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Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production

Bunushree Behera, Anwesha Acharya, Iragavarapu Akhil Gargey, Nazimdhine Aly, Balasubramanian P*

Agricultural & Environmental Biotechnology Group, Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, India

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ABSTRACT

Integrated carbon dioxide (CO_2) sequestration, wastewater treatment (WWT) and biofuel generation place the microalgae as a promising feedstock at all levels. Though microalgae based biofuels are acknowledged as an alternative and renewable source of energy, mass cultivation and subsequent bioprocessing of microalgal species are the most challenging steps from a technological perspective. Hence, this article attempted to summarise the ecological and bioprocess principles involved in microalgal cultivation, with detailed discussion on various influencing factors for biofuel production. Further, it explored the real trait of microalgae through both thermochemical and biochemical conversion processes for the production of various biofuels such as biodiesel, bioethanol, biomethane, bio-oil, biohydrogen and other products. The integrated zero waste microalgal biorefinery concept was discussed briefly to promote sustainability on the commercial implementation of the microalgal biofuel technology. Future research challenges on microalgal biotechnology for commercial biofuel production were highlighted based on the existing limitations of bioprocess principles.

1. Introduction

Currently, energy security issues associated with the use of conventional fossil fuels along with environmental consequences of global warming has led to a paradigm shift towards renewable fuels (Maity et al., 2014). Greenhouse gas (GHG) emissions are the primary cause of global warming, consisting of mainly carbon dioxide (CO_2) are expected to increase to 35.6 billion metric tonnes by 2020 and 43.2 billion metric tonnes by 2040 (Li et al., 2017). Solar, wind, hydroelectric, geothermal and biomass energy are categorized under renewable sources and are increasingly popularised due to lower environmental jeopardies. To diversify and decentralize the energy supply, it is essential to exploit these resources using less capital-intensive technologies. However, underlying financial and land acquisition issues along with site-specific availability associated with solar, wind, hydroelectric and geothermal energy limits their use at the global scale (Kandpal and Broman, 2014).

Biomass refers to the plant-derived organic matter that acts as a promising sustainable source of bioenergy, paving the way for carbon neutral economy. Currently, biofuels (mainly first and second generation) accounts for 10% of the global energy needs (Maity, 2015). The first generation of biofuels from food crops poses a severe threat to food security triggering food versus fuel dilemma. Alternate energy from second generation biofuels derived from lignocellulosic biomass even though addresses the above-mentioned problems, however, the extensive energy and costs associated with the pretreatment technologies and the land acquisition issues restrict their use (Brennan and Owende, 2010). Based on the current technical projections, the next best suitable alternative is the third generation biofuels using microalgae as they are economically viable with higher yields and avoids the drawbacks associated with first and second generation biofuels (Sharma and Singh, 2017).

Microalgae are sunlight-driven cell factories which can utilize sunlight more efficiently and have a faster growth rate due to the less cellular complexity and efficient carbon sequestration compared to terrestrial plants (Chisti, 2007; Sharma and Singh, 2017). Microalgal biomass has been proposed as a promising raw material with the diversified biochemical content of lipids, carbohydrates and proteins that can be processed via a variety of thermal and biochemical conversion routes into biodiesel, biohydrogen, biomethane, bio-oil, bio-crude oil, etc. (Singh and Olsen, 2011). Adopting an integrated biomass conversion system localising the microalgal production inside industrial premises using effluent rich flue gas and wastewater as nutrient pool, would produce biomass for meeting the ever increasing energy

* Corresponding author.
E-mail address: biobala@nitrkl.ac.in (B. P).

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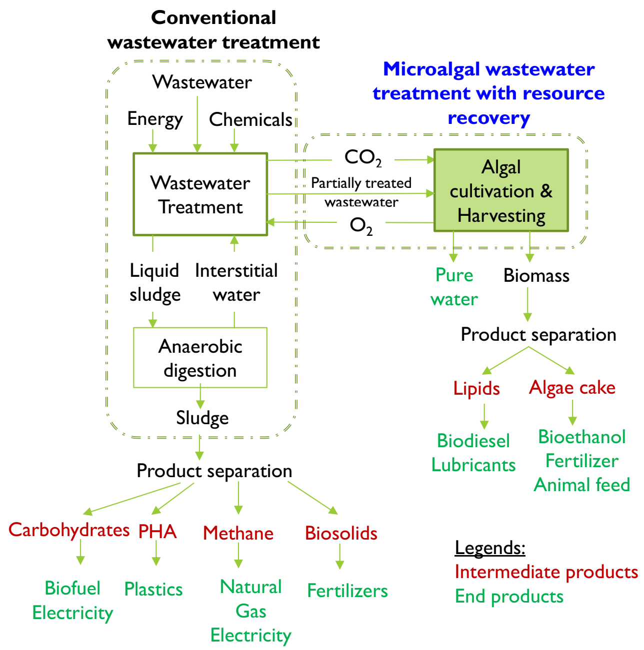


Fig. 1. Integration of microalgal wastewater treatment with resource recovery for maximizing the derivable products.

demands, with the added benefits of wastewater treatment (WWT) and emission control (Milano et al., 2016). This integrated approach (as illustrated in Fig. 1) is postulated to provide resource recovery based monetary benefits as well as 800–1400 GJ/ha/year energy which can be a source of energy at the community level (Mehrabadi et al., 2015).

The large scale production of microalgae dramatically depends on the culture conditions such as light availability, nutrients and other operational parameters associated with reactor configuration (Chisti, 2007; Pruvost et al., 2016). The available large scale cultivation technologies, of open ponds, are often outshined by the lacunae of control over the operating conditions resulting in low biomass yield and over dominance of invertebrates. Closed photobioreactors though outperform open systems, but none of the current design available could sustain at commercial scale (Singh et al., 2011). Further, the harvesting and extraction technologies currently in use, being energy intensive and costly are seldom recommended at large scale (Chen et al., 2011). Research is going on to find out the cost-effective methods of producing algal biofuels. It is essential to understand the biology of microalgae along with the biotechnological principles influencing the reactor operation and biofuel production to tackle the mentioned uncertainties. Nevertheless, to date, several researchers have reviewed in detail each of the individual aspects of strain selection (Ghosh et al., 2016); cultivation (Mata et al., 2010; Pruvost et al., 2016); harvesting and extraction (Brennan and Owende, 2010; Pragya et al., 2013; Mubarak et al., 2015); and conversion to biofuels (Singh and Olsen, 2011; Mehrabadi et al., 2015; Milano et al., 2016). Practical scale application of microalgae for bioenergy requires consolidation of the bioprocess principles influencing the biomass productivity with that of the synthetic microalgal physiology. The authors have earlier reported the effect of geographical coordinates (Aly and Balasubramanian, 2017) on microalgal growth and the effect of photoinhibition in open ponds (Aly and Balasubramanian, 2016) as well as in fixed and trackable photobioreactors (Aly et al., 2017).

The present paper attempts to integrate the biological principles governing the algal biology with that of the biomass productivity and thereby conversion into bioenergy. A detailed review of the existing large scale cultivation technologies, their design, performance along with their pros and cons has been presented. New promising photobioreactors with higher biomass productivities supplemented with much less land and water footprints have been discussed. A critical evaluation of the operational, environmental and biological parameters influencing the growth and viability of microalgae has also been presented. Finally, the review elucidates the current state of art related to the prevailing thermochemical and biochemical conversion processes routing the biomass to alternative bioenergy (i.e. biodiesel, bioethanol, biohydrogen, biocrude oil, bio-oil). The technological limitations of each of these conversion routes have been highlighted, with a special focus on integrated zero waste biorefinery approach that harbours the potential of making microalgal biotechnology a commercial reality in future.

2. Algal biology

2.1. Photosynthetic efficiency of microalgae

Microalgae are the most productive microscopic autotrophic organisms, capable of capturing CO_2 from the atmosphere and converting it into biomass by oxygenic photosynthesis (Chisti, 2007). Due to the absence of complex metabolic machinery and heterotrophic tissues, most of the accumulated energy is stored as biomass, which can be harnessed into biofuels (Sutherland et al., 2015). Photosynthetic efficiency is the rate at which chemical energy is stored in the form of biomass when the rate of incident solar radiation is about 20 MJ/m^2/day (Seth and Wangikar, 2015). Theoretically, the maximum photosynthetic efficiency of microalgae is 10–15%, but practically only 1–2% has been achieved due to reduced kinetic coupling between the light harvesting antenna and subsequent electron tran sfer processes (Perrine et al., 2012; Wobbe et al., 2016). The causes of low photosynthetic efficiency can be outlined as follows:

- Light harvesting complex (LHC) of the photosynthetic organisms can accept light in visible range which accounts for 50% of the total incident solar radiation (Seth and Wangikar, 2015);
- LHC transfers light at nearly 100% efficiency to the reaction centres photosystem (PS) II & I, where charge separation occurs. The size of reaction centres is large enough to capture light even at its limiting range. However, the size of the LHC is not yet optimized for achieving maximum quantum efficiency (Kirst et al., 2017);
- LHC captures excess electrons to drive the electron transport chain (ETC), dissipation of energy occurs in the form of heat/fluorescence by non-photochemical quenching, when the photons captured exceeds from that utilized in the ETC (Perrine et al., 2012);
- Energy wastage occurs due to photorespiration as a result of the affinity of Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCo) towards molecular oxygen (O_2) leading to oxidation of fixed carbon, which is again released as CO_2 (Hagemann and Bauwe, 2016).

Several approaches have been suggested by various researchers to improve the photosynthetic efficiency of microalgae. For instance,

- Size regulation of the LHC by genetic engineering. Shorter LHC size improves photosynthetic efficiency by allowing more light to penetrate (Wobbe et al., 2016; Kirst et al., 2017);
- Extending the waveband by including chlorophylls D and F, limiting the PAR (photosynthetically active radiation) to 750 nm, increases energy by 19% (Evans, 2013);
- Metabolic remodelling of RuBisCo enzyme to improve its catalytic efficiency (Meyer et al., 2016);
- Engineering photosystem (PS II) by genetically manipulating D1 protein to reduce photooxidative damage and achieve optimal photochemical efficiency at low as well as high light intensities (Kromdijk et al., 2016);
- Optimizing photosynthetic efficiency by exposing microalgae to

Table 1
Biochemical composition, oil yield, growth rate, CO_2 and temperature tolerance of various microalgal species reported in the literature.

Microalgae	Chemical composition (%)			Oil yield (%)	Growth rate (div/d) or g/day	CO_2 tolerance (%)	Temperature tolerance (°C)	References
	L	P	C					
Chlorella vulgaris	13.5	58.1	12.4	28	0.529 g/d	10–15	30–35	Goncalves et al. (2013); Safi et al. (2014)
Chlorella sp.	34	–	–	28–32	0.238 g/d	40	45	Chisti (2007); Singh and Ahluwalia, (2013)
Euglena gracilis	14–20	39–61	4–18	19.7	0.309 g/d	45	25–30	Kitaya et al. (2005); Singh and Ahluwalia, (2013)
Dunaliella tertiolecta	16.7–71	_	_	31–44	1.4 div/d	15	23	Mata et al. (2010); Lakaniemi et al. (2012)
Nannochloropsis gaditana	26.3	40.5	25.1	31–68	0.29–0.55 div/d	15	23	Chisti (2007); López-González et al. (2014)
Chlamydomonas reinhardtii	21	48	17	9.4	0.5 div/d	15	35	Yin et al. (2011); Singh and Ahluwalia, (2013)
Nannochloropsis oculata	22.7–29.7	_	_	31.4	_	2	30	Chiu et al. (2009); Mata et al. (2010); Yin et al. (2011)
Isochyris galbana	7–40	_	_	25–33	0.63 div/d	2–20	30	Lee and Kim (2002); Chisti (2007); Mata et al. (2010)
Scenedesmus obliquus	12–14	50–56	10–17	31.4 ± 2.06	0.56 div/d	18	30	Balasubramanian et al. (2011); Singh and Ahluwalia, (2013); Thiansathit et al. (2015)
Tetraselmis chuii	6–13	15–30	_	9.5	1.15 div/d	14	26–33	Purba and Taharuddin (2010); Lu et al. (2016)

alternating light and dark cycles, allows it to utilize intense light as the dark cycle re-oxidizes the electron transporters (Wagner et al., 2016);

- Spectral modification with photo-luminescent materials and organic dyes helps in enhancing light capture efficiency (Seo et al., 2015).

