Science of the Total Environment 766 (2021) 144236

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Review on integrated biofuel production from microalgal biomass through the outset of transesterification route: a cascade approach for sustainable bioenergy

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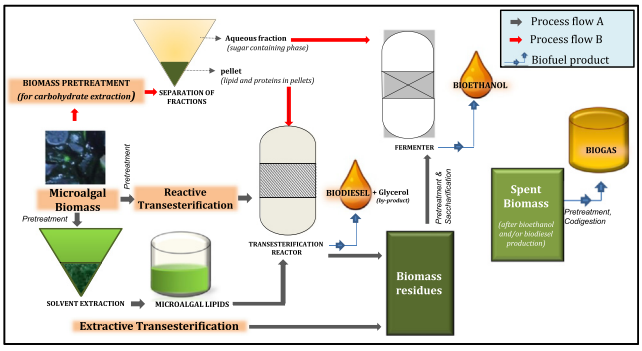
HIGHLIGHTS

- Investigation of advanced-combinatorial transesterification routes.
- 'Cascade process flow' for bioenergy-based algal biorefineries are analysed.
- 18% improvement in economic potential with joint biodiesel and bioethanol production.
- Comprehensive biogas production from spent microalgal biomass is presented.
- We discuss the technology-driven status to commercialization through TEA and LCA.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 September 2020
Received in revised form 10 November 2020
Accepted 24 November 2020
Available online 7 January 2021

Editor: Daniel CW Tsang

Keywords:
Microalgal biorefinery
Transesterification
Cumulative cascade approach
Environmental sustainability
Techno-economic analysis

ABSTRACT

In recent years, microalgal feedstocks have gained immense potential for sustainable biofuel production. Thermochemical, biochemical conversions and transesterification processes are employed for biofuel production. Especially, the transesterification process of lipid molecules to fatty acid alkyl esters (FAAE) is being widely employed for biodiesel production. In the case of the extractive transesterification process, biodiesel is produced from the extracted microalgal oil. Whereas *In-situ* (reactive) transesterification allows the direct conversion of microalgae to biodiesel avoiding the sequential steps, which subsequently reduces the production cost. Though microalgae have the highest potential to be an alternate renewable feedstock, the minimization of biofuel production cost is still a challenge. The biorefinery approaches that rely on simple cascade processes involving cost-effective technologies are the need of an hour for sustainable bioenergy production using microalgae. At the same time, combining the biorefineries for both (i) high value-low volume (food and health supplements) and (ii) low value- high volume (waste remediation, bioenergy) from microalgae involves regulatory and technical problems. Waste-remediation and algal biorefinery were extensively reviewed in many previous reports. On the other hand, this review focuses on the cascade processes for efficient utilization of microalgae for integrated bioenergy production through the transesterification. Microalgal biomass remnants after the transesterification process, comprising carbohydrates as a major component (process flow A) or the carbohydrate fraction after bio-separation of pretreated microalgae (process flow B) can be utilized for bioethanol production. Therefore, this review concentrates on the cascade flow of integrated bioprocessing methods for biodiesel and bioethanol production through the transesterification and biochemical routes. The review also sheds light on the recent combinatorial approaches of transesterification of microalgae. The applicability of

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https://doi.org/10.1016/j.scitotenv.2020.144236

spent microalgal biomass residue for biogas and other applications to bring about zero-waste residue are discussed. Furthermore, techno-economic analysis (TEA), life cycle assessment (LCA) and challenges of microalgal biorefineries are discussed.

Contents

1. Introduction

Renewable fuels sustain the environment and global economy. Biomass is an important renewable source of carbon-based biofuel which possesses the capacity of insuring the fuel supply (Owusu and Asumadu-Sarkodie, 2016). The current and future demand for biofuel cannot be practically gratified by oil from crops, cooking waste and animal fat (Chisti, 2007). Microalgae are the promising alternate feedstock for biofuel and are the nature's bioresource pool which produce lipids, carbohydrates, proteins, value-added products such as carotenoids and bio nutrients (Gerardo et al., 2015; Karpagam et al., 2018; Ruiz et al., 2016). It is important that the biomass conversions which relay on single biofuel product are not cost-effective. Based on the microalgal biorefinery concept, range of bioproducts such as biodiesel from lipids, bioethanol from carbohydrates, biogas, carotenoids can be obtained through integration of operations along with waste-utilization for algal cultivation (De Bhowmick et al., 2019). In order to achieve a comprehensive algal biorefinery process, a prior knowledge on the plausible mechanism employed by microalgae are essential. Besides processes including pretreatment, transesterification, fermentation, hydrothermal liquefaction, pyrolysis and anaerobic digestion (biohydrogen/ lipids/ biochar production) are crucial for bio-valorization of algal biomass (Kumar et al., 2020a). This can enhance the resource recovery, efficiency and cost effectiveness (Gerardo et al., 2015; Shahid et al., 2020; Venkata Mohan et al., 2020).

1.1. Microalgae: CO_2 and waste bioremediation

Microalgal biomass cultivation can be coupled with CO_2 bioremediation and wastewater treatment as an integral process (Cai et al., 2013; Collotta et al., 2018; De Bhowmick et al., 2019; Ge et al., 2017; Venkata Mohan et al., 2020). Microalgae have superior CO_2 capturing capacity than terrestrial plants (Valdovinos-García et al., 2020). About 1.83 kg of CO_2 gets converted into 1 kg of dry algal biomass under optimal conditions. Photoautotrophic microalgae can be employed to fix CO_2 existing in flue gases formed at power plants or other emission sources. Currently, concurrent air and wastewater treatment technology using microalgae is increasing owing to its economic and environmental feasibility (Viswanaathan and Sudhakar, 2019). In case of *Chlorella vulgaris* (cultivated using CO_2 from a thermoelectric plant in open raceway pond system) achieved a capture of 102.13 tons of CO_2/year in 1 ha cultivation area with biomass productivity of 12.7 g m^{-2} day^{-1} (Valdovinos-García et al., 2020). Similarly, *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* showed enhanced biomass production at the optimum CO_2 concentration of 5.35 ± 0.34% (v/v). Also, the optimum N and P removal efficiencies were enhanced, reaching very close to 100% in cultures performed with CO_2- enriched air streams. This emphasized the applicability of the microalgae in CO_2 capture from flue gases (Gonçalves et al., 2016). The source of CO_2 is the most appropriate factor affecting environmental impacts and the direct injection of flue gas into the algal pond and waste water usage persist as the most eco-friendly option (Collotta et al., 2018). These waste bioremediation aspects with its progress and challenges were reviewed recently (Bhatia et al., 2020; De Bhowmick et al., 2019). Recently, microalgae were found to utilize biodegradation, bioadsorption and bioaccumulation mechanisms to remove pesticides resulting in the dual benefit of wastewater treatment and bioproduct production (Nie et al., 2020). Interestingly, microalgae can perform biomineralization of CO_2 followed by calcification and calcite precipitation (Thakur et al., 2018). This enables microalgae a feasible feedstock for carbon dioxide capture and utilization for commercially viable organic products.

1.2. Bottlenecks in algal biorefinery

The bioproducts from microalgae can be branched into two types (i) high value-low volume products such as nutraceuticals, food

supplements, antioxidant pigments and (ii) low value-high volume products include biofuels (biodiesel, bioethanol and biogas) (Schiano di Visconte et al., 2019). The major bottlenecks of algal bioproduct production are higher net energy requirement and cost investment. Thus for the cost-effective production, simultaneous production of 'high-value-low- volume' product and 'low-value-high-volume' product can be employed (De Bhowmick et al., 2019). On the other hand, there are realistic bottlenecks present for the integral production of aforementioned two types of bioproducts from microalgae. For an instance, the regulatory requirements differ based on the bioproduct type (Schiano di Visconte et al., 2019). In this way, it was reported that the stability/reliability of large microalgal cultures avoiding contamination and crash of the cultures are essential in terms of food and feed applications such as nutraceuticals, feed supplement and antioxidants production (Enzing et al., 2014). Importantly monitoring of water quality used for biomass production is required particularly for heavy metals, pesticides, antibiotics, radioisotopes, toxins and microbial load including coliform contamination. In such a way that, microalgal biomass production for nutraceutical applications has to be done in water appropriate for human consumption as per the recommendations by pollution control boards and/or municipal corporations of various states/countries (Nethravathy et al., 2019). These regulatory and safety issues may generate possible difficulties on the integration of waste-utilization of microalgae for food application. With regard to the various unit operations in microalgal biorefineries, bottlenecks are present in upstream and downstream processing steps. For an instance for nutraceutical production, downstream processing with appropriate cell disruption and cascade extraction methods are needed to preserve the protein and carotenoids functionality (Gifuni et al., 2019; Safi et al., 2014). Establishing a suitable connection between the input and output streams for the various bioproducts, as well as for the services to be provided are the logical factors to be considered for algal biorefinery concept (Trivedi et al., 2015). Coupling the production of all type of bioproducts from algae may not prove to be a feasible option since there are main discrepancies flanked by the quality control and regulatory requirements between pharmaceutical/nutraceutical products and that are required for waste water treatment, bioremediation or feedstock production (Schiano di Visconte et al., 2019). Whereas in terms of bioenergy applications, microalgae can be readily and effectively combined with waste utilization (Karpagam et al., 2015; McGinn et al., 2011; Pittman et al., 2011; Sibi, 2018). In the context of bioremediation of waste, aspects of cultivation of microalgae in wastewater for biomass production, pollutant removal and atmospheric carbon mitigation were reviewed comprehensively in previous review (Shahid et al., 2020). The use of livestock wastes for biomass generation using microalgae and its bioremediation aspects was recently reviewed (Lu et al., 2020). In order to pay more attention on maximum resource recovery from microalgal biomass, use of mild and non-invasive technologies for microalgal biomass fractionation methods and process technologies are needed. At the same time, integrating and co-optimizing different operations strategies specific to the range of bioproducts is essential (Gifuni et al., 2019). Therefore, cascade approaches focusing on bioenergy-based biorefinery pathways would have reliable benefits to the biofuel-based microalgal industries for sustainable bioenergy.

