the growth of exoelectrogenic bacteria in anodic chamber of MCC. Hence, it is necessary to set apart the anodic chamber from the cathodic chamber rich in oxygen, and here separator plays a major role in design of MCC. An ideal separator should have higher proton conductivity, ion transport number, ion exchange capacity, water absorption along with minimal oxygen diffusion, resistance, acetate crossover and biodegradability (Tanaka 2015). Among the different cation exchange membranes commonly used, Nafion is most popularly used membrane (Huang et al. 2017a); however, bipolar membranes (Kim et al. 2017), chitosan-graphene oxide mixed-matrix membrane (Holder et al. 2017), glass wool (Venkata Mohan et al. 2008), SPEEK membranes

(Ghasemi et al. 2016), ceramic membranes (Daud et al. 2018) and clayware membranes (Ghadge et al. 2015) were also used. The mostly used membrane separator in MFC is the Nafion membrane; however, it has few limitations, for example, oxygen diffusion, cation accumulation, substrate loss, durability and high cost. These confinements have prompted tremendous attempts in the advancement of a suitable material that can viably fill in as a low-cost PEM, and the researches are still going on in search of suitable replacement.

4.4 Other Factors

Along with major factors, other parameters such as mixing conditions, immobilization of biomass, etc. affect the performance of MCC in terms of electricity generation and wastewater treatment. Turbulence (mixing) affects the growth positively by increasing rate of mass transfer between nutrients and algal cell; it also helps in removal of metabolites (e.g. oxygen) from the growth media. Similar studies also suggested immobilization of microalgae on glass beads or polymeric or biopolymeric matrices are capable of producing high algal cell concentration, resistance to hazardous matter, stable and flexible operation and longer period of operation with stable voltage due to longer logarithmic growth phase (Jin et al. 2011; Bashan and De-Bashan 2010). The rate of cathodic reactions was enhanced by concentration of oxygen as terminal electron acceptor, and hence growth kinetics of algal culture under given operating conditions is important to overcome the cathodic limitations.

5 Bottlenecks and Perspectives

Even though remarkable progress is evident in the field of MCC research, there are still certain challenges that need to be overthrown in order to commercialize this technology. Integrating algae in MFC will make MFC a complex system, whose performance will depend on several factors most of which have been already discussed in this chapter. Since in MCC the oxygen produced by the microalgae plays a major role, increasing the photosynthetic efficiency of algal species is of utmost importance. As discussed earlier, different microalgae strains respond differently to different growth conditions provided, and hence it is difficult to optimize these conditions to a specific species. Also, in MCC the algae relies upon the CO_2 received from the anodic chamber; hence, studies need to be done to quantify the flow of anodic off gas towards the algal chamber, and further anodic oxidation will be limited by the O_2 released by photosynthesis; hence, its role is important. MCC developed must be able to treat wastewater having different strengths and compositions; handle variations in pH, temperature, etc.; and operate without any adverse impact to the environment. Modelling studies and optimization of various factors affecting the performance of MCC are of great importance. For enhancing power generation, development/synthesis of low-cost cathode catalyst that is not toxic to algae is yet to be investigated.

Design parameters including the materials used and MCC configurations are equally important in increasing the efficiency of MCC. Configuration of MCC should be such that it gives no or minimal loss of CO_2 while moving from anodic chamber to cathodic chamber. Along with transport of CO_2, transport of protons from anodic chamber to cathodic chamber is equally important to complete the redox reactions. A low-cost membrane separator with minimal oxygen and acetate diffusion along with high proton conduction is prerequisite for efficient performance of MCC, which is yet to be synthesized. Similarly, the voltage generated has a positive correlation with the dissolved oxygen concentration of catholyte (Neethu et al. 2018), which fluctuates with the day and night cycle. This can come out as a great challenge faced by MCC, when thought to be operated under natural sunlight condition, which needs an immediate solution. Considering the complication of MFC system alone, coupling or integrating algal system to MFC makes overall process complex for commercialization. The high capital cost for fabricating MCC, considering all dependable components and their lower efficiencies, is also by far the major factor contributing to the limited commercialization of MCC technology. In order to compete realistically with other prevailing feedstock yield for biodiesel production and power generation technologies, as well as offering wastewater remediation, MCC should turn out to be more powerful from the viewpoint of both effectiveness and cost.

6 Summary

Application of MFC in wastewater treatment and bioelectricity generation is a well-known concept in present scenario; however, utilization of algae for oxygen supply, biomass production and other product synthesis along with providing polishing treatment to wastewater for removing nutrients is a major breakthrough in BES research domain. The performance of the system as a whole depends on the electrochemical reactions that occur between substrate oxidation to the final electron acceptor. Factors including algal growth kinetics, density, light intensity, CO_2 supply and other operating conditions govern the performance of MCC. Proper selection of these parameters and finding optimum condition for these for the algal species being cultivated in cathodic chamber of MCC can take forward this technology to an advanced level for its real-life applications. Moreover, presence of algae in cathodic chamber of MCC, apart from providing oxygen for cathodic reduction, also can serve as a substrate for anodic oxidation, a methanogen inhibitor to enhance the CE, harvested algal biomass for biodiesel production and other byproduct recovery, which can make MCC a cost-effective and sustainable solution for wastewater treatment as compared to conventional wastewater treatment methods.

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Microalgal Systems for Integrated Carbon Sequestration from Flue Gas and Wastewater Treatment

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1 Introduction

Despite the steady reduction in emissions in the previous years (2014–2016), 2017 is estimated to record 2% increase in global carbon emissions based on the initial data. Scientists warned that by 2030, the world should be ready with successful carbon sequestration methodologies to keep the temperature rise below 1.5 °C (Guardian 2017). If sequestering carbon or emission reduction strategies begin by 2018, we need to achieve the emission reduction of 2% in the next 2 years (i.e. by 2020) to check temperature rise lower than 2 °C. On failure to achieve this target by 2020, the emission reduction target will get higher and higher every year (Le Quéré et al. 2017). The major cause of these anthropogenic emissions is the burning of fossil fuels in transportation and coal power plants. Conservative usage of fossil fuels for vehicles and power generation could bring down the carbon dioxide and other greenhouse gas concentration in the atmosphere. Recently, utilization of renewable energy is on the rise; however, it is to be noted that they cannot completely fulfil the present energy requirements. Hence, there will be burning of fossil

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fuels at least for another 10–15 years, and it will continue to add carbon dioxide to the atmosphere. Leak-proof, economical and highly efficient sequestration techniques need to be in place to achieve the desired reduction in emission and keep the temperature rise in check. There are different methods of carbon capture and storage (CCS) strategies available which involve permanently storing the CO_2 in used mines, deep aquifers and under the oceans, chemical conversion of the captured CO_2 to mineral carbonates and using the captured CO_2 to extract oil. However, almost all of them suffer from some disadvantages like higher costs of construction, installation and maintenance, the threat of CO_2 leakage and convincing the local population and making them aware of its urgency and uses. Biological carbon sequestration provides the natural remedy of storing carbon in biomass, which could be later used for the production of certain valuables.

Microalgal CCS stand apart as the simplest and easier method to trap CO_2 from the flue gases. Microalgae are the group of oxygenic photosynthetic organisms, which naturally utilize CO_2 as their carbon source. Microalgae refer to the prokaryotic *Cyanophyceae* (e.g. *Spirulina* sp.), eukaryotic *Chlorophycean* (e.g. *Chlorella* sp.) and diatom (*Bacillariophyta*) members. These organisms were one among the first few organisms to photosynthesize. Microalgae have significantly better carbon fixation rate than the higher plants, and it makes them most appropriate agents for biological carbon sequestration. Microalgal CCS is gaining interest in the recent times. Even though there are very few dedicated sequestration sites, many algal biomass producers are utilizing flue gas as their carbon source. Microalgal cultivation requires a huge amount of water, and it should be fulfilled without competing with the local freshwater resources for human consumption and irrigational purposes. In India, sewage generation in 2015 is estimated to be 61,754 MLD (million litres per day), and the capacity of wastewater treating plants is just 22, 963 MLD (CPCB 2016). A staggering 38, 791 MLD are discharged directly into the nearby water bodies polluting the water and the associated aquatic life. If these wastewaters could be utilized, microalgal cultivation could be very cost-effective, and it also serves the purpose of remediating the effluent. Microalgae are noteworthy in establishing growth in a wide variety of wastewaters and treating even the most toxic pollutants. They utilize the waste chemicals in wastewater as their nutrient source thus remediating the effluent. Utilizing microalgae for carbon capture through wastewater treatment (WWT) is cost-effective, requires low energy and does not need costly installations or types of machinery, and it results in a biomass that can be processed to produce various commercially valuable products. Notwithstanding its advantages, microalgal CCS is still not a preferred mode for algal biomass production owing to its limitations in biomass productivity and poor net energy ratio (NER). This chapter will analyse the recent publications in microalgal CCS and WWT and different factors associated with the process, with a special focus on the status of commercial microalgal CCS and wastewater treatment installations with a biorefinery concept.

2 Ever-Increasing Carbon Emissions

Significant increase in the carbon emission into the atmosphere lead the United Nations Framework Convention on Climate Change (UNFCCC) to frame an agreement at Paris (COP 21) where the world nations pledged to reduce their emission to keep the temperature rise below 1.5 °C. 2017 was a great year for green energy as many nations reaffirmed their commitment to renewable energy and restraint to coal burning. The United Kingdom, on April 21, 2017, generated coal-free electricity for the whole day. Probably encouraged by this, the UK has announced that it will phase out all coal-based electricity generation by 2025, and similarly Canada vows to retire all the coal power plants by 2030 (Cockburn 2017). The price of electricity generated through solar and wind plants is getting cheaper every year. For the first time, it got cheaper than the coal-generated electricity in many countries like India, Brazil and China (Lant 2017; Safi 2017). Since the Industrial Revolution, this is the first time that prices have gone cheaper than fossil fuel-generated power. With cheaper installation and production costs, more amounts of solar photovoltaic plants are being set up. Because of increasing awareness and the regulations of the Paris Agreement, the emissions of the first two largest emitters, China and the USA, decreased or remained constant for the years 2014, 2015 and 2016. However, irrespective of the increasing renewable energy generation capacity, India, the third largest emitter of CO_2, continued to show a growth in CO_2 emissions every year, while the major emitters showed a decrease. The early data for the year 2017 shows the carbon emissions to increase by 2% to the previous year (Le Quéré et al. 2017). This clearly highlights the fact that along with increasing the renewable energy capacity, we should also look at sequestering the carbon emitted from the industries. Hence, there is a strong necessity for effective and economic strategies to trap CO_2 before it enters the atmosphere.

3 Carbon Capture and Storage

Keeping the carbon emission into the atmosphere under check is one of the major objectives for the environmentalists, and carbon capture and storage (CCS) is one among the most trustworthy and achievable routes to reduce emissions. CCS involves capturing CO_2 from the industrial exhaust gases and then dumping in a permanent and safe site (natural or man-made). CCS involves three components, capturing the CO_2, transporting it and storing it for a long term. The gas is usually captured either from the coal-firing power plants or cement industries or other potential industries using any one of the following methods, like absorption, adsorption, membrane separation and cryogenic separation (Pires et al. 2011). The captured CO_2 is transported in a compressed state via pipelines or containers to a nearby storage site like a used mine, depleted reservoirs, deep oceans, mineralization plants, etc. The storage sites can be either geographical sites, ocean floors or mineralization plants, but the sites should (i) be safe, (ii) have lower impact to the environment, (iii) have verifiable storage and (iv) have storage liability that is indefinite (Lackner and Brennan 2009).

Carbon dioxide released during combustion of fuels can be captured by various systems like pre-combustion capture, post-combustion capture and oxy-fuel capture systems (Gibbins and Chalmers 2008). In pre-combustion capture, the coal or natural gas is treated prior to combustion (Leung et al. 2014). The fuel is reformed with steam and/or oxygen to release H_2 and CO_2. CO_2 in the mix is separated by combusting H_2 with air. The CO_2 from the exhaust gas is captured after combustion of the fuel in post-combustion capture (Thiruvenkatachari et al. 2009). The lower concentration of CO_2 in the exhaust gases is a main restraint when it comes to carbon capture. Oxy-fuel combustion makes use of oxygen for combustion purposes. Condensing water is used to separate CO_2 and H_2O the combustion products (Zanganeh et al. 2009). This process is advantageous owing to the low amount of nitrogen in exhaust gas that will not harm the subsequent processes and will also reduce the thermal NOx (Buhre et al. 2005). Among the three systems, post-combustion capture appears to be the most developed method for CCS projects (Bhown and Freeman 2011). However, cost wise, pre-combustion capture and post-combustion capture were found to be less costly for coal- and gas-fired plants, respectively, on the comparison of the three methods (Gibbins and Chalmers 2008).

3.1 Physical Methods

Many different strategies have been followed to capture CO_2 from waste gaseous streams, after which it will be transported for storage (Fig. 1). They include (i) absorption, (ii) adsorption, (iii) membrane capture and (iv) cryogenic distillation.

Absorption involves physical or chemical interactions in which atoms and/or molecules dissolved in a bulk phase are used to extract CO_2 from the mixture. It is very common in chemical industries where it is used in remediating gas streams with CO_2, H_2S and NOx (Majchrowicz et al. 2009). Alkylamines like monoethanolamine (MEA), diethanolamine (DEA) and potassium carbonate are some of the widely used absorbents (Hendriks 1994). MEA for its higher efficiency than the other absorbents found use (30% MEA) in a pilot absorption-based post-combustion carbon capture setup (1 t CO_2/h) in a coal-fired power plant. However, absorption has some drawbacks: (i) absorbent's limited holding capacity per cycle, (ii) equipment corrosion, (iii) excessive energy required to regenerate solvent and (iv) loss of solvent to evaporation and degradation at high oxygen atmosphere (Knudsen et al. 2009). Piperazine is an efficient alternative for MEA, and it is under research to enhance its efficiency and economics (Bougie and Iliuta 2011). Carbonation and calcination cycles using calcium oxide to extract CO_2 are also attractive alternatives. CO_2 reacts with CaO to form calcium carbonate (carbonation), and the CO_2 is then extracted from calcium carbonate at higher temperatures (calcination) (Fang et al. 2009).

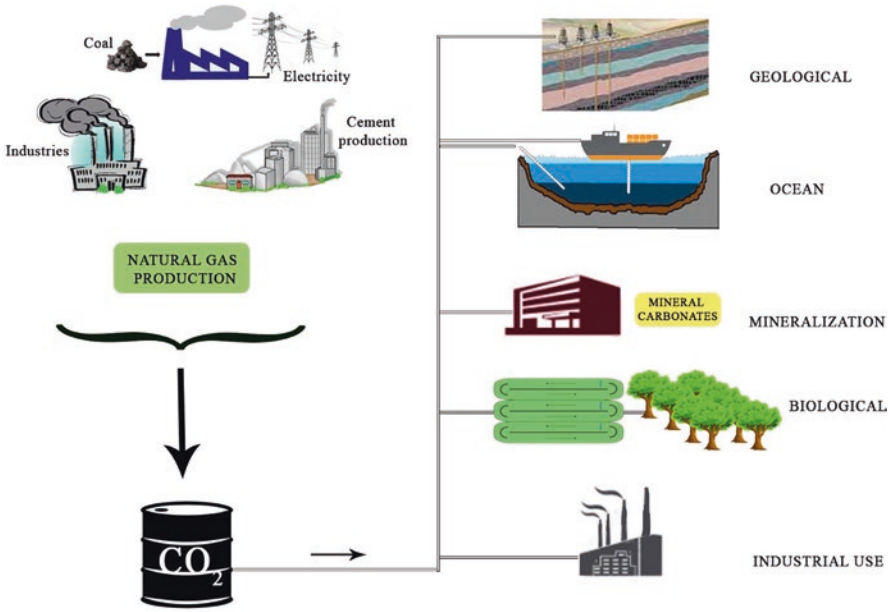

Fig. 1 Key CO_2 emitting sources and different methods of carbon capture storage and utilization

Adsorption uses a solid sorbent to bind the CO_2 on its surfaces. Sorbents are selected based on their larger specific surface area, high selectivity and regeneration ability. Molecular sieves such as activated carbon, hydrotalcites, zeolites, lithium zirconate and calcium oxides are commonly used (Leung et al. 2014). The polarity of the adsorbent and properties of the adsorbed particles will determine the efficiency of the capture process (Pires et al. 2011). High pressure is applied to adsorb the CO_2 while it is lowered to atmospheric pressure to release the captured CO_2. Meanwhile, providing excess heat could also be applied to capture carbon dioxide. Carbon-based adsorbents display excellent stability, while zeolites perform better at dry conditions; a review on different adsorbents concluded (Sjostrom and Krutka 2010).

Individual gas from a mixture of gaseous exhaust could be filtered out using membranes. It comprises of a thin polymer membrane on another thick supporting layer (Rackley 2010). High separation energy efficiency and industrial applications are some of the talking points of this process. However, one of the drawbacks in using this technique to sequester CO_2 from flue gas is that the membrane is blocked and the efficiency gets lower because of the meagre CO_2 present in the exhaust gases (Pires et al. 2011).

Cryogenic distillation is an air separation process; condensation of the gas mixture separates the components at different temperatures. The system possesses different set of filters with differing cryogenic temperature that can condense constituents at different condensation temperatures (Pfaff and Kather 2009). With this technique, it is possible to reach 90–95% efficiency in capturing CO_2 from flue gas. It consumes excess power owing to its high operating temperatures and is estimated to utilize 600–660 kWh per tonne of CO_2 recovered in liquid form (Göttlicher and Pruschek 1997). Chemical looping combustion (metal oxide) and hydrate-based separation (water with higher pressure) are the other notable techniques used to trap CO_2 from the flue gas (Leung et al. 2014).

3.2 Transportation and Utilization of Captured CO_2

The captured CO_2 is taken to depleted oil reservoirs, used mines or deep ocean where it will be stored for infinite periods. CO_2 can be transported via pipelines, tankers in rail and ships. Pipelines are the most liable method to port CO_2 but it could only be used for inshore transport. Recently the use of ship tankers for CO_2 transport using the technology for LPG carriers was considered (Aspelund et al. 2006). The captured CO_2 can be utilized in a number of ways; the industry can make use of the CO_2 for its own production processes. CO_2 finds its use in the production of food beverages, refrigerants and fire extinguishing gases. A demonstration plant used the captured CO_2 to produce urea and ammonia at the rate of 160 t per day in Luzhou, China (Dooley et al. 2009). Enhanced oil recovery (EOR) stores CO_2 for longer periods, and it is a commonly used method. On injection of CO_2 into the depleted oil/gas reservoirs, the internal pressure increases which allows the oil/gas to be extracted. By this method, the CO_2 remains stored underground for a longer period. In CO_2-enhanced coal bed methane (CO_2-ECBM), CO_2 is used to extract methane trapped in the coal seams, and the CO_2 stays in the void left over by the methane. Deep saline aquifers, which are 700–1000 m below the surface, are found around onshore and offshore that hold great potential in storing CO_2. Oceans are the natural sink of carbon dioxide sequestering 1.7 Gt annually. Owing to its higher density than the ocean water, beyond 3 km CO_2 liquefies and settles to the ocean floor (House et al. 2006). This way, ocean sequestration could provide a permanent storage for CO_2 (Adams and Caldeira 2008). However, there is a controversy with this technique; storing huge amount of CO_2 into the ocean will alter the chemistry of the water and will adversely affect the aquatic life of the oceans.

Leaking of the stored CO_2 is one serious restraint associated with carbon storage. CO_2 leaking could impair the CO_2 storage and lead to potential environmental hazards by releasing harmful pollutants and/or harming the life at the ecosystem. It could lead to acidification and pollution induced by heavy metal mobilization (Elzahabi and Yong 2001). The leakage could also occur during transportation, in carriers or in pipelines. Hence, these storage mechanisms require continuous monitoring and assessment. It is also required that the long-term effect of CO_2 on humans and other life forms be established. Leaving aside transportation, these strategies require separate plants for CO_2 capture and its storage. These methods require complex installations, heavy investments, and continuous monitoring. Hence, a simple plant with minimal maintenance costs and longer and leak-proof storage with some kind of economic returns will motivate the industrialists around the world to look up the CCS.

4 Microalgae in CCS

Microalgal carbon capture has been around for some decades, and it is gaining momentum in the last 10 years. Microalgae are uni- or multicellular, oxygen-emitting photosynthesizing microorganisms, which are the early descendants of the first photosynthetic organisms. It includes all the organisms under these five classes, Chlorophyceae, Rhodophyceae, Phaeophyceae, Cyanophyceae and Bacillariophyceae. These organisms utilize CO_2 as their carbon source, and their evolution or early records date back to 0.8 Ga (billion years ago). This illustrates the fact that the algae have strived in atmospheres rich in carbon dioxide. At present, CO_2 concentration in the atmosphere is just 0.004%, which is meagre as compared to the early years of photosynthetic algae. This lower concentration of atmospheric CO_2 is actually a limiting factor for microalgae, and a higher CO_2 concentration could enhance the growth of microalgae. Microalgae have 10–50 times faster CO_2 fixation rate and 100 times faster growth rate when compared to the terrestrial plants (Lam et al. 2012; Wang et al. 2008). Unlike the plants, marine water could be used to cultivate microalgae near seashores; arable land is not a requirement. The ability to tolerate and grow at elevated CO_2, enhanced CO_2 fixation and biomass yield and growth rate make microalgae an ideal candidate for biological carbon sequestration. A unique advantage with microalgal or biological sequestration of CO_2 is that the three aspects of CCS are unified in a single step, i.e. biomass production. Many studies have been performed on the proficiency of microalgae in CCS (Table 1). The CO_2 captured by the microalgae through photosynthesis is converted into valuable biomass. Apart from economically viable, the biomass produced could be converted to valuable products like fuels, food/feed and cosmetic products or for any other application.

4.1 Microalgae Mass Cultivation

Commercial- and large-scale production of microalgae requires an exogenous supply of CO_2, and instead of sparging commercial CO_2 into the tanks, it is advised that flue gas from the industries be utilized for the purpose. There have been many studies since the 1980s on the potentials of microalgae in growing with flue gas (Golueke and Oswald 1959; Wagener 1983; Benemann 1993). Table 2 lists different stages of a mass scale cultivation.

Selection of the microalgal strain is extremely critical to the success of the CCS strategy, and it should be capable of tolerating higher CO_2 concentration, efficient growth at mild acidic conditions and higher temperature and capable of accumulating higher biomass. Though higher CO_2 concentration could enhance the growth of the organism, exogenous supply of higher CO_2 beyond a certain concentration would inhibit the growth rate of the organism (Moroney and Somanchi 1999). The flue gas

Table 1 Growth proficiency and carbon utilization/removal efficiency of microalgae in different concentrations of CO_2 and in flue gas

Organism	Cultivation system	Volume (L)	CO_2 (%)/ flue gas	Time (days)	Biomass concentration (g L^{-1})	Biomass productivity (g L^{-1} d^{-1})	CO_2 fixation (g C L^{-1} d^{-1})/CO_2 removal (%)	Reference
Anabaena sp. PCC 7120	Bubble column and airlift PBR	1.4	5	9	1.7	NA	0.583	Nayak and Das (2013)
Chlorella vulgaris P12	Bubble column photobioreactor	0.09	6	7	10.0 ± 0.5	1.3 ± 0.0	2.22	Anjos et al. (2013)
Chlorella vulgaris LEB 104	BioFlo Fermentor	8	5	15	1.94	0.31	0.252	Sydney et al. (2010)
Anabaena sp. CH1	Photobioreactor	5	10	5	NA	NA	1.01	Chiang et al. (2011)
Scenedesmus obliquus SJTU-3	Erlenmeyer flask	0.8	10	14	1.84 ± 0.01	0.155 ± 0.004	0.050	Tang et al. (2011)
Dunaliella tertiolecta	Flask	1	2	10	0.236	0.115	0.37	Farrelly et al. (2014)
Chlorella PY-ZU1	Column photobioreactor	0.3	15	8	4.84	0.76	10.51	Cheng et al. (2013)
Ettlia sp. YC001	Bag photobioreactor	5	10	16	2.5	0.18	NA	Yoo et al. (2013)
Chlorella sorokiniana	Airlift and bubble column	1.4	Flue gas (15.65%)		1.23	0.17	2.25	Kumar et al. (2014)
Chlorella sp. MTF-15	Column photobioreactors	1	Flue gas (10–14%)	7	2.855	0.528	~ 25% (removal)	Kao et al. (2014)

Table 2 Different stages of commercial microalgal cultivation and methods available

Cultivation	High-rate algal ponds
	Column photobioreactors
	Flat panel photobioreactors
	Tubular reactors
	Biofilm reactors
Harvesting	Flocculation
	Chemicals (ferric chloride, alum, chitosan, etc.)
	Bio-flocculants (EPS, moringa seeds, microalgae extracts, etc.) Electro-flocculation
	Auto-flocculation
	Ultrasound
	Centrifugation
	Filtration
	Filter presses
	Tangential flow filter
	Gravity belt filters
	Vacuum filter
	Flotation
	Dispersed air flotation
	Dissolved air flotation
	Suspended air flotations
Drying	Sun
	Convective drying
	Spray drying
	Freeze drying
	Roller/drum drying
	Fluidized bed drying

from most of the industries usually contains CO_2 in the range of 6–15% (Yan et al. 2015). Hence, it is imperative to choose a strain with natural tolerance to higher CO_2 concentration. Published reports suggest *Scenedesmus obliquus*, *Botryococcus braunii*, *Chlorella vulgaris* and *Nannochloropsis oculata* to be the most favourable species for CCS and renewable fuel production (Singh and Ahluwalia 2013). However, the tolerance of a particular strain to CO_2 may vary even within the species; hence, it is necessary to test every strain before being applied for CCS.

A cyanobacterium, *Aphanothece microscopic Na¨geli*, grew efficiently at a wide CO_2 range from 2% to 20% (Jacob-Lopes and Franco 2013). To its contrast, for *Chlorella* sp. (a eukaryotic microalgae), CO_2 concentrations higher than 5% are found to be inhibiting (Chiu et al. 2008). However, many strains of *Chlorella* have been reported widely for its growth at higher CO_2 concentrations (Cheng et al. 2013; Ramanan et al. 2010). Though organisms could grow in higher CO_2 concentrations, they may not be able to initiate growth with flue gas sparging. Flue gas, apart from CO_2, contains carbon monoxide, nitrous oxide, sulphur oxides and other detrimental substances (Lam et al. 2012). These constituents when dissolved in water cause acidification of the growth medium which could be harmful for the growth of algae (Ho et al. 2014; Zhao and Su 2014).

Owing to its expanding potentials and simplicity in operation, there have been many academic studies about pilot and demonstration plants of microalgal cultivation through carbon sequestration from the flue gas. *Chlorella* sp. MTF-15 was cultivated in a 50 L tubular photobioreactor on site at the China Steel Corporation, Taiwan. Flue gas from three different sources, a coke oven, a hot stove and a power plant, from the same plant, was used as the CO_2 source for the organism. Cultures grown with flue gas from coke oven established a maximum growth rate of 0.827 d^{-1} which proves its efficacy to remove CO_2 from flue gas and utilize it for its growth. Cheng et al. utilized *Nannochloropsis oculata* in a 1192 m^2 raceway pond with a total volume of 310 m^3 to sequester CO_2 from coal-fired flue gas. They were able to achieve around 1/3 carbon sequestration in the biomass of the total carbon removed from the system with a CO_2 removal rate of 142.1 g m^{-2} d^{-1}(Cheng et al. 2015). The maximum potentials of microalgal carbon sequestration can only be evaluated and analysed when it is applied in commercial scale. AlgaFarm (Portugal), AlgaeTec (Australia), Seambiotic (Israel), Pond Technologies (Canada), Aljadix Technologies (Switzerland) and Algenol, A2BE and Solix Biofuels (USA) are some of the microalgal or related companies which utilize flue gas as the source for CO_2 (Cuellar-Bermudez et al. 2015). Recently, the Congress of the USA passed a bill which recommends a \$ 35 incentive for a tonne of CO_2 captured or recycled from power plants or industries using algae or other biologically based carbon capture systems (Lane 2018). Such measures around the world will inspire many more companies to capture, utilize or recycle the CO_2 produced in their premises. It has been 30 odd years that this strategy was first proposed and tested, and surprisingly the process is not commercially mature and not large enough to make a recognizable impact on the CO_2 emissions. Microalgal carbon sequestration has a number of constraints that prevent it from being a commercially profitable strategy to reduce the carbon emission to the atmosphere.

4.2 Constraints in Microalgal Cultivation

The simple explanation of the commercial shortcoming of the process is that the productivity of the microalgal biomass and the valuable products from the process are not adequate to generate profit out of it. The productivity of the valuable products can be enhanced simply by increasing the microalgal biomass productivity. A biomass productivity of 30 g m^{-2} day−1 and above is possible under optimized conditions for shorter periods but will come down in longer run owing to the seasonal variations (Borowitzka and Moheimani 2013). Maintaining a higher biomass productivity and enhanced yield of a particular strain depends on a number of interlinked factors. Some important factors that decide the algal growth rate are light (day-night cycle and irradiation intensity), temperature, nutrient concentration, O_2,

CO_2, NOx, SOx, pH, salinity, water quality, mineral and carbon regulation/bioavailability, cell fragility, cell density and growth inhibition. In addition, culture growth, reactor design and operation issues that affect growth are (a) mixing, (b) fluid dynamics and hydrodynamic stress, (c) culture depth, (d) gas bubble size and distribution, (e) gas exchange, (f) mass transfer, (g) dilution rate, (h) toxic chemicals and pathogens (bacteria, fungi, viruses) and (i) competition by other algae and harvest frequency (Razzak et al. 2013).

Microalgae could be cultivated in open raceway ponds or in closed photobioreactors. The choice of choosing the system depends on the desired products; if the final product is of pharmaceutical value, it is desired that the culture was grown in closed systems. Open raceway ponds are the better choice for production of biofuels and biomass for nutritional supplements and aquatic feed. However, the open raceway ponds have comparatively lower productivity than the photobioreactors (Tredici 2004). Recently, closed photobioreactors are considered advantageous, more productive and equally economical to algal mass culture in open systems. Henceforth, many algal startups and companies utilize photobioreactors for algal mass cultivation. Flue gas composition and the rate of the mixture will also play an important role in effecting the growth of the organism. NOx and SOx in flue gas will inhibit the algal growth and the rate at which it is mixed with air, and/or the flow rate should be adjusted to keep the algal growth medium from acidification. Flue gas dissolution in growth medium is another constraint with respect to microalgal cultivation through carbon sequestration. CO_2 concentration in flue gas is usually below 25%, and improper dissolution (eventually escape into the atmosphere) will yield very low concentration of CO_2 available to the cells. Airlift rector systems with higher gas mass transfer rates are developed and are gaining wide acceptance in algal cultivation (Sánchez Mirón et al. 2000). A cultivation system, which supports better mass transfer ratio and higher dissolution of CO_2 in the liquid medium, is preferred.

Light availability is another limiting factor of microalgal growth. Open pond raceways are operated usually at a depth of 30 cm or less (Brennan and Owende 2010). Once the cultures get denser, cells at the surface of the tank undergo a certain amount of time in darkness, preventing the cultures to reach their maximum possible productivity. Photobioreactors with enhanced light capturing improvisations and designs are proving effective and still to be tested at the commercial scale (Ugwu et al. 2008). The temperature build up inside the closed photobioreactors is one important issue to address as higher temperature could reduce the productivity of the algae. Thermoregulation of the closed systems is energy- and economically expensive (Ugwu et al. 2008). To enhance the light utilization efficiency and to avoid temperature issues, internally illuminated photobioreactors were developed (Pegallapati et al. 2014). Since we are concerned about reducing the carbon emission into the atmosphere, the process should not be emitting extra carbon into the atmosphere.

The carbon-capturing plant should be located close as possible to the power plant or flue gas source. Far located power plant and the algal plant will require the need for transport of flue gas, which will enhance the carbon emission and an expensive process (Campbell et al. 2011). The process should be at least carbon neutral if not carbon negative. The end product from the process should be easily extracted, and the downstream process should not be energy expensive. The NER of a process is the ratio of energy contained in the product to the energy utilized in the process, and for a successful method, it should be more than one.

Apart from some individual parameters that affect the growth of the microalgae, the overall economy of the process is the prime factor, which decides the success of the strategy. A successful and profitable process should at least have a 5–10% difference between production and selling cost. Failing to meet the production cost within the stipulated cost will discourage the industries to pursue microalgal production through carbon sequestration. Hence, there have been many studies and research in lab-scale, pilot-scale and demonstration-scale, and similarly, life cycle assessments were made to analyse the efficacy of the processes. Those studies recommended options for a CCS process with a positive NER, and one such option was to utilize wastewater as the growth medium for algal cultivation (Fon Sing et al. 2014).

5 Microalgae for Wastewater Treatment

Similar to the role of carbon capture and storage (CCS) systems in mitigation of elevating global carbon dioxide, wastewater treatment (WWT) plays a key role in reducing or removing nutrients, biological oxygen demand (BOD), pollutants and pathogens from domestic or industrial effluents prior to its release in the natural bodies (Grady Jr et al. 2011; Negulescu 1985). Although the mechanisms are considered separately as physical-mechanical, chemical and biological, most often they are interconnected or co-dependent as combined or successive steps in WWT process. Hence, the wastewater treatment process with all these interlinked methods generally contributes to preliminary and primary treatments for separation of water from sludge; secondary, tertiary and advanced treatments for removing water-dissolved impurities (includes at least one of the substances, viz. organic substrate, excessive inorganic plant nutrients and xenobiotics); and sludge treatment for managing slurry or solid wastes into fuel, fertilizer and/or landfill.

Biological methods are often considered as biochemical process since it is a chemical process influenced or hosted by the biological system (Negulescu 1985). In such methods, the oxidation pond utilizes the microalgal oxygenic photosynthesis in conventional secondary treatment for reducing the soluble BOD. The microalgal oxygenic photosynthesis is being exploited as a conventional tertiary treatment for the removal of excess (inorganic) plant nutrients such as nitrogen (N) in form of oxides and ammonium salts and phosphorus (P) in the form of phosphates. It was also used in various kinds of WWTs including high-rate algal ponds and rotating algal biofilm reactor as advanced secondary and/or tertiary treatment for purification of sludge-free wastewater (Von Sperling and de Lemos Chernicharo 2005). Oxygenic photosynthesis encourages the algae-bacteria combined wastewater treatment by exchange of oxygen and carbon dioxide for each other's proliferation. Moreover, this algal-bacterial wastewater treatment reduces eutrophication and also the toxic pollutants or metals in the treated water.

Although the conventional secondary/tertiary wastewater treatments in stabilization ponds, facultative ponds and maturation ponds involve microalgal photosynthesis, they were not considered as representatives of algal wastewater treatment since their design and operation does not consider the algal biomass productivity and recovery (Alcántara et al. 2015). Therefore, the wastewater treatment widely operated in high-rate algal pond (HRAP also known as raceway ponds), closed photobioreactor systems and other advanced treatments like algal biofilm reactors is considered as microalgal wastewater treatment (MWWT) in this chapter. Microalgal wastewater treatment, particularly through HRAP, was established much early in the 1950s; however, MWWT was considered with much attention only after the appraisal of elevated microalgal biomass demand with biofuel application in recent decades. Sufficient water availability, readily available nutrient sources (N and P) and eco-friendly approach with multiple benefits (including algal biomass recovery and wastewater treatment) encouraged the advanced process developments in MWWT. Thus, the practice of treating the wastewater to control eutrophication and algal bloom considerably emerged towards algae-based value-added products as an additional benefit (in municipal plants) or primary benefit (in commercial plants) (Mahapatra et al. 2018).

5.1 High-Rate Algal Pond

High-rate algal pond (HRAP) is a shallow pond with 20–40 cm (30 cm average) depth with a separating central baffle, and it is well facilitated with paddle wheels to aid flow of media and proper agitation. HRAP is more suitable for MWWT since it has less hydraulic retention time (HRT), less space requirement and more efficient nutrient removal compared to the conventional stabilization ponds. Various kinds of wastewaters such as municipal, domestic (primary/facultative pond/septic tank/anaerobic digester treated), swine manure and aquaculture wastewaters were being treated in HRAP (Posadas et al. 2017a; Salama et al. 2017; Young et al. 2017).

Organic substrate removal measured in terms of 5-day BOD (BOD_5) was reported as approximately 50% in a hectare-scale demonstration (5 ha) of HRAP (Craggs et al. 2012). Based on several pilot-scale studies, BOD_5, the total nitrogen, ammonium, phosphorus and total orthophosphate removal range between 22% and 93.4%, 26.6% and 5.7%, 21.89% and 94%, 10.48% and 97.2% and 3.75% and 71%, respectively (Young et al. 2017). Increase in pH, temperature, dissolved oxygen (DO) and solar irradiation influences significantly to control or remove the pathogens (Posadas et al. 2017a; Young et al. 2017). Heavy metals such as cadmium, chromium, copper, lead, mercury, nickel and zinc were being effectively removed (up to 90%) by active and passive mechanisms under MWWT (Hargreaves et al. 2018; Posadas et al.

2017a). Emerging organic contaminants (EOC) were also efficiently removed (>90%) by MWWT (Salama et al. 2017).

Overall, the open HRAP system has various advantages such as low operational and maintenance cost, less energy consumption, high nutrient removal, BOD removal as well by algal heterotrophic mode or algae-bacteria consortia, efficient pathogen removal and high toxic metal removal. Low photosynthetic efficiency, biomass productivity, high risk of contamination, difficulties in biomass harvesting, high HRT and land space compared to other advanced MWWT especially closed systems and evaporational water loss especially in temperate and arid regions are the constraints associated with HRAPs.

5.2 *Photobioreactor Systems*

Similar to open HRAP system, photobioreactor (PBR) system was also being simultaneously operated for the suspended or immobilized microalgal-based wastewater treatment. PBR systems are specifically preferred over HRAP to accomplish high biomass productivity with less HRT. Conventional closed suspended algal PBR system includes cylindrical stirrer-tank, tubular, bubble column, airlift, flat-panel and bag-type photobioreactors made up of Pyrex glass and various kinds of polymers (such as plastic and acrylic). Advanced immobilized closed or open algal PBR system generally considered are biofilm-based PBRs which include submerged (flat plate biofilm PBR, turf scrubber, closed biofilm PBR), periodically submerged (rotating disc PBR, rotating drum PBR, oscillating PBR, revolving belt PBR) and non-submerged (porous substrate PBR) types (Li et al. 2017). Almost every kind of wastewaters such as pretreated or untreated sludge, piggery, municipal, domestic, secondary effluent, tertiary and aquaculture wastewaters was being treated with these PBR systems (Alcántara et al. 2015; Luo et al. 2017; Sun et al. 2018; Yu et al. 2017). Table 3 provides a detailed account of various strains of microalgae employed to phycoremediate different types of wastewater.

Apart from these open and closed microalgal-based WWT, recently hybrid advanced systems integrating the HRAP with biofilm or other PBR systems and/or biogas upgrading were designed (Posadas et al. 2017b; Salama et al. 2017; Yun et al. 2018; Zhang et al. 2018).

Organic carbon (BOD_5) and inorganic carbon (CO_2) removal efficiency in PBR systems were reported to be much higher than that of HRAP. Also, total nitrogen and phosphorus removal efficiency was also much higher about >70% (mostly >85%) (Alcántara et al. 2015; Posadas et al. 2017a; Yu et al. 2017). In case of heavy metal pollutants and pathogen removal efficiencies, the effect in PBR system would be similar to that of HRAP. PBR system possesses several benefits with high nutrient removal efficiency, high biomass productivity and photosynthetic efficiency, less operational space compared to HRAP, low contamination risk and lowest HRT. Besides these advantages, high construction and operational cost and high energy consumption and scalability challenges are the general demerits of the PBR

Table 3 A comprehensive table on the capabilities of microalgae in treating wide varieties of wastewaters and its efficiency

Wastewater	Cultivation system	Organism	Volume (L)	Hydraulic retention time (d)	Aeration	Light source with intensity ($\mu mol/m^2.s$) [light (h): dark (h)]	Carbon removal (%)	Nitrogen removal (%)	Phosphorus removal (%)	Biomass productivity	Reference
Primary effluent from treated raw urban wastewater	High-rate algal pond (HRAP) for 1 year	Mixed algal population – *Chlorella* sp. dominant	470	4–8	N/A	Natural sunlight	56–93 (average = 80) (COD[i])	95–99 (average = 97) (NH_4^+)	N/A	3.3–25.8 g TSS[a]/m^2.d	Gutiérrez et al. (2016)
Primary treated fish farm wastewater and domestic wastewater	HRAP for 153 days (four stages)	Microalgal consortium	180	5–20	N/A	Natural sunlight	64 ± 16–77 ± 10 (COD)	78 ± 14–88 ± 5 (TN[c])	64 ± 19–94 ± 6 (TP[d])	0.5–5 g/m^2.d	Posadas et al. (2015)
Primary settled domestic wastewater	HRAP for 13 months	*Pediastrum* sp., *Micractinium* sp., *Coelastrum*, *Ankistrodesmus falcatus*, *Mucidosphaerium* sp. and *Monoraphidium* sp.	9540	5–8	N/A	Natural sunlight	N/A	31.5–92.1 (NH_4^+)	31.2–86.5 (DRP[e])	2.0 ± 0.3–11.1 ± 2.5 g VSS[b]/m^2.d	Mehrabadi et al. (2016)

(continued)

Table 3 (continued)

Wastewater	Cultivation system	Organism	Volume (L)	Hydraulic retention time (d)	Aeration	Light source with intensity (μmol/m^2.s) [light (h): dark (h)]	Carbon removal (%)	Nitrogen removal (%)	Phosphorus removal (%)	Biomass productivity	Reference
Primary settled domestic wastewater	High-rate algal mesocosms (HRAM) for 51 days	*Pediastrum* sp., *Micractinium* sp., *Coelastrum*, *Ankistrodesmus falcatus*, *Mucidosphaerium* sp. and *Monoraphidium* sp.	16	4–8	0.04 to 10% CO_2 in air	Natural sunlight	N/A	59–86 (NH_4^+)	39–61 (DRP)	4.7 ± 1.0–20.9 ± 2.1 g VSS/m^2.d	Mehrabadi et al. (2016)
Secondary settled municipal wastewater	HRAP for 20 days	*Chlorella* sp., *Scenedesmus* sp. and *Stigeoclonium* sp.	200–400	4–20	N/A	Natural sunlight	N/A	18.6–82.5 (TN)	32.3–89.7 (TP[^])	4.21 ± 0.48–6.16 ± 0.33 g/m^2.d	Kim et al. (2018)
Secondary effluent	Lab-scale cylindrical membrane photobioreactor (MPBR) for 130 days continuous cultivation	*Chlorella vulgaris*	4	1–6	Air at 0.5 L/min; pure CO_2 for pH control	Red/blue LED lamps (4:1), 101.5–112.3	N/A	35.9 ± 5.8–92.1 ± 6.4 (DIN[f])	76.9 ± 5.1–94.9 ± 4.3 (DIP[g])	0.049–0.027 g/L.d	Gao et al. (2018)

Microalgal digestate (anaerobic digester) diluted in secondary effluent (HRAP-treated municipal wastewater) at a rate of 1:50	Closed cylindrical photobioreactor (semi-continuous cultivation)	Mixed microbial and microalgal population with *Chlorella* sp., *Stigeoclonium* sp. and dominated by *Scenedesmus* sp.	30	8	Air at 10 L/min	Lamp, 289 [12:12]	70	100 (NH_4^+); 58 (NO_3^-)	100 (PO_4^{3-})	N/A	Arias et al. (2018)
Piggery wastewater diluted 10 and 20 times	Open photobioreactor (indoor cultivation)	Algae-bacteria population; inoculated and dominated by *Chlorella vulgaris*	3	26	Pure CO_2 only for pH 8.0 maintenance	LED lamp, 1417 ± 82 [12:12]	87 ± 2–94 ± 1 (TOC[h])	30 ± 14–56 ± 4 (TN)	99 ± 3–99 ± 5 (TP)	N/A	García et al. (2017)
Piggery wastewater diluted 10 and 20 times	Open photobioreactor (outdoor cultivation)	Algae-bacteria population; inoculated and dominated by *Chlorella vulgaris*	3	26	Pure CO_2 only for pH 8.0 maintenance	Natural sunlight	85 ± 1–91 ± 1 (TOC)	48 ± 9–72 ± 8 (TN)	81 ± 8–84 ± 13 (TP)	N/A	García et al. (2017)
Wastewater from second clarifier	(Stainless steel pot) photobioreactor without attached wall algal biofilm (cleaned) (semi-continuous cultivation)	*Scenedesmus rubescens* and *Chlorella vulgaris*	5	8	N/A	Lamp, 94.5 [12:12]	N/A	66.35 ± 4.9 (NH_4^+); 38.06 ± 5.8 (TN)	83.14 ± 8.16 (PO_4^{3-})	0.1 g VSS/L.d	Su et al. (2016)

(continued)

Table 3 (continued)

Wastewater	Cultivation system	Organism	Volume (L)	Hydraulic retention time (d)	Aeration	Light source with intensity (μmol/m^2.s) [light (h): dark (h)]	Carbon removal (%)	Nitrogen removal (%)	Phosphorus removal (%)	Biomass productivity	Reference
Wastewater from second clarifier	(Stainless steel pot) photobioreactor with attached wall algal biofilm (cleaned) (semi-continuous cultivation)	*Scenedesmus rubescens* and *Chlorella vulgaris*	5	8	N/A	Lamp, 94.5 [12:12]	N/A	73.20 ± 4.22 (NH_4^+); 42.95 ± 5.11 (TN)	98 (PO_4^{3-})	0.11 g VSS/L.d	Su et al. (2016)
Pretreated domestic wastewater	Enclosed tubular biofilm photobioreactor	Mixed algal-bacterial population	31	5–10	N/A	Fluorescent lamp, 74 ± 3 [16:8]	85 ± 5 (TOC)	80 ± 6 (TN)	68 ± 18 (TP)	N/A	Posadas et al. (2014)
Pretreated domestic wastewater	Open biofilm photobioreactor	Mixed algal-bacterial population	31	5–10	N/A	Fluorescent lamp, 74 ± 3 [16:8]	89 ± 3 (TOC)	92 ± 5 (TN)	96 ± 2 (TP)	N/A	Posadas et al. (2014)
Secondary stage municipal wastewater	Rotating algal biofilm reactor (fed-batch mode for 21 days)	Mixed algal population dominated by *Scenedesmus*, *Chlorella*, Cyanobacteria, *Oocystis*, *Ankistrodesmus* and *Synura*	8	2 and 6	N/A	Lamp, 200 ± 20 [16:8]	N/A	2 HRT: 92 (NH_4^+); 44 (NO_3^-); 77.1–93.7 (TN) 6 HRT: 100 (NH_4^+); 47 (NO_3^-); 46.7–84.7 (TN)	2 HRT: 97 (PO_4^{3-}); 39.9–81.1 (TP) 6 HRT: 79 (PO_4^{3-}); 12.3–99.5 (TP)	1.74 g/m^2.d (2 HRT) 1.64 g/m^2.d (6 HRT)	Iman Shayan et al. (2016)

Urban wastewater	HRAP with biogas upgrading [interconnected with bubble absorption column (AC)] for 98 days (continuous)	Mixed algal-bacterial population	180 L (HRAP); 2.5 L (AC)	4	N/A	Natural sunlight	59 ± 7–74 ± 7 (TOC)	80 ± 4–87 ± 4 (TN)	84 ± 5–92 ± 2 (TP)	N/A	Posadas et al. (2017)
Municipal wastewater; effluent from treated municipal wastewater	Photobioreactor-open raceway pond (PBR-ORP) hybrid system (semi-continuous for 47 days)	Mixed algal population inoculated and dominated by *Parachlorella* sp. JD076	4 (PBR); 60 (ORP)	5	Air at 6 L/min (0.1 vvm) and 1% CO_2 in air	Natural sunlight (~ 600)	N/A	N/A	N/A	17.65 $g/m^2.d$	Yun et al. (2018)

N/A not available
[a]*TSS* total suspended solids
[b]*VSS* volatile suspended solids
[c]*TN* total nitrogen
[d]*TP* total phosphorus
[e]*DRP* dissolved reactive phosphorus
[f]*DIN* dissolved inorganic nitrogen
[g]*DIP* dissolved inorganic phosphorus
[h]*TOC* total organic carbon
[i]*COD* chemical oxygen demand

system. Conventional suspended PBR system has the specific shortcoming of biofouling, whereas immobilized biofilm has a specific advantage of easy biomass harvesting.

Irrespective of the advantages and opportunities in MWWT, the number of wastewater treatment plants utilizing microalgae is very scarce. It is mostly because of the costs and workforce involved in harvesting the biomass developed, drying the biomass and further processing. Hence, integrating it with another process or making it a multi-objective process could yield more benefits, and the whole process will become economically positive. Sequestering carbon dioxide from the industrial flue gas by microalgae grown in wastewater is proving to be an excellent machinery to alleviate effects of carbon emission and simultaneously treat wastewater being discarded to the environment.

6 Concurrent CO_2 Sequestration and Wastewater Treatment

Conserving the ecosystem and safeguarding the environment is one among the important concerns, and immediate attention needs to be given to increasing water pollution (US Environmental Protection Agency 2012). Principal apprehension in today's world is to find a greener solution for cleaner water and air (Cabanelas et al. 2013). Many methods of wastewater treatment are available as of today, like physical, chemical and biological processes. However, most of these methods could not address CO_2 mitigation and effluent remediation in the same process. Microalgae provide an efficient, cost-effective platform to capture CO_2 from flue gases and simultaneously treat the industrial wastewater and other effluents. They essentially possess all the properties to bring an efficient and simple solution to the demand. They are proficient in fixing the CO_2 and utilizing the solar energy, and it could be cultivated throughout the year irrespective of the seasonal variations. These remarkable traits of microalgae could be of important service in remediating the environment. The two most essential resources required for its cultivation are nutrient-rich water and CO_2.

It is reported that to produce 1 kilogramme of algal biodiesel without reclaiming used media, 3.7 tonnes water, 330 g nitrogen and 710 g phosphorus will be required which could be expensive in mass cultivation at a higher scale (Yang et al. 2011). Wastewater could effectively solve this bottleneck and prevent crisis for water supply. Industrial effluent contains chemicals, which could initiate algal bloom that can be used for regular cultivation of microalgae. Almost every type of wastewater contains nitrogen and/or phosphorus compounds such as nitrate, nitrite, ammonia, organic nitrogen and phosphates (Razzak et al. 2017). These chemicals serve as nutrients for the growth of microalgae and are the most important input for mass production of microalgal biofuels (Pate et al. 2011; Yang et al. 2011). Microalgae are capable of initiating growth in almost any kind of wastewater provided they contain growth-promoting chemicals or externally supplemented nutrients. The preceding section elaborated the efficiency of microalgal growth in different wastewater.

The CO_2 from the industrial flue gases could serve as an effective and cheaper carbon source and help in reducing atmospheric CO_2 emissions. Microalgae are already tested and applied to capture and fix carbon dioxide from the industrial flue gases. *Chlorella* sp. MTF-15 was grown and successfully utilized to capture CO_2 from the chimneys of hot stove, coke oven and a power plant with CO_2 concentrations of 24–26% at China Steel Plant, Taiwan (Kao et al. 2014). Li et al. (2011) and Chiu et al. (2011) were also successful in cultivating *Scenedesmus* sp. and *Chlorella* sp. without any inhibition at flue gas containing 23 and 18% of CO_2. Since the CO_2 concentration in flue gas is generally more than 15%, habituating the organisms to higher CO_2 would enable the organism to have a shorter lag phase (Yun et al. 1997). Hence, microalgae could utilize the flue gas as CO_2 source and effectively combine with wastewater cultivation.