The rate of microalgal photosynthesis is crucial in treating wastewater and for accumulating biomass that can be converted into energy. A more comprehensive evaluation of the in-situ physiological changes underlying photosynthesis, with particular focus on efficiencies of light harvesting and carbon fixation will act as a significant headway in improving photosynthetic yields.

2.2. Effect of microalgal composition on biomass energy content

Algae are composed of three basic biochemical components: proteins, carbohydrates, and lipids, with a net energy content of 24 KJ/g, 17 KJ/g, and 37 KJ/g respectively (Mehrabadi et al., 2015). Biochemical composition of different algal species varies with environmental conditions (Chisti, 2007). Carbohydrates serve a structural and functional role, being the starting product of photosynthesis. Different classes of algae produce a specific type of polysaccharide. Chrysolaminarin, a linear polymer of β (1, 3) and β (1, 6) linked glucose units, is one of the common forms of starch occurring in pyrenoids of chloroplasts in almost all species of microalgae, which can be fermented to bioethanol, biohydrogen and biogas. Proteins also serve an essential structural and metabolic role, providing a framework upon which the LHC and photosynthetic apparatus are assembled. Microalgae contain almost all essential and non-essential amino acids, thus serve as an essential food additive and nutritional supplement (Safi et al., 2014). Lipids are mainly classified into two categories based on their chemical characteristics, namely polar (33–35 KJ/g) and non-polar/neutral/simple lipids (39–43 KJ/g) (Mehrabadi et al., 2015). Non-polar lipids comprise tri, di and mono-glycerides, waxes and isoprenoids whereas polar lipids comprise phospholipids, glycolipids, glycerol and fatty acid esters. This classification is highly essential as almost 30% of lipids are transformed into polar phase by esterification, and since the composition of lipids determines the quality and efficiency of the fuel (Singh and Dhar, 2011). Lipid classes in algae are composed of nitrogen, sulphur and phosphorous containing compounds that are converted into solubilized fractions following transesterification, which otherwise would have been problematic towards engine performance. When the algal cell reaches stationary phase, the overall energy content of the cell increases through the accumulation of the higher amount of neutral lipids including 18:1 and 16:0 fatty acids (Mehrabadi et al., 2015). Nutrient deprivation and temperature stress have been found to increase the accumulation of lipids by 40–50%, but it causes a shift in the biochemical content of carbohydrates and proteins due to decline in growth rate (Du and Benning, 2016). It is unfavourable if the algal biomass has to be used in biorefinery approach. Thus improved strategies for genetic and metabolic engineering are being used to increase the lipid content without compromising its nutritional status (Singh et al., 2016).

2.3. Selection of algal species

Microalgae with variable growth rate, biochemical content and viability are regarded as green gold mines for the energy sector (Ratha and Prasanna, 2012). Due to the prevailing diversity, selection of algal species is imperative for biofuel production. The essential characteristics for the selection of suitable strains are high photosynthetic efficiency and growth rate, better productivity, high CO_2 tolerance, and resistance to environmental stress (Seth and Wangikar, 2015). For fuel production at reality level, the influencing characteristics such as high lipid/carbohydrate content along with proper settling and aggregation properties are desirable to reduce the harvesting costs (Mutanda et al., 2011; Sutherland et al., 2015). Viability and sustenance of algal biofuel industries about the yield and profitability depend on a large extent over the selected strains. Table 1 shows the biochemical composition, growth rate, oil yield and CO_2 and temperature tolerance of some common microalgal strains that have been screened for biofuel production.

Microalgal ecology and diversity in open ponds are also associated with lower contamination risks and better biomass productivities complemented with greater bioremediation. Wu et al. (2014) reviewed the characteristics of microalgal species for WWT and found that *Botryococcus braunii*, *Chlorella pyrenoidosa* and *Chlamydomonas reinhardtii* showed superior performance in certain studies. However, none of the species has been proved to meet all the requirements for large-scale cultivation in wastewater. Algal bioengineering through biochemical and genetic manipulations can be used to alter the physiology as well as lipid and starch profile of potential microalgal strains. Hathwaik et al. (2017) reviewed the physical and chemical mutagenesis approaches with special emphasis on reiterative selection strategies for isolating strains with altered feedstock characteristics. Integration of biology and engineering is essential to identify the potential paradigm shifts, to optimize the growth rate and biomass productivity and increase the concentration of fuel molecules in algal cells.

3. Microalgal production systems

Bioprocess engineers have developed photobioreactors (PBRs) aiming for mass culture of microalgae. Abundant algal species are flourishing naturally in both marine and fresh waters but to extract the desired products, the growth of the biomass has to be substantial. Specific cultivating conditions are essential for achieving higher productivity and maintain monoculture ideal design parameters (Chisti, 2007). Industrially it is highly impossible to maintain all these conditions so, keeping a few crucial parameters pertaining to cell viability, enrichment, and economic feasibility numerous PBRs have been developed (Mata et al., 2010).

Photobioreactors are broadly classified into open systems and closed systems, with each having their significance. Open systems are less complicated and cost-efficient than closed systems which in turn have various shapes, designed for particular purposes (Kumar et al., 2015). Closed systems have attracted attention due to the lack of contamination and better control of operating parameters leading to greater biomass productivities as well as CO_2 abatement (Wang et al., 2012).

3.1. Open systems

Cultivation of algae in open ponds has been extensively studied over last decade (Ugwu et al., 2008). Open ponds can be categorized into natural waters (lakes, lagoons, ponds) and artificial ponds or containers. Recent technologies employ lagoons and oxidative ponds for bioremediation of municipal and industrial wastewaters which are nutrient rich and can act as a culture medium for various algal species. Lack of control over the climatic conditions and contamination by predators makes it difficult to operate it with the same productivity all round a year (Brennan and Owende, 2010).

To encounter these problems, artificial open ponds are designed. Factors considered while constructing raceway ponds are the biology of alga, cost of the land, nutrients, energy, climate (since culturing is done outdoors) and the type of final product (Mata et al., 2010; Pawar, 2016). Most large scale open raceway, circular and trough-shaped ponds operating in USA, Germany, Australia often witness the difficulties associated with sunlight and CO_2 distribution, contamination and low cell density (Laamanen et al., 2014). These issues could be overcome to a certain extent by adopting some novel methods (Mata et al., 2010; Pawar, 2016). Tank dimension is an essential criterion influencing hydrodynamic mass transfer in artificial open ponds. A recent study by Mendoza et al. (2013) recommended the tank depth of 0.2–0.4 m with multiple channels and walls built with thick fiberglass about 3 mm thickness, the length of the tank about 50 m and width up to 1 m, so that the baffles could be placed appropriately to ensure turbulent flow. Computational fluid dynamics code was developed recently in which a new bend design was introduced which reduces stagnation regions, increasing potential algal productivity (Liffman et al., 2013). A 27% decrease in bubble generation time and an equal increase in bubble residence time was observed due to the vortex flow field produced by the up-down chute baffle which in turn resulted in 29% increase in microalgae biomass yield due to better CO_2 fixation (Cheng et al., 2016). Recently, Kumar et al. (2015) and Pawar (2016) summarised the current state of knowledge in raceway ponds for biomass production and highlighted the several vital parameters influencing biomass productivity in open systems. Pires et al. (2017) have reviewed the computational fluid dynamics approaches for improving the design, hydrodynamics and kinetics of heat and mass transfer in open raceway ponds. Nevertheless, improvement in bioprocess technologies and engineering design principles concomitant with an understanding of the biological kinetics of algae would help in designing a cost-efficient open raceway pond with significantly higher areal productivity.

3.1.1. Low-cost algal production in HRAPs

High rate algal ponds (HRAPs) are open shallow raceway ponds about 30–40 cm deep, with a paddle wheel that are used for low energy wastewater treatment. The performance of HRAPs has been studied by a number of researchers (Posadas et al., 2015; Mehrabadi et al., 2017a, 2017b), who has described its capacity to be closely related to the symbiotic relationship between the bacteria and algae. Kim et al. (2014) reported biomass productivity of 0.5 g/L/day and lipid productivity of 0.103 g/L/day along with 85% and 92% removal of nitrogen and phosphorous in HRAPs using municipal wastewater. The removal efficiency of (92% and 71%) when placed indoors and (86% and 91%) in the greenhouse for total chemical oxygen demand and soluble phosphorous, respectively was reported by Hernández et al. (2016) using slaughterhouse wastewater. The same study also obtained a lipid concentration of 142 g FFA/g biomass.

HRAPs can significantly reduce the land and water footprints, along with the cultivation and harvesting costs providing low-cost WWT as well as biofuel production (Seth and Wangikar, 2015). The performance of HRAP (related to photosynthetic efficiency, biomass productivity and nutrient removal) can be significantly improved either by optimizing the reactor depth (Sutherland et al., 2014) or via recycling a small portion of harvested biomass to increase the total biomass concentration (Park and Craggs, 2014). The major drawback in integrating WWT with algal cultivation among others (as shown in Fig. 2) is the low lipid content of the biomass due to the presence of bacteria (lipid content < 10%) available in HRAPs, that reduces the biomass energy (Mehrabadi et al., 2015). A comparison showed that algal biomass cultivation through HRAPs had a lipid content of 20–40%, contrary to the expected theoretical lipid content of 10–30% (Passos et al., 2014a).

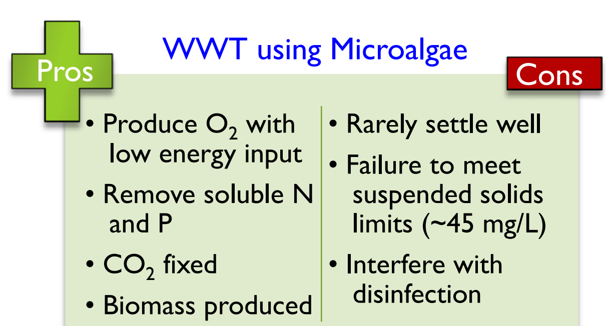


Fig. 2. Pros and cons of utilizing microalgae in WWT HRAPs.

A better insight into the approaches for improving the qualitative and quantitative lipid yields can make HRAPs a more attractive option for bioenergy.

3.2. Closed systems

Researchers with the aim to overcome the difficulties mainly the contamination problems and lack of control over the growth conditions encountered in open PBRs have upgraded their concept by developing various closed PBRs (Ugwu et al., 2008; Chen et al., 2011; Koller, 2015). The simplest design includes a quasi-state PBR formed by covering the open ponds with a transparent/translucent sheet of plastic/plexiglass thereby converting it into a greenhouse (Ugwu et al., 2008). It allows a single species to stay dominant and extends the cultivation time throughout a year. The possibility of trapping and increasing CO_2 allows more productivity. Other closed reactor configurations include mechanically stirred photobioreactors, tubular photobioreactors (TPBRs) [horizontal/tubular], column photobioreactors (CPBRs) [airlift and bubble column], flat panel photobioreactors (FPBRs), vertical photobioreactors and internally illuminated photobioreactors (Wang et al., 2012; Koller, 2015). Contrary to the open ponds, essential factors for cell viability like pH, temperature and CO_2 enrichment could be handled efficiently in the closed systems (Kumar et al., 2011).