1.3. Bioenergy-based algal biorefinery process

In the perspective of main liquid-renewable biofuel for transport, microalgae are the promising feedstock with scalability feature, amenable for biodiesel (Moody et al., 2014; Scott et al., 2010) and bioethanol (Harun et al., 2010; Wang et al., 2014) production. With regard to the scalability analysis of microalgal biofuel production, the global evaluation of lipid yields from microalgae was performed by incorporating meteorological data at 4,388 geographical sites by using a validated outdoor photobioreactor growth model. The results of this study showed that the lipid yields were ranged from 24 and 27 $m^3 \cdot ha^{-1} \cdot y^{-1}$ (corresponding biomass yields of 13–15 $g \cdot m^{-2} \cdot d^{-1}$). This implied that many non-arable land regions can supplement 30% of their fuel consumption through microalgae (Moody et al., 2014). Whereas in a study of open raceway pond cultivation in 40,000 L, higher average areal lipid productivity (5.15 g m^{-2} d^{-1} for *Chlorella* sp. L1, 5.35 g m^{-2} d^{-1} for *Monarphidium dybowskii* Y2) was achieved in semi-continuous mode. Interestingly, the financial costs of 14.18 $ gal^{-1} and 13.31 $ gal^{-1} for crude biodiesel in two microalgae were feasible for commercial production on large scale outdoors (He et al., 2016b). On the other hand, microalgae are the excellent sources of fermentable carbohydrates that could be employed for bioethanol production. A maximum ethanol concentration of 3.83 g L^{-1} was obtained from 10 g L^{-1} of lipid-extracted debris of *Chlorococcum* sp. (Harun et al., 2010). In another study, a maximum yield of 0.286 g ethanol/g total carbohydrate was obtained from wet algae of *Nannochloropsis* sp. (Rahman et al., 2019).

Various methods of biofuel production are depicted in Fig. 1. Transesterification process is the common method for biodiesel production (Takisawa et al., 2014). Microalgal biomass conversion by thermochemical methods such as pyrolysis, hydrothermal liquefaction are also employed for biodiesel production (Chiaramonti et al., 2015; Marcilla et al., 2013). Renewable diesel is a biofuel derived from biological resources by hydrotreating process (Davis et al., 2013), whereas biodiesel derived by transesterification process are fatty acid methyl esters (FAME) (Amin, 2019; Takisawa et al., 2014). Hydrotreating is a thermo-chemical process that makes alkanes out of the lipid chains and propane out of the glycerol backbone (Sustainable development of algal biofuels in the United States. Chapter 3. Pathways for Algal Biofuel Production, 2012). On comparing the fuel properties of renewable biodiesel and FAME biodiesel, renewable biodiesel has fuel properties nearer to petro-diesel than FAME biodiesel. Also, renewable biodiesel has highest cetane number and a cloud point almost similar to petroleum diesel (Amin, 2019). Thermo-chemical and biochemical conversion routes can be used for biofuel production from microalgae (Laurens et al., 2015). However, thermochemical-based routes have some disadvantages of unwanted secondary chemical reactions of the protein of the biomass components (Changi et al., 2012), which may render increased cost of bio-oil refining. Thermochemical conversion methods like hydrothermal liquefaction exhibits critical drawbacks such as higher energy requirement and inconsistency in biocrude quality, which may require further purification and upgrading (Kim et al., 2017). Whereas, biochemical conversion can selectively convert biochemical components to biofuel, leaving a biomass residue fraction rich in protein components (Laurens et al., 2015), that could be employed for biogas production through anaerobic digestion. Importantly, around the world commercial units for biodiesel production are in service that implies that biodiesel is a mature technology particularly for countries where biomass is acquirable in large amount (Amin, 2019). The most popular method of biodiesel production is transesterification, in which lipids/triglycerides are converted into fatty acid methyl ester (FAME) using methanol and a catalyst (Deshpande et al., 2017; Takisawa et al., 2014). In addition to general catalytic method of transesterification, non-catalytic transesterification using supercritical and subcritical methanol can also be employed for microalgal biodiesel production (Levine et al., 2010; Patil et al., 2011). The oil/FAME extracted microalgal biomass are rich in carbohydrates and are highly suitable for bioethanol production (Chng et al., 2016; Ma et al., 2020). The remaining residual biomass after biodiesel and bioethanol production could be utilized for biogas production by anaerobic digestion (Davis et al., 2014; Laurens et al., 2017). The spent microalgal biomass after primary fuels extraction can be employed for the production of bio-char, bioadsorbent, nutrient digestates/hydrolysates (Rashid et al., 2013). As of today, the algal biorefinery concepts (Chandra et al., 2019; Kröger and Müller-langer, 2014; Kumar and Singh, 2019; Laurens et al., 2017; Venkata Mohan et al., 2020) and its associated microalgal transesterification process (Kumar et al., 2020a; Naveena et al., 2015; Park et al., 2015; Salam

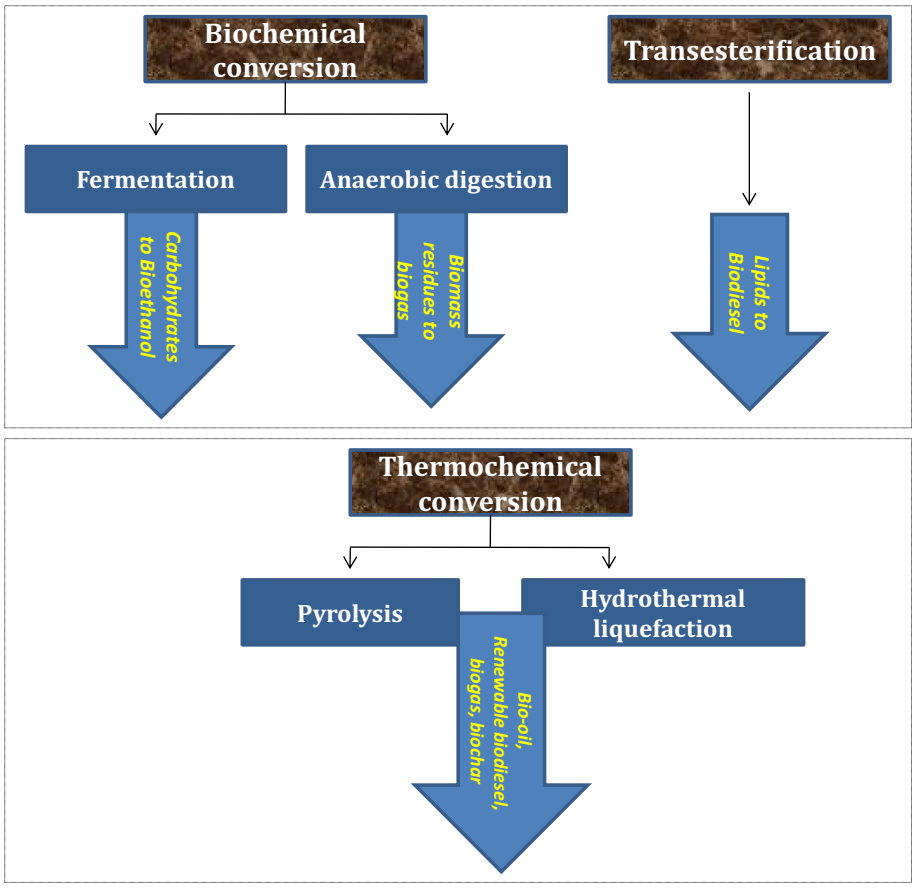

Fig. 1. Various methods of biofuel production from microalgae.

et al., 2016; Takisawa et al., 2014) are critically reviewed by several researchers. However, reports exclusively focusing on (i) the recent combinatorial transesterification approaches in microalgae and (ii) biofuel production through 'cascade flow approach' via transesterification and biochemical conversion routes are scarce. To fill this knowledge gap, this review discusses the recent methods of bioenergy-based bio-

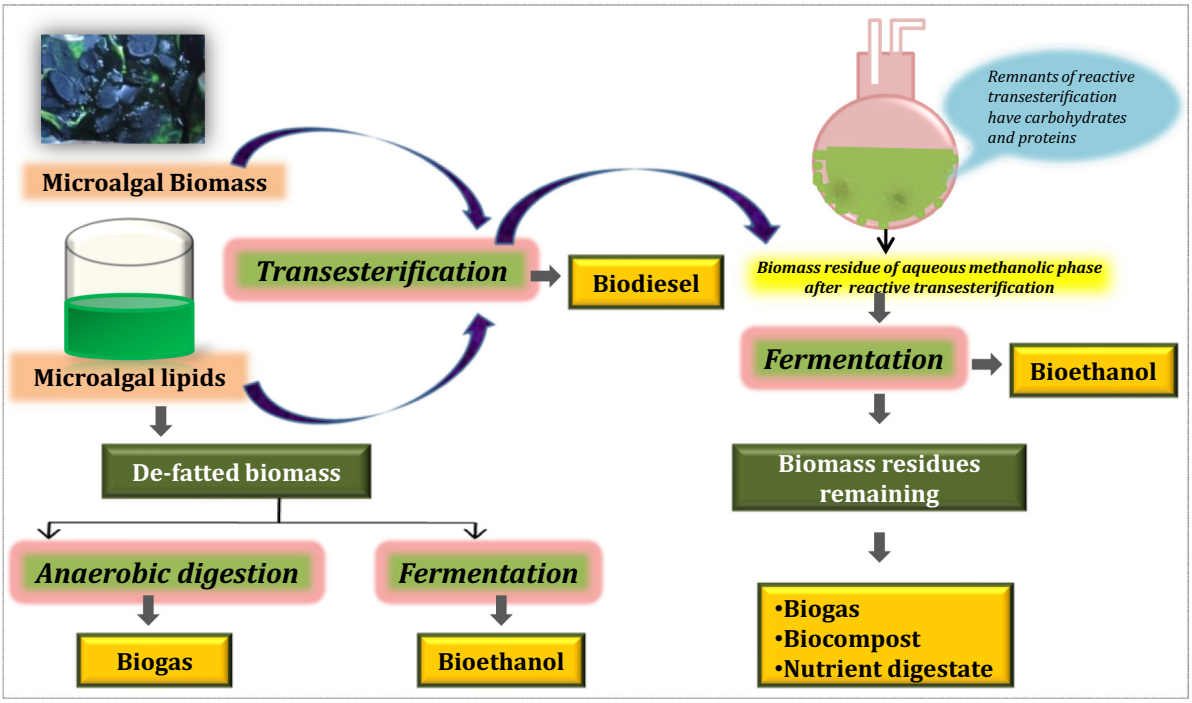

Fig. 2. Cascade flow of bioenergy production from microalgae through transesterification and biochemical conversions routes.

refineries from microalgal biomass to tune cascade-production approach with the onset of following strategies. (i) biodiesel production through transesterification route (ii) bioethanol production from remnant biomass after lipid/biodiesel extraction [depicted in Fig. 2]; and (iii) biogas or any other applications (biochar, biosorbent production etc.,) from the spent microalgal biomass residue after bioethanol production as zero-waste production approach. This review also discussed (i) the methods for integral processing for efficient joint biodiesel and bioethanol production (ii) TEA, LCA and (iii) challenges of algal biorefinery.