Coupling the availability of wastewater and flue gas to microalgal cultivation could effectively reduce the costs for cultivation, enhance net energy ratio, remediate the wastewater and reduce the atmospheric CO_2 emissions and hence more environment-friendly process (Sahu et al. 2013). Moreover, improving the biomass productivity indicates optimized algae culture, enhancing CO_2 capture and effective remediation of the wastewater.

Researchers and potential algapreneurs while designing their experimental plants and pilot plants should consider locating algal cultivation site closer to a place where wastewater and flue gas could be easily obtained from the industry (Razzak et al. 2017).

Life cycle assessment of microalgal biofuel production that considers all the bioresource utilization, energy intake and output found that the environmental impacts may not be positive as anticipated and could even yield negative energy ratio (Clarens et al. 2010).

The combined strategy allows sequestering of CO_2 from the industrial flue gas and minimizes the excess amount of CO_2 from entering the atmosphere. Exploiting wastewater for culturing microalgae allows the water to be reclaimed and used for further cultivation of microalgae after enriching it with necessary nutrients. Utilizing wastewater prevents the search for freshwater or marine water supply and saves a considerate amount of energy, costs and resources. The process offers multidimensional solutions of environmental protection and conservation of resources, viz. the effluent is treated; there is no need to dump the wastewater into water streams, hence environmental protection; and the water, a precious resource, is recycled and reused. Thus, this coupled CCS-WWT allows environmental protection in two different levels (air and water) but in the same process (Fig. 2).

Restrictions and strict regulations are already in place for the treatment and safe disposal of industrial wastewaters. With increasing carbon emission and rising concerns about the environment, it may be possible that governing bodies will also put a cap on the gas emissions. Hence, setting up CCS plant along with microalgal WWT plant seems to be advantageous. Combined microalgal CCS and WWT plant could prove to be simple in operation and maintenance and highly cost-effective. It

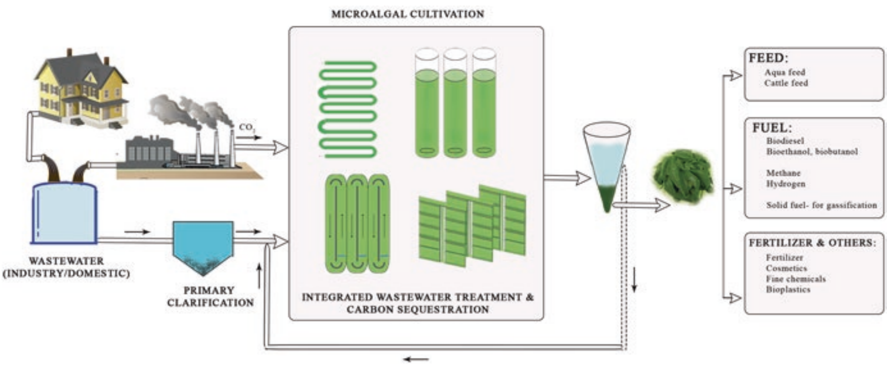

Fig. 2 Schematic representation of microalgae-mediated concomitant wastewater treatment and carbon capture and applications of the generated biomass

is already reported that excess CO_2 will enhance the biomass productivity of microalgae; hence, supplementation of wastewater with flue gas may increase the biomass production. The more biomass produced is more nutrients absorbed and faster the remediation process.

Nayak et al. (2016a) evaluated the potentials of different microalgae in treating domestic wastewater, utilizing the exogenously supplied CO_2. Later they went on to demonstrate one of the previously tested strains, *Scenedesmus* sp., to grow effectively in domestic wastewater sparged with coal-fired flue gas (2.5% CO_2). The nutrient removal properties were improved, and the biomass productivity enhanced by 36% in comparison to the ambient air (Nayak et al. 2016b). Similarly, *Spirulina* sp. was employed to test its potential in tolerating higher CO_2 wastewater treatment and biofuel production in synthetic wastewater medium (Kumar et al. 2010). A marine cyanobacterium *Phormidium valderianum* BDU20041 was utilized for removal of excess calcium and other nutrients from the ossein effluent along with un-scrubbed coal-fired flue gas sparging. Apart from the microalgal sequestration, the excess CO_2 was also sequestered as mineral carbonates (calcium carbonate) through calcification (Dineshbabu et al. 2017).

6.1 Constraints in CCS-WWT

One major constraint always associated with the microalgal culture at enhanced flue gas supply is the lower biomass productivity (Kumar et al. 2014). This is because of the combined effect of the growth-disturbing components in the flue gas, like NOx and SOx. However, this could be overcome by several schemes. The high CO_2 in the flue gas (10–20%) might harm the growth of the microalgae by creating an acidic environment in the growth medium. The dissolution of CO_2, NOx and SOx in water will result in carbonic acid, nitric acid and sulphuric acid that are highly detrimental to the cultures. With continuous sparging of flue gas, it becomes highly acidic;

hence, the cultures should be capable of tolerating the conditions and exhibit growth. On photosynthesis, they produce hydroxyl ions, which will neutralize the acidic conditions (Hulatt and Thomas 2011). Hence, there has to be a strategy where initial growth is initiated, and it could be promoted by diluting the flue gas with ambient air and/or adapting the cultures to higher CO_2 by gradually increasing the CO_2 concentration (Kumar et al. 2014). Flue gas supply could be limited only for the duration of the light period; there can also be an intermittent supply of ambient air alone after 2–3 hours of flue gas supply; the flow rate could also be adjusted if it does not affect the mixing of the cultures. These strategies would allow the neutralization of the medium pH and prevent rapid acidification of the growth medium. Table 4 provides few recent studies of concomitant microalgal CCS-WWT.

The success of microalgal CCS-WWT mostly resides in finding locations in proximity to the availability of the flue gas and wastewater. Nowadays, most of the industries are closely located, and it is highly unlikely for the industries to have unoccupied land to be allotted for microalgal cultivation (Roostaei and Zhang 2017). Microalgal CCS-WWT has to be in huge scale, in acres and hectares, if any kind of profit to be extracted from the process. Hence, it is vital to find sites that are at least close, if not in the same premises, to the industries that provide flue gas and wastewater. This will ensure higher cost and energy saving in terms of transportation charges for flue gas and effluent to the cultivation plant (Clarens et al. 2010; Soratana and Landis 2011).

Microalgal systems mostly will not provide complete sequestration of the CO_2 from the flue gas; there will be an escape of the flue gas into the atmosphere. It should be taken care that excess amount of flue gas does not escape into the atmosphere, close to human occupancies, as it will cause adverse effects on human health. Hence, the supply of the flue gas should be adjusted to the carbon removal efficiency of the system being dealt with.

6.2 Energy and Environmental Impact Assessment

The environmental and economic impact of the microalgal CCS-WWT process should be analysed through life cycle assessment (LCA) study which will include all the stages, starting from cultivation, harvesting, drying of biomass and its utilization for various products (Soratana and Landis 2011). It is a known fact that microalgal CCS-WWT should have a higher biomass productivity to achieve a positive net energy ratio. Hence, the microalgae need to be grown in hectares of land to be more economical.

The net CO_2 fixation efficiency of the system can only be concluded after nullifying the amount of CO_2 emitted at various stages of the process. For example, indirect CO_2 emissions from the electricity required for the operation, nutrients, chemicals utilized and the CO_2 emitted by the cultures. Lam et al. (2012) tabulated a number of LCA studies, which compare the performance of different cultivation modes in energy requirement and CO_2 emissions per unit amount of biodiesel pro-

Table 4 Studies on concomitant wastewater treatment with carbon dioxide fixation

Sl No	Organism	CO_2/flue gas source	Wastewater	Cultivation system and volume (L)	Days	Biomass production (g L^{-1})	Biomass productivity (g L^{-1} d^{-1})	CO_2 fixation rate/removal (g L^{-1} d^{-1})	Nutrient removal	Reference
1	*Nannochloropsis* sp.	Synthetic biogas	Biogas slurry and seawater	Bag photobioreactors; 40	6	N/A	0.245	0.067	TN – 51.78% TP – 50.58%	Zhao et al. (2016)
2	*Phormidium valderianum* BDU 20041	Coal flue gas (14–15% CO_2)	Ossein effluent	Open tank; 200	10	0.64	0.03	0.056	TN – 66.3% TP – 35.66	Dineshbabu et al. (2017)
3	*Scenedesmus* sp.	2.5% CO_2 in air	Domestic wastewater	Airlift photobioreactor	7	1.37	0.196	0.368	TN – 70.8% TP – 78.9%	Nayak et al. (2016a)
4	Microalgae consortium	10% CO_2	Septic effluent	Erlenmeyer flask; 1	17	2.27	N/A	N/A	NH_4 – 70.1 NO_3– 60% PO_4 – 20.9%	Mohamad et al. (2017)
5	*Pediastrum* sp., *Micractinium* sp., *Coelastrum*, *Ankistrodesmus falcatus*, *Mucidosphaerium* sp. and *Monoraphidium* sp.	0.04 to 10% CO_2 in air	Primary settled domestic wastewater	High-rate algal mesocosms (HRAM); 16	51	N/A	4.7 m^2 d^{-1}	N/A	NH_4 – 59–86% DRP – 39–61%	Mehrabadi et al. (2016)

N/A not available, *TN* total nitrogen, *TP* total phosphorous, *NH_4* ammonia, *NO_3* nitrate, *DRP* dissolved reactive phosphorus duced. However, the defining parameters vary in each study; it was evident that the raceway ponds were effective, economical and environmentally safe among different photobioreactors. Harvesting the biomass is an important component in microalgal cultivation and energy consuming. Even though centrifugation and filtration give more efficiency in a shorter time in comparison to flocculation, the carbon emission and energy requirements are 28–40 times higher. Hence, carefully selected flocculants, which do not have any adverse effect on the microalgae, will provide a very good energy balance and a better NER (Lee et al. 2010). Succinctly, raceway pond cultivation followed by flocculant-assisted harvesting seems to be the most energy-efficient process with less global warming potential for microalgal production.

Nutrient/chemical production for microalgal cultivation accounts to the major portion of the indirect CO_2 emissions. Clarens et al. (2010) estimated that 50% of energy spent and CO_2 emission is contributed by nutrient production. For example, production of 1 kilogramme of ammonia accounts to 1.2 kg of CO_2 emissions (Rafiqul et al. 2005). This kind of figures will contribute to the negative energy balance of the whole process. Hence, algal growth-promoting chemical-rich industrial or domestic wastewater is suggested for use as a growth medium for the cultivation of algae. With this strategy, the requirement for water and nutrients is avoided and hence lower energy use and CO_2 emissions (Mutanda et al. 2011; Rawat et al. 2011). In another study, Campbell et al. (2011) suggested using flue gas from the nearby coal-firing plant as the source of CO_2 as it could significantly enhance the positive energy balance. By far, the best possible strategy is to utilize wastewater with flue gas in raceway pond cultivation followed by flocculation to harvest the cultures. Exploiting the biomass could yield an energy-positive and commercially viable carbon capture and water remediating technique.

Either the available LCA studies focus on microalgal carbon capture or biofuel production, there are nil or limited studies when it comes to microalgal CCS and WWT. Integrated CCS and WWT needs elaborated investigations and detailed life cycle assessments to assert their prominence.

7 Conclusions and Future Prospects

Microalgal CCS is a promising and efficient mode of capturing and curbing CO_2 emissions from coal power plants. Integrating WWT to CCS will be very advantageous in terms of cost reduction, less global warming potentials and clean environment. However, this process is still in its infancy and has some serious issues to be addressed before it is commercially expanded. Along with scientific advancements, the national government should provide some incentives to the industries and encourage them to take up CCS. Improvements and innovations in microalgal CCS-WWT are required immediately, such as cultivation systems with provisions for maximum CO_2 mass transfer and higher light utilization to yield more productivity, energy- and time-efficient harvesting and drying methods and highly suitable extraction strategies to produce high-value products. Many LCA studies have to be undertaken in this approach to understand more about the commercial and environmental applicability of the strategy in hand. Significant advancements through immense research are required immediately in the above-mentioned areas to yield a noteworthy breakthrough in carbon capture and wastewater treatment.

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Industrial Wastewater-Based Algal Biorefineries: Application Constraints and Future Prospects

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1 Introduction

Algae is a biological microorganism frequently used in the treatment of wastewaters produced by many industries. Using algae to treat wastewaters is preferred because of how effective it is in removing waste as well as other purposes. For example, algae can be cultivated for many uses including the production of biofuels to replace nonrenewable and unfiltered fossil fuels. In this chapter, the use of algae as a biorefinery tool will be introduced as well as algal bioremediation for the treatment of industrial wastewaters. With the growth of the world population and industrialization of developing countries comes an inevitable increase in energy demand. The issue of overconsumption of fossil fuels without transitioning to alternative energy sources, or energy produced from sources other than crude oil and natural gas, will eventually cause a global crash in reserved fuels. Continuous extraction and burning of fossil fuels and natural gas will inevitably lead to the depletion of these nonrenewable resources. More importantly, the release of greenhouse gases into the atmosphere from combustion of fuels is a substantial environmental concern due to the intensity of global climate changes. Since the world economy is fueled by oil, depletion of this resource would have extensive effects on the economic market of developed and developing countries and their subsequent population. Because of these concerns, it is of utmost importance to develop alternative energies that are sustainable and economically feasible for a true shift to occur.

The textile industry is one of the largest industries in the world in terms of production and water pollution. The industries within the textile industry include

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dyeing, carpeting, clothing, clothe, and footwear, all of which require a significant amount of water throughout the process. This important use of water in turn causes detrimental waste and pollution, especially in developing countries where a large portion of the textile industry is located. In India alone, the textile industry makes up almost 20% of the country's exports therefore playing a large role in the country's economy (Mohan et al. 2017). The production of textiles requires the use of harsh chemicals that create pollutant wastewater which can cause harmful and dangerous effects when exposed to the surrounding environment and the local population. The significant amount of water needed in the textile industry as well as the corresponding production of wastewater is the reason why a solution for treatment of this water is crucial. For example, the process of dyeing textiles uses hundreds of chemicals that when released in wastewater, these chemicals can accumulate and be excreted into the environment. One indication of pollution is simply a change in color in a body of water. The change in color alters the amount of light which is able to penetrate the surface of the water and therefore alters the amount of light that is able to reach the plants below. This can cause significant damage to the underwater ecosystem that relies on the photosynthetic process (Mohan et al. 2017). Along with the direct effects on the aquatic ecosystem, the surrounding animals and human population that are exposed to the polluted wastewater can be harmed. If exposed to enough polluted wastewater, the accumulation of the toxic chemicals could cause sickness or death in animals and humans. If access to water is already limited in an underdeveloped country, for example, then this pollution will only worsen the issue. Using algal bioremediation could lower the amount of chemicals in wastewater and therefore could improve access to clean, drinkable water.

Another industry is the oil and natural gas industry which relies on coal and petroleum as the primary fuels. However, both coal and petroleum release harmful chemicals into the environment when burned, causing global air pollution. Because of the concern of releasing harmful chemicals, the drilling for natural gas has largely increased. Through several experiments and research, efforts in advancing the extraction of natural gas including drilling have also expanded (Entrekin et al. 2011). Natural gas serves as a "cleaner" fuel because, when burned, there are significantly less harmful chemicals released into the air. Unfortunately, the main concern with drilling for natural gas is the subsequent wastewater pollution. Historically, the method of retrieving gas has been through horizontal drilling and hydraulic fracturing. First used in the mid-1900s, hydrofracking uses high-pressured fluids that largely consists of water but also includes other chemicals in order to fracture the rock formations where the gas is located (Entrekin et al. 2011). The water and chemicals used during this drilling pose a threat to the environment due to possible runoff into streams and lakes in the surrounding area. Accumulation of this wastewater could affect the access to clean drinking water for the animal community and human population. Similar to the effects from the textile industry, the drilling for natural gas can cause buildup of polluted wastewater that affects the aquatic ecosystem and surrounding terrestrial life.

The threat from the expansion of natural gas development can cause immediate and long-lasting effects on the environment. The targeted land for natural gas extraction must be cleared before drilling can begin. This loss of habitat when essential trees and plants are torn down forces local wildlife to relocate. The displacement of these animals can cause species populations to decrease as well as cause a decline in species variation. It is also possible that wildlife will be forced into urban areas causing issues in the human and domesticated pet populations as well. With the ecosystem disruption, drilling also causes the contaminants from deeper groundwaters to be released in upper soils. Most often, drilling wells are located near some body of water, commonly rivers and streams (Entrekin et al. 2011). The contaminants from the deep groundwater and sediments from clearing the land can end up in these rivers and streams causing a decrease in water flow which would then shorten the distance the water can travel. Because of this, certain animals and plants would lose access to water altogether, contaminated or not.

A case in a Pennsylvania neighborhood in 2010 showed results of water contamination from fracking, affecting at least three households that were using the same local aquifer. The members of the households reported sediment and natural gas in their well water. One household was evacuated from their home due to vapor intrusion of natural gas reported in their basement (Llewellyn et al. 2015). This case was caused by the hydraulic fracturing for natural gas at the Marcellus Shale gas wells located in Pennsylvania. Following these reports, an investigation was initiated to determine the source of the contamination and natural gas vapors. In 2011, the gas company responsible for the wells was cited for violating the PA Oil and Gas Act and Clean Streams Law by allowing natural gas to enter the aquifer (Llewellyn et al. 2015). In response to the pollution of the aquifer, the gas company installed groundwater well replacements which eventually caused other impacts requiring that each household receives treatment. The homeowners, increasingly unsatisfied, filed a civil lawsuit and ended up having to leave their properties which were gained by the gas company as part of the monetary settlement.

Overall, oil production has always produced a large amount of water during the production phase. It is known that the volume of water produced actually increases as the age of the well increases (Wilson and VanBriesen 2012). Globally, the greatest environmental pressure from offshore oil and gas operations are found in the North Sea, with the leading areas in the Norwegian Sea and the Barents Sea (Bakke et al. 2013). One of the most impactful environmental concerns comes from the heavy metals and hydrocarbons that are released within the wastewater because of how they can alter the environment. Damage to the local environment as well as its habitants is largely to blame on polluted wastewater. Therefore, the effort in creating an efficient and successful algal bioremediation process is essential to prevent further environmental damage.

2 Biorefinery Concept and Its Application in Algal Technologies

2.1 Biorefinery and Its Generational Biofuels

Biorefinery is a broad term that emphasizes the idea of extracting and using biological compounds to function as a fuel. As the global population and its consequential demand for fossil fuels rises, research on improving biorefinery methodology is at the forefront of scientific study. In contrast to crude oil and natural gas, production of biofuels begins with processing biomass into energy precursors such as lipids, bio-oils, syngas, and sugars. In general, there are "generations" of biofuels that are produced, each varying in their starting products. In Fig. 1, the synthesis of each generation of biofuel is highlighted. In particular, third-generation biofuels are synthesized by lipids and proteins obtained from various microalgal genera (Hena et al. 2015).

Biorefinery is not a new term in the development of reducing the need for fossil fuels and other associated nonrenewable sources of fuel. Since the depletion of fossil fuel reserves, many methods to create new sources of fuel have been studied. The first few "generations" of biofuels mentioned previously were the primary focus as new energy sources. However, due to commercial limitations for arable land combined with the food crisis in the current global state, using edible and nonedible plants is failing to keep up as fuel possibilities (Ravindran et al. 2017). In response to this, research on microalgae as a potential renewable source for fuel has only grown. Microalgae thrive in nutrient-rich environments that provide adequate pH, temperature, and light conditions. They are easy to cultivate and can be mass produced for various uses. Microalgae as a biofuel source are produced in a series of steps starting with cultivation. Ravindran et al. highlight the use of open ponds/raceways or photobioreactors as the most efficient systems for cultivation. Following this, algal biomass is properly harvested, and its oil can then be extracted either through biochemical or thermal conversion processes. Transesterification and other microbial fuel cell processes are used to convert the algae into its biodiesel product (Ravindran et al. 2017). This extraction process is simplified as well as environmentally and economically beneficial compared to previous biofuel production methods.

2.2 Microalgae as a Developing Source of Fuel

In this simplified production in accordance with the numerous environmental benefits, algae have shown to be the only viable alternative to petro-diesel (Shriwastav and Gupta 2017). Microalgae is found to be one of the most efficient and renewable sources of biofuel because of its ability to remove inorganic and organic compounds as well as heavy metals, pathogens, and other contaminants (Gupta et al. 2017).

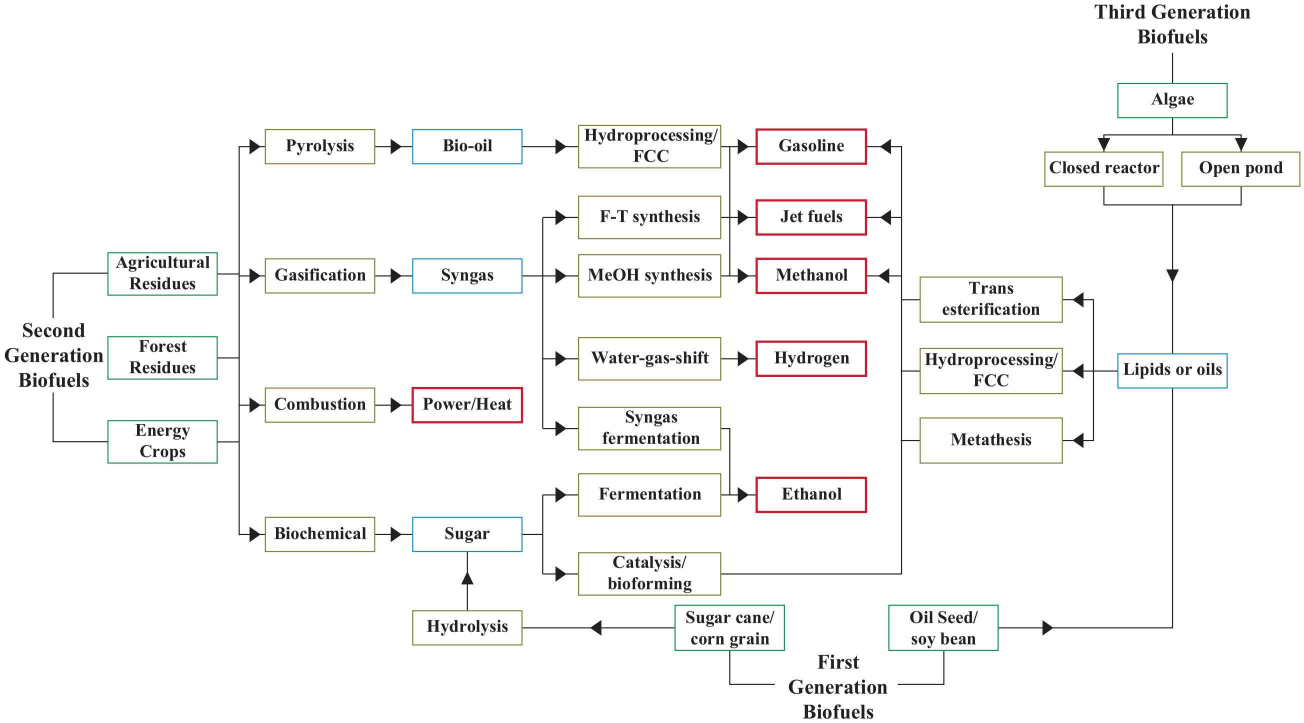

Fig. 1 This diagram illustrates the processes for producing first-, second-, and third-generation biofuels. For first-generation biofuels, carbohydrates extracted from sugar cane and corn grain crops are either fermented to produce ethanol or used as a catalyst to produce gasoline, jet fuels, or methanol. Additionally, lipids and oils from oil seed and soy beans are either transesterified, hydroprocessed using fluid catalytic cracking (FCC), or reacted via a metathesis reaction to produce gasoline, jet fuels, and methanol. For second-generation biofuels, agricultural residues, forest residues, and energy crops are either pyrolyzed or gasified to produce bio-oil or syngas, respectively, for further refinement. These biomasses can also be combusted to provide heat and other forms of power. Additionally, carbohydrates can also be produced via biochemical means. Lastly, third-generation biofuels are produced by taking advantage of microalgae that produce high amounts of lipids and oils which will be further processed as described previously. (Modified from Yue et al. 2014)

Heavy metal contamination is especially dangerous due to its non-biodegradability, high toxicity, and rapid accumulation nature (Chabukdhara et al. 2017).

Algae's ability to break down harsh pollutants is accompanied by its use as storage for valuable materials such as carbohydrates, lipids, proteins, starch, cellulose, and polyunsaturated FAs (PUFAs) (Trivedi et al. 2015). These stored biochemicals can be converted into biofuel following extraction. However, algal research has developed to show that the majority of biomass constituents in microalgae have remained unutilized due to inefficient extraction methods. Specifically, lipid and protein biomass from algal provides nearly 30–60% of residual carbon indicating that both lipid and protein extraction must be achieved in order to reach maximum biofuel yield (Ansari et al. 2017). Other products like carbohydrates, starch, and cellulose have only minimal conversion into viable biofuels. It has also been noted that using algal biofuels can reduce net CO_2 or sulfur emission, net toxic gas, significantly compared to typical petro-diesel fuels (Chabukdhara et al. 2017). Furthermore, algae have a reduced harvesting life and high solar saturation which is nearly 20 times higher lipid yield than oil crops, which can be grown on varying degrees of wastewater (Chabukdhara et al. 2017). This indicates use of phycoremediation as a tool to generate biofuels produced from microalgae. Phycoremediation is concerned specifically with removing chemicals and pollutants from wastewater produced from industrial facilities with microalgae organisms. Two species, *Chlorella* spp. and *Scenedesmus* spp., have shown diverse function in both remediation practice and biorefinery applications.

2.3 Chlorella *spp.*

Weiden Lu et al. studied and developed the concept of using *Chlorella* spp. on a large scale to remove nutrients and produce biofuels in 2015 (U.S. Energy 2016). Lab-bench scale tests were capable of removing 84.34%, in the 5% and 10% wastewater, leading to an arrest of growth. Continuous or semicontinuous cultivation methods would maintain the algae population by supplementing it with more nutrients when necessary. *Chlorella* spp. have also shown to be high in carbohydrate levels indicating possible biofuel production. Specifically, *Chlorella emersonii* contain approximately 37.9% carbohydrate biomass and *Chlorella pyrenoidosa* contain about 26% (Ravindran et al. 2016). These species and their respective protein, carbohydrate, and lipid percentages expressed on a dry matter basis are highlighted in Table 1.

Table 1 Biomass composition of microalgae expressed on a dry matter basis

Strain	Protein (%)	Carbohydrate (%)	Lipid (%)
Anabaena cylindrica	43–56	25–30	4–7
Botryococcus braunii	40	2	33
Chlamydomonas reinhardtii	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
Spirogyra sp.	6–20	33–64	11–21
Spirulina maxima	60–71	13–16	6–7
Spirulina platensis	42–63	8–14	4–11
Synechococcus 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

Excerpted from Ravindran et al. (2016)
The content percentages of each species indicate viability as biofuels. Extraction of these biochemicals and further conversion into fuel could lead to maximal production and possible replacement of fossil fuel

2.4 Scenedesmus *spp.*

Along with *Chlorella* spp., *Scenedesmus* spp. is adaptable and functional in some of the most extreme environments such as those of high temperature and high CO_2 (Ravindran et al. 2016). As mentioned, the degree of biomass largely depends on environmental conditions from light, pH, temperature nutrients, etc. However, the survivability of these species in harsh conditions could lead to studies employing algae in even the most potent wastewater. Studies by Rakesh et al. have reported nearly 77% of oil yielded from *Scenedesmus obliquus* when microwaved continually at 95 °C with solvent-hexane (Ravindran et al. 2016). This kind of treatment in addition to other forms of lipid extraction has confirmed microalgae's efficiency as a biofuel.

3 Industrial Wastewater-Based Algal Biorefineries

3.1 Use of Pulp and Paper Industrial Wastewater

Wastewater from the oil, leather, cement, steel, and textile industries falls before the pulp and paper industry for environmental pollution concerns. However, the paper industry is ever growing in demand, and production is expected to reach between 700 million tons (Mt) and 900 Mt by 2050 (Kong et al. 2016). This industry is a worldwide concern but is heavily influential in India, China, and the United States. The pulp and paper industry is also reliant on significant amounts of water in order to produce just one ton of paper. With this, it is estimated that nearly 85% of wastewater produced from the paper industry is contaminated and requires treatment solutions (Patel et al. 2017). This contamination is largely due to the pulp, which is a mixture of cellulose fibers and water formed in the initial stages of production (Patel et al. 2017). In this pulp, accumulation of toxins such as NaOH, organic solvents, phenols, lignin, and derivatives with high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) is discharged as effluents. Because of the high toxicity percentages, ingestion by animals and human populations can have detrimental health effects, according to the World Health Organization and the Environmental Protection Agency (Patel et al. 2017).

Many traditional methods have been attempted to lower contamination percentages including coagulation, flocculation, and filtration. However, these are energy and cost intensive. In light of this issue, methods utilizing bacteria, fungi, yeasts, and algae are developing as major tools of reducing pollution at a lowered cost. One study performed by Usha et al. in India in 2016 utilized two *Scenedesmus* spp. coupled together to disintegrate the pollutants formed in pulp and paper wastewater. After determining that 60% concentration of wastewater was best for adequate remediation, the coupled microalgae removed up to 82% and 75% of BOD and COD, respectively (Usha et al. 2016). Along with the removal of the organic matter and pollutants was the production of algal biomass. This adds extra economic value to the application of these species as remediation agents.

Further research and economic prospects have developed in the pulp and paper industry for biorefinery concepts. Originally, lignocellulosic material flows were the most viable option in terms of biorefinery. However, further research into microalgae biomass production has shown promise in the paper and pulp industry as well as others. It is known that algae is easy to cultivate, has high turnover rates, and can consume contaminated waters, but high production costs remain a major drawback. Experiments on reducing the conditions and costs needed to produce algae are still being researched today. Mikko Kouhia with Henrik Holmberg and Pekka Ahtila of Finland has developed a biorefinery process that uses by-products of the pulp and paper industry and converts them into microalgal matter, methane, and fertilizer. Flue gases, nutrient-rich sludge, and ash are used and converted into w-3 fatty acids containing lipids (Kouhia et al. 2015). The proposed process is intended to create as many usable algal products as possible from secondary streams in the paper industry that would otherwise end up as waste. Economic and technical feasibility of this process resulted with moderate levels of methane and oxygen but with efficient circulation for algal production and fertilizer. Kouhia et al. have future experiments in preparation to improve this process, but this is one of the first of its kind in the pulp and paper industry. A more recent study examined the effects on pulp wastewater pollution with *Chlorella vulgaris* in a mixture of both pulp and aquaculture effluents. The study performed in late 2017 by Daneshvar et al. resulted with a 60:40 ratio of pulp/aquaculture medium for the best algal growth. In addition, protein, lipid, and carbohydrate concentration was high in biomass after harvesting the pulp/aquaculture mixture containing nitrate and phosphate (Daneshvar et al. 2018). This mixture revealed a 75.68% removal of COD or chemical oxygen demand as well as 70.67% TOC or total organic carbon (Daneshvar et al. 2018). Although this industry is not the current leading pollutant producer worldwide, the demand for paper and pulp products continues to rise. This increase will require improvements in production and treatment of its wastewaters. With microalgae technologies in development, the possibility of cleaner waters is becoming achievable alongside biorefinery prospects.

3.2 Use of Wastewater from Tannery Industry

The tannery industry is important in the economies of many countries such as China, India, and Brazil. However, it is also responsible for serious environmental pollution from the effluents and solid wastes produced in the manufacture of leather products. Tannery effluents contain pollutants such as salts, dyes, detergents, nitrogen, phosphorus, and heavy metals, most significantly, chromium (Beg and Ali 2008; Saxena et al. 2017). Although tanneries treat their wastewaters before release into the environment, it is not unusual for contamination to exceed acceptable limits (Beg and Ali 2008; Verma et al. 2008). For example, one study in Unnao, India, tested the wastewater released from the common effluent treatment plant that receives and treats effluent from nearby tanneries. The study found that the treated effluent, which was discharged into a nearby river, contained concentrations of total dissolved solids (TDS), total suspended solids (TSS), sulfate, magnesium, phosphate, nitrogen, fluoride, phenols, and grease that exceeded acceptable levels set by national authorities. Furthermore, the levels of ten heavy metals were tested, and four were found at concentrations exceeding the recommended level: Cr^{6+}, As^{3+}, Ni^{2+}, and Fe^{2+}. In addition, alkalinity, biological oxygen demand (BOD), and chemical oxygen demand (COD) were well above satisfactory limits (Verma et al. 2008).

The pollutants found in this study, along with others, have deleterious effects on the flora and fauna of water bodies that receive treated effluent, the surrounding soils and crops, and, potentially, the human population. Excess nitrogen and sulfate and high BOD and COD can cause eutrophication that kill fish and aquatic animals. Heavy metals polluting irrigation water lead to decreased crop productivity and increased disease. If humans and animals consume these contaminated crops, the metals can cause cancer and other illnesses. Excess salts affect soil fertility and the quality of drinking water, while dyes block sunlight, decreasing the photosynthetic rates of aquatic plants (Saxena et al. 2017; Verma et al. 2008).

As evidenced by the continued pollution of water and soil in the vicinity of tanneries, conventional treatment techniques are inadequate to completely remove pollutants and toxins from tannery effluent. Coagulation and flocculation are closely related techniques that aim to decrease TSS, COD, and heavy metal levels by precipitation of suspended particles. Coagulates reduce the negative charges of colloids, bringing them together to form flocs. The flocs are pulled together by flocculants to form larger particles that can be removed from the effluent. The main drawbacks to these methods are sensitivity to pH and effluent composition (Saxena et al. 2017). Adsorption is an economical method for removing heavy metals from tannery effluent. Clays, activated carbon, and silica are popular materials. These can be effective in significantly reducing Cr^{3+} concentration, but, if not found locally, they may be expensive (Tahir and Naseem 2007). Biological treatment using microorganisms can take the form of either aerobic or anaerobic treatment. Aerobic treatment is highly effective in reducing BOD and COD and removing heavy metals (Ajayan and Selvaraju 2012; Saxena et al. 2017). However, it generates a significant amount of sludge. Both aerobic and anaerobic treatments can be inhibited by high salt concentrations in tannery effluent. Excessive salinity can cause cell death and bring treatment to a halt (Saxena et al. 2017). Renewable resources such as the green algae, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*, have been taken advantage of because of their biosorption properties to sequester chromium ions in tannery effluent. Chromium ions can exchange with other metal ions already present in the cell walls of algae (Maltsev et al. 2017). Additionally, at acidic pH levels, functional groups such as carboxylates and amines present within the cell walls of living and dead algae may serve as cation-exchange sites for chromium ions which can be further processed for extraction (Aravindhan et al. 2004). Figure 2 below outlines the various mechanisms of bioaccumulation of heavy metals via cellular intake.

Desorption of chromium ions is achieved by exchanging the chromium ions from the algae with hydrogen ions from sulfuric acid to recover it in the form of chromium sulfate. At concentrations of 4 M sulfuric acid, the greatest maximum uptake of chromium achieved was 14 mg of Cr(III)/g for *S. condensata* and 11.81 mg of Cr(III) for *R. hieroglyphicum* within tannery wastewaters. At concentrations of 0.1 M sulfuric acid, the desorption efficiency of chromium ions was 75% (Onyancha et al. 2008). Adsorption and desorption of chromium ions – whether it be through ion exchange, functional group interactions, or cellular uptake – will require further optimization to achieve maximum efficiencies.

While conventional treatments can be effective in reducing certain pollutants, many of them are narrowly focused on a few specific pollutants. A more holistic approach is needed to remediate multiple toxins with a single treatment. Phycoremediation with microalgae is a promising alternative, though more research is needed before it can be implemented on a commercial scale. Several factors make microalgae a good option for addressing pollutants in tannery effluent. First, algae

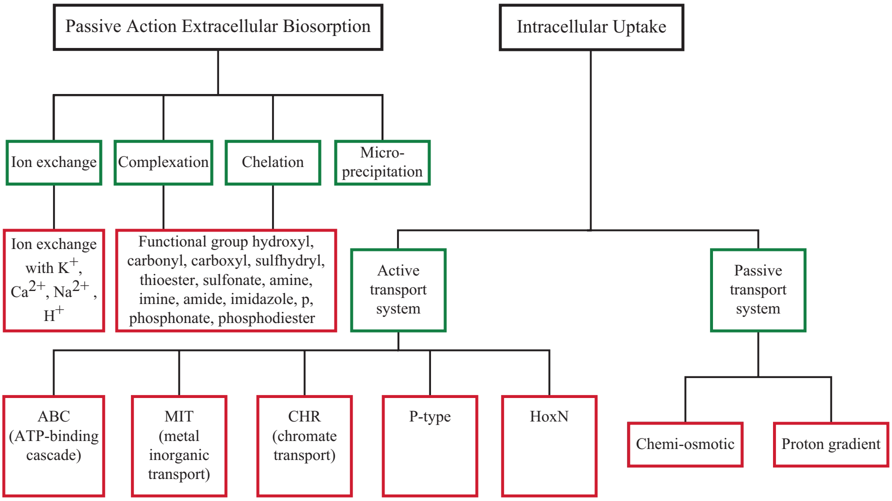

Fig. 2 Various mechanisms that allow cells to adsorb or intake heavy metals. Passive action extracellular biosorption occurs via ion exchange, complexation, chelation, or micro-precipitation. Ion exchanges occur with K^+, Ca^{2+}, Na^{2+}, and H^+. Complexation and chelation occur with various functional groups. Intracellular uptake occurs via an active or passive transport system. Active transport systems include ABC (ATP-binding cascade), MIT (metal inorganic transport), CHR (chromate transport), P-type, and HoxN. Passive transport systems include chemiosmotic diffusion and proton gradients. (Redrawn from Jais et al. 2016)

require high levels of organic and inorganic substances, including nitrogen, which they assimilate into biomass. This property ensures that BOD will be lowered and decreases the incidence of eutrophication caused by tannery effluent. Second, algae eliminates sludge rather than produce it (Rao et al. 2011; Gupta et al. 2017). A study using *Chlorella vulgaris* to treat waste from a tannery in India found that levels of most pollutants had decreased after 7 days of treatment. The pollutants measured included potassium, nitrogen, ammonia, and phosphates. In addition, BOD, COD, and salinity decreased, and sludge was nearly eliminated (Rao et al. 2011). The figure below (Fig. 3) illustrates the conventional wastewater treatment process employing activated sludge.

Another study investigated the use of two algal species, *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*, for the removal of chromium from tannery effluent. The results showed that at low concentrations (<100 mg/L), *S. condensata* adsorbed 55% of chromium from treated effluent, while *R. hieroglyphicum* adsorbed 65%. However, the algae required relatively low pH to achieve this efficiency; *S. condensata* was most effective at 5.0 and *R. hieroglyphicum* at 4.0 (Onyancha et al. 2008). Because tannery effluent is basic, these findings seem to indicate that certain algae may be a poor choice for heavy metal removal (Saxena et al. 2017). More research is needed on the metal adsorbing performance of various algae in tannery effluent.

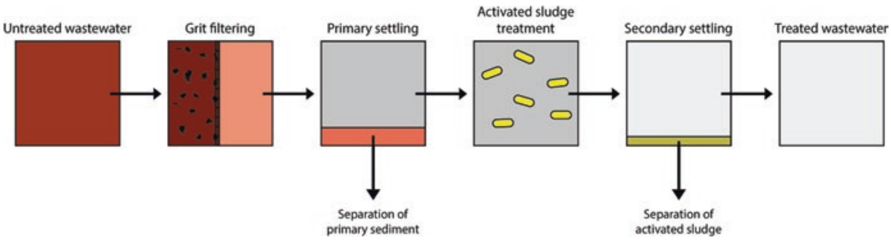

Fig. 3 Steps in treatment method typically used for sewage or industrial wastewaters. Wastewater is passed through a filter to separate any loose particles and grit. The wastewater is then inoculated with a microorganism which serves as biological floc to sequester/degrade organic compounds present in the wastewater thereby reducing BOD. Depending on the subsequent use of treated wastewater, it can be further denitrified used anoxic conditions

3.3 Use of Textile Industry Wastewater

The textile and dyeing industry is the industry with the largest consumption of water and therefore creates the highest output of chemically polluted water into the environment. The copious amounts of dyes and other chemicals provide an array of unique substances that can harm the natural environment. Although these consequences demand action, the textile industry continues to flourish and increase in demand. A study in 1995 by the American Dye Manufacturers Institute (ADMI) calculated that the capital expenditure by domestic dyeing companies has increased to nearly $2.9 billion in recent years (Patel and Vashi 2015). The textile industry has been around for centuries and has only refined the process with time. With 14% of the sector located in India, the textile industry provides about 27% of India's foreign exchange (Bhatia et al. 2017). Indonesia is found to be the second country among the list of others that has the highest level of pollutants that contributed more than 20% of the registered levels for water pollution produced by the textile industry following behind Turkey (Soeprobowati and Hariyati 2017). The toxicity from textile wastewater has been a significant issue in countries like India and Indonesia largely due to the history of dumping the waste in natural water systems surrounding the dyeing plants. Additionally, the dyeing wastewater generates the largest portion of the total wastewater from a typical textile plant. It contains excessive salt, resin residues, softeners, and ranges in color, which when combined, can be detrimental to the surrounding environment (Patel and Vashi 2015). Because of these environmental and health impacts from wastewater, several methods of treatment have been attempted. Typical treatments include the separation of solids from liquids in various techniques, physical, chemical, and biological. Other methods entail flocculation, coagulation, ion-exchange processes, batch treatments, sludge conditioning, acid-base neutralization, and many more (Bhatia et al. 2017).

The use of microalgal species for phycoremediation of textile wastewater is a developing technology. Algae is of beneficial use for reasons including lower cost, no secondary pollution, no carbon needed for nutrient removal, and converting algal biomass into products such as biogas, bioethanol, and bio-oils (Soeprobowati and Hariyati 2017). A study in 2017 utilizing algal species *Chlorella pyrenoidosa*, *Arthrospira platensis*, and *Chaetoceros calcitrans* by Soeprobowati and Hariyati identified successful bioremediation of textile wastewater. Heavy metals like Cr, Cu, Pb, and Cd were reduced by *C. pyrenoidosa* by nearly 80% (Soeprobowati and Hariyati 2017). Furthermore, the table below (Table 2) depicts a limited list from Kumar et al. in 2015 identifying certain algal species that are capable of absorbing heavy metals.

A study by Khataee et al. reported significant biodegradation of C.l. Basic Red 45 (BR46) solution using algae *Enteromorpha* sp. under optimum growth conditions (Holkar et al. 2016). Another case researched in 2014 by Meng et al. demonstrated *Shewanella* algae (SAL) to reduce azo dye (acid red 27) decolorization in the presence of NaCl and different quinones or humic acids (Holkar et al. 2016). Color removal as well as other chemicals with the use of microalgal species is becoming a promising method of wastewater treatment in the textile industry. Furthermore, the conversion of these nutrients into biofuels is at the forefront of environmental science.

Textile wastewater is useful for algal biofuel production because it contains the necessary nutrients for algal growth and organic dyes which can act as potential carbon sources for algae cultivation. Employing textile waters for algae cultivation and further as biofuel conversion could reduce cost and waste into the environment. After proper algae cultivation and harvesting steps have been concluded, lipid extraction for biodiesel production is begun. Certain species of algae including *C. vulgaris*, *Scenedesmus ecornis*, and *Dunaliella* have shown to provide the extraction of triglycerides (TG), free fatty acids (FFA), glycolipids, and phospholipids (Fazal et al. 2018). These lipids can then be transformed into biodiesel through transesterification. Physical and chemical methods have been enabled for oil extraction of algal cells. Cell drying through intense sunlight and cell lysis through several methods such as high-pressure homogenization, microwave, ultrasonication, bead beating, electroporation, expeller pressing, and more have proven to be effective methods of disrupting algae cells for oil extraction (Fazal et al. 2018). However, the efficiency of each method is dependent of species type and associated cell size. Once extraction is completed, conversion into biofuel can occur. Microalgae treating textile wastewater as well as other industrial wastewaters is most effective due to its similar physical and chemical characteristics to petroleum (Fazal et al. 2018). In contrast though, biodiesel is a much cleaner source of renewable energy. The process of transesterification into biofuel is reversible, requires less energy, and has a low molar ratio of alcohol to oil and easy recovery of esters (Fazal et al. 2018). Overall, the use of textile industry wastewater is an unfortunate side effect of a booming industry. But, with the proper algal bioremediation and cultivation technologies, this polluted water can be refined and employed for a good use.

Table 2 Major algal species that can remove heavy metals including iron, mercury, nickel, lead, and zinc

Metal	Speciation	Organism	pH	Type of biomass	Metal uptake (mg/g)	References	Country
Fe	Fe^{3+}	*Spirulina* spp.		Live	0.271	Chojnacka et al. (2004)	Poland
		Spirulina sp. *(HD-104)*		Nonliving	576	Doshi et al. (2008)	India
		Synechocystis spp.	4.5	Nonliving	23.4	Dönmez et al. (1999)	Turkey
		Chlorella vulgaris	2	Nonliving	24.52	Romera et al. (2006)	Spain
		Microcystis sp.	9.2	Nonliving	0.03	Singh et al. (1998)	India
Hg	Hg^{2+}	*Chlamydomonas reinhardtii*	6	Nonliving	72.2	Tüzün et al. (2005)	Turkey
		Cyclotella cryptica	4	Nonliving	11.92	Schmitt et al. (2001)	Germany
		Pseudochlorococcum typicum	7	Live	15.13	Shanab et al. (2012)	Egypt
		Scenedesmus subspicatus	4	Nonliving	9.2	Schmitt et al. (2001)	Germany
		Spirulina spp.	7.5	Nonliving	1.34	Chojnacka et al. (2004)	Poland
Ni	Ni^{2+}	*Aulosira fertilissima*	5	Nonliving	4.16	Singh et al. (2007)	India
		Chlamydomonas reinhardtii	5.55	Cell wall	0.4	Macfie and Welbourn (2000)	Canada
		Chlamydomonas reinhardtii	5.5	Without cell wall	0.63	Macfie and Welbourn (2000)	Canada
		Chlorella vulgaris	4.5	Immobilized	111.41	Mehta and Gaur (2001)	India
		Spirulina		Live	1378	Doshi et al. (2007)	India
Pb	Pb^{2+}	*Arthrospira (Spirulina) platensis*	5–5.5	Nonliving	102.56	Ferreira et al. (2011)	Brazil
		Chlamydomonas reinhardtii	6	Ca-alginate immobilized	230.5	Bayramoğlu et al. (2006)	Turkey
		Hydrodictyon reticulatum	5	Nonliving	24	Singh et al. (2007)	India
		Microcystis novacekii	5	Nonliving	80	Ribeiro et al. (2010)	Brazil
		Porphyridium purpureum	6	Nonliving	0.32	Schmitt et al. (2001)	Germany

(continued)

Table 2 (continued)

Metal	Speciation	Organism	pH	Type of biomass	Metal uptake (mg/g)	References	Country
Zn	Zn^{2+}	*Chlorella* spp.	7	Immobilized	28.5	Maznah et al. (2012)	Malaysia
		Cyclotella cryptica	6	Nonliving	242.9	Schmitt et al. (2001)	Germany
		Phormidium spp.	5	Nonliving	9.4	Wang et al. (1998)	USA
		Planothidium lanceolatum	7	Live	118.66	Sbihi et al. (2012)	Morocco
		Spirulina platensis	6	Nonliving	7.36	Sandau et al. (1996)	Germany

Kumar et al. (2015)
The concentration of metal uptake as well as the performing researcher and their respective country is displayed in the table

4 Application Constraints and Limitations of Use of Industrial Wastewater

Microalgae has shown to be an effective treatment option of various industrial wastewaters while producing biofuels that have the potential to replace fossil fuels and other nonrenewable resources. Algae can simultaneously breakdown pollutants and other chemicals in wastewater while producing the biomass necessary for biofuel production. Because of this, wastewaters from varying industry types are useful for algal growth. However, alongside the benefits of this biomass production from industrial wastewaters are the drawbacks and limitations.

Although the environmental benefits are substantial, the cost-effectiveness of using algae has not yet been achieved. Production conditions, such as light, pH, and temperature, must be set perfectly for algae to cultivate, which in turn raises the expenditure on supplies. Controlled temperatures must be maintained throughout to obtain high yields of biofuel product. This limits the algal growth causing a smaller harvest and fewer biofuels. Additional factors to consider when using microalgae are the type of cultivation system, net energy input and output, environmental impacts, and economic analysis. Specifically, the cultivation techniques depend on the type of algae species utilized, which can range in cost from $5 to 10 per kilogram (Katiyar et al. 2017). In order for commercialization, the physical land space, water, and fertilizers would have to be substantial to produce mass quantities of algae (Katiyar et al. 2017). In contrast, simply maintaining a large land space of algae requires a significant amount of work and is often unfeasible. A study by Philip Kenny and Kevin Flynn in 2017 identified certain physiological constraints during algal biofuel production. Kenny and Flynn emphasize the physical process of algal growth. Specifically, the accumulation of surplus carbon (C) within the cells that would be harvested for biofuel occurs mainly during the growth phase, which is when the supply of nitrogen (N) is limited (Kenny and Flynn 2017). This in turn limits cell proliferation and intended maximum yield. It is also known that phosphorous (P) is an essential fertilizer for algal production. Without nutrients like C or P, microalgae productivity is greatly reduced. The relationship between algal biomass and the TP, or total phosphorous concentration, is hyperbolic and direct in nature, according to a study by Smith and McBride in 2015.

Additionally, microalgal cells require optimum light for photosynthesis, and in commercial-sized production farms, this is often unachievable. Large accumulations of algae prevent natural light from reaching every cell surface. This minimization of natural light inhibits the performance of photosynthesis and further slows the rate of production. Because of this, models that predict ideal conditions and product output have often fallen short when attempted in reality. Kenny and Flynn also address the idea that many cost analysis research have focused only on lipid content and production in microalgae without considering the life cycle and its requirements of the species at hand. If microalgae is not being reproduced at the intended rate, then biofuel production is surely to be on the low end of the predicted parameter. Smith and McBride suggest the term nutrient use efficiency or NUE. This is defined as a direct, quantitative measure of the nutrient demands of algal production, which, in turn, measures the associated economic cost of biofuel production (Smith and McBride 2015). They suggest the NUE is varied widely among different algal species and should be used as the forefront of microalgae biofuel theoretical models for cost and performance effectiveness.

Certain application constraints have been identified based on the type of wastewater being treated. For example, the use of algae to treat the wastewaters of the textile industries has attributed a fair amount of success so far because of its effectiveness in removing nutrients and other chemical pollutants. However, textile wastewaters typically have higher levels of heavy metals and lower levels of nutrients such as nitrogen and phosphorus (Cai et al. 2013). This poses an issue because only a limited number of algal species have been studied for heavy metal removal in wastewaters. This limits the amount of algal growth in the wastewater of the textile industry and thus reduces the benefits of using algae for bioremediation. Wastewater from the oil industry can contain high levels of solids or bromides in the source water, especially if the rivers being used have a low flow rate (Wilson and VanBriesen 2012). They can also contain salts which alter the alkalinity and pH of the water that the wastewater is released into. This can modify the conditions of the wastewater, therefore inhibiting ideal microalgal growth which also reduces biofuel production. Additional components from the oil industry include hydrocarbons, sulfur-reducing bacteria, and heavy metals such as cadmium, copper, chromium, lead, mercury, nickel, zinc, and arsenic, which can further contaminate wastewaters (Bakke et al. 2013). The microalgae in these wastewaters would not produce substantial or sustainable biofuel products because of these additional pollutants.

With an understanding of these limitations and continued research into bioremediation of industrial wastewaters, the possibility of algal biofuels replacing traditional fossil fuels will become more and more attainable.