Wang et al. (2012) and Koller (2015) has extensively reviewed the design considerations, mass transfer characteristics, economic and energy consideration for increasing the performance of closed PBRs. Aeration rates, shear sensitivity and light penetration, are some of the significant parameters which are essential in operating closed PBRs. The aeration system consists of a sparger or an air diffuser placed at the bottom that not only prevents shear stress but also increases the mass transfer efficiency, preventing O_2 build up in the course of photosynthesis thus improving the overall mixing pattern (Singh and Sharma, 2012). Static mixers installation in TPBRs resulted in improved biomass yield from solar energy partly due to a better distribution of light accounted by movement of cells and partly due to the lowered dissolved oxygen level (Ugwu et al., 2008; Wang et al., 2012). TBPBRs laid horizontally in series, forming an array of tubes, provide a better illuminating surface area (Chen et al., 2011). Improvisation of the current horizontal TPBR involves a series of tubes placed on a framework, tilted at an angle (suggested 45°) that increases bubble rise velocity; gas hold up and gas transfer coefficient (Wang et al., 2012). In case of tilt angle bioreactors, the optimal tilt angle is changed throughout the year depending upon the position of the sun (Koller, 2015). Closed PBRs can be used either outdoors or even indoors where they are artificially illuminated with fluorescent lights, xenon lamps, even lasers (Hamed, 2016). Mechanically stirred PBRs have also been modified with the use of internally illuminated lights like fluorescent lights and light emitting diodes (Pegallapati and Nirmalakhandan, 2013). Use of optical fibers that enhances the average irradiance of light, providing a shorter light path and increasing the depth to which the light reaches has also been tried but they have the disadvantage of hindering the mixing pattern (Heining and Buchholz, 2015). Plastic and flexible tubes are coiled around a supporting frame to form a helical TPBRs used for the growth of microalgae (Zhao et al., 2015). Raes et al. (2014) cultivated *Tetraselmis* sp. in raceway ponds and helical TBRs and demonstrated volumetric productivity of 85 mg/L/day (ash-free dry weight (AFDW)) with a CO_2 addition in helical TPBRs which was 5.5 times higher than that of raceway ponds. The mass transfer may be an underlying problem that one may contend with while using helical PBRs due to lack of gas hold up capacity that lead to lower CO_2 transfer rates compared to bubble column and airlift reactors.

3.3. Other promising PBRs

Even though significant efforts have led to the design of numerous open and closed PBRs, none of the above technologies has achieved practical productivity at par with that of the theoretical ones. Sustainable bioenergy production, demands the need of upgrading the PBRs concepts to the next level, with regard to higher productivities, less energy and water input has led to numerous new bioreactor concepts.

Attached PBRs represent a promising technology for oil rich microalgal biomass production. It consists of multiple layers of microalgal films attached to a supporting matrix to keep the cells viable by supplying nutrients and moisture (Hoh et al., 2016). It provides a sizeable illuminating surface area for receiving the diluted sunlight, thus decreasing photo-damage and increasing photosynthetic efficiency (Zhang et al., 2016). Projected advantages of this method also include easier scale-up, better harvesting, low water consumption and less contamination. Rotating biological contactor (Blanken et al., 2014) and algal turf scrubber (Genin et al., 2014) are the most common type of attached PBR. Hoh et al. (2016) have discussed the various attached PBR configurations, their design parameters, operation strategies, and performance. Tao et al. (2017) have demonstrated a novel airlift attached PBR system that provided volumetric biomass productivity of 15.93 mg/L/day and lipid productivity of 4.09 mg/L/day with *Chlorella vulgaris*. Limitations of these reactors include O_2 build up and inefficient CO_2 transfer with continuous deposition of algal layers (Zhang et al., 2016). A recent study by Genin et al. (2015) provided insight into newer aspects of designing attached PBR, to overcome the mass transfer limitations.

Most of the photobioreactor configuration available, even though operated at optimal dilution, suffers from washout problems, which decreases the biomass productivity. Further, with wastewater (having a low concentration of nutrients, the biomass concentration further declines, which adds up to the harvesting costs due to poor settleability (Drexler and Yeh, 2014). The increasing problems related to water scarcity have led to the development of membrane photobioreactors (MPBRs) which decouples dilution rate (related to hydraulic retention time [HRT]) from biomass (sludge) retention time [SRT], this could be efficiently used in WWT (Bilad et al., 2014). Bilad et al. (2014) and Drexler and Yeh (2014) have reviewed the application of MPBRs in microalgae coupled WWT and inorganic carbon capture with a special focus on reduction in harvesting costs. MPBRs have large surface area membranes allowing transfer of gas and liquids is avoiding extraneous turbulence, further the submerged membrane module act as a solid-liquid separator, enabling it to operate at high media flow rates (Gao et al., 2014; Kim et al., 2015). Compared to conventional PBRs, nutrient removal and CO_2 fixation efficiency were found to be 0.94–5.40 times higher, owing to higher biomass productivity (Gao et al., 2014). Najm et al. (2017) studied the O_2 evolution efficiency of *Chlorella vulgaris* grown in MPBRs integrated with another aerobic membrane bioreactor forming a hybrid MPBRs under different ratios of organic/inorganic carbon and ammonia/nitrate concentration. Specific O_2 production efficiency of 17.31 mg O_2/g MLSS/h was reported in MPBRs which on recirculation into aerobic MBR, resulted in 100% utilization of phosphate and inorganic carbon from wastewater. The only problem encountered in MPBRs is membrane fouling due to the release of extracellular polysaccharides (EPS) and soluble microbial products (SMPs), which decreases the separation efficiency with time (Luo et al., 2015). The inclusion of immobilized microalgal technology with MPBRs have reduced the concentration of EPS and SMPs from 20 mg/L to < 6 mg/L resulting in significant reduction in membrane fouling (Luo et al., 2015). Plastic bag reactors are especially attractive given their low economic maintenance and sterility due to high film extrusion temperatures. Large polyethylene bags have been used as PBRs over long times. Moheimani (2013) have reported biomass productivity of 110 and 140 mg/L/day for *Tetraselmis suecica* CS-187 and *Chlorella* sp. respectively grown in 120 L hanging plastic bag reactor for 11 months. Simple designs of hanging plastic bags have been automated to reduce the maintenance costs. Ojo et al. (2014) described the use of an orbitally shaken single use PBR, illuminated from below and studied the effect of different influencing parameters like hydrodynamic flow regimes, working volume and shaking efficiency on biomass productivity. Pagliolico et al. (2017) have demonstrated the use of disposable plastic bag PBRs with square cubicles for the cultivation of *S. obliquus* as static screens for windows with a maximum specific growth rate of 0.006 to 0.009 h^{-1}. Nevertheless, the problem with mixing arises and scaling up industrially may lead to a potential problem for disposal of plastic bags. An advancement to the above concept is Offshore Membrane Enclosures for Growing Algae (OMEGA) that uses plastic PBRs filled with wastewater coastal outfalls, floating in seawater that reduces the land and water footprint, further the sea water provides buoyancy for structural support, and natural heat sink to control the temperature (Carney et al., 2014). Kim et al. (2016) have postulated the incorporation of internal partitions could create media circulation inside OMEGA system using the wave energy of sea, which could reduce the cost associated with the use of static mixers and mechanical aerators. Use of biodegradable foul resistant plastics in future would assist in making this technology more economical and sustainable.

Other approaches of improvising the reactor design include improving the light utilization efficiency in closed PBRs. To enhance the light to biomass conversion rate, flashing light effect was incorporated through a novel photobioreactor concept, with the outer surface periodically shaded by the light-shielding material at a pre-set interval to create sequential light/dark cycle that increased the biomass productivity by 21% compared to conventional TPBR (Liao et al., 2014). Janoska et al. (2017) developed a packed bed bubble column PBRs with large hydrophobic beads, where algae can grow in liquid channels of liquid foam stabilized by Bovine Serum Albumin. The novel reactor concept has been postulated to reduce the harvesting costs due to high biomass densities, improved mass transfer and low pressure drop in foam bed photobioreactor.

Reactor designing is evolving rapidly with the cons of old design being outwitted by the new designs. Over last decades, numerous improved varieties of PBRs have been developed, with a few discussed in the current review to achieve a mass concentration of algal cells. Each of the PBR has their perks with some typical disadvantages (as outlined in Table 2), which can be accounted shortly taking PBRs efficiency to the next level of substantial economic feasibility and massive productivity.

4. Parameters affecting energy production in PBRs

The concept of industrial symbiosis, with synergistic effects of achieving WWT and biofuel production, is well-known. High nutrient removal capacity (including removal of toxic metals) and potential to grow at the broad regime of nutrient concentrations, temperatures and light conditions, along with the ease of maintenance have attracted the use of algae in WWT (Wang et al., 2016). Aslani et al. (2018) reported the importance of evaluating different criteria like land requirements, CO_2 supply, climatic conditions, nutrient and process techniques for culturing microalgae. The algal growth rate is dependent on the complex interaction of various environmental, operational and biological factors as shown in Fig. 3. To achieve the desired yields, it is essential to understand the effects of these parameters (discussed in subsequent sections), as a preliminary step towards bioprocess optimization and design of PBRs.

4.1. Environmental factors

4.1.1. Light

Light of the visible region of the solar spectrum provides the required energy to facilitate microalgal photosynthesis (Huesemann et al., 2016). The efficiency with which an algal cell absorbs light depends on its size as well as its intracellular pigment concentration. Light harvesting pigments organized in LHC absorbs light energy (Perrine et al., 2012; Kirst et al., 2017). Excess energy absorbed, is dissipated in the form of heat/fluorescence. LHC synthesizes more pigments to absorb more light that leads to self-shading and light attenuation (Wobbe et al., 2016).

Since light is the basic energy source for photosynthetic microalgae, its availability and intensity play a pivotal role in maintaining algal cultures. Guedes et al. (2010) showed that algal cultures grown at the low light intensity of 9 W/m^2, produced significant quantities of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. High light intensity to a threshold range increases the productivity by modifying the lipid profile leading to more amount of monounsaturated and saturated lipids accumulation (Gim et al., 2016). The net growth at low light intensities is almost zero, as light intensity increases photosynthetic efficiency increases and reaches a saturation point after which it starts decreasing due to photo-oxidation and photodamage. As ETC gets saturated and LHC absorbs a greater amount of photons than required, mutual shading occurs in the pond, which could be overcome by selecting appropriate pond depth, mixing and with reduction in light path length of the system to increase the light penetration (Singh and Dhar, 2011; Perrine et al., 2012). It is often suggested that intermittent light intensity and flashing light effect with light/dark cycle increase the light utilization efficiency, decreasing photodamage, thus enhancing productivity. At cellular level light intensity has been proposed to be interrelated to other abiotic factors, thus more studies are essential to uncovering the relationship to optimize the productivity.