2. Production of biodiesel from algae through transesterification route

Biodiesel is the monoalkyl esters of long-chain fatty acids derived usually from transesterification of lipids from renewable biomass feedstocks (Ambat et al., 2018). Transesterification consists of a number of consecutive reversible reactions in which lipids/triglycerides are reacted with alcohol (generally methanol) for the stepwise conversion to diglycerides, monoglycerides, and finally glycerol, in successive reversible reactions with the release of moieties of fatty acid methyl ester (FAME) i.e. biodiesel at each step (Kumar et al., 2020a; Takisawa et al., 2014). On considering microalgae as a biodiesel feedstock, microalgae can accumulate lipid moieties during late growth or stationary phase, under stress conditions and the lipid accumulation strategies employed were extensively reviewed (Alishah Aratboni et al., 2019; Sharma et al., 2012; Zhu et al., 2016). Reactive transesterification (direct/*in-situ* process) represents direct conversion of microalgal biomass harboring lipids into FAME, whereas extractive transesterification (indirect process) represents FAME conversion from the extracted lipids (Park et al., 2015). Various methods of pre-treatment such as high-pressure homogenization, ultrasound sonication, microwave irradiation (Howlader and French, 2020), osmotic shock, water bath, Triton-X-100 treatment, shake mill (Ramola et al., 2019) can be used to disrupt the cell wall of microalgae during lipid extraction. In such a way, lipids from *Spirulina* was extracted by solvothermal techniques in a microwave at 60 °C for 30 min and 750 W (Singh et al., 2019). Lipid extraction from microalgae is usually performed by chloroform/methanol methods (Bligh and Dyer, 1959; Folch et al., 1957); methods using methyl-tert-butyl ether (Matyash et al., 2008) and hexane (Ramola et al., 2019; Shin et al., 2018). Combination of hexane and methanol in the ratio of 1:1 was effective for large-scale lipid extraction of microalgae (Shin et al., 2018). Also, use of ionic liquids for lipid extraction was found to be efficient. In such a way, lipids from *Chlorella vulgaris* biomass were extracted using mixtures of ionic liquids and methanol (1:1) v/v and compared with conventional Bligh and Dyer's method. A mixture of ionic liquid [Bmim][CF_3SO_3] and methanol extracted 12.5% and 19.0% of the lipids, respectively (Kim et al., 2012). Interestingly, on using switchable solvent *N*,*N*,*N*′,*N*′-tetraethyl-1,3-propanediamine (TEPDA) for lipid extraction from wet *Nannochloropsis oceanica*, a 5% higher extraction efficiency than chloroform–methanol was attained. In addition to lipid dissolving ability, it was found that minor amount of TEPDA was dissociated into tertiary amine ion. This cation functioned as a surfactant to aid in cell disruption and lipid separation. FAME yield from lipids through the transesterification of lipids extracted with TEPDA increased by 9%, whereas FAME yield from lipids extracted with chloroform and n-hexane decreased by 41% and 65%, respectively (Cheng et al., 2020). In another study, cell disruption was integrated with lipid extraction using n-hexane/formic acid as mixed solvent. On comparing with the chloroform/methanol extraction supplemented by ultrasound, microwave, hydrothermal, and dilute nitric acid pre-treatments, the FAME contents increased by 79%–99% by using n-hexane/formic acid at the ratio of 9:2 (v/v) at 80 °C for 2 h (Xia et al., 2020).

Transesterification of microalgal oil or microalgal biomass can be performed in two steps. In two-step process, acid esterification is carried out to reduce the free fatty acid (FFA) content (Dong et al., 2013; Suganya et al., 2013). In this way, first step of transesterification of algal oil by acid transesterification had reduced the FFA content from 6.3% to 0.34% with optimized parameters of 1.5% H_2SO_4, 12:1 methanol–oil ratio, 400 rpm at 60 °C and 90 min of reaction time. In the second-step, the maximum biodiesel yield of 90.6% was achieved from base transesterification through optimum conditions of 1% NaOH, 9:1 methanol–oil ratio, 600 rpm and 60 °C temperature for 70 min (Suganya et al., 2013). Though most of the *in-situ* transesterification were performed as a single step reaction (Ghosh et al., 2017; Kwon and Yeom, 2015; Patil et al., 2011; Tang et al., 2016), *in-situ* transesterification process can also be performed in two steps (Dong et al., 2013; Ma et al., 2015b). Two-step *in-situ* process in *Chlorella sorokiniana* (UTEX 1602) had resulted in a total FAME recovery of up to 94.87 ± 0.86%, whereas with the one-step *in-situ* process, the FAME recovery was reached up to 60.89% after a 70 min reaction at 90 °C. In this study, prior to the base-catalyzed transesterification, pre-transesterification was carried out using heterogeneous acid catalyst, Amberlyst-15 (Dong et al., 2013). FAME yield of 35.5 ± 1.27 mg/g biomass was obtained from two-step *in-situ* transesterification of *Chlorella vulgaris* at the optimal conditions as follows: in the first step, 10 wt. % of solid acid (Amberlyst BD20) in 50 min reaction time and in the second step, 4 wt. % of KOH for 40 min reaction time. This FAME yield was three times more than one-step alkali-catalyzed *in-situ* transesterification (Ma et al., 2015b). Biodiesel production in microalgae by *in-situ* transesterification process are gaining focus since it has the main advantage of minimal processing steps by means of avoiding costly biomass drying and lipid extraction steps (Ghosh et al., 2017; Kim et al., 2015; Ma et al., 2019; Mandik et al., 2020; Park et al., 2017) (Fig. 3).

2.1. Methods and factors affecting microalgal transesterification

The key process variables affecting transesterification efficiency are alcohol to oil molar ratio, moisture, stirring rate, reaction time, temperature, microalgal cell wall and catalyst type (Salam et al., 2016). During transesterification reaction, the most important variables affecting the methyl ester yield are molar ratio of alcohol to oil and reaction temperature (Degfie et al., 2019). Transesterification reactions are performed through catalytic and non-catalytic methods. Catalytic methods employ the use of acid, base or enzymes as catalyst (Vasić et al., 2020). Conventionally, homogenous acid (HCl, H_2SO_4) or homogenous bases (NaOH) are used as catalyst for transesterification reaction. On the other hand, heterogeneous catalysts such as solid catalyst, nano-catalyst, ion-exchange resins are gaining focus and the advantages of the heterogeneous acid/base catalysts in biodiesel production industry such as ease of fuel separation, reusability and recyclability of catalysts were reviewed previously (Aransiola et al., 2014; Galadima and Muraza, 2014). FAME percentage is the main parameter for the analysis of optimum reaction parameters and kinetics of transesterification (Kumar and Sharma, 2016). Till date, many reviews are available on various transesterification methods for biodiesel production (Aransiola et al., 2014; Guldhe et al., 2015; Hidalgo et al., 2013; Park et al., 2015; Thangaraj et al., 2019), nevertheless, this review embrace on the advancements on various transesterification methods employed and reaction parameters were discussed specific to microalgal feedstock, followed by the recent reports on advanced-combinatorial approaches for microalgal transesterification.

2.1.1. Acidic catalysis for microalgal transesterification

Usually oil from microalgae has high acid values that decrease the biodiesel yield with base catalyst owing to soap formation. Thus the microalgal oil has to be processed with either acid catalyst or by two-step approach of esterification and transesterification (Guldhe et al., 2014). Acidic catalysts have many advantages in case of *in-situ* transesterification of microbial biomass in terms of FAME conversion rate (Ehimen et al., 2010). However, direct transesterification of *Chlorella* with acid catalysts was inhibited by the presence of water. Also

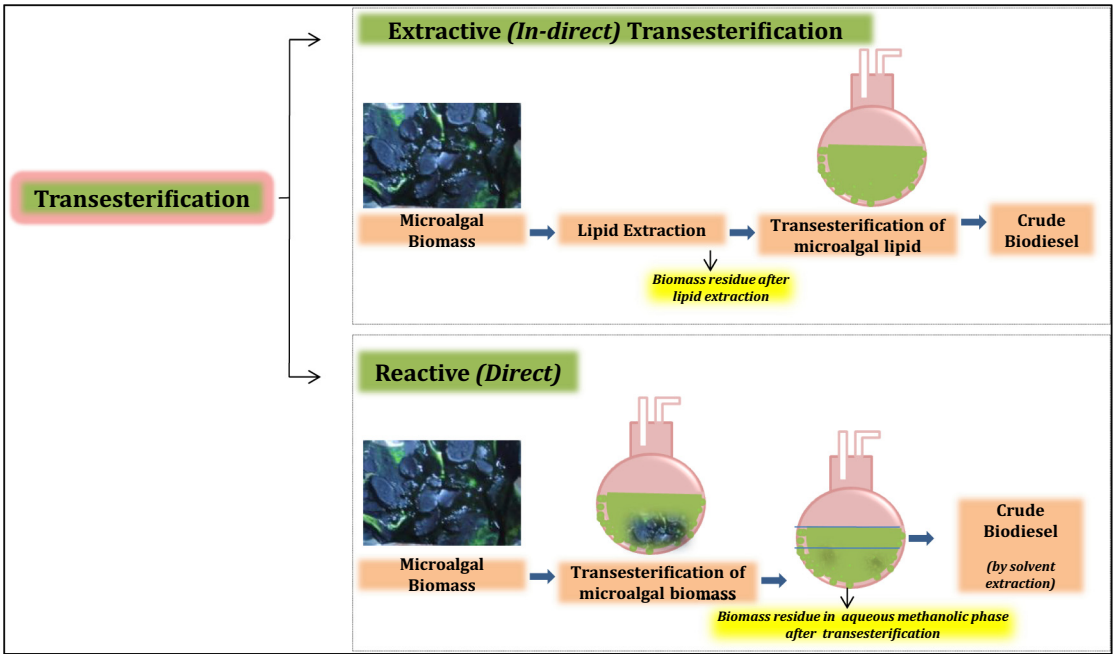

Fig. 3. Extractive and reactive transesterification.