5 Economic Aspects of Industrial Wastewater-Based Algal Refineries

The overarching goal motivating algal refineries is the idea that the extracted components from the algae can be used in various ways. Initially, algae were solely considered as a source of food for invertebrates, fish, and other small vertebrates, but with the adaption of industrial power, many other opportunities for algae have conspired over time. Algae was used as a food source by native people for thousands of years due to its rich composition of proteins, lipids, and carbohydrates. However, since the 1950s, algae use has been shifted in consideration as a possible large-scale replacement for oil-based fuel (Spolaore et al. 2006). This has led to vast environmental benefits and cost reductions, but algae can act as much more than this. With the advancement of biofuels, algae have also been commercialized into many products, including human food, animal feed, cosmetics, use in aquaculture, formulas and nutritional supplements, pigmentation, and more. For instance, microalgae have shown natural antiaging capabilities in two species, *Arthrospira* and *Chlorella vulgaris*. Each has shown potential in skin tightening and collagen synthesis allowing for tissue regeneration and support (Spolaore et al. 2006). Cultivating and manufacturing algae for products like these are promising for the commercial market and high economic value. This also means algae can act as a more natural replacement for harsh chemicals in skin products and/or foodstuffs. Using wastewater-based cultivation combined with biorefineries to extract the essential biochemicals from algae could lead to further large-scale commercialization.

The cultivation of microalgae is one of the many reasons it is used as a replacement for other petroleum-based fuels. However, studies examining the economics and environmental benefits of algae cultivation have shown that only certain cultivation technologies are truly cost-effective. It was found through a techno-economic assessment (TEA) performed by Thomassen et al. in 2016 that a special membrane to recycle the growth medium accompanied with an open pond cultivation was the most economically feasible option (Chew et al. 2017). Currently, given the cost of cultivation harvesting materials and raw material costs such as water, the technology available is in favor of standard fossil fuels. However, with further research and development on improving designs, microalgae could eventually become more economically feasible.

The key input variables, or KIV, for large-scale algae production include the following: evaporation rate, water cost, water depth, days of operation, medium cost, carbon dioxide, algae production rate, and oil content of algae (Richardson et al. 2010). These are the components that will determine the economic viability of an algae farm. The raw materials are mostly composed of the water cost because other nutrients can be recycled, and carbon dioxide is considered freely available (Chew et al. 2017). However, more can be done to sequester costs. A study in 2016 by Judd et al. focused on a microalgae culture technology, or MCT, that removes the nutrients from wastewater combined with the requisition of flue gas CO_2 in a single process (Judd et al. 2016). MCT has been covered by many articles as a potential for increasing algae cultivation meanwhile reducing the environmental concerns. MCT utilizes the combination of two of the most important aspects of algae production which in turn limits expenditure of the entire process. The simple diagram (Fig. 4) below highlights the idea of MCT.

Although microalgae biorefineries are still in the trial-and-error phase compared to other forms of fuel, it shows drastic potential. The lowered cost with the environmental benefits and mass production qualities for commercial products are only a few of the reasons why microalgae is being utilized so highly in the refinery community. It is possible with the proper technologies that algae could replace all petroleum-based fuels entirely and at a lower expenditure.

6 Conclusions and Future Prospects

In conclusion, various algal species are able to use wastewater that is generated from industries to cultivate and mass produce themselves. The biomass that is collected is then able to be used as a biofuel source creating biodiesel, biogas, and bio-oil. With these more environmentally friendly fuel sources, it is possible that nonrenewable and harmful fossil fuels could eventually be replaced. Algal ponds are less energy consuming, more cost-effective, and environmentally positive. Treating industrial wastewater, from a variety of industries, with algal species could become a conventional method in the future. Furthermore, using algae for biofuels could decrease the world's dependency on crude oil and natural gas. Research has shown that algal biofuels function equally as well to traditional fossil fuels but at a lower cost to the environment. Further refinement of this biotechnology could eliminate the use of traditional petroleum-based fuels and therefore reduce environmental pollution and degradation.

The future of algal biotechnology as a remediation tool and biofuel producer is bright. Current research in industrial wastewater cleanup with algae has been successful in several industries including the textile, pulp and paper, and tannery industry. However, there are still several other areas of commerce that will require changing to this more environmentally friendly solution. The iron and steel as well as the mine and quarry industry are two major industries that are being prospected for microalgae use. Furthermore, the conversion of microalgae into viable biofuels has also been rather successful. This transformation process is still being perfected but shows large potential for reducing the dependency on standard petroleum-based oils. Phycoremediation and biorefinery using microalgae are an up-and-coming biotechnology that has the potential to have a significant impact on both the environment and the economics of the large industry business.

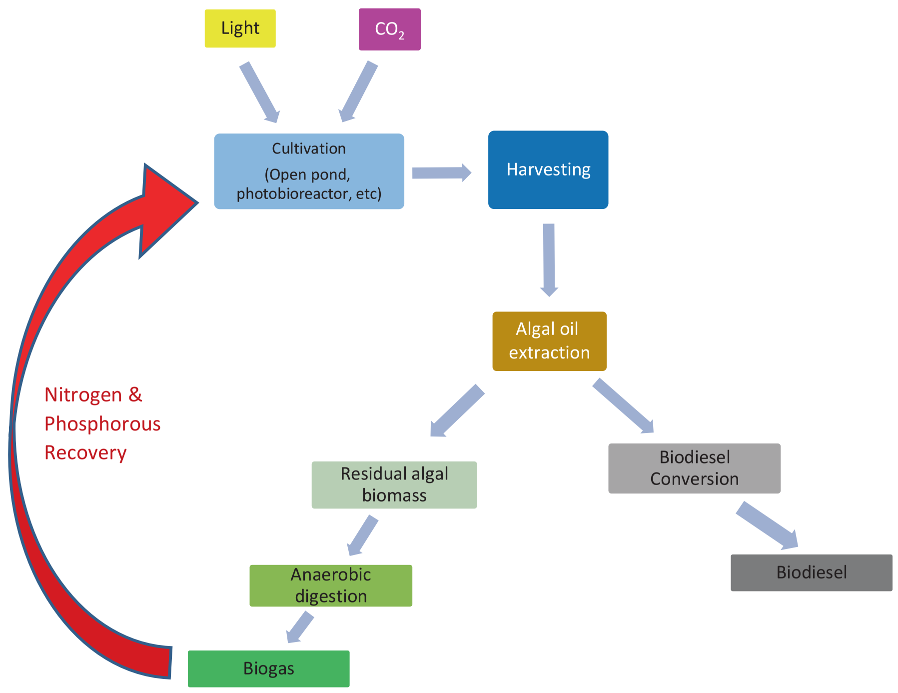

Fig. 4 This MCT flowchart displays the combined one-step process of nutrient and CO_2 substrate complexes into algal cultivation. (Redrawn from Judd et al. 2016)

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Valorization of Nutrient-Rich Urinal Wastewater by Microalgae for Biofuel Production

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1 Introduction

Microalgae biomass is the emerging substrate used for biofuel production (Hattab and Ghaly 2015). It has valuable chemical compositions such as protein, carbohydrate, lipid, and nucleic acid (Kavitha et al. 2017a). Due to its chemical composition, it can be used to produce energy (Chew et al. 2017). In addition, microalgae biomass counterattack the emission of greenhouse gases, which will reduce global warming issues (Kannah et al. 2018). In contrast microalgae biomass has an ability of splitting proton and neutron from water by utilizing the solar radiation as light energy (Ramanna et al. 2017), and it also captures a huge volume of carbon dioxide from the atmosphere for its effective growth (Nayak et al. 2016). Biofuel production from microalgae biomass involves cultivation, harvesting, disintegration or extraction, and biofuel conversion processes (Islam et al. 2017). The cultivation of microalgae biomass under favorable condition for lipid accumulation is a significant task for effective biofuel generation. The cultivation of microalgae biomass in the fresh water needs additional supply of nutrient for their growth (Slade and Bauen 2013).

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Developing integrated approaches like microalgae biomass cultivation using waste stream will result in the creation of economically viable path to sustainable development (Cheah et al. 2016). A huge quantity of valuable macro- and micronutrient enriched with organic matter are readily available in wastewater (Ge et al. 2017). However, conventional wastewater treatment systems are still inefficient in the removal of nutrient to a greater extent (Rajasulochana and Preethy 2016). Many researchers have approached the wastewater as nutrient source for cultivation of microalgae (Muylaert et al. 2015), which results in the increase of biomass productivity and decrease of nutrient pollution up to the discharge standard level (Delgadillo-Mirquez et al. 2016). Several researchers have analyzed the effect of microalgae cultivation using different culture media such as domestic (George Thomas et al. 2016), dairy (Hena et al. 2015), industrial (Mohd Udaiyappan et al. 2017), agricultural (Tango et al. 2018), paper mill (Ekendahl et al. 2018), and anaerobically digested effluent (Dębowski et al. 2017; Zhang et al. 2018). In several countries, microalgae biomass was successfully practiced in wastewater treatment facilities due its dual benefits like nutrient recovery and biomass yield for biofuel generation (Hupfauf et al. 2016; Gómez-Guzmán et al. 2017).

Recently, decentralized concept for source-separated urinal wastewater has gained more attention toward cultivation of microalgae biomass (Capodaglio 2017). However, domestic wastewater was the major source for supply of excess nutrient to the receiving water body or wastewater treatment facilities. This kind of wastewater generation was directly linked with population rate; if population rate increases rapidly, then the wastewater generation rate also increased accordingly (Naden et al. 2016). Essential human activities such as washing, cleaning, bathing, flushing, urinating, etc. lead to the generation of domestic wastewater. In general, the domestic wastewater was distinguished as black and gray wastewater. Figure 1 shows the flow of domestic wastewater stream, classification, and their nutrient composition. Black wastewater is the combination of both yellow and brown wastewater, and gray wastewater is the combination of wastewater generated due to activities like cleaning and personal care. However black wastewater is a major source of excess nutrient when compared with gray wastewater. According to Höglund (2001), nutrient composition of individual wastewater was listed in Fig. 1. On seeing the nutrient composition of urine and fecal wastewater, urinal wastewater accounts high quantity of nutrient (Simha and Ganesapillai 2017). In domestic wastewater stream, urinal wastewater is less than 1% in its overall volume.

According to Randall and Naidoo (2018), urinal wastewater is otherwise known as liquid gold of wastewater. It holds excess amount of valuable nutrients such as 82% of N (nitrogen), 56% of P (phosphorus), and 62% of K (potassium). The developed and developing countries have implemented no-mix toilet basin for separation of urine. Some are successfully turning the liquid gold wastewater into fertilizer, which results in profitable economy. Figure 2 shows the microalgae biomass valorizing the nutrient-rich urine for effective biofuel production. Bearing all this in mind, researchers have turned their interest toward valorizing nutrient-rich urinal wastewater by microalgae due its dual benefits, namely, nutrient removal and

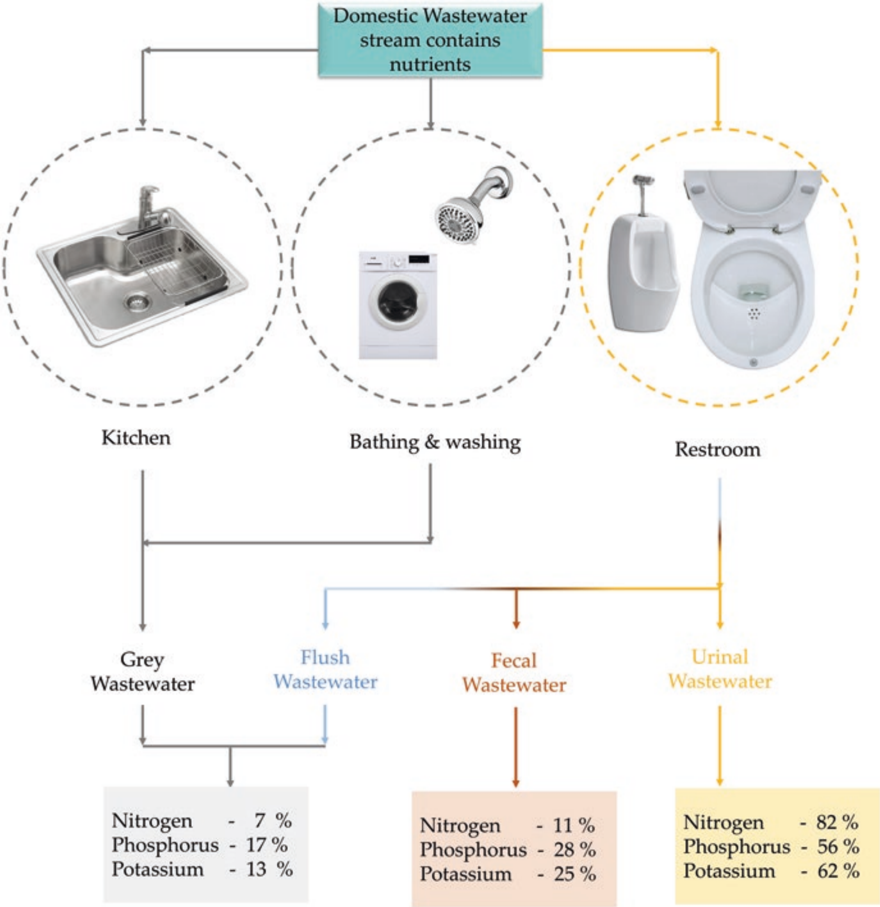

Fig. 1 Flow of domestic wastewater stream, classification, and their nutrient composition

biomass for biofuel yield. The following novel approach will safeguard the environment for sustainable development, by minimizing global warming issues created by conventional fossil fuels. Figure 2 demonstrates microalgae biomass valorizing nutrient-rich urine for effective biofuel production. Initially source-separated urinal wastewater was collected and used as culture media. Immobilized microalgae biomass valorizes nutrient and increases the biomass productivity with the help of solar radiation. The effluent from the culture tank can be utilized for flushing and gardening. On the other hand the raw urinal wastewater can be used in constructing wetland and agricultural field for crop production due to its nutritional composition. The harvested biomass was effectively disintegrated using different pretreatment technologies, and this process is known as hydrolysis. Pretreated biomass can be processed for the desired type of biofuel through the corresponding process techniques.

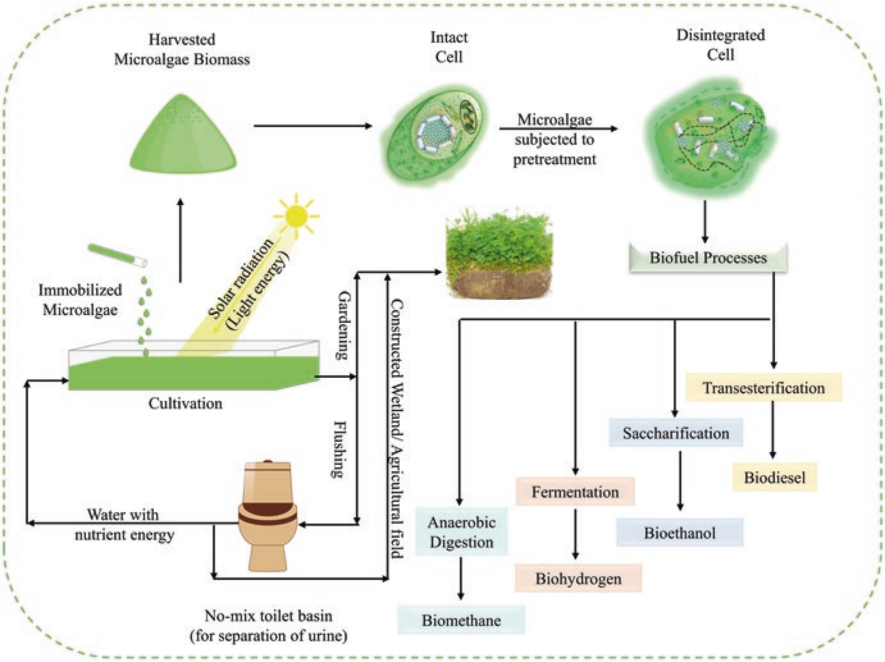

Fig. 2 Microalgae biomass valorizing the nutrient-rich urine for effective biofuel yield

The objective of this chapter is to educate about microalgae valorizing urinal wastewater for biofuel generation. Nutrient removal efficiency and biomass yield for biofuel production depend on the following parameters: microalgae species, mode of cultivation, and nature of urinal wastewater. The impact of urinal wastewater, types of microalgae species used for valorizing of nutrient-rich urinal wastewater, various immobilization technologies followed for biomass yield, and different kinds of microalgae pretreatment subjected for biofuel production will be discussed in the subsequent titles.

2 Urinal Wastewater and Their Impacts

Urine is a pale yellow liquid waste that is produced by the human kidney to excrete the waste components from the blood stream. The major component of urine is water, which accounts for 91% to 96%, and the remaining 9% to 4% consist of organic solute along with pathogens, pharmaceutical residue, and hormones. Urine from normal person is odorless and under sterile conduction. The pH and specific gravity of urine are in the range of 5.5–7.0 and 1.002–1.037, respectively. Table 1 shows the physical characteristics of urine.

Table 1 Physical characteristics of urine

Physical characteristics	Values	References
Color	Pale yellow to deep amber	Putnam (1971) Kirchmann and Pettersson (1994) Gethke et al. (2007) Randall and Naidoo (2018)
Odor	Odorless	
Volume	750–2000 mL/day	
pH	4.5–8.0	
Specific gravity	1.003–1.032	
Bacterial load	Nil (sterile)	

Table 2 Elemental composition of urine

Elements	Values	Units	References
Carbon	6.6 to 6.9	g/L	Putnam (1971) Kirchmann and Pettersson (1994) Maggi and Daly (2013)
Hydrogen	1.2 to 1.5	g/L	
Nitrogen	8.1 to 9.2	g/L	
Oxygen	7.9 to 8.3	g/L	
Sulfur	0.21 to 0.3	g/L	

According to Putnam (1971), a healthy human can discharge urine in the range of 600–2500 mL/day. The concentration of nutrient in urinal wastewater may differ from person to person, based on their health condition and diet. For example, a normal human being can be able to generate 25–35 g of nitrogen per day and 2–2.5 g of phosphorus per day. Even though the volume of generation is less, the concentration of nutrient in urinal wastewater is higher when compared to other sources of domestic wastewater. Table 2 shows the elemental composition of urine. Urinal wastewater has its own positive and negative impacts on environment as listed below.

Positive impacts

- Urinal wastewater is used as culture media for microalgae biomass yield.
- Urinal wastewater replaces the use of industrial fertilizers for agriculture crop production.
- Urinal wastewater acts as an alternative energy source (hydrogen).
- Urinal wastewater is used to generate electricity using microbial fuel cell technology.
- Urinal wastewater is used to prepare bio-stones.

Negative impacts

- Urine generated from unhealthy people may cause offensive odor, and it may contain harmful pathogens.
- Direct discharge of urinal wastewater in nearby water bodies leads to eutrophication because of excess nutrient concentration.
- Urinal wastewater causes pharmaceutical pollution on the environment; human cannot be able to metabolize all pharmaceutical drug intakes.

- The occurrence of inorganic salts, persistent organic matter, genetic hormones, and heavy metals leads to water and soil pollution.

3 Nutrient Composition in the Urinal Wastewater

Urine consists of both organic and inorganic components with 14–18% nitrogen, 13% carbon, 3.7% phosphorus, and 3.7% potassium (Strauss 1985). Potassium, calcium, sodium, chlorine, and sulfur are also some of the important components in urinal wastewater. Table 3 shows the nutrient composition in urine. The presence of trace elements like boron, copper, zinc, molybdenum, iron, cobalt, and manganese is also observed (STOWA 2002).

Apart from the essential nutrients, urine is also enriched with pharmaceutical residues, hormones, and bacterial load. Heinonen-Tanski and van Wijk-Sijbesma (2005) measured the dry contents of urine to be 4.7–10.4 g/L. There are about 65–85% of organic matter in the dry solids of urine (Strauss 1985), and among the total solids present, there are about 75–85% of volatile solids (House 1981). Urea is produced as a result of metabolism of nitrogen in the protein, and hence it forms the major constituent of the total solids of the urine (about 50%). The chemical oxygen demand (COD) in the urine is usually due to the decomposed organic matter. The COD content in the urine is usually 12 g per person per day (Jönsson et al. 2005). Table 4 shows the chemical composition of urine. The factors such as age, gender, and calorie intake determine physicochemical properties of urine. The major nutrients of urinal wastewater are discussed in detail in the following sections.

Table 3 Valuable nutrient composition of urine

Nutrient composition			
Nitrogen	Phosphorous	Potassium	References
70%	40%	60%	Coppens et al. (2016)
69%	40%	60%	Zeeman and Kujawa-Roeleveld (2011)
70%	40%	60%	Zeeman et al. (2008)
80%	50%	–	Piltz and Melkonian (2018)
80%	50%	90%	Dodd et al. (2008)
80%	50%	–	Larsen et al. (2004)
60%	80%	–	Herrmann and Klaus (1997)
80%	50%	–	Adamsson (2000)
80–90%	50–70%	60%	Hanæus et al. (1997) Niwagaba et al. (2009)
>80%	>55%	–	Gethke et al. (2007)

Table 4 Chemical composition of urine

Chemical	Ranges	Unit	References
NH_4-N	2500–8100	mg/L	Heinonen-Tanski and van Wijk-Sijbesma (2005) Maurer et al. (2006)
TN	8000–10,000		
Urea	5000–9000		
TP	700–2000		
COD	8000–10,000		
Boron	500–3300	μg/L	Rodushkin and Ödman (2001) Gòdia et al. (2002)
Iron	34–540		
Copper	42–50		
Zinc	270–850		
Manganese	0.12–20		

3.1 Nitrogen

Urine contains nitrogen in the form of urea (CO $(NH_2)_2$), formed as an end product during the metabolism of amino acids. The urea concentration ranges between 9 and 23 g/L; it is a soluble salt with a weak base. Within 4 days, about 64% of the urea is broken down into ammonia (NH_3) and carbon dioxide (CO_2) by microorganisms. According to Udert et al. (2003), when urine is maintained under non-sterile condition, the presence of urease enzyme metabolizes urea into ammonia and bicarbonates.

$$CO\left(NH_2\right)_2 + 2H_2O \rightarrow NH_3 + NH_4 + HCO_3$$

This process is known as urolysis, and the formation of ammonia increases pH, which results in the toxic nature of urine and causes nitrogen loss and has negative impacts on the environment. Uric acid is also present in urine in a small amount as 0.7 g per person per day. The fraction of nitrogen-rich compound in urine is creatinine, and each day 1.6 g is produced. Human urine has more ability for the cultivation of microalgae. The increase in ammonia concentration inhibits the growth of microalgae.

3.2 Phosphorous

Phosphorus in urine is directly proportional to phosphorus intake in diet, and it also depends on the amount of calcium and magnesium eliminated. About 6% of the phosphorus is consumed by a human of age group 1–17 years and will be accumulated in the growing body. When the population comprises more of this age group of people, the excretion of phosphorus in the urine will be less. Thus the average volume of phosphorus excreted in urine is about 1 g per person per day, and most of the phosphorus exist in urine as inorganic phosphates (Jönsson et al. 2004). There is an increase in pH, which forms crystal of phosphate of calcium and magnesium. The by-products obtained are struvite ($MgNH_4PO_4.6H_2O$), calcite ($CaCO_3$), and hydroxyapatite HAP ($Ca_5 (PO_4)_3 OH$) (Bhuiyan et al. 2008). The recovery and reuse of phosphorous from human urine are one of the options to sufficiently supply it to the future generation. About 22% of the universal phosphorus requirements are present in human urine which can be recovered as manure. Thus nutrients in urine can be utilized as fertilizers and substitute industrial fertilizer production with an environmental gain.

3.3 Potassium

Potassium is the mineral or electrolyte that is crucial for life. It is ingested through food and excreted through urine. It helps to balance the quantity of water and electrolytes in the body. Potassium has inverse actions compared to sodium in the body. The decentralized sanitation concept is the method where the urine is source separated and the nutrients were recovered in a sustainable way. Source-separated treatment offers effective energy recovery and high nutrient removals or incorporates the nutrients into microalgae cell. The urine separated at its origin has greater ability to be used as a liquor manure for plants since it is rich in nutrients. Formerly the nutrients were recovered through struvite precipitation and ammonia stripping. The output can also be used as manures for cultivation. Ammonia stripping recovers nitrogen by supplying high energy. Human urine contains essential nutrients that can support the proliferation of algae.

Chang et al. (2013) examined the growth of *Spirulina platensis* using synthetic human urine as a culture media. The researcher stated that the autotrophic culture *Spirulina platensis* can remove about 97% of NH_4-N, 96.5% of TP, and 85–98% urea from human urine. Phosphorous was directly used by *S. platensis* and was removed effectively. Similarly Luo et al. (2017) have examined the growth of microalgae in membrane photo-bioreactors (MPBR) under fluctuating operational conditions. The removal efficiencies of N and P were varied in the range of 30–97% and 46–93%, respectively. MPBR achieved an algal yield of 50–100 mg/L.

Mbir and Mensah (2017) have studied the nutrient removal efficiency in *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*. Among them *Chlorella sorokiniana* has the ability to remove 63.2% of TN and 55.8% of TP at an N/P molar ratio of 8.5:1. In the same way, *Scenedesmus obtusiusculus* removes 45.9% of TN and 76.3% of TP at an N/P ratio of 6.9:1. Based on the above, it was evident that human urine can be used as a nutrient supplement for the growth of microalgae.

4 Potential Microalgae Species Used for Urinal Wastewater

Being a photoautotrophic microorganism, microalgae are very efficient in taking up nutrients in the existence of light and carbon dioxide. Employment of these organisms to treat municipal wastewater and to recover nutrients dates back to 60 years ago by Atkinson (1986). It is a single cellular species, mostly present in water and soil particles singly or linearly or in groups. Within a day microalgae can grow rapidly and expand its population to two times of its original population. They can grow aggressively within 3.5 h and exist in all nutrient-rich environmental conditions (Zhou 2014). Protein is a major constituent of the microalgae apart from lipids and carbohydrate. The protein content ranges from 12% to 35%, and the lipid content ranges from 7.2% to 23%.

The source-separated human urine has high nutrient contents and it supports the growth of microalgae. The toxic effect of ammonia declines the growth of microalgae (Adamsson 2000; Feng et al. 2007; Yang et al. 2008; Tuantet et al. 2014a). The composition of nutrients (carbon/nitrogen/phosphorous) in microalgae is 106:16:1 in the biomass, which was called as redfield ratio. Two independent processes have been employed to remove N and P in traditional treatment process. The coupled nitrification and denitrification process converts organic N to N_2 gas, while P is precipitated with salts. Nitrogen and phosphorous from human urine were taken up by the microalgal cell, and by using these nutrients, it generates biomass. The presence of both the nutrients are essential, since microalgae need both nitrogen and phosphorous for its effective growth, and their removal takes place simultaneously. Microalgae can remove or recover the phosphorous completely and can be able to recover a large amount of nitrogen from human urine. Microalgae use nitrogen to synthesize protein, while phosphorous is assimilated in ribosomal RNA. When one of the major nutrients is reduced, the division of cell decelerates due to the decrease in protein content in cells, but carbon procreation through photosynthesis continues. When the supply of nutrient decreases, the carbohydrates or lipid accumulates, thereby decreasing the concentration of biomass. Microalgae have the capability to accumulate nutrients excessively in their biomass. The free nitrogen remains as free ammonia at 1.2 mM and has a negative impact on microalgae growth. Table 5 shows the physical characteristics of microalgae species valorizing urinal wastewater. Table 6 shows the valuable chemical composition of microalgae species valorizing urinal wastewater, and Table 7 shows the elemental composition of microalgae species valorizing urinal wastewater.

Table 5 Physical characteristic of microalgae species valorizing urinal wastewater

Microalgae	Size (μm)	Shape	Taxonomy	References
Chlorella sp.	Ø 2–8	Spherical to slightly oval	Green algae	Bock et al. (2011)
Scenedesmus sp.	5–13 L × 2.3–6 W	Spindle	Green algae	Akgül et al. (2017)
Spirulina sp.	2.8–5.5 Ø; 150–400 L	Filament	Cyanobacteria	Dadheech et al. (2010)

Table 6 Valuable chemical composition of microalgae species valorizing urinal wastewater

Microalgae species	Protein	Carbohydrate	Lipid	References
Chlorella sp.	51–58	12–17	14–22	Hattab and Ghaly (2015)
Scenedesmus sp.	35–40	8–9	15–18	Miranda et al. (2012)
Spirulina sp.	41–56	8–13	3–5	Jena et al. (2011)

Table 7 Elemental composition of microalgae species valorizing urinal wastewater

Microalgae species	C	N	O	H	S	References
Chlorella sp.	46.8	9.7	26.3	6.9	0.5	Ekpo et al. (2016)
Scenedesmus sp.	48.5	7.8	27.1	7.1	0.3	Broch et al. (2013)
Spirulina sp.	48	11.4	33.7	7.0	–	Falco et al. (2012)

Certain microalgae species have the capacity to accumulate phosphorous in the form of polyphosphate granules, when the nutrient supply is high. It absorbs about 3% phosphorous in the biomass. *Chlorella* has the biomass nutrient concentration ranging between 5.0 and 10.1% for N and 0.5 and 1.3% for P and in *Scenedesmus* 2.9–8.4% for N and 0.5–1.7% for P. The microalgae, which show efficient growth and maximum recovery of nutrients in human urine, are *Chlorella vulgaris*, *Spirulina platensis*, *Chlorella sorokiniana*, *Scenedesmus acuminatus*, and *Scenedesmus obtusiusculus.*

4.1 Chlorella vulgaris

It is an emerging strain, which holds extensive application in the field of biofuel production. Its rapid growth is the major reason for its predominance in most wastewaters. It survives in all climatic conditions. When urea was given as a nitrogen source, higher biomass yield was observed. There is no significant growth in ammonium since growth ceases due to high pH. Jaatinen et al. (2016) have cultivated *Chlorella vulgaris* in batch mode with five different dilution ranges from 1:25 to 1:300, and their results revealed that the biomass yield is higher in 1:100-diluted urine supplemented trace elements. Likewise similar biomass yield of 0.52 and 0.48 g VSS L^{-1} was obtained for the dilution ratio of 1:25 and 1:300, respectively. Ge et al. (2018) showed that through biomass absorption, it is possible to remove both nitrogen and phosphorus (>99% for both total nitrogen (TN) and PO_4^{3-}-P) completely.

4.2 Spirulina platensis

Spirulina platensis is also known as *Arthrospira platensis* and has high concentrations of coloring matter, lipids, and proteins and can reproduce within its cells. It is rich in nutrition and can be substituted for conventional food. It has a slender cell wall and shows higher metabolic rate. It not only consumes P and N in human urine but also consumes Cl, K, and S. The uptake of nutrients can be accelerated under mixotrophic conditions using molasses since it is rich in readily biodegradable organic matter. Yang et al. (2008) have obtained a nitrogen recovery of about 99% and a phosphorus recovery greater than 99.9%, with 1.05 g biomass and 12.5 mL human urine. *It can also absorb* N, Cl, K, and S, and its absorption could reach up to 99.9%, 75.0%, 83.7%, and 96.0%, respectively. Chang et al. (2013) have showed the removal of nutrient from urine by microalgae under autotrophic conditions. It is responsible for 97% of NH_4-N, 96.5% of total phosphorus (TP), and 85–98% of urea removal.

4.3 Chlorella sorokiniana

It is known as the king of microalgae. Under mixotrophic conditions, it attains maximum growth; it can tolerate high temperatures and can be suitable for outdoor cultivation. Tuantet et al. (2014a) have reported that concentrated urine can be used as a culture media to achieve a growth rate of 0.104 per hour. Mbir and Mensah (2017) showed that *Chlorella sorokiniana* has the capacity to remove 63.2% of total nitrogen and 55.8% of phosphorous at lower C/N/P ratio of 8:5:1. Zhang et al. (2014) stated that *Chlorella sorokiniana* can increase its growth from 0.44 to 0.96 g/L using 62.64 mg/L of nitrogen and 10.64 mg/L of phosphorous, when urine was supplemented. Using *Chlorella sorokiniana*, they have achieved nitrogen and phosphorous recovery of 80.4% and 96.6%.

4.4 Scenedesmus acuminatus

It is the green algae which grows in wastewater and is the dominant species in primary ponds. It is best suited because of its high growth potential and because it is easy to handle. It can be able to grow in all forms of nitrogen, but growth is restricted when its concentration is high. It promotes the survival of zooplankton by reducing the toxic nature of urine. It is a promising strain which is rich in lipids and can generate biodiesel. Adamsson (2000) stated that the green algae *Scenedesmus acuminatus* along with the *Daphnia magna* survives and reproduces efficiently in 0.5% urine solution. About 67% of N and 36% of P were removed at the end of the first phase. Similarly 98% of N and 97% of P removal were attained at the end of the second phase.

4.5 Scenedesmus obtusiusculus

It can be able to remove COD, nitrogen, and phosphorous in wastewater. The efficiency of microalgae biomass depends on the following factors: temperature, pH, and the concentration of nutrients. Mbir and Mensah (2017) have showed that *Scenedesmus obtusiusculus* can remove 27.1% of TN, 99.7% of NH_4^+ (ammonium), 88.6% of TP, and 89.3% of orthophosphate from the urine. The bacterial population was dominated by large amounts of microalgae and removes about 90% of total nitrogen and phosphorus. The bacteria present in urine show some bad effects on microalgae present in the waste, and it inhibits the growth of microalgae; however it can be overcome by controlled wastewater flow.

5 Immobilized Microalgae for Nutrient Removal

Microalgae are the microorganisms which are present freely in the wastewater and are the major solar energy converter. Microalgae can generate secondary metabolites as by-products while treating the wastewater and improve treatment efficiency. The wastewaters are usually rich in nutrients, heavy metals, and some foreign chemical substances. It can be a suitable culture media for the effective microalgae biomass yield. Harvesting of biomass is the major challenge since it is very difficult to process. Several methods have been tried out for harvesting microalgae. Among them is low-frequency ultra-sonication which is gaining attention, where separation takes place by agglomeration followed by settling. Dissolved air flotation was also used frequently to harvest microalgae, where separation happens through compressed microbubbles. These methods have limitations and are not cost-effective. To conquer the harvesting issues, immobilization techniques have been adopted, and the high-valued biomass was obtained. Immobilization is the aggregation of microalgae over the surface or inside the particles (Fierro et al. 2008; Jia et al. 2011). In other words immobilization of cell is the restriction of movement of the living cell naturally or unnaturally from its initial state to other places in a liquid phase (Eroglu et al. 2012). To improve the efficiency of immobilized microalgal cells, there are some basic requirements: viability retention, capability to photosynthesize, high cell density, and high biomass yield. Microalgal immobilization aims at keeping the cells inside a gel mold where the biological process takes place with restricted movement. The advantages of immobilization methods are:

- Accumulates higher biomass and can be used as derivatives.
- Filtering of wastewater is not needed and can be used as raw wastewater.
- Combat the toxic matters in wastewater which is treated.
- Microorganisms more than one can be easily immobilized.
- Easy to handle by nonprofessionals.
- The treatment was made easier by keeping the cells to live for a long period inside the matrix.

5.1 Microalgal Immobilization Techniques

There are two types of immobilization techniques: inactive immobilization and active immobilization. In inactive immobilization, microalgae biomass was attached over the surface of the natural or synthetic supporting material through adsorption process. In active immobilization, microalgae biomass was entrapped or encapsulated using various agents such as flocculants and chemical and gel media. Table 8 lists efficient microalgae species used for active immobilization.

Inactive Immobilization

Microalgae have the capability to get attached and grow on the surface (Robinson et al. 1986). These processes are easily reversible. The major transporters of passive or inactive immobilization may be natural and synthetic. Loofah sponges have been

Table 8 Efficient microalgae species for nutrient removal

Microalgae sp.	Culture media	Biomass yield	Mode of cultivation	Nutrient removal	References
Chlorella vulgaris	Wastewater	1.89 ± 0.07 g/L	Photo-bioreactor reactor	99.0% TN ~100% TP	Ge et al. (2017)
	Urinal wastewater	0.73 gVSS L^{-1}	Continuous reactor	>99% of TP 92% of TN	Jaatinen et al. (2016)
Spirulina platensis	Urinal wastewater	1.75 g/L	Photo-bioreactor reactor	97% of TN 96.5% of TP	Chang et al. (2013)
Chlorella sorokiniana	Urinal wastewater	0.96 g/L	Sequencing batch reactor	93.0% of TN 97.5% of TP	Zhang et al. (2014)
	Urinal wastewater	0.8 g-dw L^{-1} h^{-1}	Batch reactor	70% of TN >99% of TP	Tuantet et al. (2014b)
	Urinal wastewater		Erlenmeyer flask	63.2% of TN 55.8% of TP	Mbir and Mensah (2017)
Scenedesmus acuminatus	Urinal wastewater	39 ± 17 mg dw L^{-1} min^{-1}	Batch reactor	97.7% of TN 96.6% of TP	Adamsson (2000)
Scenedesmus obtusiusculus	Urinal wastewater		Erlenmeyer flask	45.9% of TN 76.3% of TP	Mbir and Mensah (2017)

adopted as a natural transporter. Loofah sponges are fruiting bodies of the plant genus *Luffa*. The pericarp tissues are removed, and the sponge is derived from dried fruits. This transporter has the characteristics of being nonpoisonous, insensitive, inexpensive, and mechanically stable for a long time (Liu et al. 1998). *Chlorella sorokiniana* was effectively immobilized to enhance the removal of nickel (II) from the solution using cost-effective loofah sponge by Akhtar et al. (2004). The accumulation of 25% of nickel was obtained when it was exposed for 20 min. Ogbonna et al. (1996) increased the accumulation process of cells on the loofah sponges by using chitosan.

In inactive method, synthetic materials are widely used. Urrutia et al. (1995) have investigated the effect of nitrate removal using immobilized *Scenedesmus obliquus* cells in polyvinyl and polyurethane and compared the survival of surface-assimilated cells with entangled cells by blending the concentrated cells with one of the polymers. Huang and Wang (2003) showed that the immobilized microalgae *Chlorella pyrenoidosa* in polyvinyl acetate (PVA) lead to the removal of nitrate and phosphate. In addition, it multiplied fastly within PVA gel matrix at varying pH in the range of 5–10. At pH 7, the removal of nitrate was 80% and for phosphate was 88%. Travieso et al. (1999) immobilized *Chlorella vulgaris* and *Scenedesmus acutus* in polyurethane foam for the removal of chromium. Canizares et al. (1993) achieved 90% ammonium removal efficiency with *Spirulina maxima* immobilized in polymers.

Active Immobilization

Active immobilization was carried out using the following agents, namely, flocculants and chemical and gel entrapment. Details of these agents are explained below.

Flocculant Agents

In order to avoid complicated centrifugation, flocculant agents were used to immobilize algae. Chitosan is one of the flocculant agents, which is mostly used. Chitosan is a narrow amino biomolecules of β-D-glucosamine (2-amino-2-deoxy-β-D-glucan) units connected by one to four linkages (Oungbho and Müller 1997). It is achieved by removing the acetyl group in chitin in alkaline medium. Cell walls of the microalgae contain polysaccharides and are known for their compatibility with the outer surface of chitosan. In addition, microalgae surface was enriched with negative charge, and it favors electrostatic attraction toward positively charged amine group of chitosan (Lubian 1989). Due to its biodegradable nature, it can be used for nutrient supply. The major disadvantage of chitosan in this method is its less strength (Gualtieri et al. 1988). Moreira et al. (2006) found low growth rates of immobilized *Phaeodactylum tricornutum* in alginate while adding chitosan as a hardener. They also found stunted growth of *Phaeodactylum tricornutum* while using $CaCl_2$ or $SrCl_2$ as a hardener. *Scenedesmus* sp. immobilized in chitosan alone achieved higher growth rate and removed 70% nitrate and 94% phosphate in half a day when it is maintained in a normal temperature.

Chemical Attachment

Ion attraction method is an effective method, which depends on the pH and ionic strength of the enclosing area, and it does not have any negative effect on living organism (Codd 1987). Due to the chemical intervention, the cellular surface gets damaged, and the life of the cells reduces when the cells are immobilized. Seki and Suzuki (2002) removed inert microalgae which absorb Cd and Pb in liquid medium by using bio-absorbents. *Heterosigma akashiwo* is an unlimited and alluring microalga because it creates red tides, and so it is attracted by the bio-absorbents. Bio-absorbents are usually formed by using two materials, namely, milk casein and glutaraldehyde.

Gel Entrapment

Algal immobilization is mostly achieved by gel entrapment. It was done by two methods, namely, natural and synthetic polymers (Codd 1987).

Synthetic polymers

The pores of the synthetic polymers are smaller than the size of microorganism. Once microalgae are entrapped inside these polymers, only fluid can pass through the pores, and the biological process continues displaying higher growth (Cohen 2001). The free-celled microbes are combined with the polymers and solidified to form a polymeric matrix. The biopolymer which is formed by connecting the compound to each other is then combined with the living organisms which make the microbes inactive. Polymerization can be achieved by physical and chemical treatments. Hardening is obtained by connecting the monomers with multivalent cations. The heat and the chemical reactions can affect the hardening process (Cohen 2001). The strength of the polymer is increased with increase in monomer concentration and combining medium. The syringes can be used to make the beads of spherical shape by dropping the polymeric algal mixture. A specific instrument has been designed for this purpose. The beads can be used as such, or it can be multiplied in a medium to increase the growth of microorganism inside the polymers De-Bashan et al. (2002). When the biomass is dead, the beads are dried and are used in agriculture as inoculants (De-Bashan et al. 2002).

Natural Polysaccharides

It is one of the widely used methods for algal entrapment. The natural polysaccharides, which are most commonly used, are carrageenan, agar, and alginate. Carrageenan is rich in carbohydrate derived from the red algae by alkaline extraction method. It consists of alternating 3-linked-β-D-galactopyranose and 4-linked-α-D-galactopiranose units. In the presence of cationic compounds, carrageenan is converted into a gel (Tosa et al. 1979).

Agar is extracted from the species of red algae and has sulfated galactan. It is a thermally reversible gel. The component which induces the agar to form as a gel is a linear chain of (1–3)-linked-β-D-galactopyranosyl units and (1–4)-linked-3,6-anhydro-α-D-galactopyranosyl units (Burdin and Bird 1994).

Alginate is the mostly used gel to absorb the microalgae. It belongs to a family of nonlinear copolymers of 1–4-linked-β-D-mannuronic acid and α-L-guluronic acid in varying quantities and supports the function of the cells and tissues (Smidsrød and Skja 1990). Brown algae belong to the genus *Laminaria* (*L. hyperborea*, *L. digitata*, *L. japonica*) and the genus *Sargassum* which were exploited for the production of alginate. All the brown algae have alginate in varying proportions and its dry weight is about 40% (Ertesvåg and Valla 1998). One of the major advantages of using alginate for algal entrapment was that its physicochemical properties do not intervene the activity of immobilized cell. It is highly permeable in nature, nontoxic, and easy to handle. These properties of the alginate provide a favorable environment to the cells which are immobilized (Ushani et al. 2017a, 2018).

5.2 *Immobilized Microalgal Cells for Urinary Wastewater Treatment*

The immobilized microalgal technique is vastly employed to treat the urinal wastewater containing high amount of nutrients. The most common species employed to remove the nutrients from the urinal wastewater are *Chlorella*, *Scenedesmus*, and *Spirulina*. The major limitations of using free cells for treatment of urinal wastewaters are cost of free microalgal cells and energy demand for harvesting cells from wastewater. In order to overcome this problem, immobilization techniques are employed. Immobilized cells can valorize inorganic nutrients such as P and N to the greater extent than free cells.

In a study conducted by Jia et al. (2011), they compared the nutrient removal efficiencies of free and immobilized microalgae, namely, *Scenedesmus obliquus* and *Chlorella vulgaris*, for treating urine. The growth was appreciably good in both cells. The immobilized cells consumed nitrogen in the rate of 9.6–53.2% per cell in *S. obliquus* and *C. vulgaris* by 14.0–28.1% per cell. In addition immobilized cells have high tolerance level against ammonia concentration. When the ammonia concentration increases from 25 to 100 mg N/L, the absorption of nitrogen declined to 34% and 36% for *S. obliquus* and *C. vulgaris*, where as it was 16% and 20% in immobilized cells.

C. vulgaris immobilized in alginate beads yielded higher rate of nutrient removal compared to the immobilization by polyurethane foam (Travieso et al. 1996). Similarly Jimenez-Perez et al. (2004) have immobilized *Scenedesmus acutus* and *Scenedesmus obliquus* cells in kappa-carrageenan beads for removing nutrients efficiently. About 90% ammonium removal was achieved within the first 4 h, and only trace amount of phosphate was removed. De-Bashan et al. (2002) co-immobilized *C. vulgaris* with plant growth-enhancing bacterium *Azospirillum brasilense* in alginate beads which yielded higher ammonium and phosphate removal as compared to immobilized *C. vulgaris* cells without *Azospirillum*. The immobilized microalgae can also be cultivated using porous substrate bioreactor in urinal wastewater because of its high production, low water content, and good gas exchange properties. The percentage recovery of nitrogen and phosphorous from urine using this bioreactor was 87.1% and 87.5%, respectively (Piltz and Melkonian 2018).

6 Microalgae Hydrolysis for Biofuel Production

Microalgae biomass has gained more attention in the last two decades for biofuel generation due to its high photosynthetic activity and capability of lipid accumulation. Moreover it can easily grow in saline, brackish urine and wastewater depending on their nutrient composition for high biomass yield (Park et al. 2011). Various types of biofuel can be processed using microalgae, for example, biomethane via anaerobic digestion (Kavitha et al. 2017b), biohydrogen via fermentation (Sharma and Arya 2017), bioethanol via saccharification (Martín Juárez et al. 2016), and biodiesel via transesterification (Hena et al. 2015). Figure 3 illustrates the pathway of microalgae biomass to biofuel.

Hydrolysis is the process of breaking complex substrate into simple digestible matter, which will result in improving the biofuel yield. This process is otherwise known as pretreatment or disintegration of biomass. Hydrolysis is the rate-limiting step for converting the complex substrate into different types of biofuel under favorable anaerobic condition. Pretreatment of biomass was considered an essential factor for enhancing the efficiency of biofuel production. Pretreatment process was classified into four major groups such as physical, chemical, mechanical, and biological. Table 9 shows the effect of various pretreatments on microalgae biomass for biofuel production. Figure 4 shows various types of pretreatment adopted for microalgae biomass. In the following subdivision, pretreatment of microalgae was discussed only for specific species of microalgae that are capable of growing in urine.

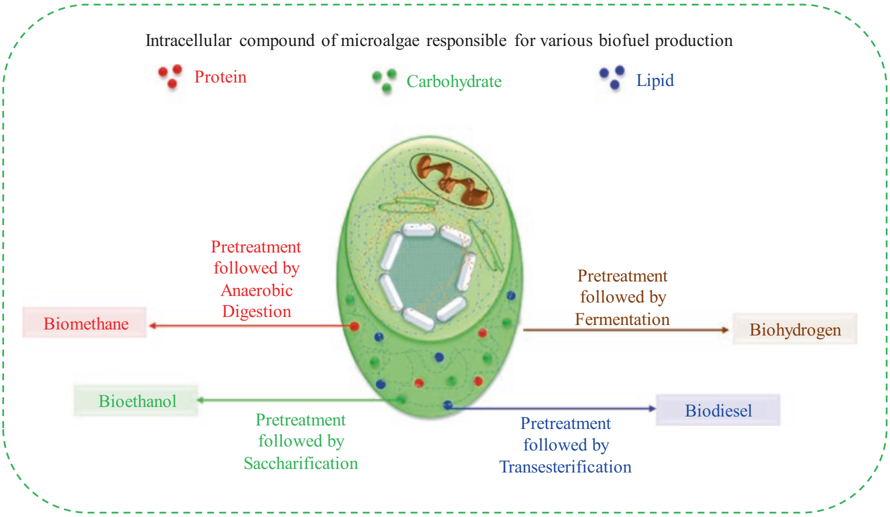

Fig. 3 Pathway of microalgae biomass to biofuel

6.1 Physical Pretreatment

Physical pretreatment is the method of using physical agents to break down the complex structure of microalgae cell wall (Hernández et al. 2015). The physical pretreatment extends the surface area of microalgae biomass for effective biodegradation. Physical pretreatment is future classified into two groups such as thermal (Passos and Ferrer 2014) and microwave pretreatments (Passos et al. 2013).