4.1.2. Temperature

Temperature is a crucial parameter that dramatically affects microalgal photosynthesis and growth. The optimal temperature range for most algae is 25–35 °C (Chisti, 2007). The deviation from the optimal temperature range could cause cell death and rapid loss of algal biomass (Ras et al., 2013). At sub-optimum temperatures photosynthesis dominates at low light intensities and temperatures greater than the optimum, photorespiration increases (Sutherland et al., 2015). Therefore, it is vital to achieve an optimal temperature during the day and to quickly reduce it at night, to maintain high productivity (Singh and Dhar, 2011). Also, over time different algal species acclimatize and develop strategies like cell shrinking and energy rebalancing, to combat the effects of the above optimal temperatures (Ras et al., 2013). Munoz and Guieysse (2006) have discussed that combining different microalgal strains having similar characteristics but varying growth temperatures, can be used to keep the temperature at an optimal level.

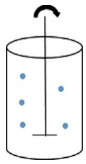
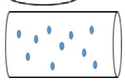
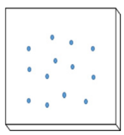
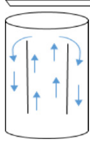
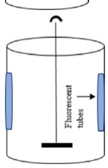
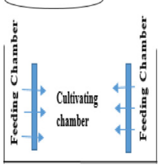
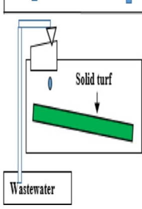
Increase in temperature increases the rate of glucose consumption as well as heterotrophic productivity (Zhao et al., 2015). The nutrient removal efficiency of algae is also increased with the rise in temperature (Sutherland et al., 2015). Temperature is often postulated to act in cohort with light intensity and CO_2 tolerance, thus more regression studies based on the Arrhenius equation is essential to establish their synergistic influence on microalgal growth rate.

4.1.3. pH

pH changes during the day, rising with carbon being drawn as a result of photosynthesis and can reach a value of 10, which has the potential to cease the growth rate. In general, a pH of 8–9 has been reported to be more conducive to maintaining the viability of most algal species and reducing the growth of other invading organisms (Bartley et al., 2014). High pH causes dissociation of ammonium ions to free ammonia which in turn inhibits photosynthesis by intervening the capacity of the RuBisCo enzyme (Sutherland et al., 2015).

Han et al. (2013) have reported enhanced lipid productivity of microalgae in a semi-continuous culture mode via coupling nutrient limitation with pH regulation. pH impacts nitrogen and phosphate removal, due to ammonia volatilization and orthophosphate precipitation at high pH (Munoz and Guieysse, 2006). Effect of pH on microalgal growth rate via supplementation with an organic or inorganic carbon source has been studied by White et al. (2013). pH control at large scale requires a detailed analysis of the isolated impacts of carbonate buffers

Table 2
Various types of microalgal photobioreactors along with pros and cons.

Reactors	Cross-section	Features	Advantages	Disadvantages	References
Stirred PBRs		Mechanically agitated stirrers used to stir the growth medium for mass transfer	Simple and easy to operate Cost efficient	Heavy stirring generates shear forces damaging cells. Low stirring causes eddies resulting in insufficient mass transfer & decreasing productivity	Wang et al. (2012); Saeid and Chojnacka (2015)
Tubular PBRs		Long thin tubes made up of glass or plastic, media circulated by using air pump/sparger at the bottom Oriented either vertically/horizontally	Suitable for outdoor cultures Oriented on direction of sunlight	Oxygen build up Mass transfer limitations	Kumar et al. (2011); Oncel and Kose (2014)
Flat panel PBRs		Made up of glass or transparent thin acrylic sheet, with one end open, stirred by aerator at bottom	Large illuminating surface area on both sides Low O_2 build up	Fouling Hydrodynamic stress build up Difficult to control temperature	Koller (2015); Hamed (2016)
Airlift PBRs		Vertical tubes of glass/acrylic where air and media are circulated in the riser and down-comer	High mass transfer efficiency Better biomass productivity	Costly to maintain Scale up difficult	Fernandes et al. (2014); Wong et al. (2016)
Internally illuminated PBRs		Stirred tank PBRs internally illuminated with fluorescent lamps on sides	Energy efficient Controlled light regulation	Wall growth & fouling Technical barriers during scale up	Pegallapati and Nirmalakhandan (2013); Heining and Buchholz (2015)
Membrane PBRs		Large surface area with hollow fiber immersed for ultrafiltration to separate end products/metabolites aiding liquid & gas transfer	Low energy requirement No shear stress	Membrane fouling Difficult to clean & maintain Cost intensive to scale up	Gao et al. (2014); Bilad et al. (2014)
Algal turf scrubber		Consists of an inclined attachment surface over which wastewater flows resulting in growth of algae that can be harvested by scrubbing	High biomass productivity per unit foot print area	High operating costs High evaporative loss	Genin et al. (2014); Hoh et al. (2016)

(extracellular pH) and intracellular pH (Ying et al., 2014). A recent study by Wang and Curtis (2016) have demonstrated the effects of intracellular stoichiometric proton imbalance through assimilation of different nitrogen sources can be used redirect the metabolic flux towards pH control in the absence of buffering through organic/inorganic carbon.

4.2. Operational factors

4.2.1. Land and water availability

Water and land are two basic requirements of growing biomass for energy. However, fertile land and fresh water are scarce in the present era and in the coming decades, the stress on these natural resources is expected to increases globally due to economic development and population growth. Further, with the increasing urge of replacing the fossil fuels with biofuels, it is essential to analyse the potential land and water demands of different energy crops. The first and second generation biofuels have drawbacks, like large land and water use (Singh et al., 2011). On the other hand, algae have the potential to grow all year round not necessarily on arable lands, as algal culturing facility can be co-localized even on wastelands and industrial premises using wastewater as a source of nutrients which is not feasible in case of crops (Chisti, 2007; Mehrabadi et al., 2015). Depending on the nature of strain, microalgae can be grown either in saline water, treated/untreated wastewater from industries and water containing heavy metals and toxic wastes. Thus, the algal biofuels as a whole have much lower ecological footprints compared to other energy crops (Singh et al., 2016).

4.2.2. Hydraulic and solid retention time

Feasibility of large scale microalgal cultivation necessitates dynamic process control over operational parameters like hydraulic retention time (HRT) and solid retention time (SRT) (Béchet et al., 2016). They synergistically affect the growth rate, light regime and nutrient load of the system (Sutherland et al., 2015). Algal recycling with shorter HRT facilitates floc formation, thereby settleability (Park et al., 2013). Shen et al. (2016) have reported a proportional increase in phototrophic biomass with an increase in SRT from 3 to 9 days in a chemostat.

HRT also affects algal population dynamics, as it influences specific growth rates of algae, which inturn affects the biochemical composition of microalgae (Mehrabadi et al., 2015). HRT in HRAPs usually ranges from 3 to 9 days and can be modified by altering the pond depth or by diluting the culture media (Sutherland et al., 2015). Shayan et al. (2016) have reported significant removal of nitrogen with hyper-accumulation of lipids (20%) with 2 day HRT and domination of phosphorous removal with 6 days HRT concomitant with an increase in

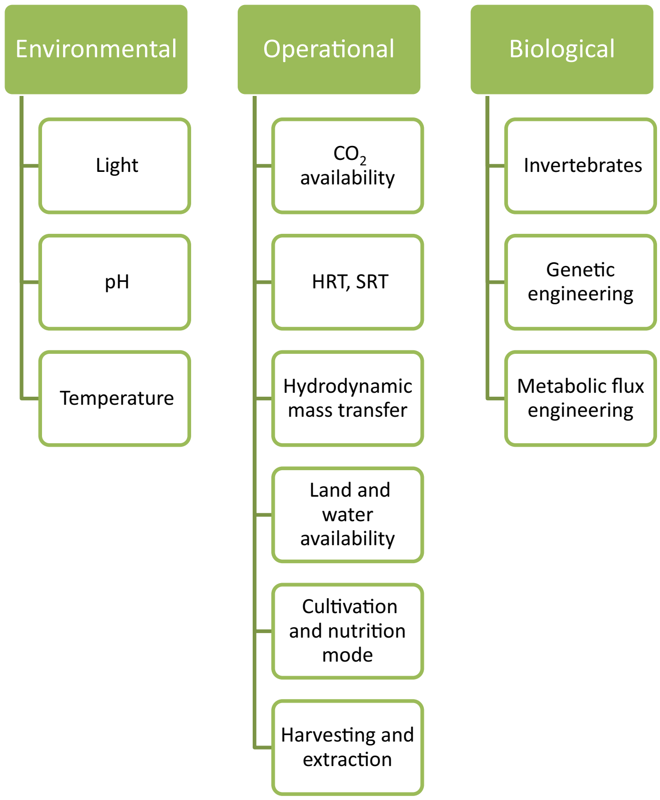


Fig. 3. Parameters influencing microalgal growth and associated energy production in WWT HRAPs.

starch (27%). Optimizing HRT, SRT and design criteria of PBRs is crucial for maximizing algal productivity and reducing harvesting costs for commercialisation of algal biofuels.

4.2.3. Hydrodynamics and mass transfer

Hydrodynamics and mass transfer, largely influenced by mixing rate affects microalgal viability (Kumar et al., 2011). Various mixing systems available for different reactors includes paddle wheels in open ponds, impellers in stirred tank reactors, air circulation through sparger or porous membrane in bubble column reactors and draft tubes in airlift reactors (Ugwu et al., 2008). Mixing helps in maintaining a homogenous cell concentration and nutrient distribution, eliminating thermal stratification, improving gas exchange as well as reducing the degree of mutual shading and photoinhibition by inducing light-dark cycles (Wang et al., 2012). Paddlewheels and impellers though provide cost-effective, efficient mixing, but often result in shear damage, thus substituting these by airlift systems resulted in a 75% increase in microalgal productivity (Munoz and Guieysse, 2006). Studies have also suggested the significance of vertical mixing on algal cultivation systems as the algae could travel from ahotic to photic zone of the open pond (Prussi et al., 2014; Huesemann et al., 2016). Approaches such as optimizing the bends in raceway ponds, engineering flow field, improving surface geometry while designing the raceway ponds could enhance the vertical mixing (Kumar et al., 2015).

Gas flow velocity is another essential parameter which on increasing accounts for better mixing and mass transfer between liquid and gas transfer regimes. Higher gas flow rate increases the diameter of bubbles formed which are then intercepted by impellers that break them down into smaller ones, resulting in better mass transfer rates and higher biomass productivity (Prussi et al., 2014). Apart from gas flow velocity, solid and liquid flow velocity is also essential hydrodynamic parameters that must be considered for efficient mass transfer (Ugwu et al., 2008). The gas-liquid mass transfer is essential for CO_2 fixation and for preventing O_2 build-up that inhibits photosynthesis. Karemore et al. (2015) have proposed hybrid membrane sparged helical TPBR reactor design for better CO_2 retention and reducing O_2 hold. These contemporary advancements along with computational fluid dynamics studies provide promising future insights for achieving higher biomass productivities.

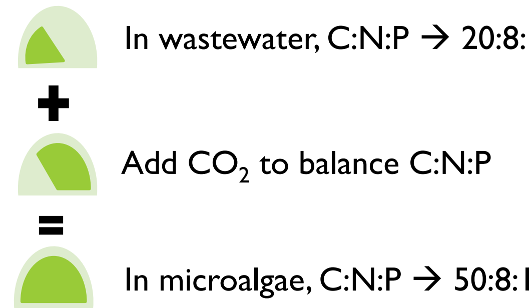


Fig. 4. Integrated CO_2 sequestration and biomass production by microalgae.

4.2.4. CO_2 availability

Microalgae are autotrophic organisms that capture CO_2 present in the air for oxygenic photosynthesis. Because of high tolerant capability to elevated levels of CO_2 in the atmosphere, microalgae are preferred over other carbon sequestration methods.