the stronger negative influence on the equilibrium FAME yield was found when the water content of the biomass was higher than 115% w/w (based on oil weight) (Ehimen et al., 2010). The effect of water content on direct transesterification of microalgae was studied and was clarified that the deactivation of acid catalyst can occur due to competence for available protons in the reaction by water molecules (Sathish et al., 2014). Similarly heterogeneous acid catalysts had demonstrated enhanced FAME yield in microalgae and were previously reviewed (Galadima and Muraza, 2014; Vasić et al., 2020), however corrosion in the engines parts by solid acid catalyst leachate (Singh et al., 2012), lesser biodiesel yield with reuse are the disadvantages (Guldhe et al., 2014). For direct transesterification of microalgae using conventional acid catalysis, methanol and sulfuric acid are required in larger amounts, at the same time, the amount of methanol and sulfuric acid should be reduced to avoid reactor corrosion by sulfuric acid. Thus at these conditions, pentane and diethyl ether can be used to lessen the methanol volume by enhancing the reaction yield, as these solvents in conjugation with methanol aid in improved diffusion of the microalgal oils across the cell wall (Ehimen et al., 2012). Recently, dodecylbenzenesulfonic acid (DBSA) catalyst in the packed bed reactor was employed for biodiesel production from *Chlorella* sp. oil. DBSA was found to be a highly active catalyst for the transesterification than H_2SO_4 catalyst. DBSA catalyst with the residence time of only 30 min can make the transesterification process more cost-effective compared to the batch process of using sulfuric acid catalyst with a residence time of more than 12 h (Jazie et al., 2020).

2.1.2. Base catalysis for microalgal transesterification

Generally, transesterification reactions with homogeneous base catalysts are faster than the homogeneous acid catalysis under optimum reaction conditions. It was reported that maximum biodiesel yield of 97.66% was obtained from *Chlorella protothecoides* oil at the optimum conditions of 0.5% NaOH catalyst conc. (w/w), methanol to oil molar ratio of 7:1, reaction temperature of 60 °C and reaction time of 60 min (Kumar and Sharma, 2016). Homogeneous alkaline catalysis (using NaOH and KOH) is the most used transesterification route for biodiesel production, as high conversion yield can be achieved in a short time, at low temperature and atmospheric pressure (Park et al., 2015). Base catalysis is faster than acid catalysis, but it is more selective based on the lipid type that are transesterified. For an instance, high concentration of FFA of the microalgal lipid causes partial saponification and soap formation, which lessens the biodiesel yield (Laurens et al., 2012). *In-situ* transesterification of microalgae using homogeneous base catalysts in *Chlorella vulgaris* had yielded a maximum FAME recovery of 77.6 ± 2.3 wt% obtained at a reaction time of 75 min, using a catalyst: lipid (NaOH) molar ratio of 0.15:1 and a methanol: lipid molar ratio of 600:1. However, on using an acid catalyst ratio of 0.35:1 for longer reaction times resulted in higher conversions of up to 96.8 ± 6.3 wt% since acid aided in the breakage of microalgae cell walls. In particular, the base-catalyzed *in-situ* transesterification of algal biomass using a lower ratio of base catalyst can accomplish high conversion in less time than an acid catalyst (Velasquez-Orta et al., 2012). Moreover, pre-esterification process using heterogeneous catalyst can reduce the content of FFA of algae prior to the base-catalysed transesterification (Dong et al., 2013). However, alkaline catalysts are generally not suggested for *in-situ* transesterification of microalgae as the microalgal biomass is FFA rich (Ehimen et al., 2010). Heterogeneous base-catalyzed reaction involves heterogeneous bases either the Lewis or the Bronsted basic sites of the catalyst with a monohydric alcohol. The generated alkoxide mixture reacts with triglyceride ester in the oil to yield fatty acid alkyl ester (biodiesel) and glycerol in the subsequent steps. The robustness of the basic sites determines the formation of the alkoxide species and therefore the overall reaction rate would be increased. Solid basic catalysts such as zeolites, oxides such as ZnO, CuO, basic polymers, oxides and carbonates such as CaO, MgO, SrO, BaO, $CaCO_3$, $MgCO_3$, $SrCO_3$ and $BaCO_3$ are reported as putative heterogeneous base catalyst (Galadima and Muraza, 2014). A study on direct transesterification of dried *Nannochloropsis* sp. using a heterogeneous base catalyst reported a maximum conversion of 28% at 10% (w/w) alkaline heterogeneous catalyst (Mg-Zr) in methanol: dichloromethane (3:1, v/v) at 65°C for 4 h (Li et al., 2011). In yet another study, production of biodiesel from *Spirulina* oil using synthesised heterogeneous base catalyst (Barium–Calcium–Zinc mixed oxide) was reported. The maximum FAME conversion obtained was 98.94% under the optimised conditions of 2.5 wt % catalyst, 1:18 molar ratio (methanol/oil), 600 RPM stirring speed, and 65 °C temperature for 120 min. The catalyst was found to be reusable for six cycles (Singh et al., 2019).

2.1.3. Enzyme catalysis

Lipases are hydrolases (EC 3.1.1.3) that have the ability to catalyze transesterification of both triglycerides and FFAs to give esters (Guldhe et al., 2015; Taher et al., 2011). On considering the extractive transesterification process, enzyme catalysis mediated transesterification were carried out from extracted microalgal oil, catalyzed through the lipase enzyme (Lai et al., 2012; Surendhiran and Razack, 2015; Tran et al., 2012; Xiong et al., 2008) or whole cell lipase system (López et al., 2016). *Chlorella pyrenoidosa* lipids catalyzed by *Penicillium expansum* lipase (PEL) had produced 90.7% biodiesel yield in ionic liquid solvent as the reaction medium (Lai et al., 2012). The factors affecting the enzymatic transesterification are water content, inhibition by alcohol, temperature, inhibition by glycerol, pretreatment for improving lipase stability and the effect of solvent (Guldhe et al., 2015). The mechanisms of lipases, immobilization techniques, and factors affecting enzymatic catalysis were extensively reviewed previously (Guldhe et al., 2015; Taher et al., 2011). Also certain enzyme pretreatments have revealed auspicious outcomes of improved lipase stability and this enhanced stability makes extended reuse of immobilized lipases, which can improve the process economy. These pretreatments include lipase enzyme treatments using glutaraldehyde, methanol and salt solutions (Guldhe et al., 2015). In the context of *in-situ* transesterification of microalgae using enzyme catalysis, cell wall disruption through appropriate pretreatment is required (Tran et al., 2013). Pretreatment of algal cells allow cell wall disruption by homogenization, sonication, autoclaving, lyophilization, acid treatment and so on (Karemore and Sen, 2016). It is worth mentioning that the biodiesel conversion from sonicated wet biomass of *Chlorella vulgaris* ESP-31 by using immobilized *Burkholderia* sp. C20 lipase as the catalyst had produced the maximum biodiesel conversion of up to 97.3 wt% oil as compared to conversion of extracted lipids (72.1 wt% oil). Without a substantial loss of original activity, the immobilized lipase was repeatedly used for six cycles (or 288 h) in this study (Tran et al., 2012).