Thermal pretreatment is the process of applying conventional heat to substrate for breaking the chemical bond of the cell matrix, which results in enhancing the cell compound solubilization and increasing the solubilization of intracellular compound in the aqueous solution (Kavitha et al. 2015). Thermal pretreatment was referred as heat treatment (Raj et al. 2013), and it was future classified into two groups such as low and high thermal pretreatments. Low thermal pretreatment refers to the temperature below 100 °C (González-Fernández et al. 2012), and likewise high thermal pretreatment refers to temperature above 100 °C (Ometto et al. 2014). The efficiency of thermal pretreatment depends on biomass concentration, treatment time, physicochemical nature, and temperature of the substrate. Mendez et al. (2013) have applied high thermal pretreatment of 120 °C for 40 min on *Chlorella vulgaris* to yield a biomethane of 267.7 mL CH_4/g COD. Similarly Ometto et al. (2014) have carried out thermal pretreatment on microalgae species, namely, *Chlorella* sp., *Scenedesmus* sp., and *Spirulina* sp. They have applied 165 °C for 30 min of high thermal pretreatment on the above-stated species and yield of biomethane around 393, 381, and 250, 267.7 mL CH_4/g VS, respectively. Yang et al. (2010) have examined high and low thermal pretreatment on *Scenedesmus* sp., for effective biohydrogen production. On seeing the effect of biohydrogen yield under

Table 9 shows the effects various pretreatment on microalgae for biofuel yield

Microalgae species	Pretreatment	Pretreatment condition	Type of biofuel	Biofuel yield	References
Chlorella vulgaris	High thermal	120 °C for 40 min	Biomethane	267.7 mL CH_4/g COD	Mendez et al. (2013)
Scenedesmus sp.	Low thermal	80 °C for 3 h	Biomethane	128 mL CH_4/g COD	González-Fernández et al. (2012)
Chlorella sorokiniana	High thermal	165 °C for 30 min	Biomethane	393 mL CH_4/g VS	Ometto et al. (2014)
Scenedesmus sp.				381 mL CH_4/g VS	
Spirulina sp.				250 mL CH_4/g VS	
Scenedesmus sp.	High thermal	121 °C for 4 h	Biohydrogen	35.38 mL H_2/g VS	Yang et al. (2010)
	Low thermal	100 °C for 8 h		35.58 mL H_2/g VS	
Chlorella vulgaris	Microwave	100 °C, 2450 MHz for 5 min	Biodiesel	10.7 g/ L Lipid content	Lee et al. (2010)
Scenedesmus sp.				11.8 g/ L Lipid content	
Chlorella vulgaris Scenedesmus sp.	Microwave	900 W 2450 MHz for 9 min	Biomethane	307.11 mL CH_4/g VS	Passos et al. (2013)
Chlorella sorokiniana	Microwave	150 W for 40 s 10 min cooling repeated same condition three times	Bioethanol	21 mg/g DW	Hernández et al. (2015)
Scenedesmus sp.				2 mg/g DW	
Scenedesmus sp.	Alkaline	NaOH for 24 h at 27 °C	Biohydrogen	16.89 mL H_2/g VS	Yang et al. (2010)
Chlorella vulgaris	Alkaline	4 M NaOH (pH 10) for 40 min at 120 °C	Biomethane	240 mL CH_4/g COD	Mendez et al. (2013)
	Acid	4 M HCl (pH 2) for 40 min at 120 °C		228 mL CH_4/g COD	
Spirulina sp.	Alkaline	NaOH (pH 11) for 1 h at 150 °C	Biomethane	0.24 m^3 CH_4/kg VS	Samson and Leduy (1983)
	Acid	HCl (pH 3) for 1 h at 150 °C		0.16 m^3 CH_4/kg VS	

(continued)

Table 9 (continued)

Microalgae species	Pretreatment	Pretreatment condition	Type of biofuel	Biofuel yield	References
Scenedesmus sp.	Alkaline	10% NaCl for 48 h	Biodiesel	8.7 g/L Lipid content	Lee et al. (2010)
Chlorella vulgaris				10.8 g/L Lipid content	
Chlorella vulgaris	Acid	1% H_2SO_4 20 min	Bioethanol	11.7 g/L	Ho et al. (2013)
Chlorella sorokiniana	Acid	1% H_2SO_4 at 121 °C for 45 min	Bioethanol	97 mg/g DW	Hernández et al. (2015)
Scenedesmus sp.	Acid	1% H_2SO_4 at 121 °C for 60 min	Bioethanol	88 mg/g DW	
Chlorella vulgaris	Ultrasonication	10 kHz for 5 min	Biodiesel	6.7 g/L Lipid content	Lee et al. (2010)
Scenedesmus sp.				7.2 g/ L Lipid content	
Chlorella sorokiniana	Ultrasonication	100 W, 24 kHz for 8 min	Biomethane	320 mL CH_4/g VS	Ometto et al. (2014)
Scenedesmus sp.				333 mL CH_4/g VS	
Spirulina sp.				203 mL CH_4/g VS	
Algae	High-pressure homogenizer	10,000 rpm, 30 min	Biohydrogen	45.58 mL H_2/g COD	Kumar et al. (2018)
Scenedesmus sp.	Ultrasonication	70% power amplitude for 30 min	Biomethane	153.5 mL CH_4/g VS	González-Fernández et al. (2012)
Algae	High-pressure homogenizer	12,000 rpm, 30 min	Biomethane	0.11 g COD/g COD	Tamilarasan et al. (2017)
Scenedesmus sp.	Ultrasonication	2200 W for 15 min at 40 kHz	Biohydrogen	1.9 mol H_2/mol glucose	Choi et al. (2011)
			Bioethanol	0.257 g ethanol/g biomass	
Chlorella vulgaris	Enzyme	Cellulase 40 °C for 24 h	Biomethane	0.08 g COD/g COD	Kavitha et al. (2017b)

(continued)

Table 9 (continued)

Microalgae species	Pretreatment	Pretreatment condition	Type of biofuel	Biofuel yield	References
Mixed microalgae (Chlorella vulgaris dominant)	Enzyme	Protease, amylase + cellulase 40 °C for 42 h	Biomethane	0.27 g COD/g COD	Kavitha et al. (2017c)
		Protease, amylase 40 °C for 42 h		0.21 g COD/g COD	
		Cellulase 40 °C for 24 h		0.24 g COD/g COD	
Chlorella vulgaris	Enzyme	*Bacillus licheniformis* 37 °C for 60 h	Biomethane	415.6 mL CH_4/g VS	He et al. (2016)
Scenedesmus sp.	Enzyme	Mixed enzyme 50 °C for 24 h cellulase esterase and protease	Biomethane	1669 mL CH_4/g VS	Ometto et al. (2014)
Chlorella sorokiniana				1296 mL CH_4/g VS	
Spirulina sp.				1996 mL CH_4/g VS	
Scenedesmus sp.	Enzyme	Cellulase	Biomethane	1425 mL CH_4/g VS	
Chlorella sorokiniana				1158 mL CH_4/g VS	
Spirulina sp.				1461 mL CH_4/g VS	
Scenedesmus sp.	Enzyme	Esterase and protease	Biomethane	1065 mL CH_4/g VS	
Chlorella sorokiniana				868 mL CH_4/g VS	
Spirulina sp.				1545 mL CH_4/g VS	

high thermal pretreatment (121 °C for 4 h) and low thermal pretreatment (100 °C for 8 h), results were 35.38 mL H_2/g VS and 35.58 mL H_2/g VS, respectively. The outcome of both pretreatments has significant difference in treatment time and energy consumption, but unfortunately the yield has no significant difference. This could be due to the formation of recalcitrant compounds during high thermal pretreatment. González-Fernández et al. (2012) have yield a maximum biomethane of 128 mL CH_4/g COD under low thermal pretreatment with 80 °C for 3 h.

Microwave pretreatment is a well-known technique for improving solubilization (Ebenezer et al. 2015; Kavitha et al. 2016a). Uniform electromagnetic wave was applied to disintegrate the biomass. This results in the release of organics in the medium (Uma Rani et al. 2013; Kavitha et al. 2018). Budarin et al. (2012) have investigated microwave pretreatment for microalgae biomass, to break the strong

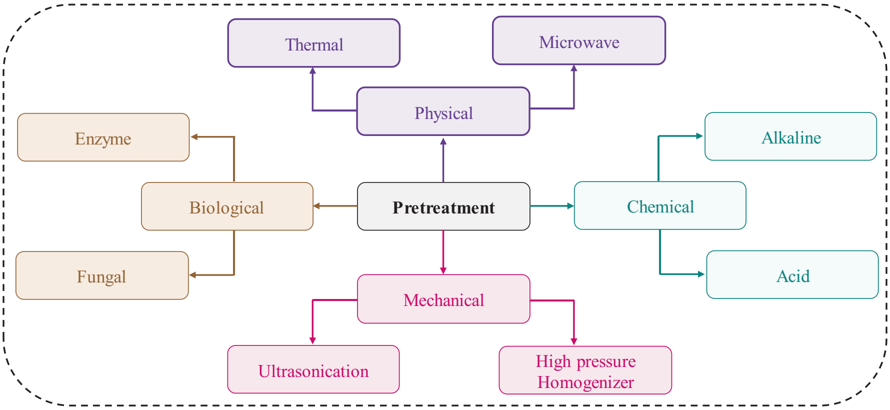

Fig. 4 Various types of pretreatment adopted for microalgae biomass

cellulose cell wall by utilizing low energy for achieving higher solubilization. The efficiency of microwave pretreatment depends on biomass concentration, treatment time, microwave intensity, temperature, irradiation, and physicochemical nature of the substrate. Passos et al. (2013) have suggested microwave pretreatment for microalgae biomass to generate biomethane. A maximum yield of 307.11 mL CH_4/g VS was achieved at optimized condition of 900 W, 2450 MHz for 9 min. Hernández et al. (2015) have investigated the effect of microwave pretreatment for bioethanol production. In this research work, two kinds of microalgae species were selected, *Chlorella sorokiniana* and *Scenedesmus* sp., for pretreatment at optimized power of 150 W for 40 s; the same condition was repeated for three times. The maximum bioethanol yield was achieved as 21 mg/g DW and 2 mg/g DW for *Chlorella sorokiniana* and *Scenedesmus* sp., respectively. Similarly Lee et al. (2010) have followed microwave pretreatment for lipid extraction from the following microalgae species: *Chlorella vulgaris* sp. and *Scenedesmus* sp. The pretreatment was very effective at 100 °C, 2450 MHz for 5 min, and resulted in 10.7 and 11.8 g lipid content/L.

Advantages

- Increase the release of intracellular polymers
- Easy to operate and implement
- Easy for scaling up and easy to commercialize
- Enhance biofuel yield
- Less toxic compound formation

Disadvantages

- High investment cost
- High energy demand
- Skilled labor required for operation and maintenances
- Formation of recalcitrant compounds at harsh condition

6.2 Chemical Pretreatment

In chemical pretreatment, disintegration of biomass was achieved by using strong chemical agents (Banu et al. 2012; Mendez et al. 2013). The chemical pretreatment increases solubilization and improves anaerobic degradation process for biofuel yield (Rani et al. 2012a). In general chemical pretreatment is further classified into two groups based on pH condition: (i) acid pretreatment (below pH 6.5) (Harun and Danquah 2011) and (ii) alkaline pretreatment (above pH 7.5) (Hernández et al. 2015). Both acid and alkaline pretreatments are cost-effective and have less energy demands for achieving higher biomass liquefaction, which improves the efficiency of biofuel production.

In alkaline pretreatment chain linkages between intercellular polymers and complex cell wall were broken into easily biodegradable substances (Kavitha et al. 2014). Several researchers have suggested that the following alkaline chemicals are used for effective pretreatment: sodium hydroxide (NaOH), potassium hydroxide (KOH), and calcium hydroxide ($Ca(OH)_2$) (Rani et al. 2012b; Kavitha et al. 2015). The efficiency of alkaline pretreatment depends on biomass concentration, treatment time, alkaline dosage, temperature, and physicochemical nature of the substrate. Biohydrogen production from *Scenedesmus* sp. was enhanced using strong alkaline sodium hydroxide (NaOH) for 24 h under 27 °C and achieved 16.89 mL H_2/g VS (Yang et al. 2010). Lee et al. (2010) have affirmed that biodiesel production using strong base like sodium chloride (10% of NaCl) with 48 h treatment time was shown to be effective. In this research work, two kinds of microalgae species, *Chlorella vulgaris* sp. and *Scenedesmus* sp., were selected for alkaline pretreatment which results in 10.8 and 8.7 g lipid content/L, respectively. Mendez et al. (2013) have stated that *Chlorella vulgaris* was pretreated using strong alkaline of 4 M NaOH for 40 min under 120 °C for biomethane production. At the optimized alkaline pretreatment condition, the maximum biomethane yield of 240 mL/g COD was achieved. Samson and Leduy (1983) have followed similar alkaline pretreatment (NaOH for 1 h at 150 °C) on *Spirulina* sp., for effective biomethane production, and achieved 0.24 m^3 CH_4/kg VS.

During acid pretreatment, long-chain structures of cellulose and hemicellulose are disintegrated into simple and digestible substances like glucose and other monosaccharides (Hernández et al. 2015). Several researchers have suggested that the following acidic chemicals are used for effective pretreatment: sulfuric, hydrochloric, and nitric acid (Harun and Danquah 2011; Ho et al. 2013). The efficiency of acid pretreatment depends on biomass concentration, treatment time, acid concentration, temperature, and physicochemical nature of the substrate. Mendez et al. (2013) have suggested acid pretreatment (4 M HCl for 40 min at 120 °C) on *Chlorella vulgaris* for biomethane production. In the optimized acid pretreatment condition, a maximum biomethane yield of 228 mL/g COD was achieved. Samson and Leduy (1983) have followed similar acid pretreatment (HCl for 1 h at 150 °C) on *Spirulina* sp., to achieve a maximum biomethane yield of 0.16 m^3 CH_4/kg VS. Ho et al. (2013) have reported the effect of bioethanol yield through acid pretreatment. Carbohydrate-enriched *Chlorella vulgaris* was used as a substrate; the strong acid (sulfuric acid) (1% H_2SO_4) was selected for cell disintegration with effective 20-min treatment time. The maximum bioethanol yield of 11.7 g/L was achieved. Similar acid concentration (1% H_2SO_4) was followed by Hernández et al. (2015) on two kinds of microalgae species (*Chlorella sorokiniana* and *Scenedesmus* sp.), for bioethanol production. However, the maximum bioethanol yield of 97 and 88 mg/g DW was achieved at 121 °C with an effective treatment time of 45 and 60 min.

Advantages

- Low energy demand
- Extent of the biodegradability and nature of the substrate
- Easy to operate and implement
- Easy for scaling up
- Improve biofuel yield

Disadvantages

- High investment and chemical cost
- High formation of inhibitory and toxic substance
- Skilled labor required for operation and maintenances

6.3 Mechanical Pretreatment

It has a dual effect, as it not only reduces particle size but also increases the surface area of biomass (Kavitha et al. 2016b,c). Effective solubilization of organic biomass via mechanical pretreatment demands high energy and cost (Kavitha et al. 2016a). In this pretreatment method, biomass tends to grinding, milling, and chipping using ultrasonication (Ushani et al. 2017b) and high homogenizer pressure (Tamilarasan et al. 2018). The mechanical pretreatment has the following benefits: particle size reduction, increased biomass lysis rate, and enhanced efficiency of biofuel yield.

Ultrasonic pretreatment is the method of propagating sound energy to disintegrate the rigid cell wall of microalgae biomass which improves biomass liquefaction and the process of degradability (Cho et al. 2013). During ultrasonic pretreatment, rigid biomass is broken down into smaller fragment with the help of acoustic cavitation induced by an ultrasonicator (Uma Rani et al. 2014). The acoustic cavitation was more effective at the frequency of 5–40 kHz. The efficiency of ultrasonic pretreatment depends on biomass concentration, treatment time, ultrasound intensity, treatment temperature, and physicochemical nature of the substrate. Increasing the biodiesel production with the help of ultrasonication pretreatment was suggested by Lee et al. (2010) for two types of microalgae species such as *Chlorella vulgaris* sp. and *Scenedesmus* sp. The ultrasonication pretreatment was effective at frequency of 10 kHz for 5 min and resulted in 6.7 and 7.2 g lipid content/L. Choi et al. (2011) have suggested ultrasound pretreatment for biohydrogen and bioethanol production using *Scenedesmus* sp., as a feedstock. Ultrasonication pretreatment was effective at 2200 W for 15 min at 40 kHz. Maximum biohydrogen and bioethanol yield were achieved as 1.9 mol H_2/mol glucose and 0.257 g ethanol/g biomass, respectively. Ometto et al. (2014) have selected three different microalgae biomass (*Chlorella vulgaris*, *Scenedesmus* sp., and *Spirulina* sp.), for the yield of biomethane. The ultrasonic pretreatment shows effective at the optimized power of 100 W, 24 kHz frequency of 8 min treatment time. Maximum biomethane yield was achieved as 320, 333, and 203 mL CH_4/g VS for *Chlorella vulgaris*, *Scenedesmus* sp., and *Spirulina* sp., respectively.

High-pressure homogenizer pretreatment (disperser) is the well-known method of mechanical pretreatment in particle size reduction (Tamilarasan et al. 2017). Homogenizer pretreatment is the process of blending or slicing the substrate with the help of high-pressure and rotational speed (Kumar et al. 2018; Rajesh Banu et al. 2018). The efficiency of high-pressure homogenizer pretreatment depends on biomass concentration, treatment time, pressure, rotational speed, physico-chemical nature, and treatment temperature of the substrate. Kumar et al. (2018) have studied the effect of high-pressure homogenizer for marine algae biomass disintegration. Disperser pretreatment has more advantages in biomass size reduction when compared with other mechanical pretreatment techniques. In the above-mentioned research work, disperser alone shows very effective in 10,000 rpm of rotational speed along with 30 min of treatment time and resulted in maximum biohydrogen yield of 45.58 mL H_2/g COD. Similarly Tamilarasan et al. (2017) have adopted the disperser pretreatment on marine algae for biomethane yield. The pretreatment was effective in solubilizing the algae biomass at 12,000 rpm with 30-min treatment time and extent of anaerobic biodegradability of biomass at 0.11 g COD/g COD. However the disperser pretreatment plays a vital role in enhancing the biofuel yield through biomass size reduction and increased solubilization. These actions favor the anaerobic microbes for effective generation of biofuel.

Advantages

- Reduce particle size and increase surface contact
- Increase solubilization of the substrate
- Improves biofuel yield
- Easy for scaling up
- Less formation of recalcitrant substances

Disadvantages

- High energy demand
- Skilled labor required for operation and maintenances

6.4 Biological Pretreatment

Biological pretreatment is cost-effective (Kavitha et al. 2017a) and has low energy demand for effective hydrolysis of the rigid cell wall of microalgae biomass (Kavitha et al. 2017b). Biological pretreatment is further classified into two: (i) enzyme (Kavitha et al. 2017c) and (ii) fungal (Liu et al. 2015) pretreatments. Hydrolysis of microalgae biomass before processing of biofuel will upsurge the yield to a greater extent. However the biological pretreatment faces problem due to occurrence of contamination during on-site enzyme and fungal production for biomass disintegration. On the other hand, the scaling up of this process is easy, and it demands low energy for effective solubilization. Biological pretreatment has advantage of solubilizing the organic substrate to a greater extent without occurrence of inhibitory compounds.

Kavitha et al. (2017b) have suggested enzymatic pretreatment for effective disintegration of rigid microalgae cell wall. In the abovementioned research work, a familiar microalgae species *Chlorella vulgaris* was chosen as a suitable substrate for biomethane production. The rigid cell wall of *C. vulgaris* contains complex substances like cellulose and hemicellulose. In order to break rigid chemical bond of cellulose between cell walls, cellulose-secreting enzyme was introduced. *Cellulose*-secreting enzyme was effective at 40 °C for 24 h which results in higher cell solubilization and enhances the anaerobic biodegradability of the substrate for effective biomethane yield of 0.08 g COD/g COD. Similarly Kavitha et al. (2017c) have suggested another potential enzyme for solubilizing the biopolymer accounts in the cell wall. In the abovementioned research work, mixed microalgae species was selected in that *Chlorella vulgaris* was found to be dominant. The cell wall of microalgae contains sufficient amount of biopolymers such as protein, carbohydrate, and lipid. These biopolymers have a vital role in the conversion of biofuel production, since they are the major substances found in the microalgae cell wall. In order to hydrolyze target substances, enzyme-secreting (*protease and amylase*) bacterial strains were selected. The researchers have compared the effect of enzyme solubilization in the following order: *cellulose* alone (40 °C for 24 h), *protease and amylase* alone (40 °C for 42 h), and combination of both *cellulose* and *protease and amylase* (40 °C for 42 h). The maximum anaerobic biodegradability of the substrate for biomethane conversion was enhanced to 0.24, 0.21, and 0.27 g COD/g COD.

He et al. (2016) have selected *Chlorella vulgaris* as feedstock for biomethane production, and to increase the anaerobic biodegradability of the substrate, enzymatic pretreatment was followed. *Bacillus licheniformis* was chosen for effective cell disintegration under 37 °C with treatment time of 60 h and achieved maximum biomethane yield of 415.6 mL CH_4/g VS. Ometto et al. (2014) have investigated the effect of enzymatic pretreatment on various microalgae species (*Scenedesmus* sp., *Chlorella sorokiniana*, and *Spirulina* sp.), using three different enzymes. For this pretreatment, *cellulase*, *esterase and protease*, and mixed (combination of both *cellulase* and *esterase and protease)* were chosen; these enzymes were found to be very effective at 50 °C with treatment time of 24 h. The highest biomethane yield of 1296, 1669, and 1996 mL CH_4/g VS for *Chlorella sorokiniana*, *Scenedesmus* sp., and *Spirulina* sp. was achieved after mixed enzyme for disintegration. In contrast, *cellulase* enzyme alone effectively solubilizes the complex cell wall of microalgae and results in maximum biomethane yield of 1158, 1425, and 1461 mL CH_4/g VS for *Chlorella sorokiniana*, *Scenedesmus* sp., and *Spirulina* sp., respectively. Similarly *esterase and protease* enzymes alone degrade the complex cell structure of microalgae biomass which results in maximum biomethane yield of 868, 1065, and 1545 mL CH_4/g VS for *Chlorella sorokiniana*, *Scenedesmus* sp., and *Spirulina* sp., respectively.

Advantages

- Low energy demand
- Easy to operate and implement
- Increases biofuel yield
- High selectivity and easy for scaling up
- No inhibitory compound formation

Disadvantages

- High investment and enzyme cost.
- High contamination occurred during on-site enzyme production.
- Skilled labor required for operation and maintenances.

7 Conclusion

Valorization of nutrient-rich urinal wastewater by microalgae is an emerging technology for cost-effective biofuel production. The effect of nutrient valorization is based on the selection of microalgae species. Microalgae species such as *Chlorella* sp., *Spirulina* sp., and *Scenedesmus* sp. show more effectiveness in nutrient valorization and biomass productivity. Urinal wastewater is a better suitable substrate for microalgae cultivation than other wastewaters, because of its excess nutrient composition. However urinal wastewater contains less amount of biological contaminant which makes a challenging task for microalgae growth. The suggested approach will be more effective in lessening nutrient pollution load, burning of fossil fuel, greenhouse gas emission, and rapid increase in the usage of renewable energy. In future research, the following aspects have to be considered such as techno-economic analysis and the approaches have to be commercialized as user-friendly.

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Comprehensive Overview of Biomethane Production Potential of Algal Biomass Cultivated in Wastewater

Carmen Mateescu and Traian Zaharescu

1 Introduction

Increasing worldwide demand of energy along with the depletion of conventional fossil fuel reserves has grown the concern in the world ability to ensure the energy security, as well as the global interest in developing alternative sources of energy (Murthy 2001). In this context, the valorization of natural resources in terms of biomass and residual biological matter, as well, for producing energy while improving the environment, can contribute to a better solving of the energy and environmental issues. Since the past few decades, algae-derived biogas technologies have become a research and economical area much promising for bringing feasible alternatives to the fast depletion of fossil fuels and oil reserves (Montingelli et al. 2015). According to the potential of algal mass for the generation of biogas (González-Fernández et al. 2011), the energy production via digestion of microalgae has a promising caloric values of output power (Saratale et al. 2018). Various kinds of microalgae as the third generation for biofuels ensure the production of methane based on the biomass cultivation in wastewater (Ansari et al. 2017) or ponds (Fernández et al. 2016). There are many advantages that the third-generation biofuel feedstock, represented by micro- and macroalgae, can offer compared to the feedstock to be used for the first and second generation of biofuels (Montingelli et al. 2015). Amongst the key advantages, it is important to note that microalgae show high growth rate, superior environmental adaptability, high nutrient removal ability, no competition with food or arable land, year-round cultivation, higher lipid productivities and photosynthetic efficiencies compared with other terrestrial plants or microorganisms (Chen et al. 2018). The conversion of microalgae mass into fuel gases is designed as a continuous chain of biochemical and technological operations

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through which the molecular fragmentation occurred in the digester section (Wall et al. 2017; González-González et al. 2018) and a significant methane amount (100–500 L CH_4 g^{-1} VS) is produced (Jankowska et al. 2017). For the near future, the fossil fuel-based electrical supply will be substituted with at least 3.6% by the biogas production EC (2016). There are various algae which generate high amounts of methane (Chen et al. 2018) by anaerobic processing as it is suggested by Fig. 1.

The multistage production of biomethane starts with harvesting microalgae which are separated from cultivation environments by various technologies, centrifugation, flocculation, flotation, filtration, gravity sedimentation or ultrasonication before their anaerobic digestion, each of them presenting specific advantages and disadvantages. (Mohd Udaiyappan et al. 2017). After separation, biomass is converted into biogas (primarily methane and carbon dioxide) through digestion of intermediate compounds (carbohydrates, fatty acids and proteins) (Dobre et al. 2014). This processing technology may be modified, and secondary valuable bioproducts like pharmaceuticals, food supplements and biofertilizers are manufactured. The certain sequence of processes, hydrolysis, acidogenesis, acetogenesis and methanogenesis, are the main steps of biogas production. The type of algae feedstocks can be rigorously selected (Ward et al. 2014) for attaining satisfactory production of bioenergy starting from algal biomass. Many factors such as applied pretreatment, cultivation environment and temperature determine the conversion yields. A detailed analysis on the essential consequences of anaerobic digestion of algal biomass is presented elsewhere (Alzate et al. 2012).

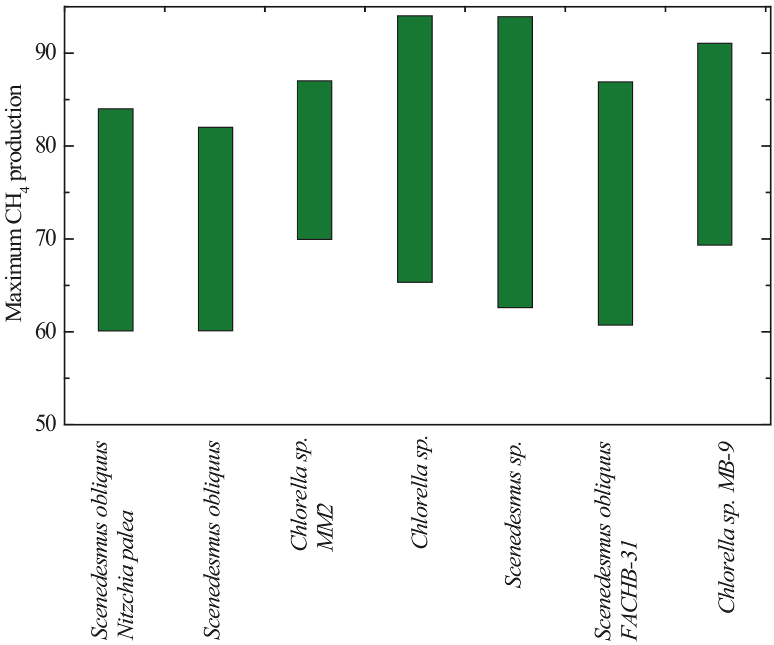

Fig. 1 The maximum production of biomethane by the anaerobic processing of various algae

The foreseen expectations of biomethane production are tightly related on the energetic scenarios and balances which take into account the overall considerations of involved factors: raw algae, energetic consumption, output methane specific volume and quality, variety of presumed bioactive products, size of equipment correlated with the level of biorefinery investment, applied technology and product separation (Murthy 2001; Wu et al. 2016; Maga 2017). The essential factor that influences the production of biomethane is the intracellular accumulation of convertible components, lipids, carbohydrates and proteins. The concentration of nutrients as well as the season harvesting is the external factor that characterizes the viability of biomass conversion (Perazzoli et al. 2016).

2 Valorization of Algal Biomass for Biomethane Production

Conversion of algal biomass into biomethane has become one of the top priorities amongst technologies for energy recovery from natural resources. The technological cascade is based on the foreseen sort of algae cultivated in specific environmental conditions (Raslavičius et al. (2018). The economical restraints have a strong impact on the extension of technologies which comprise a complete chain of production from cultivation and harvesting up to collection and storage of evolved biomethane (Bastiaens et al. 2017).

The microalgal biomethane production represents a conceptual extension of the generation of biofuels and bioproducts (Awais Salman et al. 2017) (Fig. 2).

Several technologies have been proposed for the conversion of microalgal masses into combustible biogas (Wang et al. 2013; Montingelli et al. 2015; Tartakovsky et al. 2015; Awais Salman et al. 2017; Posadas et al. 2017). The generation of energy starting from algae is thoroughly analysed as pathways of feedstock transformation in relation with economical potential, environmental impact and life cycle of biogas production (Fubara et al. 2018). The connection between gasification yields and size of investment is characterized by the foreseen utilization purposes, which are related to the optimized design (Fubara et al. 2013). The evaluation of inlet algal biomass for the digestion and gas conversion can be satisfactorily done by the transformation of slurry solid materials, whose volatile fraction determines the processing efficiency (Kunar Prajapati et al. 2014b). The association between algal biomass and the recycling of nutrients from wastewater streams, as well as the separation of carbon dioxide from the biogas stream, represents the general overview on the efficiency of methanization according to the "closed-loop process" practice (Tartakovsky et al. 2013; Tran et al. 2014; Kunar Prajapati et al. 2014a, b). The growth way of microalgae (photoautotrophic, heterotrophic, mixotrophic, photoheterotrophic) may significantly influence the ratios of the main processing products (Chojnacka, Marquez-Rocha 2004) because the light amount absorbed by the cultivated algae turns the distribution of carbon onto specific composition.

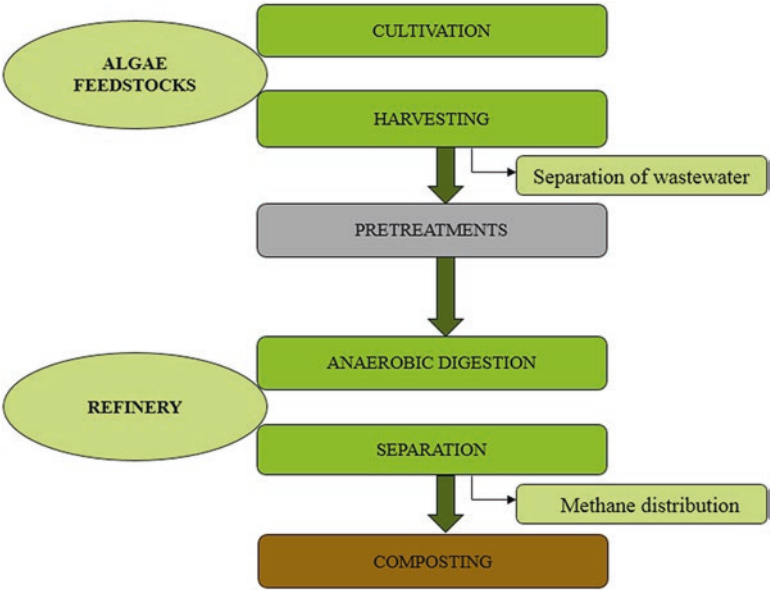

Fig. 2 General flowsheet for the production of biomethane from algae grown in wastewater

The specific methane yield is one of the main features that characterizes the production of volatile solids associated with the applied pretreatment in the technological flowsheet (Park and Li 2012). An advantageous situation where two or more substrates have a combined synergistic effect on the conversion represents a better solution for attaining good C/N ratios for certain processing route (Yen and Brune 2007).

In the pretreatment stage of algal biomass as an initial step of microalgae methanization, a combination between sludge inoculation and mechanical conditioning of feedstock by Hollander beater contributes to the enhancement of methane generation by the increasing of feedstock/inoculation (F/I) ratio and the beating periods (Rodriguez et al. 2018). The smaller F/I ratios and longer beating time length, the higher methane production (Fig. 3).

Another efficient pretreatment which improves the biomethane from algal digestion is the addition of enzymes into the reactor feeding (Bohutskyi and Bouwer 2013; Córdova et al. 2018). Their first effect is the degradation of cell wells, followed by the conversion of intermediates into 55–70% methane. For various types of algae, other secondary products containing nitrogen, sulphur and organic compounds are generated (Wang et al. 2017). For the mechanical pretreatment of algae, other shredders such as ball mill, knife mill and hammer mill could also be used (Kratky and Jirout 2011). In the sense of synergism, the simultaneous thermal and enzymatic treatments provide a significant increase of biomethane production from processing of algae.

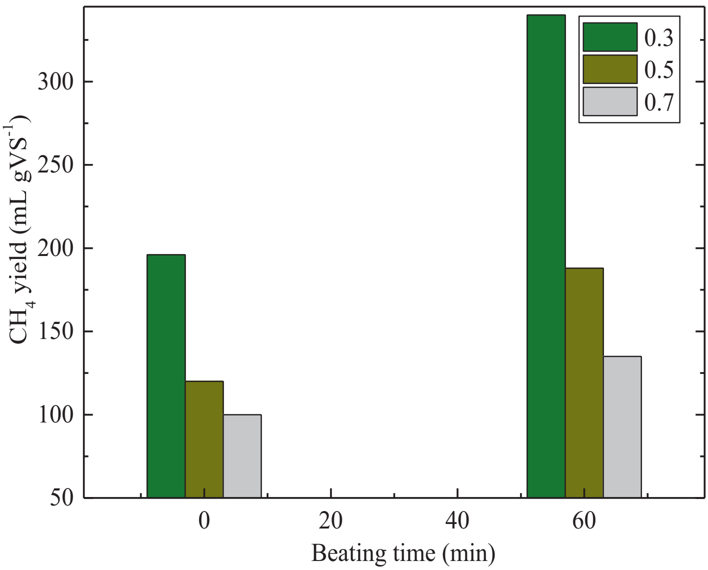

Fig. 3 Amounts of biomethane produced by inoculated algae at various feedstock/inoculation ratios

The thermal treatment applied to algal biomass grown in wastewater environment showed that the increased processing temperature (30 min at 160 °C) produces higher gas amounts (Rodriguez et al. 2015). The heating at 30 °C and 60 °C during anaerobic digestion enhances effectively the biomethane yield. The maximum biomethane amount (0.35 m^3 CH_4 kg VS^{-1}) was obtained for an inlet raw material of 2.49 kg VS m^{-3} d^{-1} when the leaded sludge was pretreated at 60 °C and (or to) 160 °C. If the preheating temperature becomes 180 °C, the methane yields drop down suggesting that a partial decomposition of intermediates takes place.

Various enzymes (β-glucanase, xylanase, cellulose or hemicellulose) are used for the increase of transformation yields of algal biomass into biomethane (Mahdy et al. 2014b; Wirth et al. 2018). For example, the involvement of protease in the anaerobic digestion of *Scenedesmus obliquus* and *Chlorella vulgaris* enhances the produced CH_4 amounts of 1.72-fold and 1.53-fold.

The sludge of wastewater-cultivated algae comprises the volatile solids that are subjected to degradation, they being the source of biomethane. The thermophilic anaerobic degradation in the continuous flux with thermal treatment at 160 °C represents the key of biomethane production, which allows the degradation process at superior levels exceeding 50% (Han et al. 2017). For the conversion of 100 TS ton/d, the process efficiency based on thermal treatment simultaneously conjugated with thermophilic anaerobic transformation 0.27 m^3 CH_4/kg VS or 8560 kcal m^{-3} CH_4 (32% conversion for electrical purposes) is obtained (Han et al. 2017).

An interesting and efficient solution for the methanization of *Spirulina platensis* is the mixture of basic material with enzymatically shifted switchgrass

(El-Mashad 2015). This formulation doubles the biomethane estimated in caloric equivalent (6774 MJ $tonne^{-1}$ for modified material instead of 3904 MJ $tonne^{-1}$ for untreated raw switchgrass).

An overview on the effects of thermal treatment coupled with hydrothermal operations applied to algal biomass for the improvement of effectiveness of processing shows the modification range of biomethanization for a large sorts of wastewater-grown algae (Passos et al. 2014a, d). As the consequence of applied pretreatments, the soluble volatile solids increase from 33.2 mg L^{-1} for algal mass control up to 50.4 mg L^{-1} for thermally pretreated materials, 105.9 mg L^{-1} for cellulase pretreatment and 114.0 mg L^{-1} for enzyme mix (cellulase/glucohydrolase/xylanase) addition (Passos et al. 2014b). Due to the advantageous contribution of enzymes on the conversion of biomass into oxygen-containing products followed to the methanization step, the digestive pretreatments based on this kind of transformation factors are seldom selected (Mahdy et al. 2014a; He et al. 2016; Kavitha et al. 2017).

The microalgal mass subjected to anaerobic digestion for production of biomethane passes through different processing stages that lead to the accumulation of final products relative to the foreseen purpose. The processing flowsheet must be adapted to the treatment strategies that are adapted to the yield level (Collet et al. 2011).

The co-digestion is other alternative for the increase of evolved amounts of methane. In this sense the applied pretreatments play the role of vector that directs the processing mechanism onto the accumulation of desired product in reactors. The multistage procedures operate in the efficient biogas genesis through which biomethane can be collected as the main product. The co-digestion allows the significant yield enhancement of 25–400% relative to the mono-digestion of the same separate substrates (Ali Shah et al. 2015).

The use of algal biomass co-digested together with hay and maize when the production of biomethane increases with more than 50% in respect with the non-combined formulations is a good practice for the generation of gaseous combustible (Dębowski et al. 2013). The highest values of conversion by the fermentation of these combinations are 373 and 386.8 m^3 CH_4 of dry matter under static conditions and in the experiments achieved in flow reactors (Dębowski et al. 2012).

The valorization of algal biomasses for energetic purposes, the production of biomethane and biodiesel, supposes a certain sequence of operations through which organic components are fragmented and converted into gaseous final products (Ward et al. 2014). The basic stage is the concentrating operation when waste components are removed. The concentration level determines the size of equipment whose dimensions influence the efficiency of processing and energetic requirements. The next stage where the raw components are fragmented draws a primary image on the conversion yields. The mode and amplitude of undergone disruption, as well as the biomass anaerobic digestion, will determine the technological efficiency.

Based on the elemental analysis of the algal feedstock, the theoretical biomethane production can be estimated. In practice, the biomethane production is lower than the value obtained by theoretical calculation since the organic material is degraded in a proportion that is lower than 100%.

Type of algae and quality of the crop feedstock are fundamental aspects to be considered for predicting the energy benefits in relation to costs prior to the technological development stage.

3 Overview Analysis on Digestion Parameters

Microalgal biomass is a useful energetic resource that is cultivated with a great efficiency in wastewaters containing various nutrients (Pittman et al. 2011). The feedstock having growing features is essentially characterized by the conversion level of organic mass into biomethane during processing. The cultivation and harvesting are two main primary stages which influence the proportion of generated biomethane in respect with other by-products. The double purpose of involving wastewater in the growing of microalgae is the remediation of industrial-derived fluids accompanied by the proper rising of raw biomasses. The most efficient systems in which microalgae can be obtained are algal ponds (Park et al. 2011), where the feeding with wastewater allows the optimization of nutrient compositions.

The cultivation of algal mass for the production of biomethane is a proper option for obtaining of high-quality feedstock. General practice choices for this initial stage of methane generation include open ponds and photobioreactors (Harun et al. 2010). Compared to open ponds, photobioreactors have several advantages in terms of controlling nutrients type and concentration, as well as processing parameters such as temperature, amount of dissolved carbon dioxide and contaminants, pH, yield of converted solid biomass. Unfortunately, some disadvantages, amongst which the most relevant are the high investment expenses and the specificity of microalgae nature, are responsible for the limitation of methane yield.

Several ways through which microalgae are transformed into biomethane are illustrated in Fig. 4. The conditions for growing algae are rather different from one scientific report to another (Harun et al. 2010; Fistarol et al. 2012, Cortés-Carmona et al. 2018; Mohammadi et al. 2018).

Consequently, it is difficult to conclude about some general issues of algae cultivation strategies. However, the third-generation biofuels are well considered and promoted due to their high production yields of biomethane. An average production of 0.2–0.5 m^3 CH_4/kg VS is satisfactory in terms of energy balance.

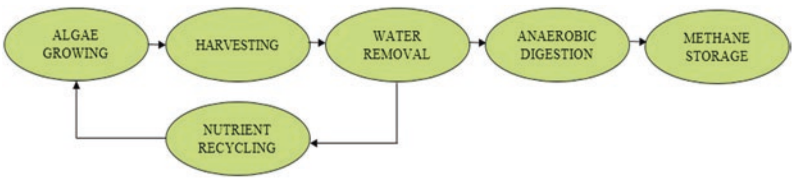

Fig. 4 Processing routes for the conversion of algal mass into biomethane

Table 1 Development of algal mass under various conditions

	Optical density (a. u.)			
Cultivation period (d)	0 mL nutrient	1 mL nutrient	5 mL nutrient	10 mL nutrient
0	0	0	0	0
2	0	0.001	0.025	0.1
6	0.0001	0.07	0.17	0.33
8	0	0.13	0.15	0.31
11	0	0.17	0.17	0.34
13	0	0.18	0.25	0.33

A detailed overview on the effects of algal biomass cultivation and growing conditions for biomethane production has been reported in relation to the gas yield efficiency (Rawat et al. 2016). The essential role of nutrients is emphasized by the increase in the algal biomass content established by the spectroscopic investigation (Odlare et al. 2011). In Table 1 the optical densities (OD) of investigated biomass are listed.

The significance of wastewater cultivation conditions defines the terms under which algal biomass is grown for an improved biomethane production (Monfet and Unc 2017), where the CH_4/CO_2 ratio should exceed the unit value. In fact, the production of methane is kinetically controlled by the distribution of nutrients, which influence the accumulation of methane-generating components (Hoang Nhat et al. 2018).

The effectiveness of biogas production in the technologies based on the conversion of algal biomass is evaluated by the conversion mechanisms (Dębowski et al. 2013), and it is strongly affected by the digestion process, a challenging issue being represented by the high resistance of cell walls. In fact, the cultivation systems and conditions make possible the advances and limitations involved in the biogenesis of methane, where the contribution of environmental aspects is decisive (Jankowska et al. 2017; Wayne Chew et al. 2018).

In the technological analysis of biogas production from algal biomass, the gas productivity shown by the feedstock species producing methane is often defined by the organic loading rate (OLR) whose level characterizes the limits through which the conversion is placed (Montingelli et al. 2015). The correspondence between OLR and evolved methane amounts is strongly dependent on the type of algae. In addition, the degradation of lignocellulose biomass during pretreatments has a major contribution on the improvement of CH_4 final quantities (Montingelli et al. 2015).

There is a great difference between the behaviour of macroalgae and microalgae related to the proportion of methane produced during the algae digestion because the cellulose loading slowing down digestion rate is quite unlike.

The economic analysis showed that an improved efficiency of biomethane production from algal biomass digestion could be achieved before the operation of lipid extraction (Capson-Tojo et al. 2017). The sampling and monitoring of the generated biogas during the algae digestion processes indicated different values of biomethane composition for thermophilic and mesophilic temperatures as shown in Fig. 5.

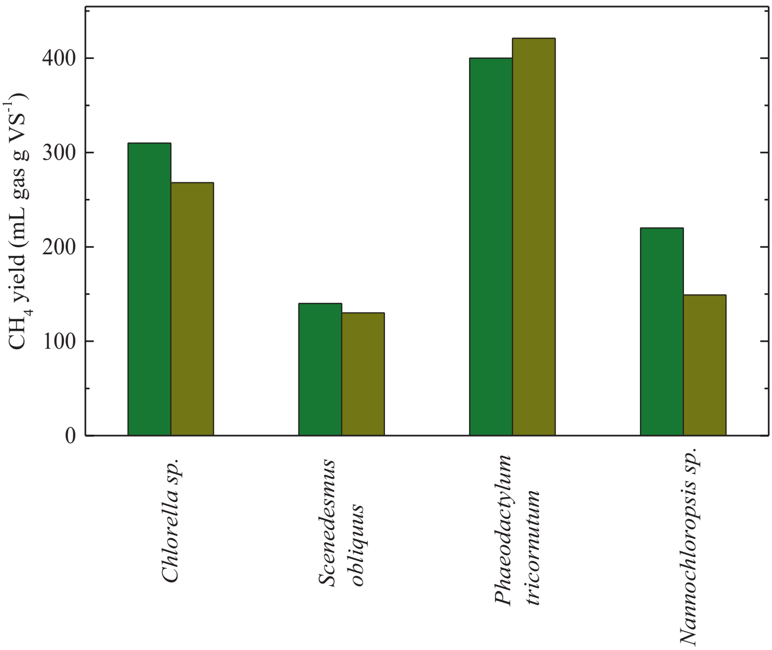

Fig. 5 The biomethane yields from two different anaerobic digestion procedures: in green, thermophilic method (55 °C); in olive, mesophilic method (35 °C)

A recommendable solution of algae pretreatment that can be applied for the increase in the biomethane production is heating the algal suspensions for various periods. The increased of temperature from ambient value up to 90 °C produces 75.3% CH_4 for 65.1 g VS/L algae suspension, while the experiment run at room temperature produces 73.9% CH_4 for 65.0 g VS/L algae suspension on the same processing time (4 hours) (Marsolek et al. 2014; González-Fernández et al. 2013).

The association of two types of pretreatments, heat and microwave, is a versatile option for the increase in the biomethane generation from microalgal biomass (Passos et al. 2014a, d). The synergic activity of the two applied treatments explains the collaboration between microwave exposure providing cell wall disruption and increased thermal agitation which determines a favourable probability of reactions (Passos et al. 2014c).

The linearity of this dependency and similar conclusion on the correlation between biomass solubilization and received energy (Passos et al. 2014a) highlights the proper reason for the application of pretreated algal biomass for energetic purposes (Fig. 6).

Different methods of pretreatment associated with the modification of anaerobic digestion conditions represent the suitable alternative for the transformation of microalgal sludges into bioenergetic supplier. The important parameter that establishes the level of algal growth efficiency is dry weight of processing mass by which the conversion yield is calculated with a correlation factor of 0.9916 (Cho et al. 2011):

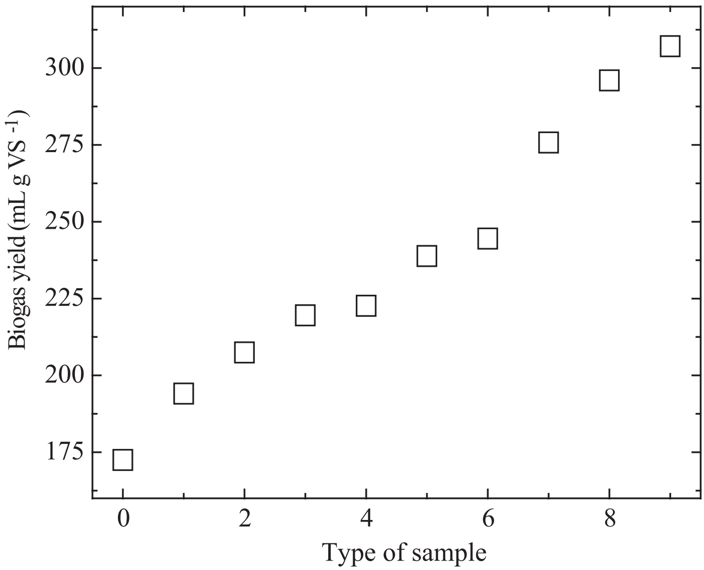

Fig. 6 Evolution of biogas production from pretreated microalgal biomass grown in wastewater pond after anaerobic digestion

Table 2 Contribution of sterility and paper biosludge on the CH_4 production

	Biomethane amount (L CH_4 kg^{-1} VS_{added})			
Processing time (d)	Digestate	80 °C digestate	Sterile digestate	80 °C sterile digestate
5	2.58	2.84	3.61	4.47
10	2.93	3.09	4.04	5.16
20	3.18	4.13	5.33	6.20
30	4.12	4.73	5.33	6.19
45	4.47	4.81	5.45	6.19

$$\text{dry weigh}\left(\text{mg L}^{-1}\right) = 600.94 \times OD_{660}$$

where OD_{660} is the optical density measured on the solubilized algae mass. This spectroscopic method is a fast and accurate route for the evaluation of cultivation and harvesting effectiveness.

For the improvement of solubilization of microalgal population growing in wastewater, either an accelerating factor like manure, sludge, terrestrial plant biomass, preheating or both of them may be required. Table 2 presents the effect of heat and paper biosludge on the CH_4 production from algal biomass (Kinnunen and Rintala 2016). A steady state of processing is attained after about 20 days.

The specific rate for biomethane production from algal biomass follows an asymptotic growth with higher values on the first 5 days of production (Arias et al. 2018).

Table 3 Cumulative biomethane over time of digestion

Period (d)	Specific methane volume (L_N g VS^{-1})	
	Microalgae grown in N-/P-deficient medium	Microalgae grown in N-/P-rich medium
0	0	0
2.5	150	185
5	200	340
10	225	365
15	250	380
20	265	390
25	–	390
30	–	390

This behaviour is illustrated in Table 3, where this processing parameter is listed against the bio-CH_4 production periods.

The reliable explanation is related to the higher rate conversion of proteins and lipids than other biodegradable structures. Another aspect that must be revealed is the contribution of environmental composition that influences the accumulation of proteins and lipids capable to provide biomethane by fermentation.

The characteristics of raw microalgae involved in the production of biomethane are main input parameters that must be taken into consideration for the evaluation of biogas production. In Table 4 the proof for the difference in the biomethane production for crude and pre-processed algal biomass is presented (Yang et al. 2018). The applied pretreatment brings about a diminution of convertible compounds, even though the production of by-products is an advantage. The improvement in the values of cumulative biomethane volumes indicates the involvement of lipids in the conversion processes of methanogenesis. However, the potential of anaerobic digestion of microalgae has been demonstrated by the consequence of solubilization of solid biomass. The similarity in the moderate rate of cumulative improvement confirms the contribution of protein content to the generation of biomethane.

The efficiency of biomethane production having two main contributions given by harvesting (filtration screening and staining, sedimentation, flotation) and anaerobic digestion depends strongly on the seasonal conditions (Ometto et al. 2018). Each type of algae grows specifically, influencing the cumulative biomethane yield (Table 5).

Important results are expected to be achieved in the area of anaerobic digestion of microalgae biomass cultivated in wastewaters both in terms of energy production and the cleaning of wet environments which are loaded in various organic pollutants coming from industrial units.

Table 4 Cumulative biomethane from different processed algal masses

Processing time (d)	Cumulative biomethane (mL g^{-1} VS_{input})	
	Raw microalgae	Lipid-extracted microalgae
0	17	0
5	49	35
10	150	75
15	225	170
20	245	200
25	260	210
30	273	212

Table 5 Changes in biomethane production vs seasonal conditions

Season		Biomethane amount (NmL g^{-1} VS)			
		F. vesiculosus	*A. nodosus*	*S. latissima*	*A. esculenta*
2014	August	40	50	295	198
	October	40	24	231	232
2015	February	20	25	190	–
	May	80	73	358	260

4 Energetic Impact Assessment of Biomethane Production

The algal biomass is considered a suitable source for the production of biomethane which can successfully replace natural gas in the same domestic and industrial applications, thus protecting the environment and saving oil deposits.

The production of gaseous biofuel is spread globally (Cucchiella et al. 2017) as the result of selective options of alternative technologies. Starting from process modelling (Wu et al. 2016; Milledge and Heaven 2017), the recovered energy by conversion of algal biomass into biomethane is a consequence of the stored energy in the microalgal biomass by photosynthesis; this makes it possible to extend the production of biomethane as a potential source of power (Zhu et al. 2018). After the application of pretreatments and a suitable anaerobic digestion, the generation of biogas may be placed on the range from 0.24 up to 0.63 L g^{-1} VS, corresponding to 6.88–18.80 MJ kg^{-1} VS (Zhu et al. 2018).

Since the composition of biogas predominantly illustrated by biomethane (40–75%) includes carbon dioxide (15–60%), moisture (1–5%), nitrogen (0–5%), hydrogen sulphide (0–3%) and other components whose caloric powers are inferior to CH_4, this gaseous biofuel has lower caloric value (23.1 MJ m^{-3}) in comparison with natural methane which provides 38.7 MJ m^{-3} (Bharathriraja et al. 2018). The large variety of microalgae would produce different energetic contributions (Zhu et al. 2018) as presented in Fig. 7.

The energetic considerations regarding the extension of biomethane production from algal biomass are based on the efficiency of anaerobic digestion that recovers

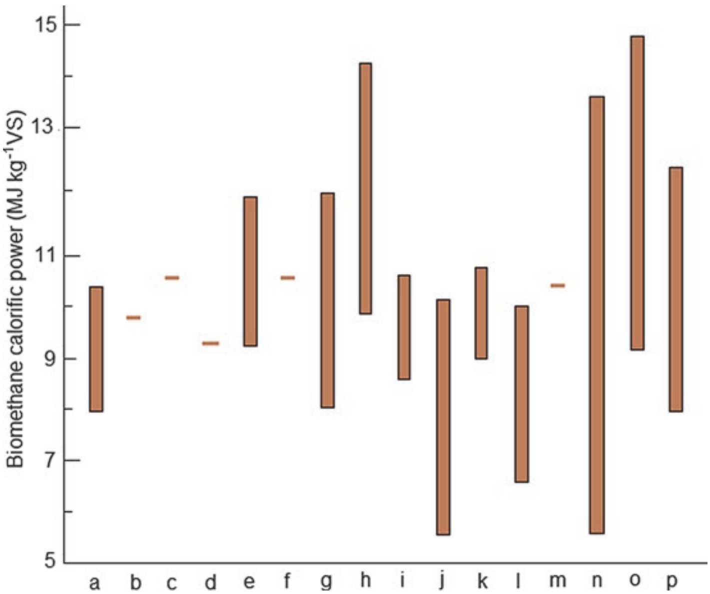

Fig. 7 Energetic production of various microalgae (a) *Anabaena cylindrical*; (b) *Aphanizomenon flos-aquae*; (c) *Chlamydomonas reinhardtii*; (d) *Chlorella pyrenoidosa*; (e) *Chlorella vulgaris*; (f) *Dunaliella salina*; (g) *Euglena gracilis*; (h) *Porphyridium cruentum*; (i) *Scenedesmus obliquus*; (j) *Spirogyra* sp.; (k) *Arthrospira maxima*; (l) *Spirulina platensis*; (m) *Synechococcus* sp.; (n) *Scenedesmus dimorphus*; (o) *Prymnesium parvum*; (p) *Chlorella zofingiensis*

the sun energy converted via photosynthesis. At the end of the year 2016, European Biogas Association reported 17,662 biogas and biomethane plants, including the units based on microalgae processing.