Heterotrophic metabolism of organic matter present in the WWT HRAPs triggers the release of CO_2 which can supply about 25–50% of the dissolved inorganic carbon (DIC) essential for microalgal growth (Rawat et al., 2011). Microalgae are composed of 50% carbon, and when the demand of carbon exceeds the supply, it limits the photosynthetic growth as well as the nitrogen removal capacity (Sutherland et al., 2015). CO_2 addition as represented in Fig. 4, increases the C: N ratio of the wastewater, making it more suitable for microalgal growth. Mehrabadi et al. (2017b) have reported that CO_2 augmentation maintains a pH of 6–7, that bears a directly proportional relationship with biomass productivity, especially in summer months. Further, it helps in increasing the content and saturation level of fatty acids in algal lipids, thereby improving its quality and quantity (Mehrabadi et al., 2015). Xie et al. (2018) reported that adding 10% of CO_2 in inorganic form can trigger biomass productivity and accumulate more lipids. Substituting conventional CO_2 with exhaust gases from industries and automobiles has been suggested as one of the possible methods of improving algal productivity concurrent with industrial wastewater treatment (Singh et al., 2016). However, the addition of flue gases releases inorganic contaminants which negatively impacts growth rate and biomass productivity, thereby decreasing lipid yields (Hess et al., 2017). An integrated approach of adding CO_2 produced by digestion of the organic solids in wastewater or by the flue gas generated via combustion of biogas, would reduce the impacts of harmful inorganic contaminants and potentially waiver the cost of algal biofuels.

4.2.5. Cultivation and nutrition mode

Cultivation mode is a major factor that influences the microalgal growth rate and productivity. There are two types of cultivation modes namely batch and continuous mode. Observation showed that batch cultures tend to produce microalgae which need low nutrients to grow and have lower productivity compared to continuous modes (Mehrabadi et al., 2015). Also, it has been reported that fed-batch and continuous cultivation produces greater lipids than conventional batch methods (Wang et al., 2016).

Microalgae usually have three basic modes of nutrition like phototrophic (uses sunlight as energy and CO_2 as carbon source), heterotrophic (uses sugars and organic compounds as carbon source and occurs in the absence of light) and mixotrophic (uses both CO_2 and organic compounds as carbon source, with simultaneous occurrence of both respiration and photosynthesis) (Singh and Dhar, 2011; Cheirsilp and Torpee, 2012). Heterotrophic mode synergistic with WWT offers the advantage of increased lipid accumulation with significant quantities of saturated fatty acids as compared to phototrophic mode, while the latter has the benefit of reducing GHG emissions (Mohan et al., 2015). Several researchers have shown that mixotrophic mode provides greater biomass productivity and better quantity and quality of lipids (reduction in polyunsaturated fatty acids and increase in saturated fatty acids) along with significant amount of reducing sugars compared to the phototrophic and heterotrophic method (Ratha et al., 2013; Chiranjeevi and Mohan, 2016). Ren et al. (2016) have reported that supplementing glucose in heterotrophic/mixotrophic mode redirects the metabolic flux towards the accumulation of neutral saturated lipids rather than starch at cellular levels. Sustainable biofuel production necessitates symbiotic association of reactors operating under different cultivation modes connected in a closed loop to provide an exchange of CO_2 and O_2, thus improvising the desired yields.

4.2.6. Algal recycling

The biomass produced in WWT HRAP can be used as a substrate for conversion into biofuels by algal recycling, making the process economically sustainable (Park et al., 2013). Maintaining the dominance of highly productive species, which are easily settleable and have high energy content, helps in increasing the amount of final energy obtained. Recycling 10% of the harvested biomass back to the WWT HRAP, maintained the dominance of *Pediastrum boryanum* leading to enhancement in biomass productivity, harvestability, energy content and the net biomass energy yield by 66% (Park and Craggs, 2014). Microalgae recycling (~10% weight (wt.)) increased the biomass recovery rate by 94%, with a significant reduction in total suspended solids, chemical oxygen demand and biological oxygen demand (Gutiérrez et al., 2016). Contrary to the above findings, Zhang et al. (2016) have shown that sequential recycling of cultures of *Nannochloropsis oceanica*, resulted in a decrease in growth rate and biomass productivity due to the presence of inhibitory polysaccharides of humic and fulvic acids. They have also reported an increase in biomass and lipid productivity, by removal of growth inhibitors from recycled media by activated carbon. Thus, a more detailed study and characterization of the biochemical composition of recycled media is an essential aspect to be focused before going for resource recycling.

4.2.7. Nutrients

The availability of nutrients highly influences the algal diversity, biochemical composition and productivity of microalgae (Mehrabadi et al., 2015). Nitrogen and phosphorous are the two most important nutrients that comprise > 10% of the algal biomass (Maity et al., 2014). Various sources of wastewater are well-known reservoirs of these macronutrients. However, the Redfield ratio of 16:1 (N:P) for an average of a variety of species shows that even though nutrients may be present, addition might be obligatory (Marcilhac et al., 2015). The form in which the abovementioned macronutrients are assimilated, along with their kinetics of removal, underlying cellular metabolism affecting biomass and lipid productivity has been discussed by several researchers (Mujtaba et al., 2017). It has been projected by a number of studies that the lipid content increases under nitrogen depletion and phosphorous limitations (Liang et al., 2013; Chu et al., 2014). Nitrogen starvation alters the enzyme balance resulting in a decrease in oxygen evolution, CO_2 fixation, chlorophyll content, and tissue production which thereby decreases the biomass yield, diverting the energy towards the synthesis of neutral lipids (Chu et al., 2014). Kumar et al. (2018a, 2018b) reported that the initial population density of microalgae/inoculum also plays an essential role in influencing the uptake of nitrogen and thereby the biomass and the lipid content. Excess phosphorus assimilated is often stored within the cells in the form of polyphosphate granules, which is used when phosphate reserves are low (Marcilhac et al., 2015). Thus, the growth rate is not immediately affected by changes in external phosphorous concentration unlike the response in case of changes in other growth parameters (Marcilhac et al., 2015; Solovchenko et al., 2016). Under phosphorus limited growth conditions, there is a reduction in the rate of light utilization required for carbon fixation and subsequent reduction in the synthesis and regeneration of substrates in the Calvin-Benson cycle leading to neutral lipid accumulations (Liang et al., 2013; Chu et al., 2014; Solovchenko et al., 2016).

Apart from macronutrients, trace metals (iron, copper, cobalt, zinc, nickel, manganese) present below the supra-threshold levels (< 4 ppm) affects algal physiology (Juneja et al., 2013). They essentially influence the normal growth and metabolism via effects on photosynthesis and respiration (Liu et al., 2008). A significant increase in lipid levels might be subjected to an appropriate concentration of trace metals (Huang et al., 2014). Studies have reported an increase in lipid content of microalgae, supplemented with iron. (Liu et al., 2008). Under autotrophic mode, supplementing the media with Mg^{2+} also increased the growth rate and lipid accumulation (Huang et al., 2014). Recently, Miazek et al. (2015) have reviewed the effects of different metals, metalloids and metallic nanoparticles on the growth of microalgae. Process engineering through nutrient management to manipulate the biochemical composition of microalgae is an essential strategy to increase the growth as well as the quality and quantity of lipids.

4.2.8. Harvesting and extraction

One of the major challenges, averting large scale microalgal technology is the energy and cost associated with the available downstream process options for harvesting and extraction. A two-step process of bulk harvesting and thickening via dewatering is very often used to concentrate the microalgal biomass (Brennan and Owende, 2010). However, the process efficiency is substantially low, due to the low density of algal broth and small size of microalgal cells (3–30 μm), which is negatively charged are stably dispersed in the media (Feng et al., 2016). The various types of harvesting methods currently in use can be broadly categorized into mechanical based (centrifugation, gravity sedimentation, filtration, & floatation), chemical based (coagulation and flocculation) and electrical based (electrocoagulation and electroflotation) (Kim et al., 2013). Brennan and Owende (2010); Kim et al. (2013); Barros et al. (2015) have reviewed pros and cons of each of these methods as well as the physiological factors affecting the above-mentioned processes. Choice of harvesting method depends on the algal species to be harvested. However, there seems to be not a single method or combination thereof which could be applied to all algal species without compromising the energy and cost demands (Barros et al., 2015). Thus, deployment of eco-friendly, energy extensive and cost-efficient harvesting methods are essential for improving the applicability of algal biofuels. Various novel strategies have been introduced to supplement the currently used mechanical and chemical harvesting methods mainly concentrating on the use of eco-friendly bioflocculants (Kothari et al., 2017), reduction in membrane fouling by use of axial vibration (Zhao et al., 2017) or via the use of polymeric matrices (Kotte et al., 2014). Biological harvesting methods like co-pelletization (Hom-Diaz et al., 2017), synthetic lichen concept [mycoalgae biofilm] (Rajendran and Hu, 2016) are under research and are likely to transform the algal harvesting platform in the future for bioenergy applications. The advantages and limitations of recently introduced harvesting strategies have been summarised in Table 3.

In the context of bioenergy from microalgae, lipid and carbohydrate extraction methods currently in use also act as the major bottlenecks in lieu of commercialisation. The available mechanical and chemical methods along with their principle, energy and cost requirements have been summarised in Table 4. Several studies have discussed in details the oil extraction methods along with their environmental and economic sustainability issues (Pragya et al., 2013; Kumar et al., 2015; Dong et al., 2016). Improvements to the conventional methods involve

Table 3
Novel harvesting strategies with their advantages and limitations.

Harvesting Methods	Distinctive Features	Advantages	Limitations	References
Bio-flocculation	Coagulants of plant based or microbial based origin are used act as a polysaccharide inter bridge framework, thus electrostatic interactions with the negatively charged algal surface often leads to agglomeration of algae	Cheap and environmental friendly coagulants Increase in harvesting efficiency with less energy consumption	Inappropriate amount of data hinders application at large scale	Salim et al. (2011); Kothari et al. (2017)
Pelletized cell cultivation	Co-culture of filamentous fungal cells with algae resulting in co-aggregation with fungi, leading to their immobilization within pellets Pellets (2–4 mm) are then easily harvested by filtration due to increase in size, contrary to the small size of microalgae	Decreases the viscosity of media Increases the mass transfer efficiency Ease of harvesting 90% increase in efficiency of harvesting Recycling of media Reduction in overall cost of cultivation	Lack of detailed knowledge about large scale industrial application	Zhang and Hu (2012); Zhou et al. (2013)
Synthetic lichen concept	A supporting matrix of polymers or stainless steel mesh is used to augment the microalgae and fungi co-culture to form an artificial lichen biofilm (mycoalgae biofilm)	Increases the harvesting efficiency upto 99.94% contrary to cell pelletized cell cultivation method Energy efficient low cost cultivation method	Need to identify suitable matrix to grow mycoalgae at carbon limited conditions	Rajendran and Hu (2016)
Photo-biological H_2 mediated auto-flotation	H_2 gas produced by heterocysts of cyanobacterial cells co-cultured with microalgae, produced buoyancy resulting in settling of algal biomass	Reduction in production costs due to the absence of chemical coagulants & gas bubbling	Feasibility of the technology at industrial scale is not established	Feng et al. (2016)
Polymeric foul resistant matrices	Polymeric matrices of polyvinylidene fluoride (PVDF), surface modified can be used to harvest algae via membrane filtration	Increase in flux and recovery rate by 100% Ease in maintenance due to less fouling	Lower water flux and pore rating with molecular weight cut-off of 10 kDa	Hwang et al. (2013); Kotte et al. (2014)

using the end product (biodiesel) with methanol (Huang et al., 2017) or triethylamine/methanol mixture (Huang and Kim, 2017) combined with mechanical stress as lipid extractant. A significant increase in lipid yields and reduction in energy due to the absence of drying and solvent recovery was also reported with the above-mentioned approaches.