2.1.4. Supercritical and subcritical solvent methods

Non catalytic transesterification process uses supercritical and subcritical solvents. At critical temperature and pressure, the densities of both liquid and gas phases become identical, and the distinction between the phases disappears to form supercritical fluids (SCFs). This requires higher temperature and pressure. The physical properties of these supercritical/subcritical solvents display extraordinary capabilities for extraction, reaction, fractionation and analysis processes (Bernal et al., 2012). Various solvents including methanol, ethanol and isopropanol affecting biodiesel yield at supercritical condition were studied (Huang et al., 2015). FAME conversion by direct transesterification was demonstrated even for the wet algal biomass of *Nannochloropsis* sp. (CCMP1776), containing about 90% water content (w/w, wet basis) under supercritical conditions. A maximum of 85.75% of FAME yield was obtained by employing RSM (response surface methodology). Further, the reaction parameters were optimised as follows: wet algae to methanol (wt./vol.) ratio of around 1:9, reaction temperature of about 255 °C, with 25 min reaction time at a fixed reaction pressure of 1200 psi. Thus, supercritical method of transesterification has advantages such as shorter reaction time than catalytic transesterification process, functioning of water in wet algae as the accelerator of the reaction, simple purification of products and maximum conversion (Patil et al., 2011). However, SCFs technology requires costly equipment, such as strong durable reactors, high pressure pumps, efficient control devices, etc., (Bernal et al., 2012). Non-catalyst *in-situ* transesterification under subcritical methanol can be considered as an equivalent method, although the reaction conditions such as temperature and pressure were not as high as supercritical conditions, except for the prolonged reaction time. In such a way, from *Chlorella* sp. biomass, under subcritical ethanol condition, maximum FAME conversion of 95% was obtained at 175 °C, 20/1 (g/g) methanol to biomass ratio with 50% water content in biomass with the reaction time of 12 h (Phong et al., 2016). In another study, from *Chlorella vulgaris* with 80% water content, the maximum FAME yield of 14.52% with respect to the amount of dry biomass (77.72% if with respect to the maximum obtainable FAME) was obtained at a reaction temperature of 220° C, reaction time of 2.6 h, and 8 mL methanol per gram of dry biomass through optimization studies (Felix et al., 2017). In a two-step process, in the first step, under subcritical conditions, the intracellular lipids of wet microalgal biomass of *Chlorella vulgaris* was hydrolyzed to FFA. In the second step, FFA-rich wet biomass solids were subjected to supercritical direct transesterification and the effects of reaction time (60 or 120 min), temperature (275 or 325 °C), and ethanol loading (≈2−8 w/w EtOH/solids) were examined on the biodiesel yield. Longer reaction time, higher temperature and higher volume of ethanol that enhanced crude biodiesel and fatty acid ethyl esters (FAEE) yields, which ranged from about 56−100% and 34−66%, respectively, on the basis of lipid in the hydrolyzed solids (Levine et al., 2010). Use of supercritical carbon-dioxide (SC-CO_2) as the lipid extraction solvent and reaction solvent for enzymatic transesterification has been proposed to replace conventional solvent extraction and the biodiesel can easily be separated from the SC-CO_2 that allows easy separation of the product (Taher et al., 2011). With SC-CO_2 as a lipid extractant, the lipid extraction yield was increased by 20% in *Scenedesmus* sp. at a temperature of 50° C and a pressure of 350 bar, when compared to n-hexane (Taher et al., 2014b). In another study, SC-CO_2 as a reactant, optimum transesterification yield of 80% was obtained in the same microalgae at 47 °C, 200 bar, 35% enzyme loading and 9:1 methanol to oil molar ratio after 4 h reaction in the batch system. In the continuous integrated extraction-reaction system, with the 10:1 methanol to oil ratio, 78 % of enzyme activity was retained for six continuous cycles (Taher et al., 2014a). In yet another study, SC-CO_2 used as an extraction solvent and reaction medium with immobilized lipase as the catalyst in *Scenedesmus* sp., the maximum biodiesel production yield was found to be 19.3% at a temperature of 35° C and at an methanol: oil molar ratio of 8:1 (Shomal et al., 2019). Non-catalytic supercritical transesterification of microalgae using ethanol system resulted in >95% of conversions in 50 min at 593 K, whereas with the ethyl acetate system, >60% of conversions were obtained in 50 min at 653 K. The pressure was maintained at 20 MPa and the ratio of algae to ethylating agent (wt./vol.) was 1:10 (Mani Rathnam et al., 2020). Both catalytic and non-catalytic methods of transesterification process in microalgae can be enhanced through the addition of (i) co-solvent, (ii) microwave and (iii) ultrasound or through its combinations. In recent years, combinatorial-transesterification methods are being explored in the transesterification reaction system to increase the process efficiency by means of enhancing the FAME yields from microalgae. The various conventional and advanced methods of transesterification are being depicted in Fig. 4.

2.1.5. Cosolvent mediated methods

Addition of cosolvents, such as hexane and chloroform, into the transesterification reaction system enhances the conversion efficiency by means of enhancing the reaction system homogeneity between the substrates and the catalysts (Dianursanti et al., 2015; Liu et al., 2018). A co-solvent separates reaction phase from water, extract lipid and enable the mass transfer of reactant. In such a way that, in mixed solvent dosage (n-hexane to 75% ethanol volume in the ratio of 1:2, being n-hexane as the co-solvent) at the optimum reaction parameters of temperature 90 °C, reaction time of 2.0 h and catalyst volume 0.6 mL, the direct transesterification process of *Chlorella* biomass resulted in a high conversion yield of up to 90.02 ± 0.55 wt.% (Zhang et al., 2015). *In-situ* transesterification of *Spirulina* using the selected co-solvents, toluene, dichloromethane and diethyl ether, as well as the solvent combinations petroleum ether/toluene, toluene/methanol and dichloromethane/methanol were evaluated. Among all, the toluene/methanol co-solvent system, 2:1 by volume ratio, demonstrated the highest overall biodiesel yield of 76% (Xu and Mi, 2011). In another study, ethyl

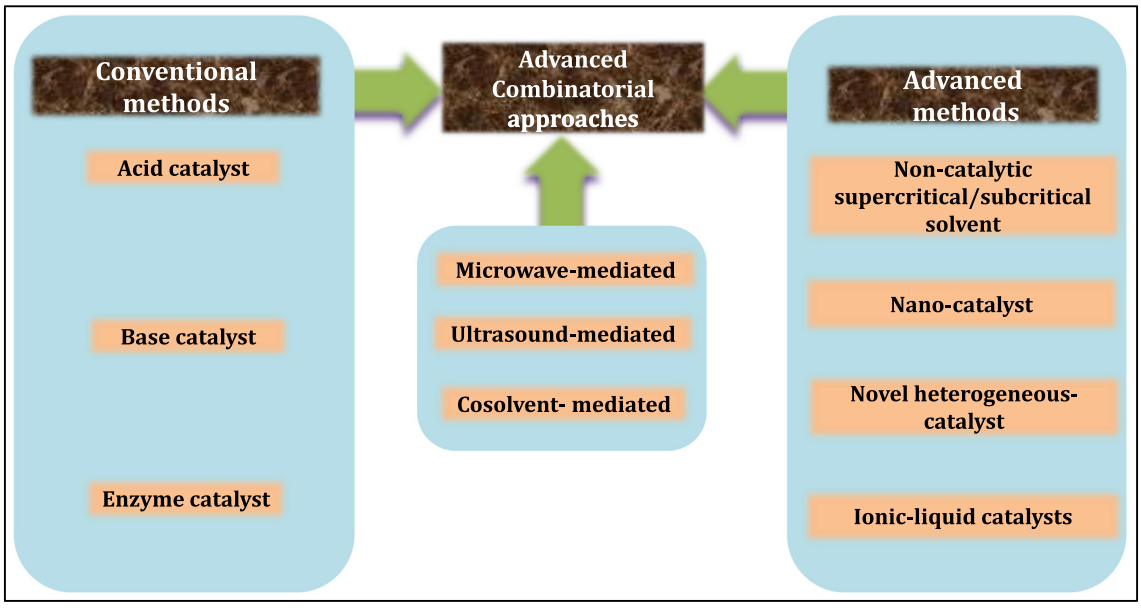

Fig. 4. Conventional and advanced-combinatorial approaches employed for the transesterification of microalgae.

acetate as a single reactant and co-solvent provided enhanced FAEE yield and more saccharification of carbohydrates. In this study, ethyl acetate and acid catalyst were mixed with wet microalgae of *Nannochloropsis gaditana* and heated in one pot for simultaneous lipid extraction and transesterification. This resulted in the optimum biodiesel yield of 97.8 wt% at 114 °C and 4.06 M catalyst with 6.67 ml ethyl acetate/g dried algae. This study enunciated the suitability of ethyl acetate as the effective co-solvent, than ethanol/chloroform system (Park et al., 2017). In a microreactor system for direct transesterification of wet microalgae, the reaction conditions were optimized to be 100 °C, 10 min and 35% PEG 4000 (2:1 volume ratio), i.e. the volume ratio of the PEG-methanol solution to H_2SO_4-methanol. This study emphasized the usage of PEG 4000 as suspension/co-solvent agent, since PEG is non-toxic and is easier to use in sample treatment than the co-solvent, chloroform (Liu et al., 2018). Recently, a highly ecological process of biodiesel production employing green solvents, 2-methyltetrahydrofuran (2-MeTHF) or cyclopentyl methyl ether (CPME) as co-solvents in the acid-catalyzed direct transesterification process was proposed. Direct transesterification using 2-MeTHF or CPME as cosolvents achieved enhanced biodiesel yields than using chloroform (de Jesus et al., 2020).

2.1.6. Microwave and ultrasound-mediated methods

Microwave radiation can be easily absorbed by methanol and the transesterification process can be enhanced by dipolar polarization and ionic conduction under microwave irradiation. This causes instantaneous and localized superheating of the reaction materials, which ultimately shortens the reaction time of transesterification (Nomanbhay and Ong, 2017). Microwave heating aids in algal cell disruption and extraction in the direct transesterification process. A single step conversion, shorter reaction times, reduced chemical and energy consumption are the main advantages of microwave-mediated transesterification methods (Patil et al., 2012). Microwave assistance in both lipid extraction and transesterification processes in *Chlorella* sp. produced maximum yields. The biodiesel yields were 20.1%, 20.1%, and 13.9% for microwave, microwave with hexane and Bligh and Dyer methods respectively; while the FAEE conversion of the algal lipids were 96.2%, 94.3% and 78.1% respectively (Martinez-Guerra et al., 2014). Similarly, biodiesel production yield was higher using microwave heating with one-step, acid-catalyzed direct transesterification in *Chlorella pyrenoidosa*. Biodiesel yields (% of dry biomass) through (i) conventional heating with two-step method; (ii) Microwave heating with two-step method and (iii) Microwave heating with one-step method were 8.34 ± 0.23; 8.92 ± 0.55 and 10.51 ± 1.37 (%) respectively (Cheng et al., 2013).

Ultrasonication is an effective process to enhance mass transfer between immiscible phases (Hidalgo et al., 2013; Naveena et al., 2015). Ultrasonication can enhance emulsion formation with immiscible liquids that are generally used during biodiesel production. It can improve the extraction rate by the formation of microcavities which facilitates enhanced mass transfer leading to the higher yield of the product (Naveena et al., 2015). Use of ultrasound agitation of 24 kHz for the acid catalysed *in-situ* transesterification of *Chlorella* sp., with co-solvent use (n-pentane and diethyl ether) had significantly improved the methyl esters conversion with reduced reacting methanol volumes (Ehimen et al., 2012). Direct transesterification of *Nannochloropsis occulata* using KOH as catalyst and methanol as a solvent resulted in highest yield of biodiesel produced of 30.3% under the optimum conditions of 1:15 algal biomass to methanol (molar ratio); 3% catalyst concentration at temperature 40° C after 30 minute of ultrasonication (Kalsum et al., 2017). Under higher ultrasound power (180 W) a high ester content (96.9%) and a relatively high extraction yield (26%) was obtained through *in-situ* alkaline transesterification of *Spirulina* (Martínez et al., 2017). Microwave-mediated and ultrasonication-mediated transesterification processes were comparatively studied and reported (Guldhe et al., 2014; Koberg et al., 2011). Higher biodiesel conversion of *Scenedesmus* sp. (~71%) was obtained with sonication compared to ~52% with microwave, using tungstated zirconia (WO_3/ZrO_2) as a solid acid catalyst. It was reported that sonication-assisted transesterification was performed at low reaction temperature (50° C) that relay on the cost-effective biodiesel production (Guldhe et al., 2014). However, microwave oven method was appeared to be more simple and efficient method than ultrasonication for the one-stage direct transesterification of *Nannochloropsis* using SrO as a base catalyst (Koberg et al., 2011). In yet another study, heterogeneous KF/CaO catalyst (calcinated at 900 °C with 25 wt.% KF loading prepared by the wet impregnation method) was used to assist biodiesel production from *Chlorella vulgaris* under the combination of ultrasound and microwave (US–MW) irradiation, that resulted in the maximum FAME yield of 93.07 ± 2.39%, significantly higher than that attained by means of US or MW irradiation individually. In this study the optimum performance was obtained with 12 wt.% of catalyst and a methanol to biomass ratio of 8:1 at 60 °C for 45 min of reaction time (Ma et al., 2015a).