The mathematical expression that is applicable for the calculation of energetic contribution of algal biomass in the generation of energy was reported (Milledge and Heaven 2015):

$$OP = \frac{34.1\,C + 102\,H + 6.3\,N + 19.1\,S + 9.85\,O}{100}$$

where OP is output power (MJ kg^{-1} dry fuel) and C, H, N, S and O are the content percentages of carbon, hydrogen, nitrogen, sulphur and oxygen, respectively (%). This expression reflects the component concentrations in the anaerobic digested mass resulting from the harvested microalgae. Many other papers report quantitatively the amounts of biomethane provided by various species of microalgae which represent the input information for biogas systems designers.

The energetic considerations related to the conversion of microalgae into biomethane, when a new industrial unit starts its production, are based on the costs involved in the processing of renewable matter of energetic sources as the third generation of biomasses. The injection of minimum 0.133 € per kg VS of processed mass justifies a profitable investment in the industrial units for the production of biomethane from microalgae (Zamalloa et al. 2011). The recovery of investment would be reliable, if the utilization possibilities of wastewaters to be cleaned by microalgae, as well as the production of by-products required by various economic sectors, were added to the biomethane-generation technological chains.

5 Conclusions

Algal biomass has been recommended as third-generation renewable feedstock for sustainable biogas production. Anaerobic digestion of algal biomass has multiple benefits for the energy and environmental sectors, providing nutrient recycling and producing sustainable biomethane. Algae cultivation for the removal of nutrients such as nitrogen and phosphorus in the wastewaters has become a research topic of increasing interest in the past decades (Saratale et al. 2018). Biomethane production from biodegradation of algae is commonly reported to range between 100 and 400 L-CH_4 (kg VS)$^{-1}$. Variations in biomethane yields are linked to microalgae species-specific differences in cellular chemical composition (Perazzoli et al. 2016). The quality of the algal biomass depends on the cell wall digestibility, which could be enhanced by various pretreatment techniques including mechanical, physical, chemical or biological methods (Jankowska et al. 2017).

Besides the overall benefits that the algae-to-biogas technologies can bring to the society, there are also several important challenges of using algae for wastewater treatment, amongst which the expensive harvesting and processing technologies of the algal suspension, internal shading, high suspended solid content, etc. Despite this, the application of algae biomass systems in the treatment of industrial wastewater requires low operation and maintenance costs (Mohd Udaiyappan et al. 2017). It was reported that production cost of biomethane from algae is higher compared to other biomass. The integrated processes that combine algae cultivation and wastewater treatment system for methane production can be the most suitable approach to reduce production cost and make it more profitable (Mohammadi et al. 2018; Mohd Udaiyappan et al. 2017).

Biomethane that is generated from the anaerobic digestion of the algal biomass can be used as fuel gas and also be converted to generate electricity (Harun et al. 2010). Biogas majorly constitutes of methane and CO_2 with 40–75% and 15–60% volume, respectively. Upgrading treatment intents at reducing the concentration of CO_2 in biogas which would yield an increased level of biomethane. When 1 m^3 of raw biogas at standard temperature and pressure containing 60% CH_4 is burnt, it gives a heating value of 23.1 MJ, but, in the same condition, pure CH_4 gives 39.8 MJ. Purification of biogas for biomethane enrichment is one of the major tasks for cost optimization amongst the trials to make the algae-to-biomethane technology a feasible option, in the context of the rapid increment in the price of fossil fuels (González-González et al. 2018).

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Microalgal Biotechnology Application Towards Environmental Sustainability

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1 Introduction

Algae are one among the foremost photosynthetic living groups beside plants and bacteria. Algae are composed of eukaryote cell. They are usually found in marine and fresh water with the size extending from a couple of micrometers to a couple of

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many micrometers. Green growth called algae have an excellent part in nourishment and agribusiness and in misusing microbial exercises for creating significant human items, producing vitality, and tidying up the earth. Green growth can be additionally separated into microalgae and macroalgae in light of their size. Those with diameters below 50 μm are called microalgae and are usually monocellular organisms, while algae with larger sizes are macroalgae. Macroalgae or "seaweeds" are multicellular plants growing in salt or fresh water. They are often fast growing and can reach sizes of up to 60 m in length (McHugh 2003).

Microalgae are minute unicellular species, prokaryotic or eukaryotic, photosynthetic microorganisms that exist in both aquatic and fresh water. Microalgae are among the quickest developing photosynthetic creatures on earth. Their photosynthetic system is like land-based plants; however because of a straightforward cell structure, and submerged in a watery situation where they have proficient access to water, CO_2, and different supplements, they are for the most part more productive in changing over sun-based vitality into biomass. They can be totally presented to light, so every cell can lead photosynthesis at full speed and twofold inside a few hours. The photosynthetic productivity of microalgae is normally more than ten times that of the higher plants and macroalgae (Hemschemeier et al. 2009; Kamyab et al. 2016a).

The most frequently used microalgae are *Cyanophyceae* (blue-green algae), *Chlorophyceae* (green algae), *Bacillariophyceae* (including the diatoms), and *Chrysophyceae* (including golden algae). All cells require wellsprings of carbon and energy for growth. Chemoorganotrophs, chemolithotrophs, and phototrophs utilize natural chemicals, inorganic chemicals, or light, respectively, as their wellspring of energy. Autotrophs utilize CO_2 as their carbon source, while heterotrophs utilize natural compounds (Xu et al. 2006). Numerous microalgae species could change from phototrophic to heterotrophic growth. As heterotrophs, the green algae growth depends on glucose or other utilizable carbon sources for carbon digestion and energy. Limited algae can also grow mixotrophically.

Microalgae have been the focus and interest of researchers, government, and industries because of (i) their growth rate and simple structure; (ii) cellular components such as carbohydrate, proteins, and lipids which can be utilized for biotechnological applications like creating biofuel and product of nutritional, pharmaceutical, and cosmetic value; and (iii) the shift toward the development of green processes. Microalgae represent very interesting natural sources of bioactive compounds such as carotenoids, fatty acids, vitamins, and sterols (Plaza et al. 2009; Chacón-Lee and González-Mariño 2010). They have been generally utilized for different applications such as biofuels, animal feed, food supplements, cosmetics, pharmaceuticals, nutraceuticals, CO_2 capture, etc. (Nesamma et al. 2015; Kamyab et al. 2017). In addition, microalgae are additionally utilized as a part of wastewater treatment for toxin and supplement expulsion. That is on the grounds that microalgae developments are subject to supplement focus and in addition light, temperature, saltiness, and pH (Venkatesan et al. 2015). Microalgae-construct wastewater treatment depends with respect to the capacity of phototrophic microorganisms to supply oxygen to vigorous natural toxin degraders and upgrade the evacuation of supplements and pathogens. It is generally known that microalgae plays an important role in self-purification of natural waters and therefore offers an alternative means as a tertiary treatment of organic wastewater (Venkatesan et al. 2015).

Microalgae are recognized as one of the oldest living microorganisms in earth (Lam and Lee 2011). They are prokaryotic or eukaryotic photosynthetic microorganisms that can develop quickly and live in brutal conditions (Mata et al. 2010; Resdi et al. 2016). Biologists have categorized microalgae in a variety of classes, mainly distinguished by their pigmentation, life cycle, and basic cellular structure (Khan et al. 2009). Microalgae are available in all current earth biological systems, not just aquatic but also terrestrial, instead of a major assortment of groups living in an extensive variety of ecological conditions, such as lakes, rivers, ponds, wetlands, and deserts, and even living in the North and South Poles (Mata et al. 2010). It is estimated that more than 50,000 species exist, but only a limited number of around 30,000 have been studied and analyzed (Mata et al. 2010). Like plants, microalgae require basically three parts to develop: daylight, carbon dioxide, and water. Photosynthesis is an imperative bio-compound process in which plants, microalgae, and a few microorganisms change over the vitality of daylight to synthetic energy. Among others, microalgae consist of lipids and unsaturated fats as membrane segments, stockpiling items, metabolites, and wellsprings of energy. Lipids are characterized as any of a gathering of natural compounds that are insoluble in water but dissolvable in natural solvents. The synthetic highlights are introduced in an expansive scope of molecules, for example, unsaturated fats, phospholipids, sterols, sphingolipids, terpenes, and others (Fogg 1959; Fahy et al. 2011).

2 Classification of Microalgae

According to Fogg (1959), microorganisms are classified according to their morphological properties, the nature of their life cycle, the chemical nature reserve photosynthetic products (intracellular accumulation product), and pigmentation. Various algae species can be found growing in lakes, oceans, rocks, and soil. There are basically two groups of microalgae, prokaryotic and eukaryotic. Prokaryotes microalgae are unicellular organisms that lack a membrane-bound nucleus (karyon), mitochondria, or any other membrane-bound organelles and rarely have cellular organelles. Under its categories is *Cyanophyceae*. *Cyanophyceae* is also called *cyanobacteria*. It is photosynthetic bacteria that are found in fresh and salt water and have chlorophyll a and phycobilins. *Cyanophyceae* are thought to be blue-green microalgae which have a high tolerance to extreme temperatures (Pulz and Gross 2004). The best known species are *Spirulina* (*Arthrospira*) *platensis*, *Nostoc commune*, and *Aphanizomenon flos-aquae*. Eukaryotes are uni- or multicellular organisms which have a complex structure comprising a core surrounded by a membrane and many intracellular organelles. These microorganisms are additionally inexhaustible in fresh water. A few types can likewise live in terrestrial area, developing on wet soils, wet rocks, and soggy wood. They can show up as single cells or as colonies. Eukaryotes are ordered into an assortment of classes for the most part characterized by their pigmentation, life cycle, and fundamental cell structure (Khan et al. 2009). The most vital classes are green growth algae (*Chlorophyta*), red algae (*Rhodophyta*), and diatoms (*Bacillariophyta*). These microalgae usually contain three main types of pigments which are chlorophylls, carotenoids, and phycobiliproteins. Microalgae signify to an exceptionally intriguing asset as normal wellsprings of bioactive compounds, for example, carotenoids, unsaturated fats, vitamins, and sterols (Plaza et al. 2009; Chacón-Lee and González-Mariño 2010). To sum up the classes of microalgae, there are 11 different divisions of microalgae, which are *Cyanophyta*, *Prochlorophyta*, *Glaucophyta*, *Rhodophyta*, *Heterokonta*, *Haptophyta*, *Cryptophyta*, *Chlorarachniophyta*, *Dinophyta*, *Euglenophyta*, and *Chlorophyta*. The largest groups are *Chlorophyceae* (green algae), *Bacillariophyceae* (diatoms), *Phaeophyceae* (brown algae), *Chrysophyceae* (golden-grown algae), *Pyrrophyceae* (dinoflagellates), and *Rhodophyceae* (red algae) (Milano et al. 2016). Green growth algae are the transformative progenitors of current plants, yet dissimilar to plants, they are basically aquatic. These microorganisms have the filamentous forms. The best known species are *Chlorella*, *Chlamydomonas*, *Dunaliella*, and *Haematococcus* (Pulz and Gross 2004). The main storage compound of these algae is starch, although oils can also be produced.

3 Culture Parameters

3.1 Temperature

Most of the microalgae are able to survive at a temperature range between 16 and 27 °C. *Chlorella sorokiniana* had an upper growth limit at 38–42 °C (Kessler 1985). For a multi-specific microalgal biomass, as studied by Golueke et al. (1957), the rate of microalgae biodegradability from 5% to 10% can be enhanced by a temperature increase from 35 to 50 °C. However, maximal methane productivity at 40 °C that was found by Chen et al. (1996), confirms most mesophilic temperatures considered as optimal conditions for methane production.

3.2 Nitrogen Concentration

Nitrogen-limiting conditions were in fact reported to significantly increase the lipid fraction of many microalgae (Illman et al. 2000). Nitrogen is vital for peptides, proteins, enzymes, chlorophylls, ATP, DNA, and other cellular constituents' synthesis. The increment of lipid fraction occurred due to the reduction of nitrate in growth medium, although of an almost constant growth rate, thus doubling the productivity of the oil (Converti et al. 2009).

3.3 Light Intensity

The limiting factor for microalgal growth is light intensity. Consequently, a suitable light intensity for microalgal cultivation needs about 1/10 of amount of light from direct sunlight. This photosynthetic microorganism produces microalgal biomass by utilizing the sunlight, water, and carbon dioxide. Twenty-five percent consumption of the biomass produced during daylight might be consumed during the night to sustain the cells until sunrise (Chisti 2007). Some microalgae are fit for heterotrophic development on monosaccharide or natural acids. This method of development offers the likelihood of extraordinarily enhancing the profitability of microalgal culture using fed-batch and high cell-density strategies, which can't be connected to photosynthetic frameworks. These strategies are routinely connected to bacterial and yeast cultures to product cell densities in the request of 150–200 g 1 dry weight (Suzuki et al. 1985).

3.4 pH

Acid or basic condition is the suitable pH for microalgal growth. Carbon dioxide consumption may lead to the increasing of pH in the medium. Therefore, CO_2 sparging maintains the pH of the medium. Culture pH was not affecting *Chlorella sorokiniana* when the value was higher than pH 4.0, and the growth rate was inhibited drastically at pH 3.0 (Morita et al. 2001).

3.5 Culture Parameters

The most critical parameters controlling algal growth by methods for photosynthesis are ecological conditions, for example, light power, pH, temperature, turbulence, saltiness, and supplements (Kayombo et al. 2003). The ideal range and the tolerable scope of working conditions depends on different species, which could associate with different components.

3.6 Nutrients

Microalgae production requires high concentrations of essential nutrients (C, N, P, S, K, Fe, etc.). The main focus is on the three most significant nutrients, i.e., carbon, nitrogen, and phosphorus; supplements are normally taken up in the inorganic shape; however a few natural types of them are additionally assimilable. A few supplements don't show any restraint impacts on microalgal development, while others, for example, NO_2 or NH_3, have inconvenient impacts when exhibited in high focuses. Supplements in the vaporous frame, for example, CO_2 and NO, face a noteworthy impediment which is connected basically to their mass exchange from the vaporous to the fluid state (Markou et al. 2014).

3.7 Carbon Sources

According to the mode of cell growth (heterotrophic, autotrophic, or mixotrophic), microalgae can utilize organic and/or inorganic carbon sources for cell growth (Feng et al. 2011). From the perspective of microalgae cultivation, the most common organic carbon sources for heterotrophic and mixotrophic cultivation of microalgae are glucose, sucrose, lactase, lactose, acetate, glycerol, ethanol (Liang et al. 2009; Perez-Garcia et al. 2011), and other sugars derived from starch, sugar cane, lignocellulosic biomass, and other sugar sources (Perez-Garcia et al. 2011).

3.8 Phosphorus

Phosphorus is another component that has huge pertinence to the cell development and metabolism of microalgae. It is one of the basic components containing DNA, RNA, ATP and cell layer materials, and so on. It is significant that, as a constituent component of ATP, phosphorus is fundamental to the cell forms identified with vitality exchange (e.g., photophosphorylation). On another pertinent idea, photosynthesis requires a lot of proteins, and the proteins are orchestrated by phosphorus-rich ribosomes (Ågren 2004). Phosphorus-containing ATP/ADP are fundamental for photophosphorylation. As a result, confinement of development by phosphate starvation may severely affect different parts of microalgal digestion, including photosynthesis and lipid aggregation. Phosphorus is especially absorbed as inorganic phosphates as H_2PO_4 - and HPO_4^{2-} (Martinez et al. 1999).

3.9 Other Elements

Magnesium, sulfur, iron, and different components are additionally key for the development of microalgae (Zhang et al. 2000). Magnesium is required for nitrogenase movement utilizing a creatine phosphate−/kinase-/ATP-producing framework as one of its parts in cell digestion (Roden et al. 1996). Sulfur is a fundamental part of cysteine and methionine. Without sulfur, protein biosynthesis is hindered, and the photosynthetic framework PSII repair cycle is blocked (Zhang et al. 2000). Iron is associated with electron spill out of H_2O to $NADP^+$ (Roden et al. 1996). According to Raven et al (1999), some metals could enter in (noncyclic) photosynthetic electron transport systems.

3.10 Factors Limiting Microalgae Growth

A few natural parameters, for example, light's source and intensity, photoperiod, temperature, saltiness, pH, blending, and so forth, impact the development of microalgae (Atta et al. 2013; Singh and Singh 2015). Therefore, it is suggested to adjust and keep up these parameters amid the cultivation time frame. Table 1 indicates parameters and their ideal ranges.

4 Culture of Microalgae

The growth qualities and arrangement of microalgae are known to essentially rely upon the cultivation conditions (Chen et al. 1996). There are four noteworthy sorts of development conditions for microalgae: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic cultivation (Chen et al. 1996). Microalgae, however, developed under pressure conditions, for example, supplement starvation, high saltiness, high temperature, and so forth, aggregate extensive sums (up to 60–65% of dry weight) of lipids or sugars alongside a few auxiliary metabolites (Markou et al. 2014).

4.1 Phototrophic Cultivation

Phototrophic development happens when the microalgae utilize light, for example, daylight, as the energy source, and inorganic carbon (e.g., carbon dioxide) as the carbon source to shape of chemical energy (e.g., polysaccharides, proteins, lipids,

Table 1 A generalized set of conditions for culturing microalgae

Parameters	Range	Optimum
Temperature (°C)	16–27	18–24
Salinity (gl^{-1})	12–40	20–24
Light intensity (Lux)[a]	1000–10,000	2500–5000
Photoperiod (light/dark)	–	16:8 (minimum) 24:0 (maximum)
pH	7–9	8.2–8.7

[a]Depends on volume and density

Adapted from Singh and Singh (2015) and Kamyab et al. (2016b) and hydrocarbons) through photosynthesis (Chen et al. 1996; Huang et al. 2010). This species is the most regularly utilized development condition for microalgae growth (Gouveia and Oliveira 2009; Yoo et al. 2010). Rather, both lipid substance and biomass generation should be considered simultaneously. Thus, lipid profitability, considering the consolidated impacts of oil substance and biomass creation, is a more reasonable execution file to show the capacity of a microalga with respect to oil generation (Chen et al. 1996). The real favorable advantage of utilizing autotrophic cultivation contrasted with different kinds of development; the defilement issue is less serious when utilizing autotrophic development. In this way, outside scale-up microalgae development systems (e.g., open ponds and raceway ponds) are normally worked under phototrophic development conditions (Mata et al. 2010).

4.2 Heterotrophic Cultivation

Heterotrophic development is the condition when microalgae utilize natural carbon as both the energy and carbon source. This sort of development offers a few points of interest over phototrophic development as far as end of light prerequisite, great control of the development procedure, and ease for collecting the biomass (Chen et al. 1996). Some microalgae species demonstrate higher lipid content amid heterotrophic development, and a 40% expansion in lipid content was gotten in *Chlorella protothecoides* by changing the development condition from phototrophic to heterotrophic (Xu et al. 2006; Chen et al. 1996). Zheng et al., (2013) studied on the biomass and lipid productivities of heterotrophic refined microalgae *Chlorella sorokiniana*. The authors investigated the impact of temperature and medium variables such as carbon source, nitrogen source, and their underlying fixations. Authors reported the most extreme lipid substance of 31.5% was accomplished in 96 h, and the lipid profitability was 2.9 g L^{-1} d^{-1}. Carbon sources are the most imperative component for heterotrophic culture of microalgae in the generation of lipids. Microalgae can absorb an assortment of natural carbon sources, for example, glucose, acetic acid derivation, glycerol, fructose, sucrose, lactose, galactose, and mannose relying upon microalgae species utilized for development (Liang et al. 2009). For example, (Chen et al. 1996) analyzed three carbon sources like acetic acid derivation, glucose, and glycerol for creating significantly higher biomass and in addition lipid content in cells by *Chlorella protothecoides* performance. A few investigations have along these lines concentrated on finding less expensive natural carbon sources, for example, corn powder hydrolysate (CPH) rather than sugars, bringing about high biomass (2 g/L/day) and lipid (932 mg/L/day) productivities (Plaza et al. 2009). Once more, heterotrophic development gives considerably higher lipid efficiency, as the most noteworthy lipid profitability from heterotrophic development is almost 20 times higher than that got under phototrophic development. In any case, the sugar-based heterotrophic framework much of the time experiences issues with tainting (Chen et al. 1996).

4.3 Mixotrophic Cultivation

Mixotrophic cultivation happens when microalgae experience photosynthesis and utilize both natural mixes and inorganic carbon (CO_2) as a carbon source for growth. This mentions that microalgae are able to live under either phototrophic or heterotrophic conditions or both (Chen et al. 1996). Natural mixes and CO_2 as a carbon source absorbed by microalgae, at that point microalgae discharge CO_2 by means of breath, will be caught and reused under phototrophic growth (Mata et al. 2010). Contrasted and phototrophic and heterotrophic cultivation, mixotrophic development is once in a while utilized as a part of microalgal oil generation (Chen et al. 1996). For instance, the development and lipid efficiency of a separated microalga *Chlorella vulgaris* ESP-31 were explored under various media and developmental conditions, including phototrophic ($NaHCO_3$ or CO_2, with light), heterotrophic (glucose, without light), photoheterotrophic (glucose, with light), and mixotrophic (glucose and CO_2, with light). The results demonstrated the higher lipid efficiency (67–144 mg/l/d) were obtained under mixotrophic growth along with the utilized media (Yeh and Chang 2012).

4.4 Photoheterotrophic Cultivation

Photoheterotrophic cultivation takes place when the microalgae require light when utilizing natural mixes as the carbon source. The principal distinction among mixotrophic and photoheterotrophic cultivation is that the last requires light as the energy source, while mixotrophic cultivation can utilize natural mixes to fill this need. Along these lines, photoheterotrophic development needs the two sugars and light in the meantime. Despite the fact that the creation of some light-directed valuable metabolites can be improved by utilizing photoheterotrophic cultivation, utilizing this way to deal with the delivery of biodiesel is exceptionally uncommon, similar to the case with mixotrophic cultivation (Chen et al. 1996). Algal–bacterial framework that productively debases thiocyanate (SCN^-), a lethal contaminant, and displays high lipid efficiency was created. A consortium of blended microscopic organisms (activated sludge) and microalgae was consecutively cultivated under photoautotrophic and photoheterotrophic modes. The development mode was changed to photoheterotrophic conditions in a consecutive way. Algal–bacterial culture containing *Chlorella protothecoides* and *Ettlia* sp. yielded essentially higher lipid efficiency under photoheterotrophic conditions contrasted with photoautotrophic conditions showing 28.7 and 17.3 higher productivity (Ryu et al. 2014).

5 Microalgae Harvesting Methods

Choice of harvesting technique is subject to attributes of microalgae, e.g., size, thickness, and e-value of the target products (Olaizola 2003). Generally, microalgae harvesting is a two-phase process, including (1) bulk gathering—aimed at detachment of biomass from the mass suspension. The focus factors for this activity are generally 100–800 times to reach 2–7% total solid matter. This will rely upon the initial biomass fixation and advances utilized, including flocculation, flotation, or gravity sedimentation. (2) Thickening—the point is to concentrate on the slurry through methods, for example, centrifugation, filtration, and ultrasonic accumulation, subsequently—is for the most part a more energy concentrated advancement than mass harvesting (Olaizola 2003).

5.1 Gravity and Centrifugal Sedimentation

Gravity and centrifugal sedimentation techniques depend on Stokes' law (Schenk et al. 2008), i.e., settling characteristics of suspended solids are determined by thickness and range of algae growth cells (Stoke's sweep) and sedimentation velocity. Gravity sedimentation is the most well-known harvesting method for algae growth biomass in wastewater treatment due to the extensive volumes treated and the low scale of the biomass produced (Nurdogan and Oswald 1996). However, the strategy is reasonable for expansive (ca. >70 mm) microalgae, for example, *Spirulina* (Munoz and Guieysse 2006). Centrifugation recovery (CR) is favored for gathering of high-value metabolites and expanded time frame of realistic usability concentrates for incubators in aquaculture (Heasman et al. 2000). The procedure is quick and energy intensive; biomass recuperation relies upon the settling qualities of the cells, slurry residence time in the centrifuge, and settling depth (Grima et al. 2003). The drawback point of the procedure incorporates high energy costs and potentially higher support requirements because of freely moving parts (Bosma et al. 2003). Harvesting productivity of>95% [50] and increment in slurry focus by up to 150 times for 15% aggregate suspended solids are actually feasible (Mohn 1980).

6 Growth Cycle of Microalgae

In the batch cultures, the growth bend of algae growth, as with most microbial frameworks, can be represented by the accompanying stages: (a) lag phase, (b) exponential growth stage, (c) deceleration growth stage, (d) stationary stage, and (e) demise stage (Shuler and Kargi 2002) as described in Fig. 1. The cellular structure and metabolic way can be differed amid each stage because of supplement levels inside the batch vessel (Shuler and Kargi 2002).

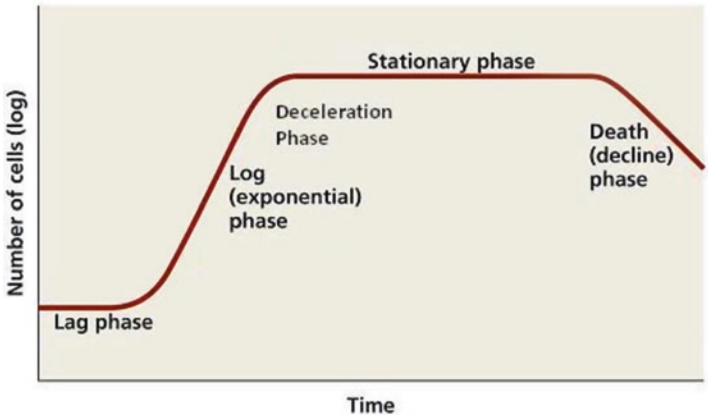

Fig. 1 Typical growth curve for microorganism population

6.1 Lag Phase

Lag stage is a time of cell adjustment in accordance with the new condition, which occurs instantly after inoculation. In this stage, cells encounter a supplement-rich condition, however altogether not the same as that of the seed culture from which the cell was exchanged. The growth occurs in the new framework; adaption happens by incorporating enzymes and co-proteins essential for cell multiplication (Shuler and Kargi 2002). Length of the lag stage depends on the measure of cells exchanged to the new growth medium, the supplement levels present, and culture age. The time duration which the inoculum has been cultured strongly affects the length of lag stage in a batch culture. Typically, the lag time frame increments with the age of the inoculums (Shuler and Kargi 2002).

6.2 Exponential Growth Phase

At this level, also called as the *logarithmic growth phase*, the cells have changed in accordance with their new environment. At the point when there is indication of growth, the development curve is moving from the lag stage into the exponential growth stage or logarithmic growth stage. After cells are adjusted to the new condition and new protein/compounds are orchestrated, cells begin to develop exponentially quickly (Shuler and Kargi 2002). Nevertheless, exponential development not just occurred after the lag stage yet additionally can occur after stationary stage (Monod 1949). This is a period-adjusted growth, in which all parts of the cells grow at a similar rate, because the normal organization of a single cell remains roughly steady during exponential period of development. During this adjusted growth, the net particular growth rate determined from either cell number or cell mass would be the same. Since the supplement focuses are vast in this stage, the development rate is free of supplement concentration. The exponential development rate is first requested (Shuler and Kargi 2002):

$$= X\,X = X0\,\mathrm{at}\,t = 0 \quad dt\,dX\,\mu\mathrm{net} \tag{1}$$

where integration X and $X0$ are cell concentrations at time t and $t = 0$.

$$T\ \mathrm{net}\ t\ \mathrm{or}\ X\ X\ e\ \mathrm{net}\ X\ X\mu\ \mu\ 0\,0\ \ln \tag{2}$$

The time required to double the microbial mass is given by Eq. 2. The exponential growth is characterized by a straight line on a semilogarithmic plot of ln X versus time:

$$\mathrm{net}\ \mathrm{net}\ d\mu\ \mu\tau = \ln 2 = 0.693 \tag{3}$$

where τd is the doubling time of cell mass.

Similarly, a doubling time based on cell numbers and the net-specific rate of replication may be calculated:

$$Rd\mu\,\tau' = \ln 2 \tag{4}$$

where $\tau' d$ is the doubling time based on the replication rate. During balanced growth, τd will equal $\tau' d$, since the average cell composition and size will not change with time.

6.3 Deceleration Growth Phase

Supplements are expended, and poisons are discharged into nature, in this stage. Space or volume required for cell multiplication may wind up restricted. Assumptions in the exponential stage are never again substantial. As such, expanding lethality and diminishing supplement level decrease the growth rate of cell. Additionally the cell morphology and physiology may change (i.e., size, cell structure, and metabolic pathways) (Shuler and Kargi 2002). Additionally, this stage takes place as quickly as changing conditions results in unbalanced development, in which τd, and $\tau' d$ will not be equivalent. This stage is called deceleration development stage before the net growth rate achieves zero. The model created on the exponential growth stage cannot be utilized to foresee precise biomass fixation without redesigning the condition (Shuler and Kargi 2002).

6.4 Stationary Phase

Stationary stage resulted in light of the fact that microorganism might not grow uncertainly, in alternate words that the net growth rate achieves zero. The growth bend enters the stationary stage from deceleration growth stage. The metabolic pathway is changed from essential metabolites to auxiliary metabolites (Shuler and Kargi 2002). A few preparations can be framed in this stage, for example, aggregation of lipid (Gouveia and Oliveira 2009). In this stage, the aggregate cell fixation may remain consistent, but total practical cells may diminish. This leads the consistent decline of the net particular growth rate into the demise stage. Another exponential stage might be seen after the stationary stage with a lower net particular growth rate (in contrast with the illustration during the initial exponential growth stage). This could be because of the cell lysis, where the lysed cells could be utilized for growth (Shuler and Kargi 2002).

6.5 Death Phase

Death stage is the decay rate of the microbial populations which is higher than the growth rate. This stage occurs after the stationary stage. In any case, the outline between late stationary and early demise stage might be hard to characterize with accuracy. In the death stage, the net particular stage is negative because of reducing quantities of reasonable cells, supplement consumption, and presence of lethal pressure (Shuler and Kargi 2002).

7 Microalgae Versus Wastewater

Numerous types of microalgae can viably exist in wastewater conditions through their capacity to use copious natural carbon and inorganic N and P in the wastewater (Kamyab et al. 2014;Kamyab et al. 2015). Furthermore, the utilization of microalgae in the wastewater manufacture is still genuinely constrained. Algae growth is utilized all through the world for wastewater treatment but on a generally minor scale. This is either using regular oxidation (adjustment) ponds or the more created suspended algal lake frameworks, for example, high-rate algal lakes which are shallow raceway-type oxidation ponds with mechanical blending and have been observed to be exceedingly successful for wastewater treatment (Pittman et al. 2011). A noteworthy necessity of wastewater treatment is the need to expel high convergences of supplements specifically N and P, which generally can prompt dangers of eutrophication if these supplements aggregate in river and ponds (Pittman et al. 2011). Microalgae are proficient in evacuating N, P, and poisonous metals from wastewater (Wang et al. 2010) and in this manner can possibly assume a critical remediation part especially during the last (tertiary) treatment period of wastewater (Pittman et al. 2011). The noteworthy preferred standpoint of algal procedures in wastewater treatment over the regular substance-based treatment strategies is the potential cost sparing and the lower-level innovation (Mallick 2002; Wang et al. 2010). An extensive variety of studies have broken down the development of microalgae under an assortment of wastewater condition (Pittman et al. 2011).

Regular municipal sewage treatment comprises of an essential treatment stage for the sedimentation of solid materials, a secondary treatment stage in which suspended and dissolved natural materials are expelled, and a tertiary treatment stage in which last treatment of the water is performed before release into condition. During tertiary treatment stage, a considerable decrease in inorganic compounds occures, for example, N and P absorbs by microalgae (Pittman et al. 2011). Some unicellular green microalgae species are especially tolerant to sewage effluent condition (Ruiz-Marin et al. 2010; Pittman et al. 2011). For instance, different types of *Chlorella* and *Scenedesmus* can expel up to >80% alkali, nitrate, and aggregate P from auxiliary-treated wastewater (Ruiz-Marin et al. 2010; Zheng et al. 2013). Moreover, microalgae additionally appeared to growth and effectively expel supplements from essential settled sewage wastewater. This showing the capability of microalgae for developing and expelling of supplement is not essentially subject to treatment level (Mallick 2002; Pittman et al. 2011).

In contrast with municipal local sewage-based wastewater, agricultural wastewater, which is frequently resulted from compost, containing high N and P (Pittman et al. 2011). In spite of high supplement fixations, previous scholar investigated that effective development of microalgae on rural waste and additionally municipal wastewater, microalgae are proficient at reducing N and P from wastewater (Pittman et al. 2011). Investigations of algal-intervened supplement recuperation from dairy manure have surveyed the capability of benthic freshwater green growth (algae) instead of planktonic (suspended) algae growth because of the potential higher supplement take-up rates in a few types of benthic algae. These species incorporate *Microspora willeana*, *Ulothrix* sp., and also *Rhizoclonium hierglyphicum*. Different studies on using a semicontinuous cultivation technique where the benthic algal growth was developed in reusing wastewater with new fertilizer has been increased by the scholars. For instance, algal cultivation rates and supplement take-up were observed to be high and identical to values from algae growth cultivation on municipal wastewater (Pittman et al. 2011).

A few investigations have analyzed microalgae cultivation and supplement expulsion characteristics utilizing artificial wastewater (Aslan and Kapdan 2006;Pittman et al. 2011). Usage of a simulated medium has advantages, for example, usability for starting research facility-based examinations. It additionally takes into account a streamlined examination of the significant segments in a wastewater medium without one expecting to consider obscure factors, for example, biotic parts. Most manufactured wastewater media are made out of inorganic constituents including high concentrations of particular supplements and will lack solid natural material and other potential poisons. Hence, there might be a few disadvantages in utilizing manufactured wastewater to survey conditions in actual wastewater. Real examinations of artificial wastewater with municipal wastewater have discovered that albeit supplement expulsion rates are comparable, microalgae growth rates are higher in simulated wastewater (Ruiz-Marin et al. 2010; Pittman et al. 2011). This is likely because of expanded poisonous quality of the actual wastewaters, inhibitory or aggressive impacts of indigenous microorganisms and protozoa, and by the distinctive synthetic organization of the wastewaters (Pittman et al. 2011).

There is critical enthusiasm for the utilization of microalgae for remediation of mechanically determined wastewaters, principally for the evacuation of substantial metal contaminants (cadmium, chromium, zinc, etc.) and natural compound poisons (hydrocarbons, biocides, and surfactants), instead of N and P. Because of, for the most part, the low N and P focus and high poison fixations, algal development rates are brought down in numerous modern wastewaters. Therefore, there is less potential for using modern wastewaters for expansive scale age of microalgae biomass (Pittman et al. 2011). The wastewater incorporates process chemicals and shades utilized as a part of the mills, plus a range of inorganic components including low concentrations of metals and generally low concentrations of aggregates P and N. This wastewater was appeared to be sufficiently low in poisons and had enough P and N to help microalgae growth, with two freshwater microalgae *Botryococcus braunii* and *Chlorella saccharophila* and a marine alga *Pleurochrysis carterae*, ready to become especially well on the untreated wastewater (Chinnasamy et al. 2010). With the huge measure of wastewater accessible from this industry, a lot of biomass and possibly likewise biodiesel could be produced from this asset (Pittman et al. 2011).

8 Sustainable Algal Biomass Production with Wastewater

Algae grow naturally in a wide range of environments. Typical requirements for phototrophic algae include sunlight, CO_2, temperatures between 20 and 30 °C, water, and nutrients (primarily N, P, and K). Algae have been grown on an industrial scale for different purposes such as treatment of organic residues, nutrient recovery for animal feed and fertilizer, human food, and production of biofuels. In industrial algae production, the ideal conditions may be provided, such as artificial light with the appropriate photoperiod and wavelength, consistent CO_2 supply, optimal temperature, and essential nutrients like nitrogen (N) and phosphorous (P). Providing optimal conditions improves the algae growth rate and potentially improves the composition (oil, starch, protein) of the algae, although it increases the costs of the production. Table 2 presents a recent comparison between open and close cultivation system in terms of environmental impact, biological issues, process issues, and costs. Measuring the algae concentration and growth rate during cultivation is a critical parameter for evaluating the feasibility of algae production. Carbon sources are essential for microalgae growth. Photoautotrophic organisms are the organisms that derive their energy for food synthesis from light and are capable of using

Table 2 Comparison between open system and close systems for microalgae production

Parameter		Open Systems	Closed systems
Environmental impact	Land footprint	High	Low
	Water footprint	High	Low
	CO_2 losses	High	Low
Biological issues	Algae species	Restricted	Flexible
	Contamination	High risk	Low risk
	Biomass productivity	Low	High
	Biomass composition	Variable	Reproducible
Process issues	Temperature control	Yes	No
	Weather dependence	High	Low
	Energy requirement	Low	High
	Process control	Difficult	Easy
	Use of wastewater	Yes	Yes
	Reactor cleaning	Not required	Required
Costs	Investment cost	Low	High
	Operational costs	Low	High
	Harvesting costs	Low	High

Adapted from (Barros et al. 2015)

carbon dioxide as their principal source of carbon. Hence, photoautotrophic cultivation implies that inorganic types of carbon (CO_2 or bicarbonates) are provided to the cultures, while light energy is changed into compound energy through photosynthesis (Ren et al. 2014). Other microalgae strains can utilize natural carbon as both energy and carbon source (heterotrophic cultivation); this cultivation framework is however practiced for the creation of high-value items as it were. Mixotrophic nutrition mode is the mix of both autotrophic and heterotrophic. Table 3 shows a comparison between photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic cultivation conditions. The following section provides extra details on each metabolism system.

8.1 Application of Microalgae in Biomass Production and CO_2 Sequestration

Microalgae have been broadly utilized for different applications, for example, biofuels, animal feed, nourishment supplements, beautifiers, pharmaceuticals, nutraceuticals, CO_2 capture, and so on (Nesamma et al. 2015; Kamyab et al. 2016c). In addition, microalgae are likewise utilized as a part of wastewater treatment for contamination and supplement evacuation. That is due to microalgae growths which are subject to supplement focus and in addition light, temperature, saltiness, and pH (Venkatesan et al. 2015). The use of microalgae mainly in wastewater treatment depends on light capacity of phototrophic microorganisms to supply oxygen to

Table 3 Comparison between cultivation conditions (Chen et al. 1996)

	Cultivation condition			
	Photoautotrophic	Heterotrophic	Mixotrophic	Photoheterotrophic
Energy source	Light	Organic	Light and organic	Light
Carbon source	Inorganic	Organic	Inorganic and organic	Organic
Cell density	Low	High	Medium	Medium
Cost	Low	Medium	High	High
Reactor scale-up	Open pond/PBR	Conventional fermentor	Closed PBR	Closed PBR
Issues with scale-up	Low cell density High condensation cost	Easily contaminated High substrate cost	Easily contaminated High equipment cost High substrate cost	

vigorous natural contamination which could degrade and improve the absorption of supplements and pathogens (Kamyab et al. 2016b).

Major greenhouse gas is CO_2 leading to global warming. Most of CO_2 emission is from electrical power plants, automobiles, and industrial sources such as cement processing. Using algae-based CO_2 sequestration to reduce CO_2 is a sustainable solution to reduce global carbon footprint. CO_2 sequestration is a notion based on capturing the total CO_2 emitted from various sources by growing microalgae which are used to make biofuel (Eloka-Eboka and Inambao 2017). Many algal species such as *Chlorella vulgaris*, *Nannochloropsis* sp., *Scenedesmus quadricauda*, *Chlamydomonas reinhardtii*, and *Nannochloris* sp. have been studied to sequester CO_2. One of the most attractive features of microalgae is to trap gaseous CO_2 in ponds or photobiorectors and have higher photosynthetic efficiencies than terrestrial plants. CO_2 dissolves in water and exists in the form of CO_2, HCO_3^-, $H_2CO_3^-$, and CO_3^{2-}. Among them, microalgae transport CO_2 and HCO_3^- into cell for photosynthesis (Zhao and Su 2014). Features of microalgae such as high protein, lipid, and carbohydrate contents make them attractive feedstock for biofuel production (Pavlik et al. 2017). The algal lipids can be extracted and converted to biodiesel to be used as biofuel (Mondal et al. 2017). The potential benefit of making biofuel from algae is it reduces lifecycle of greenhouse gases (GHG), as algae biomass converts CO_2 through photosynthesis into bio-plant material which is eventually released back to the atmosphere via microorganisms when used as a fuel, via engine tail-pipe emissions (Shirvani 2012).

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An Integrated Approach of Wastewater Mitigation and Biomass Production for Biodiesel Using *Scenedesmus* sp.

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1 Introduction

Rapid depletion of fossil fuel reservoirs along with eutrophication of organic, inorganic contaminants and heavy metals in water bodies has led to energy crisis and water pollution aggravation (Shen et al. 2015; Gupta et al. 2017b). According to US Energy Information Administration, the consumption rate of petroleum has increased by 6.3% from 2007 to 2013 (Cheah et al. 2016). Further, given the growing global population and economic growth, the worldwide energy consumption rate is estimated to climb to 49% in 2035 (Bhatt et al. 2014). Indeed, such elevated utilization of existing petroleum resources is associated with an increased greenhouse gases emission resulting in the deterioration of air quality (Ben-Iwo et al. 2016). Parallel to the fuel shortage, the generation and release of wastewater from industries, domestic and agricultural sources at an unwarranted rate into the water bodies is becoming a global threat to aquatic species and mankind (Posadas et al. 2015). According to a report by the United Nations World Water Development 2017, FAO's AQUASTAT database estimated that globally the freshwater consumption occurs at rate of 3928 km^3 per year (Koncagül et al. 2017). Out of which, 44% of water is consumed by agricultural sector, whereas the remaining 56% is released in the form of municipal, industrial and agricultural drainage (Koncagül et al. 2017). It was also estimated that around 80% of wastewater is released without requisite

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treatment in the environment (WWAP 2012; Water 2015). The present conventional approaches for wastewater treatment constitute several steps such as primary, secondary, tertiary treatment and disinfection (Rawat et al. 2016). Among these, the secondary treatment requires additional aerobic technologies for providing ample amount of oxygen to bacterial species which are responsible for performing degradation of organic contaminants (Rawat et al. 2016). Furthermore, chemical treatment in tertiary step for removal of ions and performing chlorination for disinfection of wastewater are expensive, and they lead to production of large amount of sludge (Rawat et al. 2016; Gupta et al. 2017b). On the other hand, the biological tertiary treatment also adds on to the cost of wastewater mitigation (Abdel-Raouf et al. 2012).

This scenario calls for a paradigm shift towards an integrated approach for production of bioenergy coupled with wastewater treatment. Photosynthetic microalgae-based biodiesel production has emerged as one of the most propitious options. Microalgae-derived biodiesel does not have any controversial status like first- and second-generation biofuels which suffer with limitations such as competition with edible crops and requirement of arable land, respectively (Hwang et al. 2016). Moreover, small generation time and high oil yield (up to 100 times greater than oil seeds) of oleaginous microalge in comparison to terrestrial plants make the former as one of the best sources for biodiesel feedstocks and other coproducts (Jämsä et al. 2017). Other auxiliary advantages include its adaptive nature in diverse environment (high temperature/salt/light tolerance), ability to remediate wastewater, CO_2 sequestering capacity (1.7–2.4 tons of CO_2 per ton of microalgal biomass) and reduced greenhouse gases emissions on combustion (Sheehan et al. 1998; Chisti 2007; Zhou et al. 2014; Gupta et al. 2017b).

Despite such advantages, cultivation of microalgae for biodiesel production suffers certain economic limitations. Production of 1 Kg of algal biodiesel requires around 3726 Kg of water with 0.33 Kg of nitrogen and 0.71 Kg of phosphorus, which makes it a non-viable and costlier process (Yang et al. 2011). The cost analysis on microalgal biodiesel shows that production of biodiesel at a price of $200 per barrel is only feasible if the other products associated with microalgal biomass are commercialized (William and Laurens 2010). Additionally, the requirement of nutrients, large amount of fresh water, unavailability of energy-efficient techniques for harvesting and extraction of lipid form biomass are the major constraints with microalgae-based biodiesel production (van Beilen 2010; Zhou et al. 2012, 2014). In this context, the utilization of wastewater for microalgal biomass cultivation is one of the feasible ways to cut down the cost associated with it. Microalgae utilize the nutrients and organic matter present in the wastewaters for biomass generation along with lipid accumulation, which can be converted to biodiesel (Gupta et al. 2017b). Coupling wastewater remediation with microalgal cultivation has the potential to reduce the life cycle impacts and associated costs (Álvarez-Díaz et al. 2017). Further, the ability of photosynthetic microalgae to produce oxygen can be harnessed in algal-bacterial consortium avoiding cost of aeration (Oswald 2003). The reduced emission of harmful gases, reduction in hazardous solid sludge production and recycling of nutrients along with biofuel production are the other benefits of this integrated approach (Green et al. 1996; Gouveia et al. 2016). Numerous microalgal species such as *Chlorella* sp. (Lu et al. 2015; Wang et al. 2015; Gupta et al. 2016), *Scenedesmus* sp. (Sacristán de Alva et al. 2013; Shin et al. 2015; Gupta et al. 2016), *Neochloris* sp. (Levine et al. 2011), *Chlamydomonas* sp. (Arora et al. 2016) and *Cosmarium* sp. (Daneshvar et al. 2007) have been found to be potential entities for wastewater treatment coupled with biodiesel production. Among the explored microalgal species, *Scenedesmus* sp. is well documented for its rapid growth rate in wastewater, high biomass and lipid productivity (Table 1).

The present chapter is a comprehensive overview of the integration of wastewater remediation with biodiesel production using microalgal strains of *Scenedesmus* sp. genera (Fig. 1). The chapter provides historical perspective of wastewater using microalgae, the phylogenetic classification of *Scenedesmus* sp. genera and their inherent capability to thrive in different wastewaters. The studies reported till date using *Scenedesmus* sps. For different wastewater mitigation ranging from agro-industrial, industrial and domestic and heavy metal mitigation along with biodiesel production has been systematically catalogued to highlight the research gaps for successful deployment of this integrated technology.

1.1 Historical Perspective of Wastewater Treatment and Biodiesel Production by **Scenedesmus** *sp.*

The cultivation of microalgae for bioremediation of different wastewater was initiated 70 years ago (Oswald et al. 1953, 1957; Abdel-Raouf et al. 2012). The first successful implementation of microalgal cultivation in wastewater was proposed by Oswald in early 1950 (Oswald et al. 1953). The researchers inoculated *Euglena gracilis* in open pond to treat domestic sewage along with bacterial species. The motivation behind this application was to provide ample amount of oxygen to bacterial species generated by microalgae during photosynthesis (Oswald et al. 1953, 1957). Later in 1974, Palmer surveyed around 60 genera and 80 species of microalgae isolated from various waste stabilization ponds (Palmer 1974). The identified and catalogued tolerant algal species against different harmful wastes were found to be *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*, respectively (Palmer 1974). Among them, *Scenedesmus* and *Chlorella* are the most explored species due to their prevalence in various types of wastewaters and their high growth rate, tolerance towards toxic environment with high lipid accumulating capacity (Wang et al. 2015; Kim et al. 2016). Initially, removal of nitrogen and phosphorus with biomass generation and consequent harvesting was studied using *Scenedesmus* and *Chlorella* sp. (Witt and Borchardt 1960; Bogan et al. 1961). Between the two species, *Scenedesmus* has been reported to be more resistant towards grazers such as protozoans, avoiding such contamination in open treatment ponds (Borowitzka 2013).

Table 1 Summary of the wastewater mitigation profiling of *Scenedesmus* sp.

Microalgae	Wastewater	Dilution	Dry cell weight (g/L)	Lipid content (%)	Removal (%)			References
					TN	TP	COD	
S. obliquus	Piggery	–	0.24	27	60	83	–	Ji et al. (2013)
	Urban +olive mill	50%	0.3	15.3	–	–	–	Hodaifa et al. (2013)
	Synthetic brewery	–	0.75	–	11	–	68.8	Mata et al. (2012)
	Urban secondary effluent	–	148	15	100	100	–	Ruiz et al. (2014)
	Municipal +1% food wastewater	–	0.41	18.4	97	89	74	Ji et al. (2015)
	Pharmaceutical	–		–	97	100	99.6	Yu et al. (2014)
	Olive mill	20	–	36	–	–	64.8	Hodaifa et al. (2012)
	Raw sewage	–	3.45	23.26	98.5	97.9	76.13	Gupta et al. (2016)
	Artificial	–	0.66	–	73	–	–	Voltolina (2015)
	Municipal	–	1.46	–	36.26	99	–	Zhang et al. (2014)
	Synthetic brewery	–	0.90	27	20.8	56.9	57.5	Mata et al. (2013)
S. quadricauda	Swine	50%	–	–	98.4	99.5	–	Gantar et al. (1991)
	Starch	–	3.36	27.8	–	–	58.4	Zhao et al. (2012)
	Campus sewage	–	1.09	27.36	89.73	92.98	–	Han et al. (2015)
S. bicellularis	Tertiary treated	–	–	–	100	89	–	Kaya et al. (1996)
Scenedesmus sp. LX1	Secondary effluent	–	–	35	90	99	–	Xin et al. (2011)
	Domestic	–	0.11	33	98	98.5	–	
	Effluent of electronic device factory	–	0.24	–	46	100	–	Zhen-Feng et al. (2011)

(continued)

Table 1 (continued)

Microalgae	Wastewater	Dilution	Dry cell weight (g/L)	Lipid content (%)	Removal (%)			References
					TN	TP	COD	
Scenedesmus sp.	Fermented swine	3%	0.19	–	87	83.34	–	Kim et al. (2007)
	Anaerobically digested piggery	5%	1.18	31.60	82	53	–	Jia et al. (2016)
		10%	1.22	30.99	82	88	–	
		15%	1.34	26.29	91	87	–	
	Wet market	–	–	–	74	79	–	bte Jais et al. (2015)
Scenedesmus sp. *MU1*	Cellulosic ethanol	–	1.1	21.4	71.6	47.1	–	Zhang et al. (2017)
Scenedesmus sp. *ZTY2*	Domestic	–	0.4	55.3	14	35.4	40.8	Zhang et al. (2013)
Scenedesmus sp. *ZTY3*		–	0.45	70	9.8	32.7	39.4	
S. acutus	Municipal (pre)		0.08	28.3	94	66	77	Sacristán de Alva et al. (2013)
	Municipal (post)				92	64	–	
	Urban	–	3	28.8	98.2	–	–	Doria et al. (2012)
S. bijuga	Anaerobically digested food	1/10	1.18	35	73.3	73	–	Shin et al. (2015)
		1/20	1.49	30.8	86.8	90	–	
		1/30	1.30	33.8	85.3	85	–	
S. dimorphous	Agro-industrial	1:1	–	–	95	57	–	González et al. (1997)

To improve the process of waste treatment and biomass production by microalgae, Oswald and Golueke proposed high rate algal ponds (HRAP) in 1966 (Oswald et al. 1966). Further, a large-scale study with HRAP suggested the concept of using multistage algal system to fully exploit its potential to remove nitrogenous waste from industrial wastewater as well as efficient algae harvesting system (Bosman and Hendricks 1980). Along with nutrient removal, many studies also suggested potential of microalgae for removal of total coliforms and *E. coli* in HRAP (Shelef et al. 1977; Sebastian and Nair 1984). Around 99% reduction in the total coliform load in municipal wastewater was reported by using mixture of microalgae having predominance of *Scenedesmus dimorphus*, *Micractinium quadrisetum*, *Phytoconis* sp. and *Oocystis solitaria*, respectively (Shelef et al. 1977). In an another study by Sebastian and Nair 1984, the efficiency of *S. obliquus* for the total removal of coliform and disinfection of domestic sewage in HRAP was explored (Sebastian and

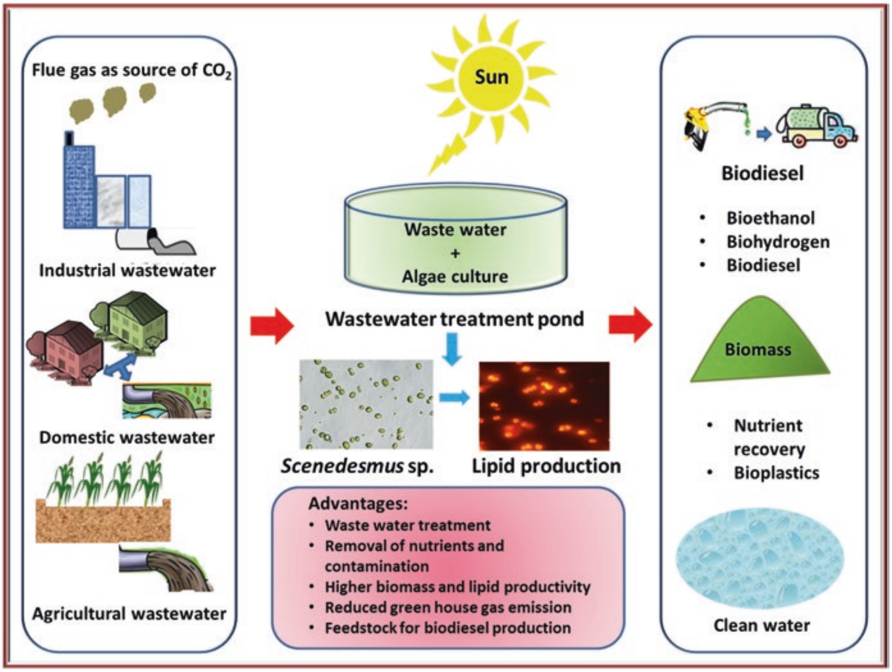

Fig. 1 Schematic showing the integration of wastewater mitigation and biodiesel production using microalgae

Nair 1984). This study suggested that an intermittently fed system comprising of 0.25 d^{-1} dilution rate at pH 11.0 for 2-day contact time is essential for 100% coliform removal.