There is a need to introduce novel extraction strategies which are not only cost and energy efficient but also are environmentally sustainable. Enzymatic disruption method for lipid extraction is a highly efficient, non-toxic and safe approach that uses enzymes like cellulase, trypsin and snailase to disrupt microfibrillar and matrix polysaccharides in the recalcitrant rigid cell wall, with minimal damage to the target product, thereby reducing the downstream costs (Mishra et al., 2017). Studies have reported higher lipid recovery from microalgal biomass compared to other conventional methods (Taher et al., 2014; Zuorro et al., 2016). Development of enzyme immobilization techniques (Fu et al., 2010) and enzyme extracts from bacteria (Guo et al., 2017) are expected to further reduce the cost of this technique making their pilot scale application a reality. Like supercritical CO_2, green solvents like subcritical water (Reddy et al., 2014) and free nitrous acid [FNA] (Wang et al., 2013; Bai et al., 2016) has been demonstrated to recover lipids as well as carbohydrates and proteins from algae. Subcritical extraction supplemented with ionic liquids (Yu et al., 2016) and microwave assisted methods (Reddy et al., 2014) has been found to reduce the extraction energy by 2–8 folds. FNA has been demonstrated to create oxidative stress resulting in cell disruption increasing the yield of lipids by 2.4 times (Bai et al., 2016). Oxidative stress induction using UV light to extract lipids has been reported by Sharma et al. (2014). Further insights into these novel extraction techniques conforming their economic viability and feasibility at field scale are expected to revolutionize algal biofuel industry.

4.3. Biological factors

4.3.1. Control of invasive species

Zooplankton is the class of organisms that consume algae as a part of their food. They include herbivores, invertebrates and pathogens which have the capacity to consume huge amounts of biomass from the time of initial inoculation especially in HRAPs (Mehrabadi et al., 2015). A variety of abiotic factors influences the growth of invasive species like temperature, pH, etc. Maintenance of moderate temperature ($> 35\,^{\circ}C$) has been found to decrease the growth of zooplankton (Gregg et al., 2009). Mehrabadi et al. (2017b) have reported that CO_2 addition in order to maintain the culture pH at 6–7 in summer and 5–6 in winter, decreases the susceptibility of microalgae towards zooplankton invasion. Montemezzani et al. (2015) have reviewed the different physical, chemical and biological control strategies for regulating the growth of invasive species in HRAPs. One of the potential mechanism of avoiding zooplanktons is polyculture (culturing multiple species altogether) that can induce over yielding effects, thus consuming the available nutrients, thereby competitively inhibiting the growth of invaders (Smith and Crews, 2014). It is essential to maintain the population of zooplankton at an optimum level, as complete eradication might cause an ecological imbalance, resulting in the growth of newer species that are not controllable. Further, an optimal population of grazers has the ability to select important algae by consuming poorly settleable unicellular algae, allowing better algae to grow (Montemezzani et al., 2015).

4.3.2. Genetic manipulation of algae to enhance the productivity

Lipids hyperaccumulation has been experimentally seen under unfavourable stress conditions of nitrogen limitations (Liu et al., 2016). However, such an approach does not provide a real-time control over the various stages of cell growth and lowers the cell density, thus declining the biomass productivity (Reijnders et al., 2014). Owing to these issues, there is a need to explore and implement noble approaches to increase the qualitative and quantitative lipid yields, without

Table 4
Mechanical and chemical extraction methods with their efficiency, energy & cost requirements.

	Principle	Lipid extraction efficiency	Energy requirement & cost involved	References
Mechanical methods				
Expeller press	High pressure squeezes intracellular oil from microalgae	About 75% of lipids are extracted, however pressure beyond a certain range decreases the lipid recovery	Energy-intensive (46–407 MJ/kg) Expensive with long processing time, the requirement of skilled labour, high maintenance costs	Cooney et al. (2009); Ranjith Kumar et al. (2015)
Bead beating	The collision of high-speed beads disrupts the cell releasing lipids	Lipid extraction efficiency similar to expeller press Use of titanium carbide, zirconium beads enhances the disruption & extraction efficiency	Energy-intensive (10.2–36.1 MJ/kg) Reduction in process costs due to lack of dewatering Overall costs may be high depending on the nature of beads	Doucha and Lívanský, 2008; Byreddy et al. (2015)
Microwave-assisted extraction	Water vapour produced by intracellular heat in the cell wall by disrupts it, thus releasing lipids	Short reaction time Increase in lipid extraction efficiency by70–90%	Energy-intensive (4.32–60.012 MJ/kg) Low operating costs due to no dewatering High maintenance costs	Patil et al. (2011); Cheng et al. (2014)
Ultrasonic assisted extraction	Oscillations cause cavitation, heat shock waves disrupt the cell wall, causing lipid extraction	Comparatively less heat generated during the extraction prevents denaturation of molecules Prolonged use generates free radicals that might alter oil quality	360–848 MJ/kg energy used, Operated at low temperature Moderately costly	Byreddy et al. (2015); Martínez et al. (2017)
Osmotic pressure method	Hyper/hypo-osmotic shock due to the pressure difference between the interior and exterior of the cell causes cell damage, exposing lipids	Simple, easy and efficient lipid extraction efficiency	Less energy required Cost effective Feasibility at pilot scale yet to be tested	Ranjith Kumar et al. (2015); Byreddy et al. (2015)
Electroporation	Electric field increases the membrane permeability, thus leading to increased lipid extraction	Improved lipid extraction efficiency in terms of time & solvent used No alteration in fatty acid profile	Less energy required (1.51–860.4 MJ/kg) Moderately costly	Ranjith Kumar et al. (2015); Garoma and Janda (2016)
Chemical methods				
Solvent-based extraction	Organic solvents disrupt the cell wall exposing lipid droplets	Easy processing Efficiency can be improved by accelerating the process via heat or pressure	Cost-intensive Environmentally toxic Health and safety issues	Taher et al. (2014); Mubarak et al. (2015)
Isotonic extraction method	Ionic solvents with arrange of hydrophobicity, solubility, specific polarity and conductivity can easily break the algal cell wall	Non-toxic Environmental friendly	Economic and technical feasibility at large scale is yet not confirmed	Kim et al. (2012); Pan et al. (2016)
Supercritical CO_2 method	Supercritical CO_2 at relatively lower pressure (72.9 bar) and temperature of 31.1 °C is used to disrupt algal cells, exposing lipids	Low flammability and reactivity	Lack of convincing data related to economic feasibility for large scale use	Santana et al. (2012); Lorenzen et al. (2017)

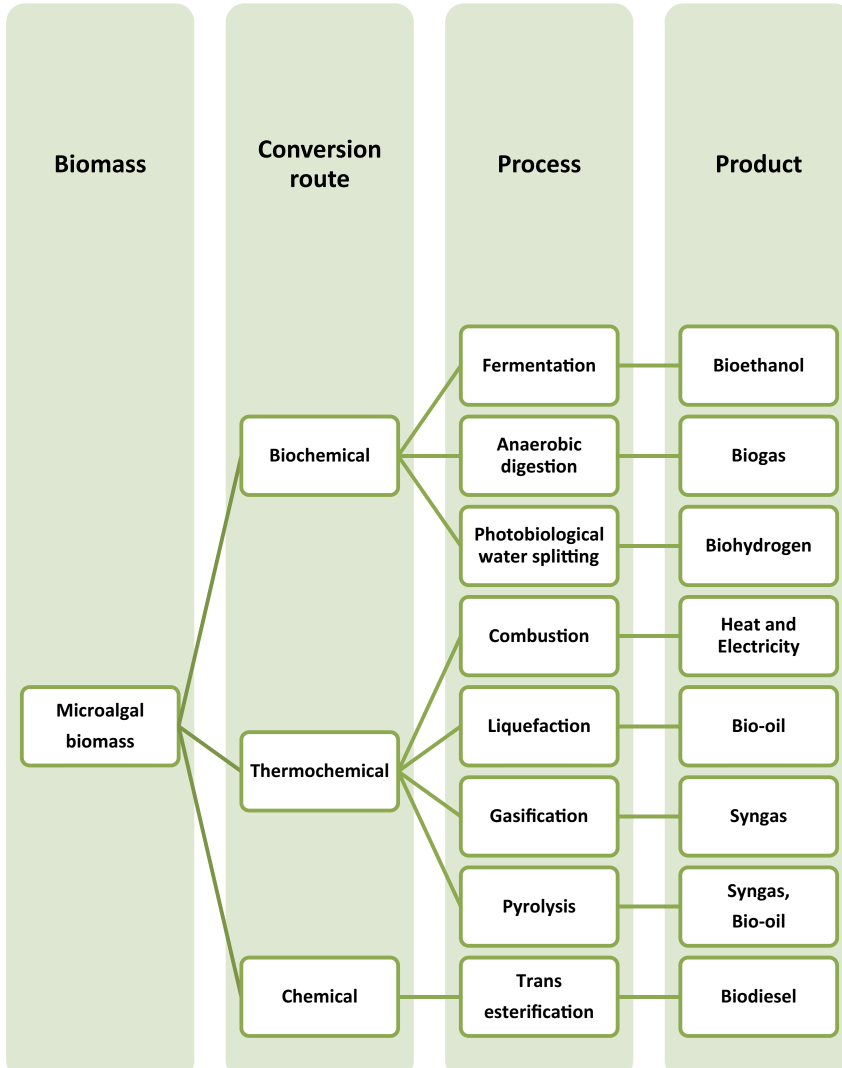


Fig. 5. Various thermochemical and biochemical routes for microalgal biofuels.

compromising the biomass productivity. Genetic transformation techniques using system-synthetic biology approaches have a great potential of exploiting eukaryotic microalgae as cell factories (Sutherland et al., 2015). Radakovits et al. (2010) have reviewed the application of different genetic engineering techniques which can be possibly used to increase the platform of biofuel production from algae. Trentacoste et al. (2013) have developed transgenic strains with increased lipid accumulation. Singh et al. (2016) have also discussed the molecular approaches and gene expression studies for increasing the lipid yields. Very recently, Kumar et al. (2018a, 2018b) have reported a significant increase in growth rate, biomass and fatty acid content of *Tetraselmis sp.*, on exposure to chemical mutagenic treatment using ethyl methane sulphonate (EMS).

Incorporation of desired genes into the host algal cells modifies them making them more resilient to environmental conditions. The efficiency of light utilization could also be improved by implementing genetic techniques (Singh and Dhar, 2011). Approaches related to gene silencing and RNA interference has been used to reduce the size and pigment content of LHC, to increase the photon utilization efficiency and reduce the photo-damage caused by the generation of free oxide radicals during the exposure to excessive light (Gimpel et al., 2013; Kirst et al., 2017). However, genetic manipulated (GM) algal strains are expected to be more vulnerable to the attack by invasive species and grazers due to inescapable ecological trade-off (Smith and Crews, 2014). Environmental and health risks associated with their long-term use remains a major challenge.