2.1.7. Nano-catalyst/novel heterogeneous-catalysts for microalgal transesterification

In addition to the applications of nano-particles for microalgal biomass harvesting, lipid accumulation, nano-particles are highly reliable for esterification and transesterification process for biodiesel production. Nanoparticles has acid/base sites that are responsible for catalytic esterification and transesterification reactions of FFA and oil. Nano-catalyst with its improved catalytic activity also encompasses the advantages of heterogeneous catalyst such as reusability and recoverability. The latest development of various nanoparticles for biofuel conversion were narrated comprehensively in the recent reviews (Hossain et al., 2019; Jain et al., 2019). Enzyme-mediated transesterification process can be improved through immobilization with nano-catalysts. The technology development and the application of nanomaterials, including nanoparticles (magnetic and non-magnetic), carbon nanotubes, and nanofibers to the nano-immobilization of enzymes were comprehensively reviewed previously (Kim et al., 2018). With regard to microalgal transesterification, a one-step extraction and transesterification process of biodiesel production from wet microalgal biomass using alkyl-grafted Fe_3O_4-SiO_2-immobilized lipase had resulted in the biodiesel conversion of over 90% under optimal conditions (Tran et al., 2013). In a recent study of enzymatic transesterification of microalgal lipids to biodiesel, the stability of lipase enzyme was improved by nanozeolites in which nanozeolites functionalized with 3-aminopropyl trimethoxysilane (APTMS) and cross-linked with glutaraldehyde was used as the solid support. Enzyme–nanozeolite complexes had resulted in higher enzymatic activities than free enzyme (non-functionalized) and FAEEs yield was above 93% using these lipase-nanozeolite complex. The catalyst was recovered and reused for next five consecutive cycles of ethanolysis transesterification (de Vasconcellos et al., 2018). To ease the nano-catalyst recovery for reuse, another study employed magnetic K/Fe_2O_3–Al_2O_3 core-shell nano-catalyst in the *in-situ* transesterification of mixed microalgal biomass cultivated in waste water. This study resulted in 95.6% ester conversion at optimum conditions of 65 °C, 12 mL g^{-1} (methanol-to-dry biomass), 4 wt% of magnetic catalyst and 6 h of reaction. The nano-catalyst was recovered and reused for several times which presented high stability with less reduction in its activity up to six runs (Kazemifard et al., 2019).

In addition to chemical substances, nano-particles are also synthesized from bio-wastes and biomass extracts as a greener approach. Recent reports on nano-catalyst mediated microalgal transesterification using iron-nano particles derived from leaf extracts (Anto et al., 2019), shell-derived nano-calcium catalysts (Ahmad et al., 2020; Karpagam et al., 2020; Pandit and Fulekar, 2017) had demonstrated its improved FAME conversion ability. Direct transesterification of nitrogen starved cells of *Coelastrella* sp. M-60 using shell-derived calcium nanocatalysts yielded improved FAME conversion on par with acid catalysis (Karpagam et al., 2020). Similarly, better FAME recovery was obtained using bio-iron nanoparticles in the direct transesterification process of microalgal biomass of *Dictyococcus* sp. VSKA18 and *Coelastrella* sp. M-60. These heterogeneous nanoparticles were synthesized using *Sargassum polycystum* (Sakthi Vignesh et al., 2020).

Different heterogeneous catalysts have been developed for biodiesel production, such as zeolites, metal oxides and ion-exchange resins (Hidalgo et al., 2013). Use of mixed bed ion-exchange resins for *in-situ* processing of *Nannochloropsis oculata* biomass within a co-solvent system resulted in highest ester yield (approaching 60% biomass lipid to ester conversion) with air-dried algae, processed at 50°C at a mixing rate of 550 rpm for 2 h (Jamal et al., 2014). FAMEs were synthesized using solid acid catalysts like ion-exchange resins (Amberlite-15, CT-275, CT-269), KSF clay and silica–alumina. FAME yields of above 90 mol% were obtained using ion-exchange resins. But these catalysts lost the activity and were regenerated by methanol and HCl washing steps (Carrero et al., 2015). Recently, novel heterogeneous catalyst derived from biological source are also employed in the transesterification process of microalgae. In such a way, *in-situ* transesterification of microalgae *Parachlorella kessleri* biomass using sulfonated Rice Husk Solid (RHS) as heterogeneous catalyst at 30 mg RHS had led to highest FAME yield after 30 min reaction time at room temperature and the RHS was very effective at room temperature compared with conventional transesterification (Wadood et al., 2020). Similarly, biochar derived from leaf waste as heterogeneous catalyst, when used along with acid catalyst in reactive transesterification had improved the FAME recovery (Anto et al., 2019). In another study of transesterification, K-pumice as novel heterogeneous catalyst with the assistance of ultrasound was able to generate up to 85% FAME yield using microalgae oil under optimum conditions of methanol to oil ratio of 12:1; 10% catalyst load; reaction time of 10 min and ultrasonic processor percent amplitude of 40% (Cercado et al., 2018).

2.1.8. Ionic liquid catalysts

Ionic liquids are organic molten salts containing anions and cations that are liquid at room temperature (Gebremariam and Marchetti, 2017; Wahidin et al., 2016). Ionic liquids are insoluble in the organic phase and thus this leads to the formation of a biphasic system at the end of the transesterification reaction. This makes easier separation of biodiesel present in the top organic phase with very minute amount of methanol (Gebremariam and Marchetti, 2017). Ionic liquids are 'green' alternatives to traditional solvents with unique properties of non-volatility, non-flammability, recyclability, good dissolving ability, thermal stability and good microwave absorbance (Wahidin et al., 2016). In such a way, direct transesterification of *Nannochloropsis* sp. by microwave technique and ionic liquid as the green solvent was investigated. Three ionic liquids, viz. 1-butyl-3-metyhlimidazolium chloride ([BMIM][Cl], 1-ethyl-3-methylimmidazolium methyl sulphate [EMIM][$MeSO_4$] and 1-butyl-3-methylimidazolium trifluoromethane sulfonate [BMIM][CF_3SO_3] and organic solvents (hexane and methanol) were used as co-solvents under microwave irradiation. [EMIM][$MeSO_4$] was found with maximum cell disruption (99.73%) activity and biodiesel yield (36.79% per dried biomass) after 15 min of simultaneous extraction-transesterification (Wahidin et al., 2016). In another study, enzymatic production of biodiesel from the oil extracted from the microalgae was investigated for two immobilized lipases, *Penicillium expansum* lipase (PEL) and *Candida antarctica* lipase B (Novozym 435), in two solvent systems: an ionic liquid (1-butyl-3- methylimidazolium hexafluorophosphate, [BMIm][PF]) and an organic solvent (tert-butanol). Under optimal conditions, both enzymes (PEL and Novozyme 435) induced significant enhanced yields in the ionic liquids (90.7% and 86.2%) relative to that obtained in tert-butanol (48.6% and 44.4%) respectively (Lai et al., 2012). Interestingly, in another study of *in-situ*, one-pot procedure of immobilized enzyme catalysis (*Candida antarctica* lipase B), use of ionic liquid as cocatalyst to a slurry of whole-cell *Chlorella zofingiensis* in water resulted in 74.8% of lipid extraction, along with 27.7% biotransformation products and up to 16% biodiesel was obtained (Bauer et al., 2017).

There are unique advantages as well as disadvantages associated with various methods of transesterification employed for biodiesel production (Table 1). Therefore, the combinatorial approaches for transesterification have proven its ability for improved biodiesel production from microalgae. In the recent years, reactive (direct) transesterification process of biodiesel production in microalgae are gaining attention for its process simplicity (Ghosh et al., 2017; Kim et al., 2015; Lee et al., 2019; Ma et al., 2019). Direct transesterification process for microalgae improves the process economics and thus lowers the biodiesel production costs (Lee et al., 2019). Some of the recent reports on advanced-combinatorial approaches of reactive transesterification employing whole microalgal biomass for biodiesel production is being tabulated in table 2.

3. Methods for combined biodiesel and bioethanol production

Utilization of microalgal biomass in its totality enhances the process energetic yields, provide economic feasibility and scalability of the co-

Table 1
Advantages and disadvantages of various transesterification methods.