Interestingly, numerous studies have illustrated the capability of various microalgal species to grow faster in heterotrophic mode which makes them more capable to utilize the organic wastewater, thus stimulating higher biomass and lipid productivity (Zhou et al. 2014). It is noteworthy that appropriate molar ratio of C:N:P (carbon/nitrogen/phosphorous) in wastewater plays an important role in biomass production. Assuming the general composition ($C_{106}H_{18}O_{45}N_{16}P$) of microalgae, N:P ratio of 16:1 (Redfield ratio) in wastewater would result in its optimum growth (Klausmeier et al. 2004; Ji et al. 2014). This N:P value can vary from 8:1 to 45:1, depending upon the species. For instance, *Scenedesmus* sp. requires N:P ratio of 30:1 to grow without any inhibition (Salama et al. 2017). Along with removal of nutrients from wastewater, microalgae are reported to accumulate good amount of lipid in comparison to when grown photoautotrophically (Chen et al. 2015). This may be due to the prevalence of carbon sources such as glycerol, acetate and glucose as main constituent of wastewater (Arora et al. 2016). Higher amount of nutrients in initial phase of cultivation lead to rapid growth of microalgae and faster depletion of nutrients in wastewater, which causes nutrient deprivation in later stage and henceforth induces lipid accumulation in microalgae (Arora et al. 2016). Further, due to nonmotile nature of oleaginous microalgae, they tend to produce lipid bodies as buoyancy compensators which regulate their position in appropriate part of the water, where they can get sufficient amount of nutrient and light. This mechanism is one of the possible reasons behind production of high amount of lipid content in microalgae under physiological stress (Gupta et al. 2017b). Microalgal cultivation in heterotrophic mode requires additional carbon source, and it is operated in dark condition (Amaro et al. 2011). Moreover, mixotrophic cultivation is combination of both autotrophic and heterotrophic condition where microalgae carry out photosynthesis in the presence of light and utilize carbon sources such as glycerol, glucose and acetate (Walker 2011; Yeh and Chang 2012; Cabanelas et al. 2013). This combined mode has many advantages in comparison to obligate photoautotrophic mode of microalgal cultivation, and it supports cultivation of microalgae in wastewater (Zhou et al. 2014). For example, *S. obliquus* showed four times increase in ammonium uptake when acetate was supplemented in autotrophic medium, suggesting positive impact of mixotrophic cultivation mode on nitrogen assimilation enzymes (Combres et al. 1994). Heterotrophic growth of *Scenedesmus* sp. was also reported in oil mill effluent with biomass productivity of 120 mg/L/D and maximum lipid content of 164 mg/L, respectively (Di Caprio et al. 2015).

Further, closed systems, e.g. photobioreactors (PBRs), were introduced to overcome the risk of contamination associated with open systems. Although PBRs provide a controlled environment, they require high capital and maintenance cost. The basic form of PBRs was devised by Davis (Davis et al. 1953), whereas the modern systems are generally based on designs of Pirt (1983). Recently scale up of PBR is widely studied for treatment of wastewater integrated with biofuel production (Hwang et al. 2016). However, the difficulties in regulating temperature in these reactors are adding up to the cost (Pruvost et al. 2016). In order to test the feasibility for growth and lipid accumulation in *S. dimorphus* in domestic secondary effluent, 1.5 L bubble column photobioreactor was used, and in this study they obtained maximum biomass of 244 mg/L and lipid content of 26.06% with simultaneous removal of 98.04% of TN and 98.72% of TP (Zhou et al. 2014). Another study involves utilization of tubular airlift pilot photobioreactor for cultivation of *S. obliquus* in urban wastewater and resulted 90% removal of TN and 89% removal of TP (Arbib et al. 2013).

Recently, use of immobilized culture (biofilm-based reactor) for microalgal cultivation has gained interest worldwide (de la Noüe and Proulx 1988; Wang et al. 2016). In contrast to suspended culture in PBRs, biofilm-based reactors have certain advantages of high nutrient removal efficiency, high carbohydrate/lipid production, low-energy cost and requirement of land space and less water resources (Robinson et al. 2001; Wang et al. 2016). Capability of *S. acutus* and *S. obliquus* immobilized in kappa-carrageenan for removal of nutrients from secondary effluent was investigated by Chevalier and de la Noue. The authors reported a 90% removal of ammonium in first 4 hours and almost complete removal of phosphate within 2 hours (Chevalier and de la Noüe 1985). *Scenedesmus* cells immobilized in chitosan beads were also investigated for removal of phosphate and nitrate (Fierro et al. 2008).

Other studies include utilization of flat surface alginate beads for entrapment of *S. bicellularis* for removal of ammonium and phosphates (Kaya et al. 1995) and *S. rubescens* entrapped within two-layered system comprised of nitrocellulose membrane and glass fibres for removal of ammonium, phosphates and nitrates (Shi et al. 2007). Various studies have also suggested the utilization of hyper-concentrated algal culture (biomass > 1.5 g/L) and flocculants such as chitosan to reduce the cost and ease the harvesting of the algal biomass (Lavoie and de la Noüe 1985; Gupta et al. 2017a). These studies established the utilization of *Scenedesmus* sp. for treatment of wide variety of wastewater coupled with lipid production offers an environmental and cost-effective approach in terms of nutrient recycling and biofuel production. However, construction of engineered microalgae with improved qualities and harnessing their capability in advanced wastewater-based cultivation systems needs to be explored in detail.

1.2 Evolution and Classification of **Scenedesmus** *sp.*

The first species of the genus *Scenedesmus* was described by Turpin in 1828 and placed in family *Scenedesmaceae* by Oltsmanns in 1904 (Hegewald 1997). The genus name was given by Meyen in 1829 and studied by many researchers for more than 100 years using light microscopy (Hegewald 1997). Initially in 1988, there were around 1300 taxa described under these genera (Hegewald and Silva 1988). However, to better understand the different taxa under these genera, Chodat (1926) revised the genus and divided them into few subgenera (Chodat 1926), and these subgenera were further reduced to three, namely, *Scenedesmus*, *Acutodesmus* and *Desmodesmus*, which were then largely accepted by other group of scientists (Hegewald 1978). However, Komarek (1983) treated some taxa as separate genera such as *Tetradesmus*, *Raysiella* and *Pseudotetradesmus* (Komárek 1983; Hegewald and Wolf 2003). *Scenedesmus* sps. mainly inhabit freshwater lakes and pond throughout the world but rarely present in brackish waters (http://www.algaebase.org/). The distinguishing characteristic of genus *Scenedesmus* is defined as flat or moderately curved coenobia of 2–32 cells that are arranged in 2–3 rows (Toledo-Cervantes et al. 2018). The cell shape can vary from spherical to ellipsoidal or elongated fusiform (Hegewald 1997; An et al. 1999; Hegewald and Wolf 2003). According to Hegewald, *Acutodesmus* is distinguished by the presence of acute cell poles, while *Scenedesmus* and *Desmodesmus* have obtuse/truncated poles (Hegewald and Wolf 2003). Furthermore, smooth hemicellulosic and sporopolleninic layered cell walls are present in *Acutodesmus* and *Scenedesmus*, while *Desmodesmus* is reported to have some microscopically visible granulations or dents also called spines (An et al. 1999). The microalgal cells are generally autosporic in nature, uninucleate, with one chloroplast and one pyrenoid (Komárek 1983; Trainor 1996).

Initially the species were categorized in three subgenera based on their cell wall ultrastructure studied by electron microscopy (Hegewald et al. 1990; Hegewald and Schnepf 1991). On the basis of electron microscopic studies, species with four sporopolleninic layers with a tiny ornament on the outermost layer were placed in *Desmodesmus*, whereas species with only three sporopolleninic layers without any structure were placed in *Scenedesmus* and *Acutodesmus* (Hegewald 1978; Komárek 1983; An et al. 1999). Nonetheless this type of differentiation did not provide a clear picture about the phylogenetic relationship among the *Scenedesmus* and *Scenedesmus* like green coccoid species. To further establish the phylogenetic relationship among the species of the genera *Scenedesmus*, the study based on 18S r DNA and ITS (internal transcribed spacer) sequence was performed. The very first study with DNA and nuclear encoded rRNA internal transcribed spacer region (ITS2) sequences of *Scenedesmaceae* was done in 1986 (Paschma and Hegewald 1986) and published by An et al. (1999; Hegewald et al. 2013). The study showed that ITS2 ribosomal DNA from microalgae was a suitable candidate to analyse the evolutionary relationship among various taxonomic levels (An et al. 1999). The authors showed a clear difference between the two subgenera *Desmodesmus* and *Scenedesmus* and raised *Desmodesmus* to genus level (An et al. 1999).

In a study by Hegewald, the ITS2 sequence of *Scenedesmus obtusus*, *S. hindakii* and *Acutodesmus regularis* was investigated to find the closest species to *Scenedesmus arcuatus* (Hegewald and Wolf 2003). The phylogenetic analysis showed that on the basis of 18S rDNA data, *S. arcuatus* was not closely related to *S. hindakii*, whereas *S. obtusus and/or S. raciborskii* and *S. hindakii* were closely related and thus grouped under *Scenedesmus*. However, the suspected *S. arcuatus* was clustered separately with *A. regular* and *A. pectinatus*, without having any morphological similarity. Thus, *Acutodesmus* includes *A. acuminatus*, *A. wisconsinensis* and *A. obliquus*, whereas *S. arcuatus*, *A. pectinatus* and *A. regularis* clustered under *Scenedesmaceae incertae sedis* (Hegewald and Wolf 2003). Additionally, a study by Kessler et al. suggested that a mean similarity of 99.3% showed doubtful separation of the two subgenera *Scenedesmus* and *Acutodesmus* (Kessler et al. 1997). This study also revealed that the three taxa which were earlier assigned to the genus *Chlorella* actually belong to *Scenedesmus*. "*Chlorella*" *fusca* var. *vacuolata* and "*C.*" *fusca* var. *rubescens* were found to be closely related to *S. obliquus* which belong to *Scenedesmus/Acutodesmus*, whereas "*C.*" *fusca* var. *fusca* is closely related to *Scenedesmus communis* which belongs to *Desmodesmu*s. The RNA data of *Kermatia pupukensis* strongly suggests its close relationship with *Scenedesmus* and the subgenera *Desmodesmus* (Kessler et al. 1997).

To clarify the taxonomic relationships with the genus, karyological study of several *Scenedesmus* species was done (Dzhambazov et al. 2001, 2003). In several studies, *S. acuminatus* Chodat and *Scenedesmus pectinatus* Meyen were used interchangeably by many authors, but the certain differences between both species were highlighted by Hegewald (1978) and An et al. (1999). Balik Dzhambazov et al. carried out karyological study to understand the taxonomic relationship among *S. acuminatus* Chodat and *S. pectinatus* Meyen and also reported changes in *S. pectinatus* when it was transferred to high salt concentration media. Interestingly, increase in salt concentration caused an evolutionary transition of *S. pectinatus* towards *S. regularis* (Dzhambazov et al. 2006). This study clearly stated that *S. pectinatus*, *S. regularis* and *S. acuminatus* are three different species and should not be used interchangeably. Further to understand the taxonomic relationship between *Pectinodesmus*, *Acutodesmus* and *Scenedesmus* sensu lato, large set of strains were studied using rRNA gene sequences (ITS1/5.8S/ITS2) and cell wall ultrastructure/morphology (Hegewald et al. 2013). This study accepted *Acutodesmus* and two new genera, namely, *Verrucodesmus* and *Chodatodesmus*. The chronological events depicting the evolution of the *Scenedesmus* sp. family have been summarized in Fig. 2.

Although applicability of *Scenedesmus* sp. in field of biotechnology especially in area of wastewater treatment and biodiesel production has gained interest worldwide, this species has been given a little attention in terms of taxonomic issues involved with it (Toledo-Cervantes et al. 2018). Due to highly polymorphic nature of *Scenedesmus* sps., they are still not clearly separated under certain taxa and are continuously reallocated (Lürling and Van Donk 1997; Leliaert et al. 2012). Recent study by Akgül et al. also highlighted the urgent need of integrated study involving morphological, taxonomical as well as phylogenetic analysis for accurate delineation of members of *Scenedesmaceae* that can support easy identification of novel species under these genera (Akgül et al. 2017). Till date, there are approximately 119 taxonomically accepted species of *Scenedesmus* (http://www.algaebase.org/), out of which only 23 have their complete 18S rRNA sequences submitted in NCBI. The present scenario requires a better understanding of molecular relationships among

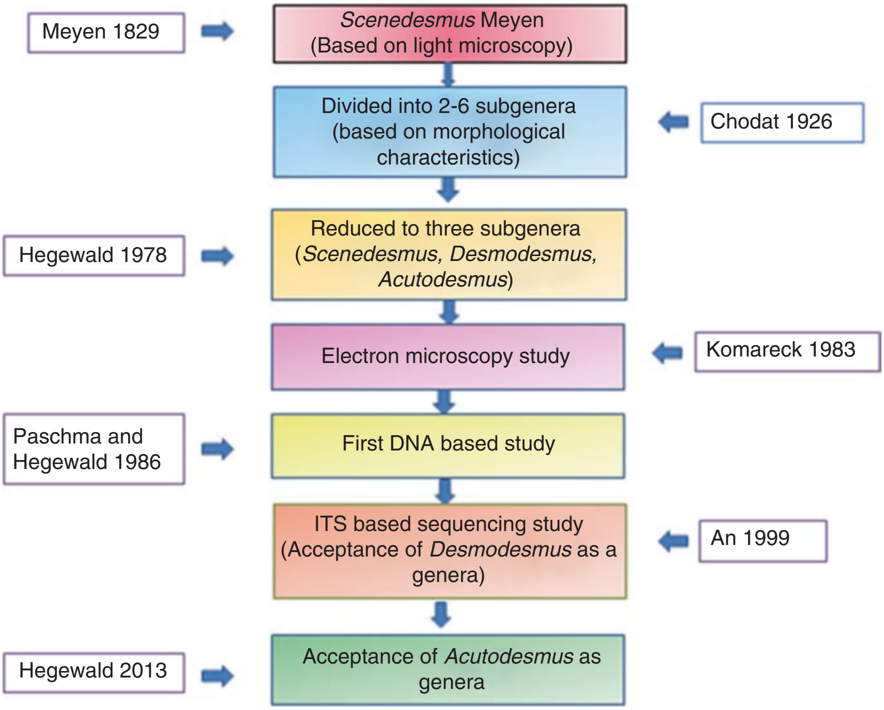

Fig. 2 Schematic showing the systematic classification of *Scenedesmus* sp.

different species to increase the information related to different genetic markers in the existing database. This will help to explore the novel species having better capabilities as well as can provide appropriate target sites that can be played with to improve their efficiency in integrated systems like wastewater treatment with biodiesel production.

2 Integration of Remediation of Different Wastewater and Biodiesel Production by *Scenedesmus* sp.

Depending on source of origin, wastewaters can be broadly classified as agro-industrial, industrial or domestic wastewater. A major difference between these wastewaters is that agro-industrial effluents have high amounts of nitrogen (particularly ammonia) and phosphorous content as compared to domestic or municipal sewage which are tertiary treated (Chen et al. 2015; Wang et al. 2016). On the other hand, industrial wastewaters essentally comprises of toxic heavy metals (Chen et al. 2015). However, irrespective of the wastewater selected for culturing microalgae, the biomass generated cannot be utilized as human food supplements or animal feeds due to toxicological concerns (Ji et al. 2013). This has diverted the research towards integration of wastewater mitigation with biodiesel production which has the potential to make the wastewater remediation and microalgae-based biodiesel production economically sustainable. Microalgae belonging to the genera, *Scenedesmus* sp., have the capability to efficiently acclimatize to different wastewaters along with efficient removal of TN, TP and COD (Table 1). Indeed, the efficiency of wastewater treatment by the microalga depends on various factors including growth rate, availability of nutrients, operating conditions and dissolved organic carbon (DOC) (Mata et al. 2012). A brief overview of different wastewater mitigation potential of *Scenedesmus* sps. Has been detailed in the following sections.

2.1 Agro-Industrial Wastewater

Agro-industrial wastewaters include effluents generated from: (a) anaerobic digestate from food and dairy processing industries; (b) animal husbandries such as swine/piggery, livestock, fish farming and slaughter house etc. (Wang et al. 2016). These wastewaters contain high amounts of ammonia which is favoured by the microalgae over nitrates and nitrites as it directly gets incorporated in the cells without any prior need for redox reaction, thereby consuming less energy (Ramanna et al. 2014). Nitrates and nitrites are only consumed by the microalga after complete exhaustion of ammonia from the growth medium. On the other hand, phosphorous reduction in the wastewaters involves assimilation by the microalga, precipitation and adsorption to the cell surface (Shin et al. 2015).

Piggery effluent is one of the major wastewater sources across the globe, especially in China and South Korea contributing to about 49 million tons (Ji et al. 2013). These wastewaters are traditionally treated using anaerobic digestion using bacteria (mesophilic or thermophilic) (Park et al. 2011). Although this treatment efficiently removes the organic carbon load and reduces the waste volume, the anaerobically digested effluent still bears high amounts of ammonia content. To date, *S. obliquus*, *S. quadricauda*, *S. accuminatus* and *Scenedesmus* spp. have been cultivated in piggery/swine wastewater for evaluating their potential for mitigation of TN, TP and COD, respectively (Table 1). Of various studies reported on wastewaters, Jai et al. have reported the integrated approach for bioremediation and biodiesel production (Jia et al. 2016). The authors utilized different dilutions of anaerobically digested piggery wastewater (5%, 10% and 15%). The maximum dry cell weight (1.34 g/L) was recorded in 15% dilution along with lipid yield of 26. 29% and lipid productivity of 27 mg/L/d (Jia et al. 2016). *Scenedesmus* sp. also showed an efficient TN removal (91%) and TP removal (87%) in 7 days. It is worth noting that the total nitrogen and in particular ammonia amount to not only the nitrogen consumed by the microalga for its growth but also losses to the air due to increase in pH during algal growth (Olguín and Sánchez-Galván 2012). Park et al. have also reported the efficiency of *S. accuminatus* to remove ammonia from anaerobically digested piggery waste under semi-continuous culture (Park et al. 2010). The authors reported that a seeding density of 1.5 g/L resulted in the maximum growth rate (56.6 mg/L/d) along with a total ammonia removal of 6.46 mg/L/d, respectively.

Food wastewater is associated to liquid food waste which is directly dumped into the oceans without any prior treatment or anaerobically digested (Shin et al. 2015). The adaptability and efficacy of *S. bijuga* to mitigate food wastewater along with biodiesel production was evaluated using different dilutions (1/10, 1/20 and 1/30) with municipal wastewater (Shin et al. 2015). Among the dilutions, the maximum TN (86.9%), TP (90%) and COD (66.4%) were obtained in 1/20 dilution along with a lipid productivity of 15.59 mg/L/d, respectively.

2.2 Industrial Wastewater

Depending on the type of industry, industrial wastewater varies significantly as individual sector generates its own mixture of pollutants. Industrial wastewaters contain low phosphorous and nitrogen along with high levels of heavy metals and toxic organic compounds such as phenols, polycyclic aromatic compounds, nitrogenous compounds, organic acids and oils which are difficult to remove (Abinandan and Shanthakumar 2015). Extensive studies have been reported on mitigation of different industrial effluents generated from olive mill, brewery, pharmaceutical, electronic device factory and tannery (Table 1). The olive mill wastewater (OMW) is generated after a three-phase extraction process of olive oil, which is high in organic matter and considerable amount of suspended solids (Hodaifa et al. 2013).

A mixture of urban wastewater (UWST) and OMW in different dilutions (0–10%; v/v) for increasing the biomass and lipid production of *S. obliquus* was utilized by Hodaifa et al. (Hodaifa et al. 2012). They reported that *S. obliquus* efficiently adapted to all the different dilution without any lag phase reaching a maximum biomass accumulation of 0.040 g/L in 10% OMV along with an average lipid accumulation of 33.7%, respectively. Moreover, the authors also evaluated the effect of daily dose of light in relation to growth of the microalga in OMW (Hodaifa et al. 2008). Microalgae are photosynthetic organisms that utilize light for their growth and lipid accumulation. Thus, to harness efficient mitigation and biodiesel production of microalgae, maximizing light harvesting is imperative (Wobbe and Remacle 2014). On cultivation of *S. obliquus* in 100% OMW for a period of 7 days, 12.9 $Em^{-2}d^{-1}$ daily dose of light showed maximum enhancement in the biomass (0.08 g/L) (Hodaifa et al. 2008). On the other hand, Di Caprio et al. utilized unsterilized OMV (9%; v/v) mixed with BG11 medium to cultivate *Scenedesmus* sp. and reported 0.35 g/L biomass with 32% of lipid yield along with 36% COD removal in 8 days (Di Caprio et al. 2015). Moreover, they reported a 6% reduction in total phenols present in OMW by the microalga which is crucial when mitigating industrial wastewaters.

Pharmaceutical wastewaters contain toxic compounds that are not completely degraded and hence not removed before being discharged into the aquatic bodies posing threat to all the living forms (Yu et al. 2014). To date, among the *Scenedesmus* genera, only *S. obliquus* has been studied for its potential for bioremediation of 16 pharmaceutical wastewaters collected from China (Yu et al. 2014). The microalga was co-cultivated with bacterium (*Vibrio fischeri*) showed exceptional removal efficiencies for total suspended solids (98.8 %), COD (99.6 %), NH_3-N (97.1 %) and TP (100 %). Brewery wastewaters are characteristically composed of sugars, soluble starch, alcohols and volatile fatty acids that are easily degradable by microalgae (Raposo et al. 2010). Cultivation of *S. obliquus* in synthetic brewery wastewater resulted 57.5% COD, 20.8% TN and 56.9% TC removal in 14 days (Mata et al. 2013). The microalga also accumulated 27% lipid content which was used for biodiesel production.

2.3 Domestic Wastewaters

Wastewater resulting from toilets, food preparation, laundry, cleaning of utensils, etc. is termed as domestic wastewater (Mara 2004). The domestic wastewater is grey turbid in colour and has an offensive odour along with suspended solids, soaps, fats, pesticides, etc., thus having a high COD ranging from 40 to 371 mg/L depending on the source (Jefferson et al. 2000). Different types of wastewaters including tertiary treated, municipal, urban, raw sewage and campus sewage have been utilized for cultivating various *Scenedesmus* sps. (Table 1). *S. obliquus* cultivated in artificial municipal wastewater having six different total organic carbon (TOC) concentrations (0, 20, 40, 60, 80 and 120 mg/L) showed an increase in dry cell weight with the TOC, reaching maxima of 0.47 g/L within 8 days along with its complete removal from the wastewater (Shen et al. 2015). Furthermore, the microalga also efficiently removed TN (14.16 mg/L/d) and TP (2.93 mg/L/d) from the wastewater along with accumulation of 11.9% lipid content (Shen et al. 2015). The authors extended their study by investigating the effect of CO_2 supplementation on nutrient removal. They reported that sparging the wastewater with 10% CO_2, though reduced the algal biomass by 5%, the TN and TP removal rates reached 99.6% and 88% with an overall augmentation of total lipid content to 17.6%. Unfortunately, when *S. obliquus* was cultivated in real secondary wastewater, the microalga failed to grow with lipid productivity of only 9 mg/L/d (Shen et al. 2015). Interestingly, the TN, TP and TOC were removed by 97.8%, 95.6% and 59.1% in only 6 days.

In another study, *S. obliquus* was cultivated in municipal wastewater (MWW) appended with food wastewater (0.5–2%) and flue gas CO_2 (5–14%) (Ji et al. 2015). Assessment of microalga growth showed maximum DCW (0.44 g/L) in MMW + 1% food wastewater +10% CO_2 due to favourable TOC (103 mg/L). Further, the removal of TN ranged between 83% and 97%, TP (82–89%) and TOC (58–74%) along with lipid accumulation of 18.4% corresponding to 9.80 mg/L/d lipid productivity (Ji et al. 2015). In a recent work, the applicability of *S. obliquus* for treatment of raw sewage and biodiesel production has also been illustrated (Gupta et al. 2016). Different concentrations of raw sewage (25, 50, 75 and 100%) were used by diluting in post-chlorinated effluent, with microalga cultivated in 50% raw sewage showing maximum DCW (3.81 g/L) along with 70.56% COD, 97.29% TN and 89.72% TP removal. Moreover, they reported nearly complete removal in the total faecal coliform from the wastewater. Further, a total lipid yield of 26.59% was reported in 50% raw sewage. On a similar note, *S. acutus* has been cultivated in pre- and post-treated municipal wastewater discharges for evaluating its potential for wastewater bioremediation and biodiesel production (Sacristán de Alva et al. 2013). In pre-treated municipal wastewater, removal TP, TN and COD were observed to be 66%, 94% and 77%. Whereas, for post-treated the same componentes were reduced to 64%, 92% and 48% respectively. The authors have reported a maximum lipid accumulation of 28.3% in pre-treated wastewaters.

Additionally, *S. quadricauda* was cultivated in campus sewage which has similar composition like domestic wastewater in conjunction with biodiesel production (Han et al. 2015). The campus sewage had characteristics of COD (240 mg/L), TP (2.40 mg/L) and TN (97 mg/L) which was efficiently mitigated by *S. quadricauda* in 16 days with removal of 89.73% (TN) and 92.98% (TP), respectively (Han et al. 2015). Further, when recycled campus sewage was again utilized to grow the microalga, 50.74% nitrate removal efficiency with 100% TP removal was reported. Interestingly, the lipid content (~27%) remains unaffected in the raw and recycled campus sewage, but due to low biomass obtained in the recycled wastewater (0.37 g/L) as compared to the raw effluent (1.09 g/L), the overall lipid productivity decreased in the recycled wastewater (Han et al. 2015). It was also interesting to note that *Scenedesmus* sp. ZTY2 isolated from artificial domestic wastewater when cultivated under heterotrophic conditions (dark) in real domestic wastewater resulted in biomass production (45 mg/L) and removal efficiency: COD (40%), TN

(14%) and TP (32.7%) along with 69% total lipid yield in 11 days, respectively (Zhang et al. 2013).

In a recent study, ultraviolet (UV) and ethyl methanesulfonate (EMS) mutants of *Scenedesmus* sp. were evaluated for the mitigation of cellulosic ethanol wastewater and biodiesel production (Zhang et al. 2017). Cellulosic ethanol wastewater contains various inhibitory compounds such as humic acids, furfurals, phenolic, aldehydes and aliphatic organic (Zhang et al. 2017). Among the mutants (MU1, MU2 and MU15), maximum biomass was achieved in MU1 (1.07 g/L) with lipid content of 21.4% along with removal efficiency of 33.5, 71.2% and 46.4% for COD, TN and TP, respectively.

3 Mitigation of Heavy Metals by *Scenedesmus* sp.

Rapid industrialization has led to escalation in the levels of toxic and carcinogenic heavy metal discharges into the aquatic bodies, disturbing the natural geochemical cycle of metals along with posing threat to living organisms. The conventional techniques deployed for mitigation of these heavy metals suffer from a number of drawbacks including high operational costs, expensive mineral adsorbents toxic discards and disposal of metal sludge (Sari et al. 2011). In this regard, bioremediation of heavy metals by microalgae has emerged has a sustainable and eco-friendly tool (Suresh Kumar et al. 2015). Microalgae have a high growth rate leading to high adsorption/absorption of the heavy metals, reusability, no production of secondary products, short operation time and metabolism of heavy metals into less toxic forms (Ben Chekroun and Baghour 2013). Bioremediation by microalgae is a two-level process, a fast biosorption of heavy metal onto algal surface by interacting with functional groups (amino, hydroxyl, carbonyl and sulphate) followed by a slow bioaccumulation within itself (Sari et al. 2011; Suresh Kumar et al. 2015).

Scenedesmus sps. have also been exploited extensively for mitigation of various metals including copper, cobalt, nickel, cadmium, zinc, lead, chromium (III, VI), arsenic (III, V) and strontium as listed in Table 2. Copper (Cu), an essential plant micronutrient, plays a pivotal role in photosynthesis and respiratory electron transport (Kováčik et al. 2010). However, due to its unwarranted usage in pesticides and fungicides, its levels in the environmental have become toxic, making its removal essential (Ma et al. 2003; Kováčik et al. 2010). The maximum removal of Cu (99.91%) has been reported by *S. abundans* when cultivated in growth media containing 10 mg/L of the metal (Terry and Stone 2002). *S. quadricauda* when cultured in *Trebouxia* medium supplemented with 25 μM copper chloride ($CuCl_2$) showed 45.9% removal within 24 hours (Kováčik et al. 2010). Further, they described that addition of salicylic acid (SA) in the growth medium stimulated the chlorophyll synthesis, thereby protecting it from toxic effects of Cu without affecting its accumulation inside the microalga. It has been also reported that addition of ethylenediaminetetraacetic acid (EDTA) and fulvic acid (FA) to the growth medium also reduces the toxic effects of Cu in *S. subspicatus* (Ma et al. 2003). Addition of EDTA

Table 2 List of heavy metals mitigated by *Scenedesmus* sp.

Microalgae	Metal	Metal concentration	IC_{50}	Removal efficiency (%)	References
S. subspicatus	Copper	–	5.4 mg/L	–	Ma et al. (2003)
S. obliquus	Cobalt	3 mg/L	–	94.5	Travieso et al. (2002)
		–	4 mg/L	–	Osman et al. (2004)
	Nickel	–	6.5 mg/L	–	
	Copper	–	0.13 mg/L	–	Hu et al. (2016)
	Cadmium	–	0.42 mg/L	–	
		50 mg/L	–	100	Chen et al. (2012)
		50 mg/L	–	93.39	Zhang et al. (2016)
		–	0.058 mg/L	–	Monteiro et al. (2009)
	Zinc	–	16.99 mg/L	–	
	Lead	50 mg/L	–	92	Abdel Ghafar et al. (2014)
S. quadricauda	Nickel	30 mg/L	–	98	Chong et al. (2000)
	Zinc	30 mg/L	–	98	
	Chromium (III)	10 μM	–	41.3	Kováčik et al. (2015)
	Chromium (VI)	10 μM	–	65	
	Copper	25 μM	–	45.9	Kováčik et al. (2010)
	Cadmium	10 mg/L	–	66	Mirghaffari et al. (2014)
	Lead	10 mg/L	–	82	
S. abundans	Cadmium	10 mg/L	15 mg/L	97.4	Terry and Stone (2002)
	Copper	10 mg/L	–	99.91	
S. incrassatulus	Cadmium	–	7.7 mg/L	–	Pena-Castro et al. (2004)
	Copper	–	6.9 mg/L	–	
	Chromium (VI)	–	2 mg/L	–	
		1 mg/L	–	43.5	Jácome-Pilco et al. (2009)
S. armatus	Cadmium	–	93 μM	–	Tukaj et al. (2007)
S. spinosus	Strontium	1 mg/L	–	76	Liu et al. (2014)
Scenedesmus sp.	Arsenic (III)	0.75 mg/L	196.5 mg/L	–	Bahar et al. (2013)
	Arsenic (V)	0.75 mg/L	20.6 mg/L	–	
Scenedesmus sp. IITRIND2	Arsenic (III)	500 mg/L	–	72	Arora et al. (2017)
	Arsenic (V)	500 mg/L	–	72	
Scenedesmus sp.	Copper	–	10 M	–	Tripathi and Gaur (2006)
	Zinc	–	25 μM	–	

(34 μM) to the microalgal cultures increased the EC_{50} value to 60 μM as compared to control (5.4 μM). However, the addition of two artificial sweeteners, sucralose (SUC) and acesulfame (ACE), in *S. obliquus* cultures did not significantly affect the Cu toxicity (Hu et al. 2016). Nalewajko et al. assessed pH tolerance in two different copper-tolerant strains of *S. acutus f. alternans* (B-4 and X-Cu) and showed that both the strains have different Cu tolerance mechanism (Nalewajko et al. 1997). X-Cu was an adaptive laboratory evolution strain and tolerated Cu by extrusion mechanism, while B-4 was a contaminated lake isolate that tolerated Cu by producing Cu-binding proteins and accumulating the metal in nuclear inclusions (Nalewajko et al. 1997). Between these two strains, B-4 was more acid tolerant as its EC_{50} value was pH 4.42, while for X-Cu it was pH 4.81, respectively.

Cobalt (Co) is also an essential micronutrient and is a component of vitamin B_{12}, cofactor of metalloenzymes (Osman et al. 2004). However, excessive levels of Co in the living system may result alteration in membrane permeability of the cells inhibiting the proper functioning. A rotary biofilm reactor for algae immobilization (BIOALGA) was engineered to remove Co from synthetic wastewater (Travieso et al. 2002). The authors reported that 3 mg/L of cobalt sulphate ($CoSO_4.7H_2O$) was removed 82% within 3 days, 93% in 7 days and 94.5% in 10 days, respectively.

Nickel (Ni) is a highly mobile toxic metal which is found in mineral processing, electronic, electroplating and steal alloys wastewaters (Osman et al. 2004). Biosorption of Ni by live *S. quadricauda* cells showed 97% removal within 5 min which increased to 99% once the contact time was enhanced to 300 min (Chong et al. 2000). The accumulation of Ni by *S. quadricauda* cultures differing in age; old culture (13 months) and young (1 month) showed that the old microalgal cells accumulated the metal twofold higher as compared to the young ones (Kováčik et al. 2016). This higher accumulation of Ni in old cultures was attributed to the change in the permeability which resulted in increased metal uptake.

Cadmium (Cd) is a non-essential/beneficial heavy metal and its highly toxic to all the life forms (Tukaj et al. 2007). Cd is reported to interact with thiol groups of the proteins, inhibiting many enzymes, thereby disturbing key metabolic pathways (Tukaj et al. 2007). The maximum (97.4%) Cd uptake has been reported by *S. abundans* when exposed to 10 mg/L of the metal and having an IC_{50} value of 15 mg/L (Terry and Stone 2002). The addition of ACE increased the tolerance of *S. obliquus* to Cd as EC50 value of 0.54 mg/L and 0.42 mg/L was reported in Cd + ACE and only Cd cultures (Hu et al. 2016). Such alleviation of the Cd toxicity in the microalga was due to the change in the permeability by addition of ACE which promotes the metal transmembrane movement. The elevation in the CO_2 levels (2%; v/v) in the *S. armatus* cultures also reduced the toxicity of Cd due to increase in the pH which lead to Cd complexes formation, rendering them inaccessible to cells (Tukaj et al. 2007). The biosorption of Cd by *S. obliquus* (0.6 g/L) at pH 6 and 30 °C resulted in 100% removal at concentration of 50 mg/L (Chen et al. 2012). Arsenic is a notorious metalloid which exists in inorganic (arsenite, arsenate) and organic forms (dimethylarsinic acid (DMA), monomethylarsonic acid (MMA)) (Wang et al. 2017). Based on human epidemiological data, it has been classified as Group 1 carcinogen with the European Union fixing a maximum limit of 10 μg ml^{-1} for its contamination in aquatic bodies (Arora et al. 2017). Recently, *Scenedesmus* sp. IITRIND2 showed ~72% arsenic (III, V) removal along with an increase in total lipid in the total lipid content (~ 50%, dry cell weight) (Arora et al. 2017). Additionally, maximum removal of zinc (98%), lead (98%) and strontium (76%) was reported for *S. quadricauda* and *S. spinous*, respectively (Table 2).

Interestingly, immobilized algal biomass in polymeric matrices has also been utilized for metal mitigation (Bayramoglu and Yakup Arica 2009; Bayramoglu and Arica 2011) Immobilization facilitates the reuse of the microalgal biomass, easy recovery of the biomass from the liquid and increase in metal removal as polymeric matrices also contain functional groups that bind to the metal (Bayramoglu and Yakup Arica 2009). Entrapment of *S. quadricauda* in calcium alginate beads showed 75.6 mg/g (Cu), 55.2 mg/g (Zn) and Ni (30.4 mg/g) in 5 days (Bayramoglu and Yakup Arica 2009).

4 Analysing the Alterations in Fatty Acid Methyl Esters (FAME) Profile of *Scenedesmus* sp. Cultivated in Wastewater

The fatty acid composition of microalgae varies depending with culture conditions such as growth media, physiological conditions, cultivation mode etc. (Demirbas 2011). The typical fatty acid composition of microalgal ranges from C12 to C20 with palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) as the major fatty acids which are similar to plant oils (Subramaniam et al. 2010). The changes in the FAME profiles of various *Scenedesmus* sps. cultivated in different wastewaters are presented in Table 3. Irrespective of the wastewater used for cultivation of any *Scenedesmus* sp., the FAME profile comprised of high proportions of C16:0 (19–57%) and C18:1 (15–38%) (Table 3). Biodiesel containing high amounts of saturated fatty acids such as C16:0 and C18:0 has been reported to exhibit high cetane number which ensures better cold start properties and minimizes the development of white smoke (Ramos et al. 2009). Further, high percentages of saturated fatty acids also lower the iodine value which has a maximum limit of 120 gI_2/100 g specified by European standards (Ramos et al. 2009). Moreover, an increase in the C18:1 fatty acid also contributes towards high cetane number, oxidative stability and low temperature property (Han et al. 2015). Interestingly, Shin et al. reported that as the dilution ratio of the anaerobically digested food wastewater increased, i.e. from 1/10 to 1/30, the percentages of C18:2 and C18:3 decreased, while C18:1 and C16:0 increased (Shin et al. 2015). A decreased C18:2 and C18:3 increases the oxidative stability, thereby enhancing the storage/shelf life of the biodiesel (Zhang et al. 2017). An extra precaution should be made towards the percentages of C18:3 and PUFA with ≥4 double bonds which has been restricted to 12% and 1% by European biodiesel standards, limiting the usage of most of the reported studies as potential biofuel feedstocks (Hodaifa et al. 2013) (Table 3). Based on the

Table 3 Overview of the FAME profiling of *Scenedesmus* sp. cultivated in wastewater

Microalgae	Wastewater	C14:0	C16:0	C16:1	C16:2	C16:3	C18:0	C18:1	C18:2	C18:3	C18:4	C20:0	C21:5	References
S. obliquus	Urban +olive oil	0.3	33.3	4.6	–	–	0	6.0	24.2	16.8	2.2	0.9	0.4	Hodaifa et al. (2013)
	Olive oil	0.57	24.2	4.60	2.51	0.48	6.03	39.4	12	6.55	0.75	0.38	–	(Hodaifa et al. (2008)
	Three-phase diluted olive	–	25	4.3	4	–	13.2	19.4	16.3	5.2	–	–	–	(Hodaifa et al. (2010)
	Synthetic brewery	–	47.78	–	–	–	8.60	21.59	11.42	10.61	–	–	–	(Mata et al. (2013)
S. bijuga	Anaerobically digested food (1/10)	0.40	22.89	5.78	–	–	1.14	26.95	19.53	23.22	–	–	–	(Shin et al. (2015)
	1/20	0.48	24.65	7.19	–	–	1.99	35.83	14.25	15.62	–	–	–	
	1/30	0.74	24.55	8.16	–	–	2.23	38.23	13.16	12.92	–	–	–	
S. quadricauda	Campus sewage (first use)	–	54.20	3.03	–	–	1.37	32.88	3.69	4.83	–	–	–	(Han et al. (2015)
	Campus sewage (second use)	–	57.07	1.19	–	–	0.91	34.02	3.0	3.82	–	–	–	
Scenedesmus sp. MU1	Cellulosic ethanol	0.37	19.99	1.28	–	–	0.98	14.72	21	23.43	–	0.08	–	(Zhang et al. (2017)
Scenedesmus sp. AMDD	Municipal	–	22.80	–	–	–	1.62	31.81	6.77	23.59	5.23	–	–	(Dickinson et al. (2013)
	Municipal + anaerobic digestate (1.6 X)	–	25.35	2.07	–	–	2.73	27.05	5.75	25.02	4.99	–	–	
	Municipal + anaerobic digestate (2.4 X)	1.53	19.38	1.01	–	–	2.40	19.92	6.92	30.90	4.45	–	–	

fatty acid profile, only *S. quadricauda* cultivated in campus sewage and *S. obliquus* grown in three-phase diluted olive-derived biodiesel complied with the international fuel standards.

5 Conclusions and Future Avenues

Microalgal cultivation in wastewaters integrated with biodiesel production is an economically viable alternative which has the potential to provide a sustainable solution to the global energy demands, curb the CO_2 emissions and remediate the wastewaters and heavy metals. Microalgae belonging to the *Scenedesmus* sp. genera have been exploited extensively for wastewater and heavy metal mitigation due to their capability to efficiently adapt and grow under stress conditions. Various *Scenedesmus* sps. Including *S. obliquus*, *S. quadricauda*, *S. abundans*, *S. acutus*, etc. have successfully established their prospective for mitigating agro-industrial, industrial and domestic wastewaters. However, very little amount of literature is reported the integrative potential of microalgal biomass generated for biodiesel production during wastewater treatment.

Indeed, there is long road ahead for making this integrated operando commercially successful which will require directed research. The first logical step should be examining *Scenedesmus* sps. strains under different wastewaters for high lipid accumulation and subsequent evaluation of the biodiesel potential. The cultivation of the selected strains should be then scaled up for larger biomass production in photobioreactors and open raceways ponds. For example, HRAPs have been demonstrated as one of the most economical outdoor systems for wastewater management and biodiesel production owing to their low capital and operational costs (Abinandan and Shanthakumar 2015). Further, due to uneven distribution of nutrients in the wastewater, mixture of effluents could be evaluated for balancing the TN, TP and COD content, thereby enhancing the biomass and lipid productivity of the chosen microalgal strain.

Overcoming the harvesting of algal biomass economically is another bottleneck towards the successful exploitation of wastewater mitigation and biodiesel production by microalgae. This could be achieved by testing of cost-effective flocculants which increase the auto-settlement property of the microalga. Nevertheless, extraction of all the coproducts such as proteins, ω^3 polyunsaturated fatty acids, sugars, etc. from the microalgal biomass at highest purity with no health hazards is quintessential for reducing the overall cost of production. Additionally, due to presence of high organic content in the wastewaters, microalgal culture crashes also pose a major hurdle. Algae-bacteria consortia can aid in enhanced wastewater removal along with maintenance of the desired microalgal strain. Certainly, this also requires a better understanding of algae-microbial (bacterium/fungal/yeast) symbiotic relationship, influence of grazers and parasitism on microalgal growth which will aid in formulating better control methods and thus warrants further research. Ultimately, an improvement in the entire integrated process is imperative for the full-scale implementation of wastewater remediation and biodiesel production.

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Microalgae and Wastewaters: From Ecotoxicological Interactions to Produce a Carbohydrate-Rich Biomass Towards Biofuel Application

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1 Introduction

The demand for potable water, as well as the treatment of water used in the various human activities, is considered the priority of the century (Cabanelas et al. 2013; Cuellar-Bermudez et al. 2017). Several biological processes are proposed for the treatment of domestic, industrial and agroindustrial wastewaters, such as bioremediation using microorganisms or even a microorganisms consortium – bacteria, microalgae, fungus and protozoa (aeration ponds, anaerobic lagoons, aerobic and anaerobic bioreactors, activated sludge, biological filters and biological nutrient removal) – as well as mycoremediation and phytoremediation (constructed wetlands, rhizofiltration, rhizodegradation, phytodegradation, phytoaccumulation, phytotransformation and hyperaccumulators) and vermicomposting or vermifiltration (Saemer 2015).

The advantages of exploring photosynthetic organisms for industrial applications, mainly in the environmental sector, are relevant for a sustainable future, as long as they contribute to the carbon cycle and, consequently, to the renewability of carbon sources. Moreover, the production of bioenergy must be significantly increased in order to compete with the energy production costs from other sources, namely, petroleum-based fuels (Cho et al. 2013). Furthermore, microalgae are a promising alternative as they present high growth rates when compared with higher plants, leading to greater biomass yields per area. Additionally, they are easily cultivated, with a wide variety of species being found in lakes and oceans, as well as

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having unique biochemical plasticity (being capable of changing their metabolism for the differentiated production of certain biochemical fractions, such as proteins, carbohydrates and/or lipids, depending on the environmental and nutritional cultivation conditions) (Silva and Sforza 2016; Vitova et al. 2015; Klinthong et al. 2015; Gonçalves et al. 2016b, Gao et al. 2016).

In the cultivation of microalgae and cyanobacteria, when using excess carbon gas in synthetic culture media, the main costs of the process are associated with the greater amount of water and gas, as well as to the nutrients added (mainly salts), and, in some cases, due to artificial light to avoid photosaturation/photoinhibition, despite the higher biomass production that might be reached. However, the valorisation of this biomass also has additional costs involved, such as the extraction and conversion/recovery step of the biomass/biomass fraction produced (Cho et al. 2013; Silva and Bertucco 2016).

Using urban and industrial wastewater would then help to eliminate the costs with the nutrients added to the culture media. Thus, the combination between wastewater treatment by algae and biofuel/biochemicals production can develop a technology to recover the contaminants (nutrients) and valorise the biomass produced.

Several effluents have been treated with microalgae/cyanobacteria, for example; whey protein concentrate (*Spirulina platensis* using 7.2% lactose and producing 60 mg L^{-1} d^{-1} of carbohydrate productivity) (Salla et al. 2016), whey cheese permeate (40% of culture medium, 1.9–3.0 g L^{-1} in 13 days of cultivation) (Girard et al. 2014), dark fermentation effluents ((*Chlorella vulgaris* ESP6, 0.25–0.3 g L^{-1} of lactate, formate, butyrate and acetate; 0.2 g L^{-1} d^{-1} of biomass productivity) (Liu et al. 2013), (*Scenedesmus subspicatus* GY-16, *Chlorella vulgaris* FSP-E and *Anistrodesmus gracilis* GY-09, 2 g L^{-1}; sodium acetate, 0.5 g L^{-1} d^{-1} of carbohydrate productivity) (Chen et al. 2016) and (*Chlorella sorokiniana*, 0.2–0.3 g_{carbon} L^{-1} of acetate and butyrate; 1.14 g L^{-1} in 10 days of cultivation) (Turon et al. 2015), glucose (*Chlorella sorokiniana*, 0.5–2 g L^{-1}; 0.1–0.6 g L^{-1} of biomass in 10 days of cultivation) (Juntilla et al. 2015), human urine (*Spirulina platensis*, urea 75 mg L^{-1} and 200 mg L^{-1} acetate; 1.7 g L^{-1} of biomass in 5 days) (Chang et al. 2013) and glycerol (*Chlorella pyrenoidosa*, 1%; 1.2 g L^{-1} of maximum biomass production with 60% of carbohydrate content) (Bajwa et al. 2016).

However, this approach aiming at carbohydrate accumulation in biomass is still not widely discussed in the literature, with studies mostly focusing on lipid accumulation. Nevertheless, the organic carbon together with the dissolved nitrogen and phosphorous in these effluents can become a relevant issue to carbohydrate accumulation by mixotrophy (autotrophy + heterotrophy – using wastewaters). With this in mind, this chapter focused on widening the discussion on the process of wastewater treatment, starting with a brief elucidation on the environment importance and on determining ecotoxicity of effluents, integrating important aspects which are frequently ignored when these microorganisms are cultivated, such as optimising the biochemical composition of photosynthetic organisms, for example, the carbohydrate fraction, as well as its importance on the valorisation of the biomass produced, combined with its environmental aspects (contaminants treatment performance, mostly chemical oxygen demand, nitrogen and phosphorus removal rate).

2 Photosynthetic Microorganisms and Ecotoxicological Experiments

The contact between untreated effluents and microalgae may be used as pollution indicators. Thus, the application of microalgae is not only restricted to the industrial sector, having several environmental roles, serving as food and as an oxygen source for aquatic species – phytoplankton (Hamed 2016). They have also been employed on ecotoxicity tests, aimed at evaluating the effects of natural or synthetic substances on living organisms, animals or vegetables. Thus, microalgae are considered a very important tool for environmental monitoring and for the treatability of these effluents (Magalhães and Ferrão Filho 2008). The experiments regarding the interaction of microalgae and ecotoxicology address the different ways in which these organisms act as a treatment form when exposed to contaminated environments. Some examples will be pointed out further ahead in this chapter.

Microalgae species (*Pseudokirchneriella subcapitata*) was used in growth inhibition tests in artificially modified cerium dioxide nanoparticle suspensions (nano-CeO_2). Several concentrations of the nano-CeO_2 stock solution were prepared, with concentrations ranging from 0.2 up to 25 mg CeO_2 L^{-1}. The nano-CeO_2 agglomerate exhibited little influence on microalgal growth inhibition, regardless of the suspension tested. Therefore, this nanomaterial presented low ecotoxicity towards microalgae, either if used on materials during cultivation or as part of the effluent to be treated (Manier et al. 2013). This microalga species was also used to assess the chronic toxicity of caffeine and its by-products on a study carried out by Zarrelli et al. (2014), which demonstrated that these compounds had no effect on the bioindicator organism.

This same species was used in a similar way by Booth et al. (2015), who studied the ecotoxicity of this organism using nanoparticles of cerium oxide (CeO_2) stabilised with poly(acrylic acid) (PAA-CeO_2) and the toxicity of pure PAA. This analysis was performed over a period of 72 hours, having then estimated the static inhibition of growth. The effective concentration – 50 (EC_{50}) of PAA-CeO_2 obtained in the results revealed inhibitions of growth of two to three orders of magnitude lower than the CeO_2 EC_{50} values presented in the literature and that the concentration of pure PAA in the exposure solutions was significantly inferior to the value of inhibition of growth EC_{10} (47.7 mg/L), indicating that it was not toxic in this study.

In 2005, Pavlic´, Vidakovic´-Cifrek and Puntaric published a study with different species of freshwater and marine microalgae in order to investigate the ecotoxicity of commercial surfactant products in shampoo (eight types) containing different combinations of these surfactants, then comparing the sensitivity of these algae to the products tested. Based on environmental effects, the surfactants and the shampoos used may be considered toxic to the *P. subcapitata* species, with some being considered very toxic towards *Scenedesmus subspicatus*, *Skeletonema costatum* and *Phaeodactylum tricornutum* species. In turn, marine microalgae were more sensitive to the surfactants tested when compared to the freshwater microalgae.