4.3.3. *Metabolic flux engineering of algal strains*

Metabolic engineering is a promising technology to expand the productivity of microalgae by redirecting the metabolic flux and cellular functions towards the synthesis of desired products (Rosenberg et al., 2008). Lipid triggers have been seen to be achieved by coercing the microalgae, by subjecting them to the lack of bioavailable nitrogen (Liu et al., 2016). Proteomics analysis has proposed an increase in energy metabolism, cell wall synthesis, fatty acid biosynthesis and subsequent decrease in lipid catabolism and translation machinery of photosynthesis, resulting in a change in the functional balance between PS I and II (Longworth et al., 2012).

Gene alteration through mutagenesis has been found to cause an inferred shift in metabolic flux which can result in the desired yield. Integrated flux balance modelling of biosynthetic pathways of Calvin-Benson cycle and Krebs's cycle has been used for tunnelling the carbon fluxes, altering the light-dark cycle, enhancing lipid synthesis (Wu et al., 2015). Banerjee et al. (2016) reviewed recent transgenesis strategies like RNAi and riboswitch engineering to metabolically manipulate and alternatively bioengineer the metabolic pathways to optimally design GM algae, to maximize the target product. Even though metabolic engineering through genetic manipulations seems a promising approach for enhancing the yields, however, associated concerns with environmental safety and health issues hinders its commercialisation. Existing expertise of algal biotechnology, bioprocess engineering combined with current legislation can be used to develop effective risk management strategies ensuring their use in future.

5. Prospects of microalgal-derived biofuels

Algal biomass has been touted as a promising source of a variety of third-generation biofuels, being an unprecedented reserve of lipids and carbohydrates along with proteins, pigments, vitamins that also adds up to a range of value-added compounds. Solar energy stored in the biomass can be converted into a series of hydrocarbon-based fuels (biodiesel, bioethanol, bio-oil, biogas etc.) through thermochemical and biochemical routes (Daroch et al., 2013; Milano et al., 2016). Chemical processes comprise transesterification of lipids to biodiesel (Park et al., 2015). Biochemical processes include the fermentation of carbohydrate to produce bioethanol (Ho et al., 2013), biohydrogen (Batista et al., 2014) and anaerobic digestion generating biogas (Passos et al., 2014a, 2014b). Thermochemical processes comprise a thermal decomposition of algal biomass to liquid and gaseous fuels (Mehrabadi et al., 2015; Chiaramonti et al., 2017). The range of biofuels generated via the above-mentioned processes have been discussed in subsequent sections and illustrated in Fig. 5.

5.1. Biodiesel

Oleaginous algae produce a significant amount of lipids (5000–100,000 L/ha/day) (Singh and Olsen, 2011; Mehrabadi et al., 2015). Biodiesel produced has an energy content of about 39–41 KJ/g (Singh and Dhar, 2011; Daroch et al., 2013). The lipid content of the algae depends on the growth conditions (Chu et al., 2014) and can be further modified by genetic (Trentacoste et al., 2013) and metabolic engineering (Wu et al., 2015) or exposure to neutron irradiation. (Liu et al., 2016). Algal lipids are converted into biodiesel either by direct transesterification in the presence of catalyst [heterogenous/homogenous] (Galadima and Muraza, 2014) or via *in-situ* (trans)esterification (Rangabhashiyam et al., 2017; Martínez et al., 2017). Studies by Daroch et al. (2013); Park et al. (2015); Skorupskaite et al. (2016) have discussed the recent technological advances in the transesterification of algal lipids to biodiesel.

The disadvantages associated with the conventional transesterification process are, generation of a large amount of wastewater containing glycerol and catalysts, and subsequent oxidation of different value-added products like nutraceuticals in the deoiled biomass (Skorupskaite et al., 2016). Further life cycle assessment and techno-economic feasibility analysis have shown that currently used harvesting and extraction techniques are the biggest challenges, as they add up to the costs and energy requirements and contribute significantly to GHG emissions (Singh and Olsen, 2011; Dutta et al., 2016). To overcome the above-mentioned issues, enzyme-mediated transesterification of algal oils to biodiesel has been accessed. Enzymatic transesterification uses lipase either in free/immobilized form as bio-catalyst, at a temperature of 40–50 °C to convert algal oil into fatty acid methyl esters at comparatively much higher efficiency, with the ease of product separation, thus reducing downstream costs (Wang et al., 2014; Kim et al., 2016). Wang et al. (2014) and Wu et al. (2017) have optimized the concentration of different alcohol substrates and co-solvents (t-butanol) to oil ratio for maximizing the enzymatic transesterification efficiency. Noraini et al. (2014) and Amini et al. (2017) have summarised in details the current state of the art related to enzymatic transesterification process. In spite of widespread research, still the market price of algal biodiesel under current production strategies is not competitive with petrodiesel. Very recently, a study by Nagappan et al. (2018) suggested via energy balance analysis that the fuel value and process economics as well as the yield up to 98% of FAMEs can be achieved via the implementation of the wet route of saponification with co-solvent under optimized conditions. Nevertheless, it is essential to bridge and tackle the technological gaps to make algal biodiesel economically viable.

5.2. Biomethane

Anaerobic digestion is the process of treating biomass/sludge through a series of four steps namely hydrolysis, acidogenesis, acetogenesis and methanogenesis, to produce biogenic gas comprised of biomethane and CO_2, with minor quantities (< 1%) of hydrogen, ammonia, hydrogen sulphide and water vapour, popularly called biogas. Harvested biomass containing a significant amount of moisture can be digested to produce biogas after lipid extraction (Mussgnug et al., 2010; Wiley et al., 2011; Mehrabadi et al., 2015). Sialve et al. (2009) have estimated a theoretical yield of 0.48–0.80 L of biomethane/g volatile solids (VS). However, the experimental yield of biomethane currently achievable is much below than the theoretically expected values. Indeed, the yield is projected to be species-specific (Mussgnug et al., 2010; Passos et al., 2014a, 2014b). Reviews by Singh and Olsen (2011); Passos et al. (2014a) and Uggetti et al. (2017) have summarised the possible yield of biomethane obtained from a series of microalgal strain.

Biogas generation from algal biomass is considered a sustainable option over other bioenergy concepts as the use of wet biomass decreases the additional energy and costs involved in drying, and the digestible matter obtained as a by-product in biogas generation can be used in the form of fertilizers (Wiley et al., 2011). The issues with anaerobic digestion are low biodegradability of the algal cell wall due to the presence of cellulose and hemicellulose, also low C: N ratio of algal biomass (5:1–10:1), that potentially decreases the methane production (Passos et al., 2014b). Co-digestion via the addition of agricultural wastes/wastepaper, enhancing the C:N ratio and cellulase activity could overcome the low productivity of methane (Uggetti et al., 2017). Passos et al. (2014b) and Uggetti et al. (2017) have discussed the increase in methanisation potential of microalgae in continuous reactors following different thermal, chemical and enzymatic pre-treatments. Nutrient starvation has been found to redirect the metabolic flux towards increased carbohydrate accumulation resulting in a higher yield of biogas (Mussgnug et al., 2010). Biorefinery approach integrated with WWT in industries is further expected to improve the environmental and economic aspects of biogas generation (Mussgnug et al., 2010; Uggetti et al., 2017).

5.3. Bioethanol

Certain species of microalgae like *Chlorella*, *Dunaliella*, *Scenedesmus* and *Tetraselmis* etc. have a significant quantity of carbohydrates (> 40% of dry weight), thus can act as an ideal substrate for bioethanol fermentation (Kim et al., 2014). Bioethanol from microalgae has better yields than other energy crops, as the carbohydrate in algae exists in the form of mainly cellulose and starch with the absence of lignin which is not easily degraded (Ho et al., 2013). It is produced by fermentation of sugars, obtained by disrupting the algal cell wall by various pre-treatments like physical (autoclave, microwave etc.), chemical (acid, alkaline) and enzymatic (alpha- and glucoamylase) (Hernández et al., 2015). Pre-treatment is an essential step in fermentation as most of the sugars exists inside the cell bounded by a cell wall. Ho et al. (2013) have reviewed the pros and cons of different pre-treatment processes. Velazquez-Lucio et al. (2018) have summarised the current state of the art related to the third generation pretreatment techniques to gelatinize and change the structural features of microalgae, to enhance the bioethanol production. Microalgae compared to other lignocellulosic biomass has a significantly different chemistry having cell wall with relatively less or no lignin, thus has the advantage that the intracellular carbohydrates can be made available with mild or less intensive pretreatment (Chen et al., 2013). However, the starch granules found in microalgae has very less water content in their crystalline form, thus is more stable compared to the starch from other biomass thus, the enzymatic hydrolysis is difficult (Velazquez-Lucio et al., 2018). Therefore, often a combination of enzymatic/biological and other thermochemical pre-treatment methods is applied under optimum conditions, to achieve the desirable products. Hernández et al. (2015) have reported that a combination of acid pre-treatment with enzymatic hydrolysis, causes a better cell disruption, resulting in higher yield of monosaccharide which could be converted into ethanol. Enzymatic pretreatment/hydrolysis involving a mixture of enzymes and polymers, enhances the hydrolytic effect of enzymes, resulting in efficient cell lysis (Zheng et al., 2016). Another, promising way of pretreatment is hydrothermal processing which involves heating the microalgal biomass in the presence of a catalyst like acid, alkali or water, at 60 °C to 180 °C for a shorter reaction time of 60 min. Ruiz et al. (2013) postulated that the use of optimized parameters like reaction temperature, time, moisture content and particle size can increase the efficiency of the process, increasing the yield of cellulose. Nevertheless, it is imperative that the choice of pretreatment depends on the microalgal species and the cells to be fractionated to obtain the desired products. Traditional fermentation process involves saccharification of the pretreated biomass using enzyme cocktails depending on biomass origin (Daroch et al., 2013). Kim et al. (2011) and Lee et al. (2015) used a multi-enzymatic mix, due to the heterogeneity of algal carbohydrates. The final step of ethanol production is fermentation mainly mediated by yeasts either in free/immobilized forms (Trivedi et al., 2015; El-Dalatony et al., 2016). Kim et al. (2014) produced ethanol from *Chlorella vulgaris* with an efficiency of 89% using immobilized yeasts.

Daroch et al. (2013) have enlisted novel strains which could potentially convert pentose sugars, unlike yeasts, thus increasing ethanol yields. de Farias Silva and Bertucco (2016) have discussed the aspects and challenges associated with recent techniques of dark fermentation and photofermentation. In general, bioethanol production is postulated to be an energy extensive process but the costs associated with enzymes and distillation (ethanol purification) act as a potential barrier to commercialisation (Singh and Dhar, 2011; Daroch et al., 2013). Use of waste (deoiled) algal biomass for ethanol production as a part of biorefinery approach is expected to reduce the process costs, which could commercialise industrial bioethanol generation.

5.4. Biocrude oil

Biocrude oil is a tarry and heavy oil comprising C-17 and C-18 *n*-alkanes and polyaromatic hydrocarbons that can be used as distillate fuel. It is produced by hydrothermal liquefaction (HTL) of biomass with 10–20% wt. of solids in water, at temperatures of 200–350 °C with high pressure and residence time of 60–120 min (Biller et al., 2015). Hydrothermal processing is an effective technique that can be used to obtain oil, even from the carbohydrates and protein fraction apart from the lipids (Ruiz et al., 2013). The yield obtained is 30–50% wt. of oil with a heating value of 30–40 KJ/g (Mehrabadi et al., 2015). Eboibi et al. (2014) and Chiaramonti et al. (2017) have reviewed the process of HTL in relation to the variation in yield with different operating conditions (holding temperature, retention time, amount of solid and moisture content). The yield of the HTL process varies with the biochemical composition of algae (Li et al., 2014). It can be operated either as a batch (Faeth et al., 2013) or continuous mode (Biller et al., 2015). Continuous HTL has been found to consume much less energy with high throughput (Elliott et al., 2015). A variety of by-products are produced by HTL in numerous phases apart from biocrude oil. Gaseous products include methane gas, CO_2, hydrogen, nitrogen, ethane, etc. Liquid products or aqueous phase includes a high proportion of nutrients which can be recycled back into the HRAPs, and residual solids are formed by about 10% by wt. (Biller et al., 2015; Mehrabadi et al., 2017a).