S. No.	Methods of Transesterification	Advantages	Disadvantages	References
1.	Homogenous acid catalysis	-Simultaneous esterification of FFA and transesterification are efficient. -Avoids soap formation. -Less inhibition by biomass water content. -Highly suitable for direct transesterification of microalgal biomass promoting cell wall lysis.	-Corrosion of the reactor. -Reaction rate lesser than base catalysis. - Demands additional separation and product purification.	Ehimen et al. (2010); Faruque et al. (2020); Lotero et al. (2005); Ma et al. (2019); Velasquez-Orta et al. (2012)
2.	Homogenous base catalysis	- Faster reaction rate than homogenous acid catalysis. - Lesser biodiesel conversion reported in case of direct transesterification than acid catalysts.	- Soap formation due to FFA. -Simultaneous esterification and transesterification process are difficult.	Ma et al. (2019); Velasquez-Orta et al. (2012)
3.	Heterogeneous acid/base catalysis	-Easy separation of biodiesel from glycerol. -Catalyst recycling/reuse. -Less energy requirement and minimum water consumption.	-Some catalysts suffer leaching in harsh reaction conditions. -Moderate biodiesel yield, mass transfer resistance, time consumption, fast catalyst deactivation. -Tedious catalyst preparation.	Akubude et al. (2019); Faruque et al. (2020); Guldhe et al. (2015); Thangaraj et al. (2019)
4.	Enzyme catalysis	-High biodiesel conversion. -Low energy requirement. -High product purity. -Reusability. -No wastewater generation, can be used in continuous process.	-Cost -Inhibition by alcohols	Guldhe et al. (2015); Nomanbhay and Ong (2017); Taher et al. (2011)
5.	Super critical/Sub-critical solvent	-Catalyst-free operation. -Faster reaction times. -Higher purity of final product. -Dewatering of biomass is not required.	-Requires high pressure and temperature. -Higher methanol consumption. -Poor process economics. -Safety concerns.	Deshpande et al. (2017); Patil et al. (2012); Takisawa et al. (2014)
6.	Co-solvent mediated	-Use of co-solvent increases the biodiesel yield. -Enhance the contact of lipids with esterification reagent.	-Ineffectiveness of some cosolvents in certain transesterification reaction conditions.	Cao et al. (2013); Liu et al. (2018); Najafabadi et al. (2015); Sawangkeaw et al. (2007)
7.	Ultra sound mediated	-Accelerate cell disruption. -Reduced transesterification reaction time.	-Chance of increased overall processing cost and time. -Low effective heating transfer ability.	Koberg et al. (2011); Ma et al. (2015a); Priyanka et al. (2020)
8.	Microwave mediated	-Accelerate cell disruption. -Reduced transesterification reaction time. -Better heating method than conventional heating. -Biodiesel conversion under ambient pressure using microwave technology.	-May not be efficient with higher solid loadings of the feedstock. -Inability to provide energy to break bonds. and thus, chance of increased overall processing cost and time.	Ma et al. (2015a); Nomanbhay and Ong (2017)
9.	Nano-catalyst	-High surface-area-to-volume ratio. -Strong selectivity, improved longevity. -Easy recovery and re-usability. -High catalytic activity.	-Nano-toxicity to health and environment. -Research in nano-catalyzed transesterification is fragmentary and needs process optimization.	Akubude et al. (2019); Jain et al. (2019)
10.	Ionic liquid catalysts	- 'Green' alternatives to conventional solvents. -Non-volatile, non-flammable, recyclability, dissolving ability. -Efficient microwave absorbance. -Thermally stable solvents	-Production of some ionic liquids are costlier. - Requirement of more alcohol for effective yield.	Akubude et al. (2019); Gebremariam and Marchetti (2017); Krossing et al. (2006); Wahidin et al. (2016)

products (Laurens et al., 2015). The methods employed for combined production of bioethanol and biodiesel from microalgal biomass can be classified into three types, (i) step by step methods (ii) Integrated methods involving lipid and carbohydrate extraction and (iii) Integrated methods involving combined processing. Step-by-step method involves lipid extraction from microalgae. Then bioethanol can be produced from lipid-extracted microalgal biomass and the extracted lipids are transesterified to biodiesel (Chng et al., 2016; Lee et al., 2013). In these methods, employing simultaneous saccharification and fermentation of the lipid-extracted biomass avoids costly pre-treatment procedures for bioethanol production (Chng et al., 2016). In this way, bioethanol was directly produced with 82% yield from the saccharification solution without additional pretreatment by enzymatic saccharification (Lee et al., 2013). Whereas, in the second type of method, simultaneous carbohydrate and lipid extraction from microalgal biomass is carried out from the pretreated microalgae. Through which fermentable sugars are recovered from the supernatant for bioethanol production, whereas lipids are recovered from pellets for biodiesel production (Karemore and Sen, 2016; Laurens et al., 2015). Thus, concomitant extraction of carbohydrates and lipid is possible with this method, which allows for concurrent FAME and bioethanol production without any down time (Karemore and Sen, 2016). For an instance, in a parallel algal processing, the sugar-rich liquor from dilute acid-pretreated microalgal biomass separated by solid–liquid separation (SLS) was used to produce ethanol via fermentation; whereas the lipids recovered from the solid fraction using hexane extraction was employed for renewable diesel or FAME biodiesel production (Dong et al., 2016; Laurens et al., 2017; Laurens et al., 2015). However, approximately 37% of the soluble sugars were lost in the solid cake after the SLS process (Dong et al., 2016; Laurens et al., 2015). This emphasis the need of integrated processing method with minimum sugar loss that can reach close to achievable bioethanol yield. On the other hand, in the other type of integrated method involving combined processing (third type of method), the onset of transesterification or fermentation process could ensue from the biomass. In such a way, a novel Combined Algal Processing (CAP) approach was demonstrated in which whole algal slurry after pretreatment was used for bioethanol production. Ethanol and lipids were successively recovered from the fermentation broth by thermal treatment and solvent extraction respectively (Dong et al., 2016). Moreover, elimination of SLS step had reduced the capital and

Table 2
Recent reports on advanced-combinatorial approaches of reactive transesterification.

Sl. No.	Microalgae	Combined methods	Transesterification experimental conditions	FAME/FAEE yield in terms of oil or biomass		References
				FAME yield (per biomass)	FAME conversion (per lipid/ transesterifiable lipid)	
1.	*Nannochloropsis* sp.	Microwave with sonication with the aid of SrO catalyst	Chloroform: Methanol (2:1) and 0.3g SrO catalyst – sonicated and microwave for 2 min each	37.1%	99.9%	Koberg et al. (2011)
2.	*Nannochloropsis* sp.	Wet algae – Supercritical methanol reactor Dry algae – Microwave methanol (with KOH catalyst)	Supercritical methanol: wet algae to methanol (wt./vol.) ratio of around 1:9, reaction temperature and time of about 255 °C, and 25 min. Microwave methanol: dry algae to methanol ratio of 1:12 (wt./vol.); and 2% KOH catalyst – microwave 800W – 4-5 min; 60-64° C;	NR	84.15% (wet-SCM); 80.13% (dry microwave)	Patil et al. (2012)
3.	*Chlorella* sp.	Microwave with ethanol solvent and base catalyst	Microwave - Algal biomass: ethanol molar ratio of 1:250–500 and 2.0–2.5% catalyst with reaction time 6 min (alternate to Bligh and Dyer method)	20.1%	94.3%	Martinez-Guerra et al. (2014)
4.	*Chlorella vulgaris*	Microwave and ultrasound with heterogeneous catalyst and methanol	12% (wt%) KF/CaO catalyst calcinated at 900° C; methanol to biomass ratio of 8:1 at 60° C for 45 min	NR	93.07%	Ma et al. (2015a)
5.	*Synechocystis* sp.	Heterogeneous nanocatalyst couple whole cell transesterification	3% (wt%) TiO_2 nanocatalyst; 2.5 mL methanol and 50 mL conc. hydrochloric acid and the reaction was carried out at 100°C for 1.5 h	36.5%	NR	Jawaharraj et al. (2017)
6.	*Chlorella* sp. and *Nannochloris* sp.	One step direct transesterification using *in-situ* supercritical conversion	Methanol to dry microalgae (vol./wt.) ratio of 10:1, at 265° C and for 50 min	NR	45.62% (*Chlorella* sp.) (21.79 % *Nannochloris* sp.)	Jazzar et al. (2015)
7.	*Nannochloropsis* sp.	Direct transesterification by microwave technique and ionic liquid as the green solvent	Ionic liquid solvent - 1-ethyl-3-methylimmidazolium methyl sulphate [EMIM][$MeSO_4$; 700W microwave; 65° C for 15 min	36.79%	NR	Wahidin et al. (2016)
8.	*Nannochloropsis gaditana*	Catalyst-free wet *in-situ* transesterification and hydrothermal liquefaction	Transesterification temperature at 185.08 °C with 4.69 mL ethanol and 1.98 mL of dichloroethane/g of dry algal cells	11.65%	91.85% (biocrude)	Kim et al. (2017)
9.	*Chlamydomonas sp.*	Microwave disruption, Sr_2SiO_4 catalyst and direct transesterification	2 g cake - 12 mL hexane -4 mL methanol solution with 0.25% (w/v) NaOH at 5°C and 600 rpm for 15 min	NR	97.2% (nearly 100% was achieved by excess NaOH loading)	Chen et al. (2015)
10.	*Nannochloropsis sp.*	Pretreatment, fermentation and ethanol-assisted liquefaction process	3% H_2SO_4 pretreated biomass – fermentation with *Saccharomyces cerevisiae* – 10% ethanol – liquefaction assisted with 15% (v/v) ethanol (2:1 ethanol to algae ratio) at 265 °C	14.16%	14.18% (Wet) 12.48% (Dry)	Rahman et al. (2019)
11.	*Nannochloropsis* sp.	Ionic liquid (IL) -microwave heating *in-situ* transesterification	1-Ethyl-3-methylimmidazolium methyl sulphate [EMIM][$MeSO_4$]; ratio of wet algae to methanol (wt/vol) was at 1:4, methanol: IL ratio maintained at 1:0.5 at reaction time of 25 min at 65 °C	42.22%	NR	Wahidin et al. (2018)
12.	*Nannochloropsis gaditana*	Acid catalyzed direct transesterification using ethyl acetate as reactant and co-solvent	1g dry biomass - 114 °C and 4.06 M catalyst with 6.67 ml Ethyl acetate	120.48 mg FAEE/g dry biomass	97.8%	Park et al. (2017)
13.	*Chlorella protothecoides*	Non-catalytic transesterification using supercritical methanol and ethanol	320° C temperature, 152 bar pressure, 19:1 alcohol to oil molar ratio, 31 min residence time, 7.5 wt% water content	NR	90.8% (SC methanol) 87.8% (SC ethanol)	Nan et al. (2015)
14.	*Nannochloropsis sp.*	Simultaneous cooling and microwave heating transesterification	Wet biomass - cold chiller at 15 °C - 800 W, 50 °C and 10 min.	NR	75%	Chee Loong and Idris (2017)
15.	*Chlorella vulgaris*	Radio frequency (RF) heating for both cell disruption and *in-situ* transesterification	RF heating (27.12 MHz and 6 kW power) for 90 °C for 30 min for cell disruption. Followed by first stage conversion [acid catalysis - 36% HCl to MeOH, v/v (5:95) - stirring and RF heating at 55 °C for 20 min]. Second stage transesterification of upper liquid phase from first conversion [alkaline catalysis – 10 mL of NaOH-MeOH solution (NaOH to MeOH, w/v, 0.5:100); RF heating at 55 °C for 20 min]	NR	79.5 ± 3.0%	Ma et al. (2020), Ma et al. (2019)