Several species of marine microalgae were used as bioindicators in the ecotoxicology experiments to predict growth inhibition when those were exposed to a tensioactive surfactant widely used in industries and on commercial products, *alquil etoxi sulfato* (AES), commonly released in aquatic environments. The contact of AES with the microalgae showed growth inhibition after 24 hours, for all species studied. The inhibiting effect was higher after 96 hours of exposure, especially for the following species: *Nannochloropsis gaditana*, *Chaetoceros gracilis* and *Dunaliella salina* (Sibila et al. 2008).

The compounds of TiO_2 and Pb are bioaccumulators, and their effects on the food chain were assessed using *Chlorella elipsoides*, microalga and copepod microcrustaceans of the order Cyclopoida. It was then verified that lead and titanium dioxide, individually or mixed, can be transferred through diet from algae to microcrustaceans (Moise and Mustapha 2018).

A mixture containing activated sludge with three microalgae genera (*Phormidium*, *Oocystis* and *Microspora*) was collected from a production tank of high algal concentration in order to analyse the potentials and limitations of microalgal-bacterial symbiosis in the removal of carbon (C), nitrogen (N) and phosphorus (P) from five different industrial effluents, originated from the industries of potato processing, fish processing, animal feed, coffee production and yeast production. The initial ratio of C/N/P in agroindustrial wastewater was then correlated with its biodegradability. It was perceived that the unbalance of these nutrients caused an inhibition of microalgal and bacterial growth resulting in either nitrogen or phosphorous residual when the carbon source was insufficient, vice versa, and the interaction between them, mainly CO_2-O_2 gaseous exchange; thus, a ratio of bacteria/microalgae biomass linked to the nutrient concentration must be adequate. It is a highlighted fact that adaptions to the effluents of these sectors before microalgal cultivation are highly necessary (Posadas 2014).

With these examples, an important question is raised regarding the treatability of raw effluents in microalgae environments (agroindustrial, chemical or emerging pollutants). In some cases, the ecotoxicological experiments showed low toxicity of certain industrial substances, while others showed almost entirely inhibited growth of these microorganisms.

This chapter will specifically discuss urban and agroindustrial effluents. These effluents have been widely disseminated in the production of microalgal biomass, stressing that in theory include all the nutrients required for microalgal development, though can also have limitations. A discussion showing the importance in considering the biochemical and cultivation aspects will be subsequently presented, conveying this approach of biomass production with high carbohydrate levels on the application of biofuel production processes.

3 Microalgae and Cyanobacteria

3.1 Biological Aspects and Classification

Microalgae are a polyphyletic group with a high diversity of organisms. Cyanobacteria (also known as phylum *Cyanobacteria* or *Cyanophyta*) are, at times, considered microalgae, though they are part of the oxygenic bacteria (prokaryotic organisms) group, presenting very distinct structural and metabolic characteristics. Thus, the designation “microalga” is restricted only to eukaryotic organisms (Silva and Bertucco 2016). Although they are widely remembered because of the flowering or bloom phenomenon, in which they release toxins highly prejudicial to animals and consequently human beings, they are commercially explored for several applications, such as biofuels, pharmacy and food, among others. *Spirulina*, *Chlorococcus*, *Gloeocapsa*, *Synechocystis* and *Synechococcus* are some examples of genera grouped into the cyanobacteria phylum, being researched mainly for industrial applications.

In turn, *Scenedesmus* (*Desmodesmus*, *Tetradesmus*), *Chlorella*, *Chlamydomonas*, *Nannochloropsis* and *Tetraselmis* are some examples of genera of (eukaryotic) microalgae. The taxonomy of these photosynthetic organisms has been based on their morphology, pigmentation and biochemical composition. However, there are still several factors which are undetermined or undergoing a validation process, given the constant changes proposed with the discovery of more modern techniques of genetic and evolutionary analyses. In fact, 25,000 species are estimated to have been recently discovered around the globe, but only approximately 70 species have been commercially explored (at an industrial scale), being of great importance in the production of food, food additives, animal feed, fertilisers and biochemicals.

3.2 Photosynthesis and Carbohydrate Accumulation

Photosynthesis is a vital process for all biocompounds, namely, biofuels, as the conversion of light energy into biomass may contain reserve products (carbohydrates and lipids, for instance) and a small amount of H_2. In green algae, the light-harvesting complex (LHC) (pigments such as chlorophyll and carotenoids) absorbs photons from solar or artificial light as chemical energy. This energy is used by photosystem II (PSII) for the catalytic oxidation of water to form protons, electrons and molecular oxygen. Low-potential electrons are transferred to the electron transport chain for the reduction of ferredoxin and subsequent formation of nicotinamide adenine dinucleotide phosphate (NADPH). An electrochemical gradient is then formed, and the release occurs after the oxidation of water in the thylakoid lumen, which is used to produce adenosine trisphosphate (ATP) by ATP synthase. Photosynthetic products (NADPH and ATP) are substrates to the Calvin cycle, where CO_2 is fixed as C_3 molecules (glyceraldehyde) that are assimilated to form sugars, lipids and other biomolecules essential for cell growth (Beer et al. 2009; Silva and Bertucco 2016).

The accumulation of carbohydrates occurs with the participation of cell compartments containing concentrated RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase), the main enzyme responsible for carbon dioxide fixation, and combined to help overcome the inefficiency of the process. Thus, RuBisCO has proved to be the main obstacle in the production of biomass, with its catalytic activity not being increased by the adjustments in the environmental and nutritional conditions of cultivation (Gimpel et al. 2013). In microalgae, these cellular compartments are the pyrenoids, while in cyanobacteria they are known as carboxysomes.

Inherently, the accumulation of carbohydrates plays some specific roles, leading to the imbalance between photosynthetic carbon reduction rate and the consumption rate of carbon reduced in the microorganism growth. Furthermore, carbohydrate accumulation helps microorganisms to survive at times of lack of nutrients and light (low amounts or photoperiods with long dark periods) (Raven and Beardall 2003).

Several environmental and nutritional factors can shift microalgal metabolism, increasing the action of certain enzymes in accumulating carbohydrates, for instance, light intensity, temperature, pH, carbon concentration, nitrogen concentration, phosphorus concentration and salinity (González-Fernández and Ballesteros 2012).

Under an industrial point of view, the efficiency of carbohydrate accumulation utilising light intensity and nitrogen concentration and the exposure time to these stress conditions (residence time in the reactor) have proved to be the quickest and with the lowest loss of biomass and carbohydrate productivity (Silva and Sforza 2016; Silva et al. 2017a, b, 2018a).

It is well known that nitrogen starvation/limitation is one of the most efficient methods for accumulation of energy reserves in microalgae (González-Fernández and Ballesteros 2012), including carbohydrates (Ho et al. 2013a); nitrogen limitation shifts the carbon fixed by Calvin cycle to produce other substances instead of nitrogen-based compounds (proteins), i.e. synthesis of carbohydrates and lipids (Vitova et al. 2015). Most green algae (Chlorophyta as *Chlamydomonas*, *Chlorella* and *Scenedesmus*) accumulate starch as carbon reserve and primary energy, whereas lipids serve as a secondary storage. As for cyanobacteria, the carbohydrate reserve is glycogen. It is also important to point out that the metabolic pathways of carbohydrate and lipid accumulation can interact depending on the nutritional and environmental conditions (Breuer et al. 2014; González-Fernández and Ballesteros 2012).

A schematic representation as to how atmospheric carbon gas (generally, dissolved in aqueous phase) reaches the synthesis of polysaccharides, mainly reserve polysaccharides, is demonstrated in Fig. 1.

4 Mixotrophy and Its Importance to Wastewater Treatment by Microalgae and Cyanobacteria

The need to diversify energy sources perceives wastewater treatment as a new potential. By using microalgae and cyanobacteria, wastewater can be treated and generate biomass. This biomass, in turn, may contain high levels of carbohydrates and lipids, which can be used on biotechnological applications, such as biofuels/biochemicals. For this, photosynthesizing microorganisms may rely on mixotrophy to effectively treat wastewater, which also still contains organic matter.

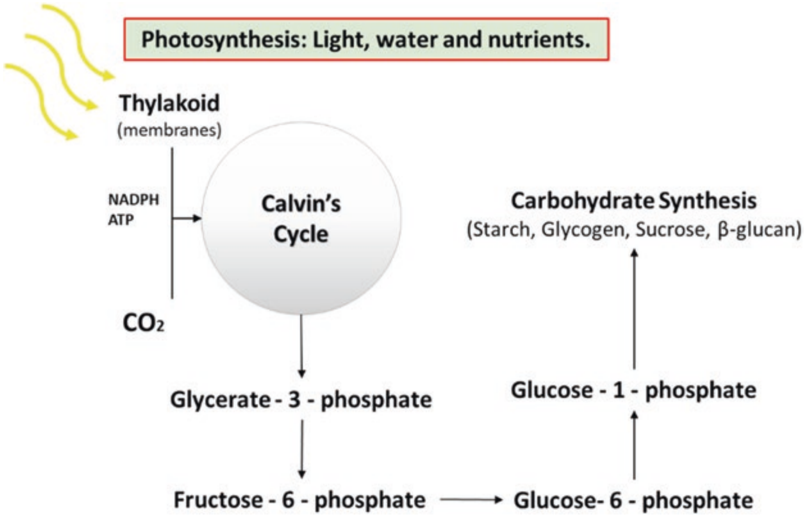

Fig. 1 Basic representation of the carbohydrate accumulation from photosynthesis in microalgae/cyanobacteria. (Adapted from: Jaeger et al. 2014; Silva and Bertucco 2016; Badary et al. 2018; Qiao et al. 2018)

A more general concept of mixotrophy is that it is the ability of both microalgae and cyanobacteria in carrying out photosynthesis (autotrophy) and heterotrophy combined, using either organic or inorganic carbon sources, thus, justifying the importance of wastewater treatment. Mixotrophy can be considered as having a combination of the advantages of both autotrophy and heterotrophy, as well as being able to overcome the disadvantages of autotrophy (Zhan et al. 2017).

Several studies have been carried out on mixotrophy, though scientists are still divided in what concerns the explanation for the high biomass rates reached by microalgae/cyanobacteria in relation to simple autotrophic and heterotrophic cultivation. Some authors suggest that microalgae mixotrophic growth rate is approximately equal to the sum of autotrophic and heterotrophic rates. Others consider that it is not simply the combination of both growth rates, but that one process may affect the other, resulting in higher biomass production (Salati et al. 2017; Zhan et al. 2017; Perez-Garcia and Bashan 2015). An example to that is the study carried out by Kong et al. (2013), who observed that the growth rate of the microalga *Chlorella vulgaris* varied between 0.48 and 0.99 (day^{-1}) and increased (i.e. twice the initial growth rate in best conditions) in relation to the control and for different concentrations of glycerol and glucose or a combination of them, therefore resulting in biomass concentrations ranging from 0.34 for the control to 2.62 g/L for the best mixotrophic conditions, with a higher carbohydrate content in biomass when mixotrophy was activated.

However, the cultivation of photosynthetic microorganisms for industrial purposes is still costly, restricting further applications, mainly for products with low-added value with respect to traditional biomasses, such as biofuels. Therefore, the economic viability tends to be the limiting factor of using such microorganisms in biofuels (Zhan et al. 2017).

One of the greatest costs is related to the microalgal cultivation media, with studies reporting that this is around 80% of average production costs. Thus, it is necessary to find substrates that have a low production cost, so that microalgae may be applied at a larger scale. Another factor that increases costs is, in some cases, the need of aeration with concentrated carbon dioxide, the use of supplements (vitamins and phosphate salts and nitrogen, for instance), besides the high energy consumption (artificial light, stirring and/or pumping) (Salati et al. 2017; Wang et al. 2016; Mitra et al. 2012; Perez-Garcia and Bashan 2015).

The main problems/challenges related to the heterotrophic/mixotrophic cultivation of microalgae are related to the cost of carbon sources, the dispute between competitive organisms such as bacteria and their high growth rates, adaption to bioreactors with low operating costs as well as the separation of the biomass produced and the process of biomass conversion/valorisation (Perez-Garcia and Bashan 2015).

4.1 Metabolic Pathway of Mixotrophy

In metabolic terms, the basic integrative representation of heterotrophy together with the photosynthetic pathway (autotrophy) for the reproduction of the main macromolecular cells (proteins, carbohydrates and lipids) is illustrated in Fig. 2. The organic carbon sources most mentioned in the literature are glucose, acetate and glycerol.

Glucose is among the most common carbon sources used in heterotrophic cultivation of microalgae. The highest growth rates are obtained with glucose, when compared with other carbon sources, such as sugars, sugar alcohols, sugar phosphates and organic acids (Perez-Garcia et al. 2011). This probably occurs as glucose exhibits higher levels of energetic content per mole in comparison with other substrates. For instance, glucose produces ~ 2.8 kJ/mole of energy compared with ~ 0.8 kJ/mole for acetate (Boyle and Morgan 2009).

The oxidative assimilation of glucose starts with hexose phosphorylation, producing glucose-6-phosphate, which is widely available for cellular metabolic pathways. Of all metabolic pathways used by microorganisms for aerobic glycolysis (complete glucose breakdown), apparently two, the Embden-Meyerhof pathway (EMP) and the pentose phosphate pathway (PPP), have been demonstrated in algae (Perez-Garcia and Bashan 2015). The PPP has a particular activity when the cultivation is under darkness, while the EMP takes a major role during growth with light (Perez-Garcia et al. 2011).

Both the tricarboxylic acid cycle (TCA) and oxidative phosphorylation have *Chlorella pyrenoidosa* and *Synechocystis* spp. activity. However, it is important to point out that many species may not be efficient on glucose removal from cultivation media, linked to the inefficiency of these metabolic pathways, as is the case of

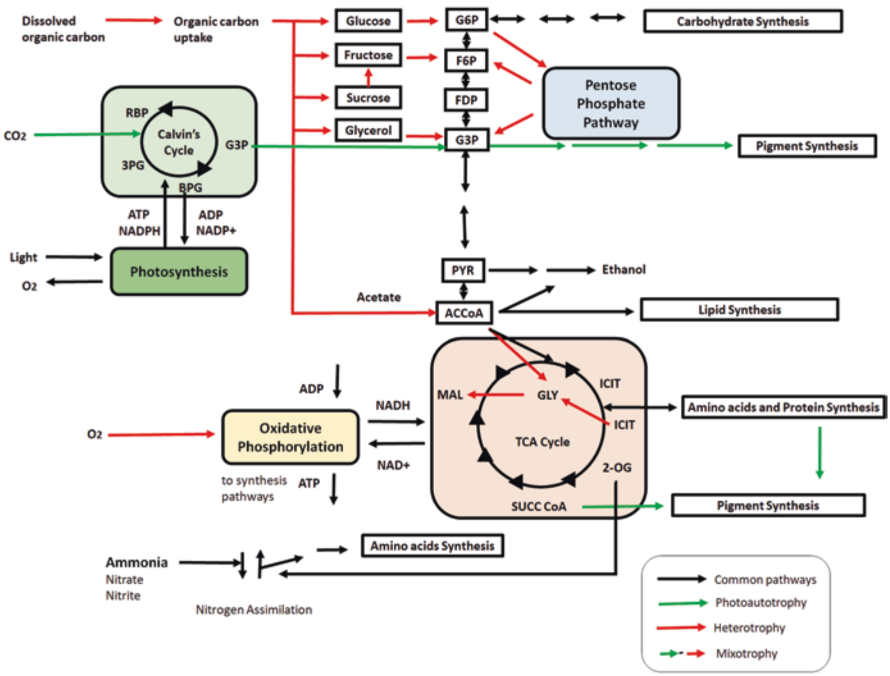

Fig. 2 Scheme of metabolic pathways for assimilation of carbon and production of energy in photoautotrophic, heterotrophic and mixotrophic microalgae. *The legend of the figure indicates that in red and green, the indicated reactions are* ***highly active*** *during photo and heterotrophic cultivation, respectively.* Compound abbreviations are subsequently specified. 2-OG, 2-oxoglutarate; 3PG, 3-phosphoglycerate; R5P, ribulose-5 phosphate; ACCoA, acetyl-coenzyme A; ADP, adenosine-diphosphate; ATP, adenosine-triphosphate; F6P, fructose-6 phosphate; BPG, 1,3- bisphosphoglycerate; FDP, fructose 1,6-biphosphate; G1P, glucose-1 phosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; ICIT, isocitrate; MAL, malate; NAD^+, nicotinamide adenine dinucleotide (oxidised); NADH, nicotinamide adenine dinucleotide (reduced); $NADP^+$, nicotinamide adenine dinucleotide phosphate (oxidised); NADPH, nicotinamide adenine dinucleotide phosphate (reduced); PYR, pyruvate; RBP, ribulose-1,5 biphosphate; SUCCCoA, succinyl-coenzyme A. (Source: Adapted from Perez-Garcia and Bashan (2015))

Prymnesium parvum, *Dunaliella tertiolecta* and *Prochlorococcus* spp. (Perez-Garcia et al. 2011).

Glycerol is a substance capable of reducing the osmotic force derived from solution/suspension, promoting osmotic equilibrium in the cell. It is also a common carbon source in economic terms (an effluent from the biodiesel industry), a compatible solute for microalgae and with low or no toxic effect on these microorganisms (Perez-Garcia et al. 2011). In metabolic terms, it is initially phosphorylated utilising ATP, with glycerophosphate being then oxidised to form triose phosphate. It may also be converted to glyceraldehyde-3-phosphate and glycerate, which are EMP intermediaries to form pyruvate that enters the TCA cycle. Nonetheless, its metabolic knowledge is still limited (Perez-Garcia and Bashan 2015).

As for acetate (carried by coenzyme A), it is usually oxidised via two different routes: (1) the glyoxylate cycle to form malate in the glyoxysomes (specialised in plastids in the glyoxylate cycle) and (2) via the TCA cycle to form citrate in the mitochondria, which provides carbon skeletons, energy and ATP, reducing energy (NADH) (Perez-Garcia et al. 2011). Yet, acetate can be toxic to several photosynthetic microorganisms (Perez-Garcia and Bashan 2015).

5 Wastewater Characteristics and Nutritional Requirements of Photosynthetic Microorganisms

As aforementioned, microalgae can eliminate phosphorus, nitrogen, sugars and organic carbons, which constitute many effluents. Therefore, the use of microalgae in wastewater treatment, besides being an alternative to wastewater treatment, may also reduce biomass production costs by reducing expenses with culture media (Perez-Garcia and Bashan 2015). Thus, it is of utmost importance that the elements required for the satisfactory growth of microalgae are made available in the effluent and in adequate amounts. Insufficient amounts may undermine growth rates and, consequently, purification rates. On the other hand, if in excessive amounts, as in some sources of organic/inorganic carbon, it may directly inhibit growth by toxicity or through rapid alterations in cultivation conditions due to the accumulation of metabolites, by altering the pH, for instance (Markou et al. 2014; Silva et al. 2017b).

In order to reach good efficiency rates, some characteristics may be taken into account when choosing the microalga to be used in wastewater treatment and for biofuel production, namely, high growth rates, contents and productivity (production/time) of lipids/carbohydrates, greater tolerance to potential pollutants (metallic ions and toxic compounds present in wastewater), more tolerance to NH_4^+ as well as superior O_2 generation rates, better CO_2 consumption capacity and robust growth properties with improved tolerance to various environmental conditions (Wang et al. 2016).

Regarding the nutritional factors for microalgal growth, carbon sources, nitrogen and phosphorus are considered as the most important. The use of inorganic nutrients (N and P, for instance) together with CO_2 and light absorption are essential factors, as this combination generates the biochemical energy required for microalgae through photosynthesis (Cuellar-Bermudez et al. 2017).

The carbon source may be found either in its organic or inorganic form. The presence of CO_2 (inorganic carbon) is directly related to the photosynthesis rate of the microalga, i.e. in case of excess CO_2, the photosynthesis rate increases slightly, even under mixotrophy. Thus, the injection of a proper CO_2 concentration in the system is highly efficient. As mentioned before, common organic carbon sources in mixotrophic tests are glucose, glycerol and acetate. However, both autotrophy and mixotrophy may be inhibited by high CO_2 concentrations, with this parameter being of utmost importance. Sforza et al. (2012) tested two different species, *Chlorella proto-*

thecoides and *Nannochloropsis salina*, in mineral culture medium supplied with 1% glycerol and 5% excess CO_2 under a day-night cycle (12–12 hours). Two scenarios were tested with the day-night cycle, aeration during 24 hours and aeration only in the irradiated part of the day. The presence of CO_2 in excess during the night photoperiod inhibited microalgae growth. Growth rates decreased from 0.867 to 0.640 and from 0.613 to 0.354 day^{-1} for *C. protothecoides* and *N. salina*, respectively.

The same was also demonstrated by Gonçalves et al. (2016a), who studied four different microalgae species (*Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*). The cultivation used different CO_2 concentrations (0–10%), with the following optimal CO_2 concentrations being obtained for each species: 5.35% for *C. vulgaris*, 4.87% for *P. subcapitata*, 5.5% for *S. salina* and 5.62% for *M. aeruginosa*; i.e. growth was diminished in concentrations higher than these obtained, demonstrating growth inhibition.

The treatment of synthetic municipal wastewater was studied by Shen et al. (2015), using different CO_2 concentrations (1–15%). The wastewater presented total organic carbon concentrations between 5–120 mg O_2/L, 25 mg/L of total nitrogen and 3 mg/L of total phosphorus, having also observed that N reduction occurred more quickly when using 5% CO_2. However, after 12 days of cultivation, all concentrations reached almost 100% of nitrogen removal. In turn, phosphorus removal occurred regardless of the CO_2 concentration used, reaching 100% after 2 days of cultivation for all concentrations used, showing the capacity of these microorganisms in removing these contaminants from wastewaters. In addition, when 15% CO_2 was used, microalgal growth inhibition occurred.

Nitrogen and phosphorus are of extreme importance for microalgal and cyanobacterial growth. Nitrogen, one of the main responsible for microalgal biomass production, may be found as NO_3^-, NO_2^- and NH_4^+, with some cyanobacteria being capable of reducing N_2 into NH_4^+, besides that present in organic molecules, namely, urea and amino acids. As for phosphorus, it is an essential macronutrient for microalgae, derived from either phosphates or superphosphates, such as sodium and potassium, for example. Yet, in wastewaters, phosphorus is present in different forms, namely, orthophosphate, pyrophosphate and metaphosphate, as well as in organic forms, though orthophosphates are more common in digested effluents. Microalgae can accumulate phosphorus inside their cells and use it in case of lack of this compound in the external medium, an advantageous characteristic of this microorganism class, being also beneficial to the removal of phosphorus from wastewaters (Silva and Sforza 2016; Markou et al. 2014; Markou and Georgakakis 2011).

The assimilation or not of nitrogen and phosphorus in these microorganisms is directly linked to nutritional and environmental factors, such as the availability of these nutrients in the external medium, being also dependent on carbon sources to combine the synthesis of cellular components, light, cultivation time and temperature, among others (Silva and Sforza 2016). Regarding nitrogen and phosphorus content in biomass, these depend on the nutritional and environmental conditions to which microalgae are submitted, varying between 4–14% for nitrogen and 0.5–4% for phosphorus (Markou et al. 2014; Silva and Sforza 2016; Silva et al. 2018a).

The same findings were demonstrated by Beuckels et al. (2015), who cultivated the species *Chlorella* and *Scenedesmus* with different concentrations of nitrogen (10–40 mg L^{-1}) and phosphorus (2–10 mg L^{-1}), having verified that a minimum ratio between both nutrients is necessary to ensure their adequate removal, being different to each species, i.e. it is species-dependent. In addition, this ratio directly influenced the biochemical composition and accumulation of carbohydrates and lipids in these microorganisms, reaching up to 40% of carbohydrates and 30% of lipids in lower concentrations, mainly of nitrogen.

Silva and Sforza (2016) and Silva et al. (2018a) observed the same effect on continuous cultivation, therefore optimising the amount of nitrogen used under a certain hydraulic retention time, light intensity and temperature while maintaining an excess phosphorus concentration. With optimised nitrogen concentrations of 150–300 μmol photons m^{-2} s^{-1} and 2.1–2.9 days at 26–28 °C, for *Scenedesmus obliquus* and *Chlorella vulgaris*, respectively, between 42% and 50% of carbohydrate content in biomass was produced, reaching maximum carbohydrate productivities rates of 0.80 and 0.37 g L^{-1} day^{-1}. Thus, would the availability of mainly phosphorus and nitrogen be enough for a mixotrophic production of carbohydrate-rich biomass, when cultivated with wastewater? This is one of the questions that will rely on advances on experimentation and theoretical discussions to be answered, as there is still inconsistency in the literature and on the mixotrophy discussion, only. This is because such discussions mostly involve the removal of nutrients and, in some cases, the amount of lipids accumulated to value the biomass produced.

In order to minimise the costs related to the supplementation of culture media, the nutrients that are still present at the end of the treatment, or the exhausted biomass after the extraction of carbohydrates or lipids/effluent from the production of microalgal biomass conversion, may still be used for nutrient recycling (Teymouri et al. 2017). Furthermore, a detailed study on the effluent, to verify the presence of the main nutrients necessary for microalgae, is recommended, as the supplementation of one or some nutrients may significantly increase the efficiency of the treatment as well as biomass production. However, researches have indicated that the capacity of using recycled nutrients varies from species to species (Markou et al. 2014).

Besides the nutritional factors, microalgal production rate is also influenced by environmental factors such as temperature and pH. These are directly related to solubility and CO_2 availability, determining the availability of essential nutrients and strongly interfering on cellular metabolism. An alkaline pH of between 7.5 and 9.0 is usually used (Subhash et al. 2014), with typical temperatures ranging between 20 and 35 °C and each species having their specific range of these parameters (Markou and Georgakakis 2011).

Studies report that mixotrophy increases bacterial contamination and fungus culture, with the latter growing more quickly than microalgae. In order to prevent contamination, some studies supplemented cultures with antibiotics such as streptomycin, chloramphenicol and penicillin; others used chlorination, though it may inhibit microalgal growth. Other scientists opted for not carrying out this form of supplementation, but to use organic carbon control, given the high competition for this compound among algae and bacteria; thus, a carbon control approach results in higher algae growth rates and, consequently, greater control of bacterial populations (Salati et al. 2017; Garcia et al. 2000; Perez-Garcia and Bashan 2015).

Table 1 presents the composition of some effluents regarding concentrations of total nitrogen, ammonia, phosphorus, BOD (biochemical oxygen demand) and COD (chemical oxygen demand). Thus, it is noted that COD and BOD levels varied between 0.06–1680 g O_2 L^{-1} and 0.007–1307 g O_2 L^{-1}, respectively, with industrial/digestate effluents mostly exhibiting the highest concentrations. When comparing concentrations of nitrogen (N) and phosphorus (P), digestate effluents have higher concentrations of these nutrients, even greater than standard culture media, such as BG-11 (Rippka et al. 1979) and f/2 (Guillard and Ryther 1962), in which nitrogen and phosphorus concentrations range between 12.5–250 and 1.5–7 mg/L, respectively. Furthermore, it is known that the assimilating capacity of microalgae and cyanobacteria can be higher with concentrations of up to 500 mg N/L and 200 mg P/L, capable of obtaining up to 7 g/L of dry algal biomass weight (Silva and Sforza 2016; Silva et al. 2018a).

Regarding nitrogen concentration, it is observed that urban wastewaters exhibited very similar ammonia content to the value of total nitrogen. In microalgal growth, ammonia is the preferred source of nitrogen, which may lead to a more rapid performance in the removal of this nutrient when compared to the other forms available, such as nitrate, nitrite and organic compounds in the form of amino acids/proteins, in comparison with industrial effluents. However, it is important to point out that high ammonia concentrations may inhibit microalgal growth under concentration ranges that are specific to each species (Markou et al. 2014; Massa et al. 2017). Interestingly, anaerobic digestion effluents (digestates) exhibited high concentrations of ammonia, also similar to the value of total nitrogen. This is due to the mineralisation promoted by the anaerobic digestion of these organic contaminants, transforming most of the nitrogen present in organic ammonia (Silva and Abud 2016; Silva and Abud 2017).

Besides ammonia, it is also important to analyse the N and P ratio (N/P), with specific species-dependent relations, as previously mentioned. This ratio influences microalgal and cyanobacterial growth, with studies indicating that phosphorus acts as a growth-limiting macronutrient (Markou et al. 2014; Massa et al. 2017), though constant growth monitoring of the culture is still necessary. Other studies also report that high BODs, as well as high N/P ratios, in cyanobacteria, opposed to microalgae, result in mixotrophic metabolism (Massa et al. 2017). Reference values for the N/P ratio are of between 8 and 45 gN/gP (gram of nitrogen per gram of phosphorous) (Cuellar-Bermudez et al. 2017).

Common phytoplankton elemental composition is based on the universal Redfield C/N/P ratio of 106:16:1. In some conditions, algae stoichiometry may diverge from this canonical ratio, thus suggesting that the cultivation media should be flexible and must be adapted to the metabolic needs of microalgae (Markou et al. 2014). The standard biochemical composition of microalgae includes protein

Table 1 Characteristics of some wastewaters

Wastewater type		COD g O_2 L^{-1}	BOD g O_2 L^{-1}	Total nitrogen mg L^{-1}	Ammonia mg L^{-1}	Phosphorus mg L^{-1}	References
Urban	Wastewater from a WWTP in Saint Nazaire, France	0.169	Not informed	65	62	5.4	Caporgno et al. (2015)
	Urban wastewater from the city of La Linea de la Concepcion	0.384	Not informed	54.58	Not informed	12.70	Mennaa et al. (2015)
	Wastewater from a WWTP in Valladolid	0.259	Not informed	80[a]	80	14.2	González-Fernández et al. (2016)
	Wastewater from a Spanish WWTP	151.4	42	92.4	Not informed	Not informed	Hodaifa et al. (2013)
	Sewage	0.095–0.126	0.030–0.050	40[a]	40	Not informed	Cheah et al. (2016)
	Slurry from a Malaysian landfill	0.68–0.95	0.97–0.184	1185[a]	1185	Not informed	
	Wastewater from a WWTP in Edinburgh, UK	0.142	Not informed	Not informed	Not informed	3.2	Evans et al. (2017)
Industrial	Vinasse diluted in water at 50%	8.93 ± 1.45	3.74 ± 0.20	21.59[b]	Not detected[c]	11.95 ± 0.35	Santana et al. (2017)
	100% Vinasse clarified	22.65 ± 2.64	9.64 ± 0.69	48.81[d]	9.25 ± 2.52	18.47 ± 3.29	
	Wastewater from the olive oil industry	0.0572	0.00687	Not informed	Not informed	Not informed	Hodaifa et al. (2013)
	Sewage from an agricultural canal in India	2.2	0.0024	105.83	21.26	3.162	Renuka et al. (2013)
	Wastewater from the palm oil industry	15–100	10–43.75	1400	80	Not informed	Cheah et al. (2016)
	Whey Cheese wastewater	147 ± 7	97 ± 4	805 ± 48	103 ± 9	400 ± 20	Salati et al. (2017)
	Glycerol	1680 ± 154	1307 ± 360	Not detected	Not detected	Not detected	
	White wine lees	181 ± 11	162 ± 13	219 ± 4	32.2 ± 1.3	150 ± 8	
Digestates	Ultrafiltered digestate	1.52 ± 0,03	0.554 ± 0.03	1377 ± 41	1155 ± 35	300 ± 18	
	Zootechnical residue (liquid digestate)	14.1	4	1630	1400	716	Massa et al. (2017)
	Vegetal residue (liquid digestate)	22.12	6.41	2890	2000	66	
	Solid municipal residue (liquid digestate)	19.8	5	3370	2650	24	
	Digestate	2661	Not informed	2667	2276	381	Cai et al. (2013)
	Digestate obtained from treatment sludge	0.715	Not informed	1311	1279	12	Caporgno et al. (2015)

WWTP wastewater treatment plant

[a] 100% of ammonia did not present nitrate or nitrite

[b] Mostly nitrate (21.49 mg L^{-1})

[c] Mostly nitrate (39.41 mg L^{-1})

[d] Lower than 5 mg.L^{-1}

(30–50%), carbohydrates (20–40%) and lipids (8–15%) (Cardoso et al. 2011; Hu 2004), but several studies have shown that lipids and carbohydrates can be accumulated under different stress conditions (mainly under nitrogen starvation), decreasing protein content, as aforementioned and here reinforced (Ho et al. 2013a, b; Hosseini et al. 2016).

6 Performance of Wastewater Treatment by Microalgae and Cyanobacteria

By comparing wastewater treatment using one of the conventional methods (e.g. the activated sludge process) and the system using only microalgae, it can be observed that the traditional process requires previous denitrification for complete nitrogen removal and usually exhibits inefficient final phosphorus concentrations to be released into water bodies if a tertiary treatment required is not performed (Cuellar-Bermudez et al. 2017). On the other hand, microalgae/cyanobacteria are capable of efficiently removing nitrogen and phosphorus by using these for their own growth, reaching the detection limit in several cases described in the literature (Markou and Georgakakis 2011; Cabanelas et al. 2013; Markou 2015; Karn 2016; Cuellar-Bermudez et al. 2017).

The main difficulty in comparing both treatment systems is that microalgal growth in wastewater is inhibited/hampered by high COD/BOD levels. Thus, a maximum COD of 5000 mg L^{-1} is usually used in microalgal cultivation, though this level is already prejudicial to the remediation performance (Wang et al. 2016); therefore, it is considered as the main disadvantage of this treatment technology.

In this context, as previously stated, most domestic and industrial wastewaters present satisfactory quantities of sources of organic carbon, nitrogen and phosphorus, being considered a favourable environment for microalgal growth. It is well known that one of the main objectives of wastewater treatment is the reduction of COD and other organic nutritional compounds. Therefore, when high levels of COD and nutrient content are observed, as is the case in agroindustrial wastewaters, either anaerobic treatments are implemented, or these effluents are adequately diluted to avoid algal growth inhibition caused by greater COD levels (Wang et al. 2016; Perez-Garcia and Bashan 2015). This section will present examples of three types of wastewater treated with microalgae: urban, agroindustrial or anaerobically digested (digestate).

In general, there are two approaches of algae application to wastewater: (1) to evaluate the depurative capacity of a specific strain or the combination of algae species (cocultivation) due to their capacity to perform mixotrophy (removal of nitrogen, phosphorous and organic matter simultaneously) and (2) the consortium between algae and bacteria, improving the efficiency of gas exchange (CO_2 and O_2), and the speed for which bacteria can remove chemical oxygen demand (COD), in respect to algae (Markou and Georgakakis 2011; Markou 2015; Cuellar-Bermudez et al. 2017).

The first approach is very important, as the depurative capacity depends of the species and variations can occur. Several species have been studied, with most of them being represented in freshwater strains to avoid some interference of the reduced/no salinity of urban wastewater, such as *Chlorella vulgaris* (Cabanelas et al. 2013; Markou 2015; Gonçalves et al. 2016b, c), *Scenedesmus* spp. (Lynch et al. 2015), *Microcystis aeruginosa*, *Pseudokirchneriella subcapitata* (Dang et al. 2012; Gonçalves et al. 2016b, c), *Synechocystis* PCC 6803 (Cai et al. 2013), *Arthrospira platensis* (Markou 2015) and *Synechococcus* spp. (Lynch et al. 2015). Nevertheless, some saline species are cited: *Nannochloropsis salina* (Cai et al. 2013) and *Synechocystis salina* (Gonçalves et al. 2016b, c). The algae-bacteria consortium will be subsequently discussed, in a separate topic.

As previously stated, microalgae remove some compounds in the effluent and are also capable of producing biomass. This chapter discusses the production of carbohydrates by microalgae, existing a great lack of studies in the literature focused on carbohydrate production, with most of them discussing lipid production, the first remarkable application of microalgae in biofuels (Cai et al. 2013). A correlation between the amount of these two compounds could be observed (they are complementary metabolisms; see Sect. 3), as indicated by the data presented in Table 2. The presence of carbohydrates and lipids after biomass production is advantageous not only for biomass production but also for using these lipids in other applications, thus increasing the energy balance of the process. By analysing the data from the same table (Table 2), it can be noted that carbohydrate percentages are different for every species, suggesting that these compounds vary with the species. Therefore, there is a great need for researches that assess the influence of nutritional and environmental factors on wastewater treatment for carbohydrate production. In addition, simulated wastewaters for this purpose (synthetic effluents) have been usually proposed, though it is important to point out that not all characteristics nor the same performance as the effluent may be reached.

As reported in Table 2, carbohydrate productivity varied between 20.3 and 100 mg L^{-1} day^{-1}. Shen et al. (2015) used different concentrations of total organic carbon (TOC), ranging between 0 and 120 mg L^{-1}. It was then observed that an increase in TOC led to greater carbohydrate productivity, indicating that this productivity may be related not only to the quantities of nitrogen and phosphorus (these were almost completely removed) but also with the quantity of carbon available. It is important to stress that some works which used different substrates report greater carbohydrate productivities, as is the case for *Scenedesmus subspicatus* GY-16, *Chlorella vulgaris* FSP-E and *Ankistrodesmus gracilis* GY-09, 2 g L^{-1}; sodium acetate, 498 mg L^{-1} d^{-1} of carbohydrate productivity (Chen et al. 2016) and glycerol *Chlorella pyrenoidosa*, 1%; 1.2 g L^{-1} of maximum biomass production with 60% of carbohydrate content (Bajwa et al. 2016).

González-Fernández et al. (2016) tested three different cultivation conditions: the first using a temperature of 23 °C and under light for 14 hours, the second at a temperature of 15 °C and 14 hours under light and a third using a temperature of 15 °C and 11 hours of light exposure. These conditions were carried out under a light intensity of 5500 lux in all scenarios. It was then observed that the highest

Table 2 Some microalgae, their composition (carbohydrates and lipids) and carbohydrate productivity

Type	Microalga/cyanobacteria	Wastewater type	Carbohydrates (%)	Lipids (%)	Carbohydrate productivity (mg L^{-1} day^{-1})	References
Urban	*Chlorella vulgaris*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*	Wastewater from a WWTP in Valladolid	35–52.6	20–30	100	González-Fernández et al. (2016)
	Scenedesmus obliquus	Simulated municipal wastewater	52.8–68.5	15.7–21	20.33–37.20	Shen et al. (2015)
	Scenedesmus obliquus	Secondary wastewater effluent from a municipal wastewater treatment plant in Lin'an	80	13	50	
Agroindustrial	*Micractinium* sp. *Embrapa|LBA#32*	Vinasse diluted to 50%	17.55	Not informed	31.20	Santana et al. (2017)
	Micractinium sp. Embrapa|LBA#32	100% clarified vinasse	21.79	Not informed	35.87	
	Chlamydomonas biconvexa Embrapa|LBA#40	Vinasse diluted to 50%	13.50	Not informed	24.58	
	Chlamydomonas biconvexa Embrapa|LBA#40	100% clarified vinasse	11.71	Not informed	26.03	
	Spirulina platensis	Soy protein	37.84	Not informed	60	Salla et al. (2016)
Digestate	*Arthrospira platensis*	Effluent from chicken anaerobic digestion (liquid digestate)	17.9–44	23.0–33.6	Not informed	Markou (2015)
	Chlorella vulgaris	Effluent from chicken anaerobic digestion	22.7–27.1	36.0–51.2	Not informed	
	Arthrospira maxima	Zootechnical residue (liquid digestate)	30.54	7.94	Not informed	Massa et al. (2017)
		Vegetal residue (liquid digestate)	28.40	10.54	Not informed	
	Tetradesmus obliquus	Zootechnical residue (liquid digestate)	18.33	23.53	Not informed	
		Vegetal residue (liquid digestate)	18.20	27.52	Not informed	

carbohydrate percentage was reached for a temperature of 23 °C, when greater COD removal occurred, thus suggesting that the higher the COD removal, the greater the ability of microorganisms to accumulate carbohydrate.

Abreu et al. (2012) studied *Chlorella vulgaris* regarding dairy wastewater treatment (whey cheese) and verified that the mixotrophic growth speed tripled in comparison with autotrophic growth; however, the lactose present in the effluent must be hydrolysed in galactose and glucose to be effectively absorbed by *Chlorella*. Considering an autotrophic process, mixotrophic with non-hydrolysed and hydrolysed effluent, the growth rates reached values of 0.13, 0.12 and 0.43 day^{-1}, respectively. However, a low carbohydrate content was observed, though no appropriate argument to this could be given, as the elements removed had not been previously quantified, namely, N and P.

Nitrogen removal varied, mostly, between 50% and 99%, while phosphorus removal ranged between 70% and 99%, reinforcing the efficiency of such microorganisms in removing these nutrients, as well as demonstrating their ability in treating wastewaters with high concentrations of N and P (Table 3). Nonetheless, during vinasse treatment, for instance, there was a slight raise in phosphorus content for both cases described by Santana et al. (2017). Furthermore, COD levels also increased, despite a 40% nitrogen removal. The authors explained this effect as being due to the use of carbon dioxide (5% air-CO_2 mixture), with no/low preference for organic carbon.

An excellent efficiency in the removal of both nitrogen and phosphorus was obtained in the study carried out by Shen et al. (2015), using synthetic municipal effluent, having reached almost 100% removal for both nutrients, with nitrogen removal being obtained after 8 days of cultivation and phosphorus more quickly, after 2 days of cultivation. Cai et al. (2013), in turn, studied a digestate (liquid fraction after anaerobic digestion) in different dilutions, between 3 and 24% (digestate), having observed that N removal exhibited an opposite trend to the concentration of digestate; i.e. while the concentration of effluent increased, nitrogen removal decreased. A possible cause to this trend could be linked to the high concentration of contaminants, exceeding the purification capacity of these species.

In turn, Evans et al. (2017) compared the removal efficiency of utilising glucose and glycerol supplementation of urban wastewater, separately. It was then noted that the removal efficiency of both nitrogen and phosphorus were similar in both cases, reaching approximately 90 and 99%, respectively. The glucose medium exhibited an increase in carbohydrate production, indicating that this substrate accumulates this energy reserve more rapidly. The efficiency of the use of CO_2 in urban wastewater was also observed, reaching a rate of 0.20 v/v per minute during 8 hours. As a result, microalgal inhibition occurred, corroborating the theory that excess CO_2 can reduce the removal efficiency of both N and P, as previously reported.

Another interesting factor concerning anaerobically digested zootechnical/vegetal effluents is that, despite exhibiting extremely high concentrations of nitrogen and phosphorus of around 1630–2890 and 66–716 mg L^{-1}, respectively, these nutrients can be almost completely removed (Massa et al. 2017). This efficiency can be attributed to the fact that effluent/residue mineralisation favours greater availability

Table 3 Performance of the contaminants removal in some effluents by microalgae/cyanobacteria

Type	Microalgae/cyanobacteria (cultivation conditions) pH, temperature, light, rotation, aeration/CO_2, cultivation time	Effluent type	Characteristics of the effluent: COD g O_2 L^{-1}	Characteristics of the effluent: N_{total} mg L^{-1}	Characteristics of the effluent: P mg L^{-1}	Removal rate: COD (%)	Removal rate: N_{total} (%)	Removal rate: P (%)	References
Urban	*Chlorella vulgaris, Scenedesmus obliquus* and *Chlamydomonas reinhardtii* (15–23 °C and 11–14 hours of light exposure, light intensity of 5500 lux)	Wastewater from a treatment plant in Valladolid	0.16	80****	4.7	56-80.5	99	97	González-Fernández et al. (2016)
	Ankistrodesmus falcatus *Scenedesmus obliquus* *Chlorella kessleri* *Chlorella vulgaris* *Chlorella sorokiniana* *Botryococcus braunii* *Neochloris oleoabundans* *Natural algal bloom* CO_2 aeration at a rate of 1 L min^{-1}, kept under a temperature of 20 ± 3 °C. Light intensity of 90 µmole m^{-2} s^{-1}	Urban wastewater from the city of La Linea de la Concepcion	0.384	54.58	12.70	Not informed	87–99	80–98	Mennaa et al. (2015)
	Scenedesmus obliquus Feeding with 5% CO_2 at a rate of 300 mL min^{-1}; under intense light at 40 µmole m^{-2} s^{-1}, at a temperature of 25 °C	Secondary effluent from urban wastewater in Lin'an	Not informed	13	1.1	Not informed	98.85	96.89	Shen et al. (2015)
	Scenedesmus obliquus Were cultivated under a temperature of 25 °C, under intense illumination of 40 µmole m^{-2} s^{-1}	Simulated urban wastewater	Not informed	25	3	Not informed	99	99	
	Microalgae-bacteria consortium Chlorophyta (mainly *Scenedesmus and Chlorella)* Sludge blanket reactor under ascending hydrolytic flow, at a hydraulic retention time of 5 hours	Municipal wastewater from the city of Barcelona	0.250	50	35	68	60	95	Passos et al. (2013)
	Chlamydomonas sp., *Chlorella* sp., *Oocystis* sp. Constant illumination (450 lx), at a temperature of 25 ± 2 °C, 130 rpm stirring	Secondary effluent form wastewater in shiraz	Not informed	190.7	19.11	Not informed	68–85	82–100	Rasoul-Amini et al. (2014)
	Chlorella vulgaris Temperature of 15 ± 1 °C, under a day-night cycle 12:12, illuminated at 100 µmole m^{-2} s^{-1}	Wastewater from the City of Edinburgh, UK	0.1461–0.4282	29[a]	3.2	67-78	90	99	Evans et al. (2017)

(continued)

Table 3 (continued)

Type	Microalgae/cyanobacteria (cultivation conditions) pH, temperature, light, rotation, aeration/CO_2, cultivation time	Effluent type	Characteristics of the effluent			Removal rate			References
			COD g O_2 L^{-1}	N_{total} mg L^{-1}	P mg L^{-1}	COD (%)	N_{total} (%)	P (%)	
Agroindustrial	*Micractinium* sp. *and Chlamydomonas biconvexa Embrapa LBA#32* 3 days at a 12 hours/12 hours regime of light/dark (light intensity of 400μEm^{-2} s^{-1}) and temperature of 37 °C ± 1 °C during the light period and 24 ± 1 °C during the dark period. Aeration with 64 L.h^{-1} of atmospheric air supplemented with 5% CO_2 supply	Vinasse diluted to 50% *Micractinium* sp.	8.93	21.59[b]	3.90	−35.27	46.41	4.29	Santana et al. (2017)
		100% Clarified vinasse *Micractinium* sp.	22.65	48.81[c]	6.02	−18.54	44.88	−3.14	
		Vinasse diluted to 50% *C. Biconvexa*	8.93	21.59[b]	3.90	−49.83	39.37	−77.21	
		100% clarified vinasse *C. Biconvexa*	22.65	48.81[c]	6.02	−23.53	43.62	17.97	
	Mix 1: Calothrix sp., *Lyngbya* sp., *Ulothrix* sp., *Chlorella* sp. *Mix 2: Phormidium* sp., *Limnothrix* sp., *Anabaena* sp., *Westiellopsis* sp., *Fischerella* sp., *Spirogyra* sp. *Mix 3: Chlorella sp.*, *Scenedesmus* sp., *Chlorococcum* sp., *Chroococcus* sp. 3-weeks cultivation at a temperature of 25 ± 2 °C, illuminated at 75 μmole photons m^{-2} s^{-1}	*Mix 1*, *Mix 2* and *Mix 3*, sequentially. Sewage from an Indian agricultural canal	2.2	105.83	3.162	72	83	98	Renuka et al. (2013)
			2.2	105.83	3.162	72	83	98	
			2.2	105.83	3.162	72	78	98	

Digestate	*Arthrospira maxima* 24 ± 1 °C, continuous illumination of 200 μmole photons m^{-2} s^{-1}	Zootechnical residue (liquid digestate)	14.1	1630	716	Not informed	98.5	90.9	Massa et al. (2017)
		Vegetal residue (liquid digestate)	22.12	2890	66	Not informed	99.5	97.5	
	Tetradesmus obliquus 24 ± 1 °C, continuous illumination of 200 μmole photons m^{-2} s^{-1}	Zootechnical residue (liquid digestate)	14.1	1630	716	Not informed	99.8	96.0	
		Vegetal residue (liquid digestate)	22.12	2890	66	Not informed	99.2	97.5	
	Arthrospira platensis	Effluent from chicken anaerobic digestion (liquid digestate)	0.528–1.874	88–313[a]	1.95–6.94	20–70	98–99	98–99	Markou (2015)
	Chlorella vulgaris		0.587–2.333	98–373[a]	2.18–8.28	58–70	95–99	98–99	

[a]Most nitrogen in the form of ammonia
[b]Lack of ammonia
[c]Most nitrogen in the form of nitrate
Negative values of COD and P removal rate indicate an increasing of these parameters

of nitrogen and phosphorus contents in the solution, namely, when converting organic nitrogen into ammoniacal nitrogen and phosphorus into orthophosphates (Silva and Abud 2016; Silva and Abud 2017). Thus, an effluent/residue with high levels of COD and concentrations of nitrogen and phosphorus, as is the case of anaerobically digested effluents, presents lower toxicity in microalgal cultivation.

Hodaifa et al. (2013) used a mixture between an effluent from the olive oil industry and another from an urban wastewater treatment plant after secondary treatment. The final cultivation media resulted in a combination of different concentrations of urban wastewater (0–50%) and olive oil industry effluent (0–10%), with the remaining volume consisting of distilled water. It was then observed that the olive oil effluent presented lower microalgal growth, when only comparing with the urban wastewater in study. In addition, microalgal growth inhibition (*Scenedesmus obliquus*) occurred with a combination 25% of urban wastewater and 5% olive oil effluent. As the microalgal cultivation conditions (298 μ E m^{-2} s^{-1}, stirring at 350 rpm and aeration at a rate of 1 v/v/min) were similar for the different concentration levels used, microalgal growth was inhibited by the characteristics of the effluent, more specifically, its composition, mainly due the sensitivity to the olive oil effluent.

An option for improving efficiency can be a consortium of several microalgae species, as it can overcome the difficulty in assimilation of one species, with another species being able to perform it more easily or interact in symbiosis, complementing each other to reach higher efficiency levels (Renuka et al. 2013).

6.1 Effluents Discussed in This Chapter

Urban Wastewater

The treatment of urban wastewater is a matter of increasing importance and needs to be addressed, especially given that after adequate treatment this water can be reused with no harm to humans or the environment. Some European countries, for instance, already limit the quantity of N and P that can be released into wastewaters, as established by the EU 91/271/EEC regulation, which states that a maximum of 2 mg L^{-1} of total phosphorus (TP) can be discharged from urban wastewaters for every agglomeration with less than 100,000 inhabitants and a maximum of 1 mg L^{-1} for agglomerations with more than 100,000 inhabitants. As for total nitrogen (TN), a maximum of 10 mg L^{-1} can be released in agglomerations with a population equivalent to more than 100,000, with COD being limited to 125 mg L^{-1} and BOD to no more than 25 mg L^{-1} (Evans et al. 2017). At the end of the study, Evans et al. (2017) verified that some of the wastewater treatments investigated were in accordance with the EU legislation. For glucose or glycerol supplementation treatments, all levels of TN, TP and COD presented final concentrations lower than that established by law. However, treatments without supplementation but with CO_2 injection, the amounts of TN and TP were still above the required, with only COD below 125 mg L^{-1}. It is possible to conclude that the technology is indeed capable for effectively treating urban wastewaters.