Techno-economic and life cycle assessments of the HTL have established the process to be sustainable, providing a higher return in terms of energy and money with substantially lower GHG emissions, compared to other routes of biofuel production (Vardon et al., 2012; Delrue et al., 2013). Recycling the aqueous phase back into the batch reactor, improves the yield by 32.6% wt., further reducing the water consumption, making the process more cost-efficient (Hu et al., 2017). Despite the economic feasibility, bottlenecks are associated with the quality of biocrude oil. The major disadvantage is that the biocrude oil produced contains nitrogen (~5% wt.) and oxygen (~10% wt.) with a high viscosity, thus making it unstable and its combustion can lead to the release of NO_x which causes acid rain (Biller et al., 2015). Hydrotreating has resulted in around 80% upgradation of oil quality with significant reductions in oxygen and nitrogen content (Elliott et al., 2015). Carbon efficiency and economics of the process can be further improved by integrating the production process with other biofuels from algae using a biorefinery approach.

5.5. Bio-oil

Pyrolysis converts algal biomass containing (< 5%) moisture, under anaerobic conditions at 400–600 °C for 30–120 min, under ambient pressure to bio-oil (Vardon et al., 2012). Based on their heating rate, it can be classified as flash [1000 °C/s], fast [10–200 °C/s] or slow [0.1–1 °C/s] (Yanik et al., 2013) and has three steps:

- Dehydration of the water present in the cells at 80–200 °C
- Volatilization at 190–600 °C, producing liquids and gases upon condensation
- Decomposition producing solid biochar at > 500 °C

The thermal decomposition and conversion efficiency to bio-oil depend on a variety of factors like the microalgal biochemical composition, operating temperature, residence time and pretreatment (Vardon et al., 2012; Belotti et al., 2014; Tag et al., 2016). Yang et al. (2014) reported a maximum bio-oil yield of 49.36% at a temperature range of 425–500 °C. Disadvantages of the process are the energy-intensive nature due to the involvement of thermal drying. Further, the bio-oil which is a complex mixture of aromatics, witnesses changes in chemical and physical properties like acidity, density, viscosity with long-term use making it unsuitable as a transportation fuel (Mehrabadi et al., 2015). Reduction in oxygen content, hydrogenation/hydrooxygenation followed by catalytic cracking/catalytic pyrolysis in the presence of Ruthenium (Ru/C) as a catalyst at 200–300 °C and 150–200 bar for 1 h, improves the stability of bio-oil, decreases the acidity and increases the energy density of the oil (Zhang et al., 2013). Yang et al. (2015) summarised the phenomenon that leads to instability of bio-oil and discussed the recent techniques for improving the properties so that bio-oil remained stable and could be easily processed. Co-pyrolysis of microalgae with sewage sludge resulted in an increase in C4, C7, C9 hydrocarbons in bio-oil, thus improving its engine performance (Wang et al., 2016). Such approaches are promising alternatives which concurrently reduces the feedstock costs, enhancing the oil qualities (higher heating value, lower oxygen content) for real-time engine applications.

5.6. Biohydrogen

Biohydrogen production through microalgae is considered an eco-friendly and less energy intensive process compared to conventional thermochemical (e.g. gasification) and electrochemical processes (e.g. water electrolysis). Biohydrogen could be produced either by direct bio-photolysis or fermentation route. Bio-photolysis route utilizes light energy to photosynthetically convert water into hydrogen. A large number of microalgae are capable of producing biohydrogen (He et al., 2016).

Microalgal biomass like *Anabaena sp.* (Ferreira et al., 2012) and

Scenedesmus obliquus (Batista et al., 2014) could be used as a direct substrate for biohydrogen production by dark fermentation. A recent study explored the biohydrogen production coupled with lipid generation by utilizing oleaginous microalgae cultivation with crude glycerol as a low-cost exogenous carbon source (Sengmee et al., 2017). Nevertheless, today biohydrogen production technologies face challenges of low-yield and high production cost. However, recent advancements have been made on biohydrogen research to improve the yield through process modifications, physiological manipulations and promoting metabolic and genetic engineering (Kumar Gupta et al., 2013). Yet, to date, only around 15% of the theoretical maximum of biohydrogen production has been achieved which further dictate the need for improving the product extraction from microalgal biomass. The technical feasibility of biofuel production from microalgal biomass is demonstrated well, however, biorefinery approaches are demanded in order to make it economically viable.

6. Integrated zero waste algal biorefinery

In spite of huge interest and research on microalgal biofuels, the realistic application at industrial scale is still limited, as the existing technologies are still not in a position to produce biofuels at an amount and cost that can compete with the prevailing fossil fuels. Thus, large-scale application of microalgal technology for production of third generation biofuels calls for the integration of different innovative technologies, in order to make the entire process economically sustainable. One of the possible routes is via the use of an integrated biorefinery approach, which efficiently combines different conversion techniques to produce a range of products and by-products. Further, the co-products and energy generated can be redirected as input to facilitate the conversion process. The biorefinery implementation scenario provides an economic advantage by reducing the requirements of energy as well as by providing a series of value-added marketable products.

The algal biorefinery is a multifaceted approach used to produce a range of biofuels including biodiesel, biohydrogen, biomethane, bioethanol or other hydrocarbon fuel variants, such as JP-8 fuel, gasoline, etc. (Batista et al., 2015). However, very few studies have investigated the possibility of deriving more products simultaneously from microalgal biomass through various integrated approaches. Kumar et al. (2013) have demonstrated an integrated concept of producing bioethanol from the leftover pulp of red alga *Gracilaria verrucosa* following the extraction of agar. It was further postulated that the residual algal biomass after fermentation was rich in organics and minerals, thus could be possibly used as a source of fertiliser. Wieczorek et al. (2014) synthesized integrated biohydrogen (by dark fermentation of *Chlorella vulgaris*) and biomethane (by dark fermentation of residues) through the two-stage fermentation process. Dasgupta et al. (2015) attempted an integrative approach for biohydrogen from *Chlorella* sp. NBRI029 and *Scenedesmus* sp. NBRI012 and biodiesel production from its residual biomass. Zhu (2015) reported an innovative framework with a biorefinery approach to attain sustainable development with microalgal biomass as a potential feedstock as illustrated in Fig. 6. Apart from energy-based products, the residual microalgal biomass can be processed into a number of value-added products like pigments, plastics, food/feed etc. based on their composition (Yen et al., 2013). Chew et al. (2017) assessed the advantages of microalgal biorefinery with value-added products, in terms of total life-cycle energy and techno-economics. Similar to the above study, the analysis by Gong and You (2015) had also projected a net GHG reduction from 5 to 63% and an overall increase in global sustainability using a comprehensive structural approach with microalgal biofuel, and value-added products like polyethylene glycol, glycerol-*tert*-butyl ether and poly-3-hydroxybutyrate. Kouhia et al. (2015) experimentally described a biorefinery approach of integrating the waste streams from the pulp and paper industry to culture microalgae, which could be processed into biogas

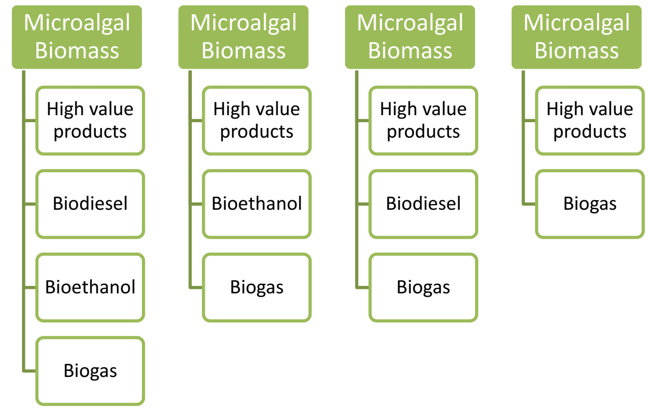


Fig. 6. Microalgal biorefinery pathways for maximizing the derivable products from microalgal biomass.

and the residual biomass being used as fertilizers and a source of omega-3-fatty acids. Trivedi et al. (2015) have highlighted the need for establishing a proper connection between the broad spectrum of various energy and non-energy products produced in an algal biorefinery to make the entire process financially viable.

7. Concluding remarks and prospects

Microalgal biotechnology has gained exceptional importance in recent decades for a wide array of applications ranging from biomass production for food, feed, fuel, fiber and other ecological applications. Realizing the enormous biodiversity, microalgae endorse as one of the most sustainable and promising sources for various products and applications. In view of ecological applications, microalgae could be helpful in treating wastewater, minimizing the CO_2 impacts on the surroundings and for producing valuable fuels which may subsequently replace the fossil fuels and in turn reduce the GHG emissions. They not only aid in overcoming the problem of depleted fossil sources but also help in reducing pollution and protecting the ozone layer.

Though algae are fast growing phototrophic organisms with high photosynthetic efficiency, the growth depends significantly on the culture and environmental conditions like light availability, pH, nutrients, temperature, pond depth, CO_2 availability and so on. Hence it is crucial to choose the microalgae that can offer maximum yield through faster growth rate and biomass productivity based on their tolerance to harsh environmental conditions. To realize the benefits of microalgal biotechnology in biofuel production, ways must be sought to make it economically feasible and to maximize biomass productivity. Further research needs to be done in the following areas as such:

- In view of strain development, bioprospecting the potent high lipid algal strains for greater biofuel production
- Understanding the adoption strategies of microalgal species to environmental stress and improve lipid productivity without affecting the photosynthetic growth
- Bioengineering aspects to improve the algal traits through synthetic biology
- Advancements in omics and metabolic engineering of algal biofuels to comprehend and control the production systems
- Nutrient recovery strategies from wastewater for overcoming the limited growth performance of algae in industrial reactors
- Improvement in algal production systems to make it competitive as well as economically feasible
- Less sophisticated as well as cost-effective harvesting methods should be sought to make biofuels available for real-time scenarios
- More attention has to be paid to biofilm-based harvesting systems as

they are least explored

- Exploring cost effective oleaginous materials and improving transesterification technologies for sustainable biofuel production
- Efficient utilization strategies of zooplanktons without harming the proliferation of dominant species
- Promotion of biorefinery concepts for production of natural coproducts and technological advances in reactor engineering has to be achieved for minimizing the production cost and improved economics
- Techno-economic and life cycle assessment of algal biofuel technologies for avoiding the outweigh off long-term consequences over short-term benefits

Many lab and pilot scale studies have acknowledged the potential of microalgae for the renewable fuel sources, still advanced research and developmental activities are needed on promoting the microalgal growth and bioprocessing at large scale systems. Bioprospecting the novel algal strains, enhancing the oil productivity, improving the bioseparation techniques, augmenting the downstream processing at industrial scale systems are to be focused on attaining the sustainability of the processes. Integration of algal cell biology with bioprocess engineering principles at field scale is to be biophysically modelled with the inclusion of site-specific metrological parameters to mimic the real-time field scale systems could minimize the field scale trials. The emerging *in-silico* based approaches with the principles of system biology and bioinformatics could comprehend the genetic and metabolic strategies to derive a few feasible solutions at the field scale.

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Disclosure statement

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