Nevertheless, in developing countries, such as Brazil, only some of these aspects are defined by law: for total ammoniacal nitrogen discharged, up to 20 mg L^{-1}, as well as BOD, with a 60% minimum removal, considering a maximum of 120 mg L^{-1} to be released in sanitary sewage. Regarding phosphorus, the environmental entity may define guidelines for this parameter when discharging effluents into hydric bodies with a recorded history of cyanobacterial bloom, in stretches with water catchment for public supply. Furthermore, there is also a regulation setting certain parameters for the classification of fresh, saline and brackish waters, with these being divided into different destination classes, such as for human consumption (after treatment), fishing activity and recreation, among others. In these criteria, there is a restriction on the amount of phosphorus and nitrogen that can vary in each class and for every water type (CONAMA, resolution 403/2011 and 357/2005).

In some countries, these standards vary according to the population of the city, being extremely important as this minimum discharge amount may influence water quality in lakes and eutrophication in rivers, for instance (Evans et al. 2017). Furthermore, norms that control wastewater treatments may also result in international accords for environmental protection, as is the case of the Helsinki Convention on the Marine Environment of the Baltic Sea Area (HELCOM), as well as national courses of action for energy production from renewable sources (according to 2009/28/EC), as is the case in Finland (Lynch et al. 2015).

Industrial Wastewater

The increase of industrial production to meet the demands of the population culminated in greater amounts of waste, sometimes improperly released without any type of treatment. Industrial wastewater is considered as being every residue originated from industries and agroindustries, as result of a manufacturing process, namely, soy protein, whey cheese and vinasse effluents. Vinasse is a dark brown liquid by-product of the ethanol and sugar industry, with high contents of organic compounds and COD levels, though its characteristics vary depending on the ethanol production technology (Silva and Abud 2016).

When characterising agroindustrial effluents, high amounts of ammonia could be noted, with algae possibly taking advantage of this nutritive environment and rapidly converting nutrients into biomass. In optimal conditions, ammonia removal can reach up to 90–95%. It is worth mentioning that NO_3^- (nitrate) absorption, specifically, depends on light supply (Massa et al. 2017).

Moreover, high COD levels can also be observed in some effluents, restricting microalgal wastewater treatment, despite being efficient in some cases. In this regard, the bacteria-algae consortium may be a potential alternative to the treatment of these wastewaters.

However, some disadvantages in relation to microalgal treatment of industrial wastewater may be cited, namely, the presence of microorganisms and bacteria in the effluent, which may compete with microalgae, thus interfering on the growth rate of the latter. Besides, the presence of suspended or dissolved solids may interfere on light penetration and, consequently, on microalgal photosynthesis (Markou et al. 2014).

Anaerobic Digestion Effluent (Digestate)

Anaerobic digestion is commonly related with organic matter stabilisation and deterioration by microorganisms under anaerobic conditions. Anaerobic treatment can boost pollutant reduction in wastewaters, namely, urban and agricultural wastewaters. In this regard, the effluent is anaerobically hydrolysed and fermented, obtaining sludge (solid/humid fraction), liquid effluent (digestate) and biogas (gas fraction, mainly consisting of H_2, CO_2 and methane). Anaerobic digestion is advantageous mainly due to low sludge reduction, as well as because of the lower amount of energy required when compared with the aerobic process, besides producing energy in the form of biogas. Yet, depending on the wastewater source, some organic and inorganic substances may inhibit anaerobic digestion, namely, ammonia, sulphate and heavy metals (Chen et al. 2008).

Additionally, organic matter is mineralised into simpler carbon forms, nitrogen into ammonia and phosphorus content being present in the form of orthophosphates (Silva and Abud 2017). These physicochemical characteristics foment the increase in microalgal and cyanobacterial treatment and help the treatability of effluents with high organic matter content, also producing energy. However, secondary/tertiary treatment may be necessary for the anaerobic digested effluent, as is the case of microalgae cultivation.

6.2 *Microalgae-Bacteria Consortium*

The microalga-bacteria consortium may be applied to wastewater treatment to prevent external oxygen and carbon dioxide supply, with nutrient assimilation in the biomass and by reducing CO_2 emissions to the atmosphere. Some studies point out that the main limiting factor of their combined use is that high efficiencies of nutrient and pollutant removals are dependent on algal growth, though, when simultaneously used, algal growth can be stimulated (Wang et al. 2016). However, an adequate control of bacterial populations is required during organic matter removal phase. Table 4 shows some advantages and disadvantages, as well as opportunities regarding the use of the algae-bacteria consortium.

Table 4 Contributions and disadvantages of the use of a microalgae-bacteria consortium

Advantages	Disadvantages
CO_2 is assimilated by the bacterial population Symbiosis of essential nutrients may happen Flocculation can be improved by bacterial association High N and P removal efficiencies, for instance, as well as high COD removal Algal oxygenation Algal organic matter serves as a source of carbon	Algaecide effects on some bacteria Increase in pH and temperature due to association of algae metabolism Antibacterial effect on some algae

Source: Wang et al. 2016; Cuellar-Bermudez et al. 2017

7 Application of a Carbohydrate-Rich Biomass for Biofuels

As previously mentioned, microalgae can be applied on wastewater treatment and consequently employed for biomass generation. However, this biomass should not be used as a nutrient or food additive, given the possible presence of pollutants from the wastewater. Thus, this resultant biomass is majorly applicable in biofuel production (Markou et al. 2014).

This biomass produced is widely cited in the literature as a result of lipid production aimed at biodiesel generation. Nevertheless, in some cases, the biomass obtained can be rich in carbohydrates, as demonstrated in some articles cited in this chapter. It is widely acknowledged that carbohydrates are quicker, when hydrolysed, to be assimilated and metabolised or, better, fermented to obtain end products. In addition, from a biofuel perspective, autotrophic cultivation is essential if the process is to be sustainable; but the effect of organic sources on carbohydrate accumulation could become a relevant issue in the case of waste stream exploitation. Besides, microalgal carbohydrate exploitation is not only limited to ethanol fermentation, as other products such as butanol, acetone, hydrogen, methane or value-added molecules can be obtained.

7.1 Bioethanol

For bioethanol, productivity is mainly determined by sugar concentration, since a high amount of fermentable carbohydrates can be converted from yeast and bacteria (e.g. *S. cerevisiae* and *Z. mobilis*). A carbohydrate-rich microalgae biomass (~50%) presents a theoretical bioethanol production of around 0.26 $g_{ethanol}/g_{biomass}$ (based on Gay-Lussac stoichiometry, 1 g monomer glucose produces 0.5111 g ethanol). However, the efficiency depends on the extraction of sugars and saccharification methods, which must not degrade the sugars and ensure high rates of monomer production. Another factor is the composition of sugars present in the biomass, as glucose (hexose), for instance, is easily fermentable by the strains mentioned above, though xylose (pentose) is not used by S. *cerevisiae* (which is usually present in considerable amounts within the carbohydrate-based microalgae biomass (10–30%)) (Ho et al. 2013a, b; Harun and Danquah 2011). Other strains are necessary in this case, such as *Pichia stipitis* (aka *Scheffersomyces stipitis*), *Pichia segobiensis*, *Kluyveromyces marxianus*, *Candida shehatae* and *Pachysolen tannophilus*, despite the low ethanol production rates when compared to a *Saccharomyces*-glucose fermentation system (Silva and Bertucco 2016). In addition, glucose is the main component of carbohydrates in the biomass, reaching values of total carbohydrates between 60 and 90%, which is good given that this monomer is easily fermentable by *Saccharomyces* strains.

The bioethanol production steps from microalgal biomass are biomass cultivation, harvesting, pretreatment and/or hydrolysis, fermentation and distillation. For microalgae, more specifically in hydrolysis, the most efficient methods are acid and enzymatic hydrolysis. Acid hydrolysis is considered advantageous as no pre-treatment is needed, while enzymatic hydrolysis requires exploded/open cells for adequate enzymatic accessibility. Nonetheless, there is still a great deal to be learned about the fermentation step before consolidating it as an industrial process, given the often low efficiency rates in converting sugar into ethanol (Silva et al. 2018b).

Some experimental values of ethanol/biomass yields obtained are 0.163 $g_{ethanol}/g_{biomass}$ (*Arthrospira platensis*, chemical hydrolysis) (Markou et al. 2013), 0.140 $g_{ethanol}/g_{biomass}$ (*Dunaliella tertiolecta*, chemoenzymatic) (Lee et al. 2013) and 0.214–0.233 $g_{ethanol}/g_{biomass}$ (*Chlorella vulgaris* FSP-E, enzymatic and chemical hydrolysis), respectively (Ho et al. 2013c).

7.2 Biobutanol

Regarding biobutanol production, it was produced with a concentration of 8.05 g/L (Gao et al. 2016) and 3.74 g/L (Castro et al. 2015) by using the residues of microalgae biomass after lipid extraction, which may still have traces of good carbohydrate contents. Moreover, butanol was obtained from *Chlorella vulgaris* JSC-6 hydrolysate (mix of glucose and xylose), with a productivity of 0.9 g L^{-1} d^{-1}, with *Clostridium acetobutylicum* ATCC824 as inoculum, incubated for 24 hours at 37 °C (Wang et al. 2014).

7.3 Biohydrogen

Biohydrogen production from fermentation generally consists of two routes: the acetate and butyrate routes, when using mixed cultures (different species together). For every mole of glucose, 4 and 2 moles of acetate and butyrate are produced. The main parameters which influence H_2 production are pH, the species involved and retention time. The major species used in biohydrogen production is usually *Clostridium* sp., though the presence of competing bacteria, namely, the *Lactobacillus* sp. and *Sporolactobacillus* sp. species, may lower the production of H_2 (Turon et al. 2016).

Between 38 and 97 mL g^{-1}, VS of biohydrogen produced from microalgal biomass was obtained using several microalgae species, such as *Scenedesmus* and *Chlorella* species (Kumar et al. 2016), as well as *Chlorella pyrenoidosa* and *Nannochloropsis oceanica*, using a mix of effluents from anaerobic digesters, mainly consisting of marine algae, as inoculum. The inoculum was then degasified at 37 °C for 7 days (Ding et al. 2016). For *Arthrospira platensis*, mixed anaerobic fermentative bacteria were used as inoculum, originated from anaerobic sludge from a digester effluent of a farm in Ireland. The sludge was subsequently pretreated at 100 °C for 30 minutes in autoclave in order to remove H_2 consumers (Xia et al. 2016).

7.4 Biogas (Methane)

Biogas is one of the final products from the anaerobic digestion of a combination of microorganisms that convert organic matter into fermentation products, being mostly dissimilated into the form of gas, namely, CO_2, H_2 and CH_4. This process occurs in four different stages: hydrolysis (breakage of complex organic matter), acidogenesis (constant breakage of organic matter, forming volatile fatty acids with consequent decrease in pH), acetogenesis (the compounds formed during acetogenesis are fermented, subsequently producing mostly CO_2, acetic acid and H_2) and methanogenesis (methanogenic bacteria use by-products from acetogenesis as a substrate to form methane). The most important parameters for the process are C/N ratio, pH, temperature, concentration and inoculum type, volatile solid concentration (VS) and hydraulic retention time.

González-Fernández et al. (2016), when using a biomass obtained from microalgal mixotrophy cultivated under different temperatures (23 °C and 15 °C), employed anaerobic digestion for 38 days at 35 °C. The microalgal biomass presented between 35 and 50% of carbohydrates, having obtained a methane production ranging between 125.1 and 153.2 mL CH_4 g^{-1} COD in 10–38 days of hydraulic retention time (HRT). The percentage of CH_4 in the biogas varied between 67.9 and 70.2%.

In turn, the study carried out by Hernández et al. (2016) obtained a variation on the production of methane between 100 and 190 mL CH_4 g^{-1} VS (volatile solids) in 42 days of HRT, from the residual biomass after lipid extraction (biochemical composition of biomass *in natura*: carbohydrates between 22 and 25 and lipids 13 and 15%, characterised as a protein-rich biomass).

Passos et al. (2013) observed methane production from a biomass produced by a mix of microalgae (*Scenedesmus* and *Chlorella*, mostly) cultivated in effluent lakes employing previous treatment with microwaves to increase digestibility and rapidness of the anaerobic process. Thus, 68% of methane in the biogas was obtained, with a production ranging between 172 and 307 mL CH_4 g^{-1} VS in 46 days of HRT, with the highest production rates being reached under higher power (energy/time). The biochemical composition of the biomass used was of 17% of lipids, 20% of carbohydrates and 49% of proteins.

Caporgno et al. (2015) then used *Chlorella kessleri* and *Chlorella vulgaris* that had been cultivated in wastewater, conducting anaerobic digestion after harvesting in order to assess their potential as substrate in the production of methane. The production varied between 310 and 400 mL CH_4 g^{-1} of VS in 30 days of HRT. The biochemical composition of the biomass was then of 35.2–36.7 of proteins and 36.2–44.6 of carbohydrates, being characterised as a low lipid content biomass (7–15%), i.e. more in line with the focus of this chapter.

It is already known that a greater lipid content, as well as an unbalanced amount of carbon and nitrogen in the fermentation environment (C/N ratio – due to many microalgal biomasses used in anaerobic digestion experiments exhibiting high protein contents), may significantly alter biogas production. It is therefore recommended that a biomass should only be used after lipid extraction, with high carbohydrate content, thus enabling to balance the C/N ratio of the anaerobic digestion. Unfortunately, there are still insufficient optimisation studies on this matter, regarding degradation of microalgal biomass, with no consistent method being found in the literature.

The experiments carried out by Zhao and collaborators (2014), when studying anaerobic digestion of several microalgae species, mostly of low lipid content (between 9 and 13%), serve as examples to indicate that although important differences did exist, the overall range among all biomasses, both whole cell and lipid-extracted, ranged from 304 to 557 mL CH4 g^{-1} VS without any clear explanation. The anaerobic digestion of *Chlorella* biomass after the biodiesel production process showed that the process efficiency was improved by 37.1% when increasing the C/N ratio of the residues from 5.4 to 24.17 (Ehimen et al. 2011). Hermann et al. (2016) studied the co-digestion of *Arthrospira platensis* and abundant carbohydrate sources (Barkley straw BS, energy beet sillage EBS, *L. digitata* LD), as the microalga exhibited a C/N ratio of only 4.3, while the other substrates had ratios of BS 145.5, EBS 41.7 and LD 28.7. Thus, the idea of combining them might be an interesting alternative to reduce the C/N ratio and may cause inhibition due to high ammonia concentrations in the digester. Still, the results showed a biogas production varying between 311 and 360 mL CH4 g^{-1} VS, being very close to the control (only with microalgal biomass).

8 A Brief Presentation of Patents and Market Situation of Microalgae for Wastewater Treatment

Although the scientific literature is very intense, we have also to consider the inventions registered in the patent data bases and the market application of this technology to have a wider vision of this field.

The search for keywords "microalgae and (wastewater or effluent) and treatment" at Derwent Innovations Index resulted in the recovery of 125 families of patents, with a great part of them deposited since 2008 and originated from China (42 recoveries, mainly the after 2010) and Korea (27). The most cited – also the oldest – is the European EP328474, where microalgae within the bed react with the organic material. This technology is supposed to remove organic contaminants, ammonia, nitrates and nitrates and organic pigments and improve water clarity (Jaubert and Jaubert 1989). Another very well-cited patent is CN102336498-A, where nitrogen-phosphorus sewage is treated using floating and mechanical pretreatment, followed by processing with batch reactor and optical bioreactor, with the microalgae powder produced being used as feed additive for livestock and poultry. This invention is classified as environmentally friendly and economically viable and resulted in water with improved quality (Cai 2012). Finally, a photobioreactor device was proposed by Ferreira (WO2008010737, 2008) to produce useful biomass (e.g. microalgae and cyanobacteria) when treating effluents, using a set of transparent tubes vertically aligned to allow the upflow direction of culture medium exposed to sunlight. The large range of uses claimed for this device include producing nutraceuticals, such as beta-carotene and astaxanthin, engineering for treating wastewater with reducing suppressing nitrogen and phosphorus and purifying effluent containing pollutants like heavy metals and/or radionuclides (Ferreira 2008). Many other inventions are directly related to the field, for example, CN103086520A (coupled bioreactors and electrochemistry), CN105417877A and CN106365318A (livestock wastewater treatment process and deep sewage treatment by serial microalgae culture, respectively). These bring the evidence or at least expectation of commercial and market interest for microalgae and water remediation/treatment technologies.

In terms of market, during the Algae for Wastewater Treatment Workshop, 2016, (Glendale, AZ, USA) organised by the Water Environment Federation, AZ Water Association and ABO (Algae Biomass Organization) demonstrated some restrictive applications in the past (decades) of the large-scale application of microalgae for wastewater treatment. However, at present, this technology is gaining more attention and is viewed in constant evolution and prospective to large-scale applications and commercialisation combining energy, environmental and biomass production issues (AWTW 2016).

A few pilot plant of biorefineries are operational, to cite: High-oil-yielding microalgae strains (Oilgae) and advanced trait algae strains (Aurora Algae), Tubular or floating photobioreactor panels to the growing and harvesting systems by UniVerve; Greenline's disruptive wastewater technology, Evodos's breakthrough centrifugal separators; Aragreen's proprietary strains of algae, Algal-bacterial photobioreactor by AlbAcqua, RENEW Process by CalPoly, LEAR (Low Energy Algal Reactor) by Aqualia, Phenometrics Environmental's Photo Bioreactor™ (ePBR™) and Matrix System (Zule et al. 2013; AWTW 2016; Roa 2018). Main reactor configurations are expected to be raceway ponds, but closed photobioreactors and algal biofilm technology are cited as well. Large-scale demonstrations have been in operation over the last 5 years, with other plants being in implementation phase in the USA (Kumar 2016; AWTW 2016; Roa 2018).

9 Conclusions and Future Prospects

With this chapter, it could be observed that microalgae might be an interesting alternative for analysing wastewater toxicity. Furthermore, their biochemical plasticity may lead to greater carbohydrate content in biomass, reaching 60% or more in some cases, which may serve as a substrate for biofuel production. It was also demonstrated that these photosynthetic microorganisms can exhibit high COD, nitrogen and phosphorus removal efficiencies in urban, agroindustrial and digestate effluents. Nevertheless, studies regarding the optimisation of carbohydrate production in effluent cultivation are still insufficient in the literature, as is also the case for researches on biofuel production with microalgae, with high carbohydrate content. With this, we hope that this chapter can raise the reader's and the community's awareness to the aspects discussed.

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Comprehensive Evaluation of High-Rate Algal Ponds: Wastewater Treatment and Biomass Production

Shashi Ranjan, Pankaj Kumar Gupta, and Sanjay Kumar Gupta

1 Introduction

HRAPs are shallow and open raceway ponds that have been used for wastewater treatment using algal species. High-rate algal ponds (HRAPs) offer self-sustainable prospects for high-efficiency wastewater treatment and value-added material and energy recovery, as well as biomass production (Park et al. 2011). As of now, thousands of communities, industries, and farms globally use HRAPs systems for wastewater treatment along with algal biomass production (Pittman et al. 2011). HRAP is gaining more attention of the scientific community and industrial practitioners to implement it for concurrent treatment of wastewater and biomass productions. Further, some key merits of this system established more space among other techniques: (1) high pollutant removal efficiency, (2) high rate of nutrient uptake/sink, (3) low cost of implementation and little maintenance, and (4) high biomass production for biofuels, i.e., produce >20 times more oil per hectare than terrestrial oilseed crops. Initially, Oswald and Golueke (1960) proposed simultaneous production of algal biofuels and wastewater treatment using HRAPs. With time the efficiency of HRAPs has been improved by incorporating the advanced facultative ponds, HRAPs, algal settling ponds, and maturation ponds (Craggs, 2005). Generally, the design of the HRAPs depends on the pollutant loads and biological oxygen demand (BOD) removal. Furthermore, the algal growth and photosynthetic activity under different environmental conditions are important to meet the maximum pollutant removal efficiency and high biomass productions. The crucial environmental (light,

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temperature, etc.), operational (pH, CO_2 concentration, and nutrient level), and biological (species diversity of zooplankton, phytoplankton, pathogens) parameters significantly affect the removal efficiency and biomass productions (Torzillo et al. 2003). To accelerate the removal efficiency, generally the controlled conditions are maintained in and around HRAPs by providing appropriate temperature, light, pH, nutrient, additional CO_2, and so on. The optimal temperature conditions help to accelerate microbial growth inside the HRAPs along the algal growth. The optimal temperature measured under conditions of maximum algal growth rate varies between algal species but is often between 28 °C and 35 °C for many algae. Increased carbon availability is ensured by the addition of CO_2 in the HRAPs to maintain the optimal pH (7.5–8.5) for algal and bacterial growth. Likewise, the light conditions affect photosynthetic activities of algal species (Park and Craggs, 2010). To compensate for the limitation of the nutrient availability, generally fertilizer is added in commercial HRAPs systems. Hence, the optimum and sustainable production of microalgae subsequently with wastewater treatment in HRAPs can be attained by overcoming the limiting conditions and through the control of algal grazers and pathogens.

A better understanding of the governing mechanisms involved in HRAPs system is required to treat diverse wastewater and biomass production. Both fundamental and field-scale research is needed to optimize algal production and harvest from wastewater treatment HRAPs while maintaining high effluent water quality. In this chapter, a state-of-the-art review of literature is presented to understand the design, governing process of HRAPs system, and role of different environmental/operational parameters on its performance. Effective implementation of HRAPs system will lead to the production of value-added products like potash in the near future. This chapter may help to frame research work related to improvement/implementation of HRAPs system for effective wastewater treatment and biomass production for fertilizers, feeds, and biofuels.

2 Design of HRAPs

HRAP is an open pond system generally installed in the outdoor area to maintain natural sunlight, referred to as raceway ponds. Some advantages of this pond are (1) low investment and operational costs, (2) easy to maintain, (3) utilizing nonagricultural land, (4) low-energy inputs, and (5) low hydrodynamic stress on algae. HRAPs have shallow configurations to prevent light limitations to algal culture. First, the concern in the design of HRAPs system is to select an area having the low impact of rainfall or flood to prevent dilution of culture. Solar power radiation an important factor for the photosynthetic activity of algal cells which is a minimum of 4.65 kW h m^{-2} d^{-1} is found necessary for algal cultivation in open raceway ponds. Further, the slopy lands having slope more than 5% are usually not suitable for pond construction as they will alleviate the construction cost (Bennett et al. 2014). The depth of HRAPs is crucial for energy efficiency of ponds, and it has been reported that the shallow ponds are highly efficient with higher biomass production. Several factors including the depth of HRAPs, presence of baffles, and paddle wheel speeds affect the power consumption, which generally ranges 1.5–8.4 W m^{-3} (Mendoza et al. 2013). Cell mixing in HRAPs is a crucial factor for the optimal growth of microalgae; it provides periodic light exposure to the cells and helps in homogeneous dispersion of nutrient and cells and the removal of oxygen generated. Usually the C/N ratio of algal cells is much higher than the incoming wastewater; thus the addition of CO_2 is recommended for higher production (Brennan and Owende 2010). The increasing pH promotes the higher CO_2 absorption rate in HRAPs; generally the pH in open ponds ranges from 7 to 8 (González et al. 2012). The design of HRAPs depends on the scale, pollutant loads, BOD removal, climatic conditions, etc. Generally, the HRAPs are of two types, i.e., raceway ponds and circular ponds. In recent years, with the advancement of instrumentation and research capabilities, several enhancements have been demonstrated and proposed for higher algal production. The focus of such modification was on enhancing the mixing efficiency and residential times of CO_2/gas bubbles, etc. (Brennan and Owende 2010). The basic design of HRAPs is highlighted in Table 1 and shown in Fig. 1.

Table 1 Summary of the design of HRAPs used in wastewater treatment

HRAPs types	Advantage	Mixing processes	Remarks
HRAPs with manual mixing	Manual mixing raceway ponds is effective if the algal species are well grown in pH >10 conditions	Nonmechanical	Limited nutrient and pathogen removal
Paddle wheel-driven HRAPs	Paddle wheel creates eddies which help to mix algae from the bottom to top circulation	Mechanically by paddle wheels	Boxlike shape was found to be the optimum in terms of energy utilization and losses and enhances the mixing (Liffman et al. 2013)
Sump-/baffle-/airlift-assisted HRAPs	Countercurrent injection of CO_2 to increase the liquid/gas contact time	Sump-assisted HRAPs do not require external energy to maintain the flow	These systems increase the gas/liquid contact time and the dissolution and utilization of CO_2
Hybrid raceway ponds	Dual system of photobioreactor and open pond system for enhanced growth	Mechanically	By utilizing the dual system, both the systems complement each other by harnessing the benefits and eliminating the demerits

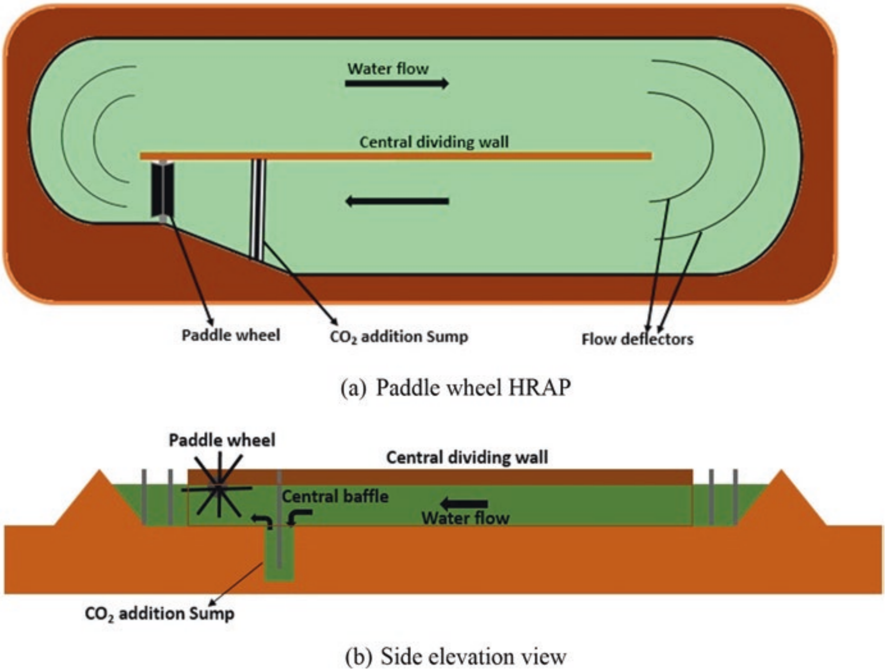

Fig. 1 Schematic diagram of HRAPs generally used in wastewater treatments. (**a**) Paddle wheel HRAP. (**b**) Side elevation view

3 Wastewater Treatment in HRAPs

The activated sludge treatment is one of the most extensively used traditional approaches for secondary wastewater treatment so far. In conjugation with anaerobic treatment, activated sludge treatment can be applied effectively to various types of wastewater, albeit there are environmental as well as economical concerns with it like external supply for O_2 for aeration as well as it lacks in the recycling of nutrient. With the increasing concerns over water scarcity and energy security, the HRAPs are the promising technology as they offer a sustainable and energy-efficient system for wastewater treatment (Kim et al. 2014). The HRAPs are basically a well-mixed shallow pond system which is designed to enhance and optimize the algal growth for a high-rate wastewater treatment. Constant mixing through paddle wheel is essential for algal culture circulation and to prevent the sedimentation of biomass. The HRAP is based on the symbiotic relationship between microalgae and bacteria, in which the oxygen required for organic matter decomposition by bacteria is provided by photosynthesis of algae and the nutrient for algal growth provided by bacterial decomposition of organic matter (Garcia et al. 2000). The algal biomass generated during the wastewater treatment can be harvested for the biofuel production, and it increases the commercial viability of HRAPs (Table 2).

Table 2 Comparisons of general wastewater treatment system and HRAPs wastewater system

Factors	General WWT system	HRAPs WWT	References
Economical aspects			
Capital cost	High (to develop multistage reactors)	Low (minimal infrastructures)	Park et al. (2011); Mehrabadi et al. (2015)
Operational/ maintenance cost	High (mainly due to aeration)	Low (maintained by algal by-product)	
Commercial applicability	Low	High (production of algal biomass for bioenergy)	
Environmental aspects			
Water footprint	Significant	None	Mehrabadi et al. (2015)
Risk of contamination	High (if advance processes are not working properly)	Less (generally pathogens are not removable)	Cuellar-Bermudez et al. (2017)
Nutrient management	Low (no uptake)	High (due to algal uptake)	Bashar et al. (2018)
Social aspects			
Acceptance	Rare in rural and remote areas	Easy and has potential for rural and remote area	Efroymson et al. (2017)

3.1 Mechanism of Nutrient Removal in HRAPs

The aerobic bacterial degradation of organic compound and nutrient removal by microalgae is a complex and mutualistic process between algae and bacteria. Microalgae can grow photosynthetically even in harsh conditions while assimilating nutrients (nitrogen and phosphorus) to produce huge amount of algal biomass. The photosynthetic aeration of wastewater in HRAPs by algae is beneficial in terms that it provides oxygen to the heterotrophic bacteria and prevent the eutrophication of aquatic environment (Delgadillo-Mirquez et al. 2016). The bacterial population takes up oxygen and facilitates the aerobic degradation of the organic matter while releasing CO_2 which is utilized in the photosynthesis of microalgae. Microalgae play a central role in nutrient removal either by direct assimilation of nitrogen or by indirect volatilization of ammonia and precipitation of phosphorus with increased pH due to photosynthetic growth of algae (Delgadillo-Mirquez et al. 2016). The nutrient uptake by microalgae depends on the biomass concentration of elements in the algae; for example, nitrogen uptake by microalgae is higher than phosphorus due to the fact that the nitrogen content for algal biomass is higher than phosphorus (Malik 2002; Whitton et al. 2015). The effectiveness of HRAPs over convention methods has been extensively reported. The nutrient removal process will be elaborated in the upcoming subheadings.

Nitrogen Removal

Nitrogen is one the constituents of wastewater that is largely responsible for eutrophication of aquatic environment, mainly because the convention treatment systems are unable to remove it below the permissible limit before discharging. Algal ponds have received so much attention due to their nutrient removal capacity. In HRAPs the nitrogen can be removed directly (nitrification/denitrification) as well as indirectly (volatilization/sedimentation) by the growing biomass of microalgae. Wastewater receives influent having high nitrogen content mainly in the form of ammonium nitrogen. High availability of $(NH_4^+ - N)$ leads to the nitrification by the autotrophic bacteria, in a two-step oxidation of ammonium: first it oxidizes to nitrite, and later nitrite oxidizes to nitrate. The most common genera of bacteria involved in the oxidation process are *Nitrosomonas* and *Nitrobacter*. The nitrate will further be used by microalgae present in the suspension. Nitrogen is one of the major constituents in the living matter, present in the form of organic nitrogen (peptide, proteins, DNA, etc.) derived from the inorganic form (nitrite, nitrate, ammonium). The process of conversion from inorganic nitrogen to organic nitrogen is termed as assimilation. During the process, the inorganic nitrogen (nitrate and nitrite) is translocated through the plasma membrane of microalgae and reduced to ammonium to finally get incorporated into amino acid (Cai et al. 2013). The overall nitrogen assimilation is a two-step process: initially the nitrate is transported into the cell where the nitrate reductase enzyme reduces nitrate to nitrite, and further nitrite is transported into the chloroplast and subsequently reduced to ammonium by nitrite reductase enzyme (Fig. 2). The resulted ammonium then is incorporated into amino acid by glutamate synthase (Sanz-Luque et al. 2015). Wastewater contains a

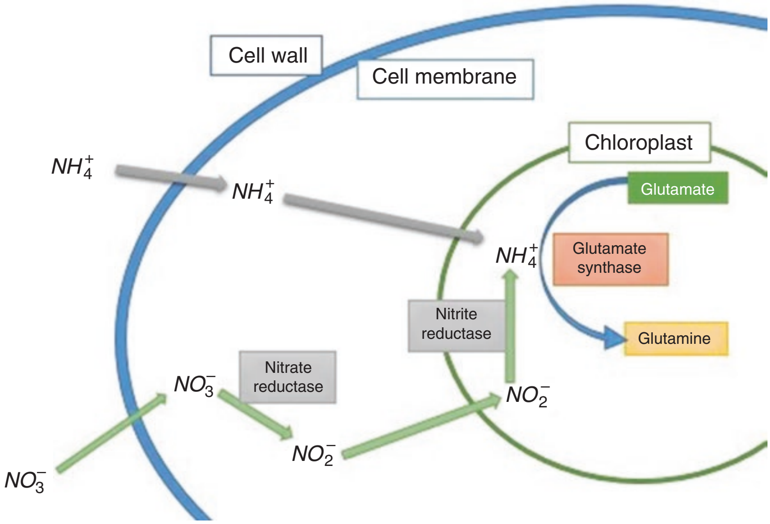

Fig. 2 Nitrogen assimilation by algal cell

high amount of ammonium available for microalgae, and most of the algal species prefers ammonium over nitrate because it requires less energy for ammonium assimilation. Ammonium is directly translocated into the algal cell and incorporated into amino acid by glutamate synthase for protein formation.

A large part of nitrogen removal from wastewater occurs through indirect means by ammonia striping mainly because of change in pH and temperature.

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O \tag{1}$$

In the daytime, due to microalgae photosynthesis, the CO_2 and carbonate ion concentrations decreases, results in higher pH (>9) of wastewater, and the raised temperature due to diurnal change, which leads to ammonium ($NH_3 - N$) striping. With increasing pH (>7), the equilibrium shifts to the right in Eq. 1, with production of NH_3 gas (Martínez 2000). Garcia et al. (2000) have reported ammonium ($NH_3 - N$) striping as high as 60% due to the combination of raised pH and temperature and based on that suggested it as the main mechanism of nitrogen removal in HRAPs. They have also reported the total nitrogen removal of 73% in which 47% by ($NH_3 - N$) striping and 26% by algal assimilation and further separation.

Phosphorus Removal

Wastewater contains phosphorus in three forms: orthophosphate (Ortho-P), polyphosphates, and organic phosphorus compounds. The latter two forms of phosphorus are hydrolyzed and decomposed, respectively, to Ortho-P, which constitutes 80% of the total phosphorus in wastewater. At normal pH, HPO_4^{-2} is the principal form of Ortho-P. In the presence of carbonate salt of calcium, magnesium, and other metal salts at higher pH (>8), the Ortho-P precipitates as the insoluble complexes (Nurdogan and Oswald 1995). The induced precipitation of Ortho-P by the addition of metal salts is termed as auto-flocculation. Phosphorus assimilation by microalgae is essential in terms that it is needed for phospholipids and nucleic acid synthesis and for the energy transfer in the cell. Phosphorus, preferably in the form of $H_2PO_4^-$ and HPO_4^{-2}, gets across the plasma membrane through active transport (Whitton et al. 2015). The Ortho-P is incorporated into the nucleotides by going through a three-step process: (a) phosphorylation, (b) oxidative phosphorylation, and (c) photophosphorylation. At the end of these processes, ATP (adenosine triphosphate) is formed from ADP (adenosine diphosphate). As compared to nitrogen, the phosphorus assimilation is quite low due to the reason that phosphorus content in algal biomass is less than nitrogen. The nutrient removal from animal waste through HRAPs shows nitrogen removal of 78% over the phosphorus removal of 54% (Fallowfield et al. 1999).

BOD Removal

A major part of wastewater consists of organic material (proteins, carbohydrates, oil, and fats) which requires oxygen to get degraded by bacteria aerobically. Oxygen provided by microalgae photosynthesis is used by bacterial consortia to oxidize the organic content to generate energy for cell synthesis and maintenance (Batten et al. 2013). BOD removal of 50–80% was reported by Craggs et al. (2012) without CO_2 addition in a pilot-scale HRAPs having influent BOD of 63 gm/m^3 of wastewater. In a mixed algal pond system, heterotrophic microalgae use organic carbon as a carbon source for their cellular growth, enhancing the BOD removal (Gonçalves et al. 2017). Apart from the nitrogen and phosphorus, microalgae can also take up the dissolved metals in aqueous phase, which was demonstrated by Oswald (1988). Majority of heavy metals like iron, zinc, cobalt, chromium, nickel, etc. can get accumulated in algal biomass. Uptake of some toxic metal limits the use of algal biomass as food supplements.

4 Production of Algal Biomass in HRAPs

The effectiveness of HRAPs as a wastewater treatment system at different scales has been reported and demonstrated in literature extensively (Craggs et al. 2012; Garcia et al. 2000). Algal farming is considered to be beneficial over the traditional agricultural crops due to high growth rates, less requirement of land and water, and ability to grow over the year. Algal biomass production in HRAPs for fertilizers, feed, and feedstocks for biofuel production is an additional advantage, and this makes it an economical and sustainable option for wastewater treatment. Most of the algal biomass for biofuel production is produced from the open raceway algal ponds, but commercial algal biomass production requires a large amount of nutrient and freshwater, which increases the cost of production. Alternately HRAPs are economically very cheap, as they take wastewater as the input and perform the dual function of wastewater treatment and biomass production. As the wastewater provides abundant nutrients (nitrogen, phosphorus, and carbon), it provides a perfect medium for the biomass production with the additional benefit of phycoremediation. The productivity in the HRAPs is reported to be almost the same as photobioreactors (20 gm m^{-2}d^{-1} of maximum biomass concentration), but due to the zooplankton grazers and pathogens, often biomass productivity is less than expected (Gera et al. 2015). Typically in raceway ponds with wastewater, the algal productivity ranges from 5 to 15 gm m^{-2}d^{-1} without the addition of CO_2. The biomass productivity increases up to 30 gm m^{-2}d^{-1} with addition of optimum CO_2 (Sturm and Lamer 2011) (Table 3).

Biomass production in HRAPs with wastewater comes with a potential drawback; the lipid content of algal biomass grown in HRAPs is generally lower than the freshwater grown. Lower lipid content is the limitation to use the HRAPs-grown algal biomass as biofuel. The production of the algal biomass for commercially viable products is subjected to different factors which include physical, operational, and biotic factors. Biomass harvesting from open pond is the limiting step in bio-

Table 3 Biomass productivity by different microalgae species under different growth medium

Microalgae species	Growth medium	Biomass productivity (gm $m^{-2}d^{-1}$)	Lipid productivity /nutrient removal	References
Chlorella vulgaris	Open pond freshwater	0.339	0.825 gm L^{-1} d^{-1}	Bhola et al. (2011)
Neochloris oleoabundans	Artificial wastewater	0.350	99% N and 100% P removal	Wang and Lan (2011)
Neochloris oleoabundans	Secondary municipal wastewater	0.233		Wang and Lan (2011)
Chlamydomonas reinhardtii	Municipal wastewater	2.0	25.5% oil content 83% N and 14.25% P removal	Kong et al. (2010)
Chlorella sp.	Secondary municipal wastewater	0.74	0.029 gm L^{-1} d^{-1} 92% N and 86% P removal	Cho et al. (2011)
Scenedesmus obtusus	Freshwater bioreactor	0.212	0.0607 gm L^{-1} d^{-1}	Xia et al. (2013)
Chlorella sp.	Secondary municipal wastewater	0.92	0.12 gm L^{-1} d^{-1} 89.1% N and 80.9% P removal	Li et al. (2011)
Chlorella pyrenoidosa	Soya bean processing wastewater	0.64	0.40 gm L^{-1} d^{-1} 88.8% N and 70.3% P removal	Hongyang et al. (2011)
Chlorella pyrenoidosa	Municipal wastewater	0.16	95% N and 81% P removal	Dahmani et al. (2016)
Chlorella vulgaris	Brewery wastewater	0.227	0.108 gm L^{-1} d^{-1}	Farooq et al. (2013)
Chlorella vulgaris	Municipal wastewater	0.195	9.8 mg L^{-1} d^{-1} N and 3.0 mg L^{-1} d^{-1} P removal	Cabanelas et al. (2013)

mass production. The life cycle of algal species varies; hence it possesses difficulties during the harvesting phase (Renuka et al. 2015). Most of the facility uses flocculation followed by gravity settling for harvesting the biomass. Chemical flocculants are used (multivalent cations and cationic polymers) to agglomerate the microalgae cells in the mixed suspension by neutralizing the negatively charged surface (Gera et al. 2015).

5 Factors Affecting Biomass Production and Nutrient Removal in HRAPs

Various factors affect the algal growth and nutrient removal in HRAPs including physical (light and temperature), operational (pH, CO_2, nutrient, dissolved oxygen, mixing, hydraulic retention time), and biotic factors (zooplankton grazers and pathogens).

5.1 Physical Factors

Light

Autotrophic microalgae obtain energy from sunlight (photosynthetically active radiance 400–700 nm) to convert inorganic carbon to organic carbon to accumulate biomass, and the process is called photosynthesis. A very small part of sunlight (12–14%) is converted into biomass, and majority is lost as heat (Larsdotter 2006). In a nutrient-sufficient condition, the increasing light intensity supports a maximum growth for microalgae till the saturation point; beyond this point, the increasing light intensity can damage the photosynthetic microalgae, termed as photooxidation. As the biomass and concentration of microalgae increase in the pond, the shadding effect decreases the sunlight penetration below 15 cm of pond depth, so optimized HRT (hydraulic retention time) and vertical mixing are required to ensure proper sunlight exposure (Lee and Lee 2001; Park et al. 2011). Light and dark cycles during waste treatment with algae affect the nutrient uptake and biomass production. Nitrate uptake and cell growth by *Chlorella kessleri* are reported to be higher under continuous light illumination, but carbon removal efficiency was better under alternate light and dark conditions (Lee and Lee 2001).

Temperature

The temperature of the mixed system and the surrounding environment influences the metabolic rate, biomass composition, and nutrient requirement. Seasonal temperature variation as well as the daily fluctuation in temperature can affect the growth of microalgae. Majority of microalgae can grow over a wide range of temperature (10–40 °C), but the optimal temperature range is 20–35 °C (Mehrabadi et al. 2015; Ras et al. 2013). The maximum growth rate for *Chlorella vulgaris* in a heterotrophic bacterial mixed system is obtained at an optimum temperature of 32.4 °C (Mayo 1997). Further, temperature is a crucial factor for the biochemical composition of the algal biomass, and a general trend of increased saturation of fatty acids has been shown with increasing temperature. Temperature also affects the total lipid content of algal biomass (Hu et al. 2008). Hence, temperature is found to be an important factor for algal growth and the biochemical nature biomass.

5.2 Operational Factor

pH

The pH of the mixed system influences the algal photosynthesis, the biomass regulation, the nutrient availability, and even the species composition of algae. The alkalinity and ionic composition of nutrient and different elements in the aqueous medium are defined by the pH. In the daytime when due to photosynthetic uptake of CO_2 and HCO_3^- by microalgae raises the pH, which in turn increases the ammonia striping and phosphorus precipitation, which was described earlier. Majority of freshwater algae grow optimally in a pH range of 7–9. Some of the species can live in higher pH; for example, *Chlorella vulgaris* grow optimally at pH 6.5, whereas the optimum pH for *Spirulina maxima* is about 9.5 (Mayo 1997). Large variations in the optimal pH can cause physiological and productivity issues in microalgae (Pulz 2001).

CO_2

During photosynthesis microalgae take up the carbon and convert it in the biomass. Carbon is assimilated by microalgae in one of either inorganic (CO_2 or HCO_3) or organic (sugar, organic acids, glycerol, etc.). Generally, most of the microalgae uses the organic as well as organic carbon, but some heterotrophic algae strictly can assimilate only the organic carbon (Gera et al. 2015). Carbon-to-nitrogen ratio of the wastewater is important for the growth of microalgae; generally it is well below (C/N 2.5–3.5:1) than what is required for the rapid growth of algae. Hence, most of the HRAPs employ additional CO_2 for the steady growth of microalgae; besides growth they also affect the fatty acid content of algal biomass. The CO_2 concentration of wastewater further alters the saturation of fatty acids. *Chlamydomonas* species was grown under different CO_2 concentrations, and the composition of the total saturated lipids was found to be 65.3% under 4% CO_2 and 58.1% under 2% CO_2 condition (Nakanishi et al. 2014).

Nutrient

Often nutrient becomes a limiting factor for the growth of microalgae; among all nitrogen is the most critical one. Algal biomass has a general composition of $C_{106}H_{181}O_{45}N_{16}P$, so nutrient in the proportion of 16:1 would be required with respect to nitrogen and phosphorus (Lannan 2011). Although N/P ratio can vary from 4:1 to 40:1 in different algal species (Craggs et al. 2014). The nutrient concentration in the mixed system defines the microalgae species dominance in the algal ponds. In general the wastewater contains excess of phosphorus for the nitrogen available in the wastewater (Mehrabadi et al. 2015). Beuckels et al. (2015) studied the effect of nitrogen supply on nutrient uptake in two species of microalgae. They found that the removal of phosphorus from wastewater is directly related to the concentration of nitrogen in wastewater. The nitrogen concentration in wastewater also affects the biochemical composition of algal biomass; at high concentration of nitrogen, the carbohydrate and lipid concentration in the algal cell decreased by around 20%. Even phosphorus limitation can lead to increased lipid content especially triacylglycerol (TAG) in several microalgae species (Hu et al. 2008).

Dissolved Oxygen

Due to photosynthesis in the daytime, the dissolved oxygen concentration in the mixed system can shoot up to 200% of saturation level albeit the increased oxygen concentration can impact the algal growth (Garcia et al. 2000). At the optimum dissolved oxygen, the photosynthetic activity and the biomass generation stay steady. At a high dissolved oxygen concentration, more than 470% of air saturation inhibits the photosynthesis, but they do not cause cell destruction (Molina et al. 2001).

Mixing

Mixing in the algal ponds promotes the balanced distribution of sunlight and homogenization of carbon and other nutrients. Stagnation in algal ponds can induce the thermal stratification and formation of a boundary layer around the microalgae cells; these can reduce the nutrient assimilation, gas exchange, and photosynthetic activity of microalgae (Mehrabadi et al. 2015). Mechanical mixing mainly achieved through paddle wheels nowadays increases the sunlight exposure of the algae in shallow algal ponds, which promotes steady photosynthetic activity throughout the algal consortia. Ogbonna et al. (1995) have studied the effect of mixing on the productivity of the algal species (*Chlorella pyrenoidosa*) and found that the productivity of algal cells can be increased with the mixing as well the cell density in the mixed system increases, although there were no effects found when the cell density was low.

Hydraulic Retention Time (HRT)

For algal ponds, HRT is an important factor, influencing the cell density, algal species, algal/bacteria ratio, and nutrient removal efficiency. HRT also determines the algal population dynamics as the growth rate of species is different, and in turn it affects the biochemical nature of the total algal biomass (Mehrabadi et al. 2015). HRT should be optimized; it should not be too long or too short. The long HRT slows the algal growth due to shading and nutrient deficiency; with shorter HRT, the algal pond will be unable to remove the nutrient from the wastewater. HRT shorter than the minimum generation time of algal cell will lead to washout of algal cells (Larsdotter 2006). For wastewater-treating algal ponds, the HRT is commonly 2–7 days.

5.3 Biotic Factors (Zooplankton Grazers and Pathogens)

In a completely mixed system, apart from the environmental and operational factors, some biotic factors like internal species competition and zooplankton grazers and pathogens also affect the algal growth and productivity. In the mixed system, the competition between the species for space and nutrient is evident. In case of monoculture system, the zooplanktons, which enter the HRAPs as pollutant, can have detrimental effect on the wastewater treatment, as the zooplankton consumes microalgae. Ciliates, rotifers, cladocerans, copepods, and ostracods are the major herbivorous zooplankton grazers in HRAPs. These zooplanktons can consume the algal biomass within a short duration, affecting the efficiency of HRAPs; thus grazer management is an essential step for effective wastewater treatment through algal ponds (Montemezzani et al. 2015).

6 Environmental and Economic Sustainability of HRAPs

Economically the production of algal biofuels and concurrent treatment of wastewater using HRAPs is one of the most possible approaches to achieve environmental sustainability. Algal productivities measured in both commercial production and wastewater treatment HRAPs range widely from 12 to 40 g/m^2/d (Park et al. 2011). Furthermore, Mehrabadi et al. (2017) reported up to 47.4% of the biomass energy (19.7 kJ/g) recovery as bio-crudes from algal production. Zhu et al. (2017) highlighted high lipid yields (up to 52%) from algal biomass. Biomass can be used for bioethanol production to enhance the biorefinery economics. Ashokkumar et al. (2015) achieved the maximum biodiesel yield of 0.21 g/g of dry weight and a bioethanol yield of 0.158 g/g of dry weight. Patnaik and Mallick (2015) obtained 38 g of biodiesel, 3 g of glycerol, 2 g of omega-3 fatty acids, 0.06 g of β-carotene, and 17 g of bioethanol from 100 g of *S. obliquus* biomass.

Wastewater treatment is a major concern of twenty-first-century world where urbanization and population significantly degrade the quality of water resources. Low socioeconomic communities, especially remote communities, are still waiting for such treatment facilities. In this situation, HRAPs are low-cost treatment techniques which provide an opportunity to replace high-cost treatment facilities.

The electromechanical secondary-level activated sludge treatment is generally costly, and the capital and operating cost are estimated to be three or four times higher than the HRAPs systems (Downing et al. 2002). On the other hand, HRAPs system is a well-reported system acting as a sink of CO_2 or greenhouse gas which may help to reduce atmospheric CO_2 concentrations (Clarens et al. 2010). The biomass from HRAPs helps to produce fertilizers having sufficient nitrogenous compounds and biofuels to ensure energy demands. The wastewater contains high amount of nutrient, which is used by the algae in HRAPs system and recycled. Biomass produced from the HRAPs wastewater treatment may further be used as bio-fertilizer and bio-feed which significantly contribute wealth to society. Thus, HRAPs system is one of the sustainable approaches to meet the effective wastewater treatment and biomass production.

7 Research Need and Future Prospects

In this chapter, an overview of HRAPs system has been discussed with special emphasis on the role of environmental and operational conditions on wastewater treatment and biomass productions. Although there is a huge potential for algal production for biofuel, the approach still needs a lot of attention and improvement, so as to commercialize the biofuel. The following points are highlighted which can possibly provide a clear idea for near future research and development.

1. *Improvement in HRAPs designs:* More engineering attention should be given on the low-energy or self-sustainable solar-powered designs of HRAPs in the near future. Further, one should have to focus on climatic suitable design of HRAPs to reduce the impact of rainfall, evaporations, floods, etc. Microalgae are photosynthetic species growing in HRAPs, and thus it is particularly important to design and develop the suitable HRAPs for the maximum production of biomass.
2. *Biotechnological/biochemical approaches:* The biological properties such as photosynthetic efficiency, high lipid production, and enhanced tolerance capacity toward the environmental factors of microalgae can be enhanced biotechnologically (Hamilton et al. 2014). Most of the open ponds cultivate the monoculture of algal species. Microalgae are diverse organisms, and it's not possible to grow all the species at commercial scale; thus species should be identified, and their enhancement through biotech will be fruitful for the improved productivity. Genetic and metabolic engineering toward improving algal properties has been widely reported in literature (Zhu et al. 2017). To improve the performance of target algal species and production of biocatalysts. It is important to identify algal strains that thrive in the HRAP environment and also important to improve the lipid extraction methods and glycerol production to accelerate algal biodiesel economics.
3. *Environmental/operational parameter optimization:* Most of the earlier studies investigated the impact of single parameters on HRAPs performance. There are urgent needs to investigate the combined role of different environmental and operational parameters on the wastewater treatment in HRAPs. The various parameters (light and temperature, pH, CO_2, nutrient, dissolved oxygen, mixing, hydraulic retention time, zooplankton grazers, and pathogens) affect the algal growth and nutrient removal in HRAPs, and to attain the better yield, a thorough understanding of these factors is required. The role of all these parameters on the algal production and their lipid content need to be investigated in large field-scale HRAPs for proper understanding.

4. *Performance evaluation for all pollutants:* Wastewater contains large numbers of pollutants like hydrocarbons, heavy metals, emerging contaminants, and so on. Low removal of heavy metals and pathogens is a disadvantage of HRAPs; thus it is needed to develop new species capable to remove these pollutants from wastewater (Rajasulochana and Preethy 2016). Thus it is important to evaluate the performance of HRAPs for all pollutants in separate and mixed forms under varying environmental conditions.

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