operating costs that resulted in simple and robust process. It was also proposed that a number of high-value co-products, such as PUFA and protein residue, may also be produced via the CAP processing concept due to its non-destructive nature of biomass fractionation (Dong et al., 2016). In yet another study, optimum biofuel (yielding 92% of methyl ester and 93% of ethanol) production was achieved when direct transesterification of microalgae (by enzyme catalysis) was performed first, followed by ethanol fermentation of remaining microalgal biomass residues (Sivaramakrishnan and Incharoensakdi, 2018). Similarly, recent reports demonstrated bioethanol production from the pretreated-residual biomass remnant after FAME extraction. In these reports, FAME production was obtained by acid-catalyzed *in-situ* transesterification process of microalgal biomass (Kim et al., 2020; Ma et al., 2020; Mandik et al., 2020). The solvents used during *in-situ* transesterification process for biodiesel production did not display significant inhibitory effects on saccharification as the solvents were volatilized during the air-drying process (Ma et al., 2020). The final reducing sugars yield from microalgal residue (after biodiesel extraction) was 54.52 ± 1.19%, which was very nearer to that of disrupted microalgal biomass (59.66 ± 3.00%) and thus represented very minimal sugar loss. This study shows the feasibility of microalgal residue (after transesterification process) for fermentable sugar production through simple enzymatic hydrolysis (Ma et al., 2020) (reaction conditions are given in table 3). Recently,

a biorefinery process for the recovery of valuable products including pigments, biodiesel, sugars and protein from oleaginous microalgal biomass was performed. It was demonstrated that prior to direct transesterification of microalgae, the pigments could be recovered. Then the lipid-free biomass residues were then converted into fermentable sugars through acid hydrolysis for bioethanol application, which was lastly left over with solid residues rich in protein (Mandik et al., 2020). Maximum FAME yield of 256 g/kg-biomass and 44.8 g sugars per kg-lipid free microalgal biomass residues were obtained (reaction conditions are given in table 3). The protein residue was also recovered after hydrolysis (Mandik et al., 2020), amenable for biogas production. Therefore, algal biorefinery process for zero-waste utilization of microalgae is also open through direct transesterification route. The reaction and yield parameters of the three types of combined biodiesel and bioethanol production methods are given in the table 3.

4. Spent microalgal biomass usage for zero-waste algal biorefinery

Conversion process of spent algal biomass residue into biogas can produce recoverable bioenergy. Biogas primarily constitutes methane and CO_2 produced by the anaerobic digestion process of biomass (Ehimen et al., 2009; Ward et al., 2014). To reclaim the remnant carbon after bioethanol and renewable biodiesel extraction from microalgae, the spent residues were utilized for biogas production by anaerobic digestion. This biogas was used for combined heat and power (CHP) production for electricity generation (Davis et al., 2014). Pretreatment by physio-chemical agents, co-digestion or governing the gross composition are the approaches that can efficiently enhance the conversion yield of the algal organic matter into methane (Sialve et al., 2009). On biomethane basis, a recoverable energy of 8.7–10.5 MJ kg^{-1} of dry microalgae biomass residue was obtained by the anaerobic conversion of lipid extracted and transesterified microalgae samples. Further co-

Table 3
Reports on combined biodiesel and bioethanol production from microalgae:

Sl. No.	Microalgae	Method	Process flow and conditions	Yield of biofuel/biofuel precursors	References
1.	*Scenedesmus dimorphus*	Step by step method	Lipid extraction and subsequent fermentation of the lipid-extracted biomass; Optimum key parameters for the fermentation were identified at an amyloglucosidase enzyme concentration of 60 units/ml, pH 5, temperature at 36° C and yeast loading of 3 g/L	a maximum bioethanol yield of 0.26 g bioethanol/g lipid-extracted biomass; Lipid yield was 14 ± 0.6 % (w/w). FAME production through extractive transesterification.	(Chng et al., 2016)
2.	*Dunaliella tertiolecta*	Step by step method	Lipid extraction and subsequent fermentation of the lipid-extracted biomass; Fermentation of the enzymatically-saccharified biomass residue after lipid extraction.	0.14 g ethanol/g residual biomass.	(Lee et al., 2013)
3.	*Chlorococcum*	Integrated method involving lipid and carbohydrate extraction.	Bead-beating followed by acid pretreatment. The recoveries of fermentable sugars from the supernatant and that of lipid from pellets.	86.5 ± 2.6% fermentable sugars for bioethanol production and 74.1 ±1.8% of lipid for FAME production.	(Karemore and Sen, 2016)
4.	*Scenedesmus* (LRB-AP 0401) and *Chlorella* (LRB-AZ 1201).	Integrated method involving lipid and carbohydrate extraction.	Acid-catalyzed pretreatment- fermentable carbohydrates from the aqueous fraction and hexane extractable lipid fraction in the residual biomass.	>90% glucose in the hydrolysate liquors and recovery of up to 97% of the fatty acids from wet biomass. Maximum theoretical combined biofuel potential at 143 gasoline gallon equivalent (GGE) for *Scenedesmus* biomass, and for *Chlorella* at 128 GGE per ton.	(Laurens et al., 2015)
5.	*Scenedesmus acutus*	Integrated method involving lipid and carbohydrate extraction.	Parallel algal processing (PAP) - fermentable carbohydrates from the aqueous fraction and hexane extractable lipid fraction in the residual biomass; Lastly, spent residual biomass utilization (anaerobic digestion) to biogas.	84.9% of FAME recovery. Total gasoline equivalent fuel yield (Renewable diesel and bioethanol) 114.4 GGE/ton.	(Dong et al., 2016)
6.	*Tribonema* sp.	Integrated method involving lipid extraction.	Acid hydrolysis with 3% H_2SO_4 was performed to saccharify cell wall into fermentable sugar and release lipid; Lipid was separated using ethanol:hexane solvent mixture (1:3, v/v). Bioethanol was produced from the hydrolysate (by fermentation using *Saccharomyces cerevisiae*).	biodiesel conversion rate of 98.47% (obtained from the extracted lipid samples); maximum bioethanol yield of 56.1% (obtained from 14.5 g/L glucose in hydrolysate).	(Wang et al., 2014)
7.	*Scenedesmus acutus*	Integrated method involving combined processing	Combined algal processing (CAP) - algal slurry after acid pretreatment was a sufficient medium for cultivating yeast to produce ethanol. Ethanol and microalgal lipids were sequentially recovered from the fermentation broth by thermal treatment and solvent extraction respectively.	87% of FAME recovery. Total gasoline equivalent fuel yield (Renewable diesel and bioethanol): 126.3 GGE/ton.	(Dong et al., 2016)
8.	*Chlamydomonas* sp. KNM0029C.	Integrated method involving combined processing	FAME production by direct transesterification route from the biomass [using methanol/chloroform (2:1) and HCl as catalyst]; Treatment of residual biomass with amyloglucosidase enzyme (after sonication) for sugar production. These treated residues were used for bioethanol production by fermentation.	a maximum yield of 0.16-g FAME/g. Residual biomass was pre-treated for bioethanol production, and the yields from different methods were compared. The highest bioethanol yield (0.22-g/g residual biomass) was obtained Approximately 300-mg biofuel was obtained, including 156-mg FAME biodiesel and 144-mg bioethanol per g dry cell weight.	(Kim et al., 2020)
9.	*Chlorella vulgaris*	Integrated method involving combined processing	*In-situ* transesterification by acid catalysis at first stage from pretreated biomass (via RF heating) for FAME extraction. Residual solid phase separation and enzymatic saccharification for bioethanol application. Second stage of transesterification of organic phase to biodiesel (alkaline catalysis)	79.5 ± 3.0% FAME extraction efficiency. Fermentable reducing sugar yield of 54.5 % was obtained after 72 h saccharification from microalgal residues after first stage of transesterification.	(Ma et al., 2020, Ma et al., 2019)
10.	*Chlorella* sp.	Integrated method involving combined processing.	One-step, acid-catalyzed direct transesterification process at the optimized conditions of 1.35:1 volumetric ratio of chloroform: methanol, 70 °C reaction temperature, and 120 min reaction time; Subsequently the lipid-free microalgal biomass residues (LMBRs) were acid hydrolyzed into sugars and the protein residues were also recovered after hydrolysis.	maximum FAME yield of 256 g/kg-biomass and maximum sugar yield of 44.8 g/kg-LMBRs.	(Mandik et al., 2020)

digestion of the microalgae residues with glycerol had recorded a 4-7% increase in CH_4 production (Ehimen et al., 2009). Similarly, co-digestion of microalgal biomass with glycerol improved the production of methane (Sittijunda et al., 2018). Co-digestion of algal sludge with waste paper (50%) was employed for biomethane production and it was observed that lesser carbon contents and higher nitrogen inhibited the methane production (Yen and Brune, 2007). It is because higher nitrogen content leads to ammonium accumulation that could ultimately affect methane production (Yang et al., 2011; Yenigün and Demirel, 2013). The inhibition process during methanogenesis is highly associated to the characteristics of the substrate to be anaerobically digested, pH, process temperature (mesophilic or thermophilic), seed sludge type (inoculum), reactor configuration and to the concentrations of ammonium and ammonia. Evaluation of ammonia toxicity to the growth of methanogens is generally performed in terms of ammonia/potassium exchange reaction and inhibition of methanogenesis. The toxicity recovery strategies such as dilution of the substrate, dilution of the reactor contents, adjustment of process pH, alteration of C:N ratio of the substrate, addition of compounds like zeolite, glauconite, and activated carbon which could increase the biomethane production (Yenigün and Demirel, 2013). In such a way that, it was reported that the incorporation of protein-rich microalgal biomass as the substrate for anaerobic digestion process had led to the formation of ammonia due to its high nitrogen content that could inhibit biomethane production. In order to alleviate the inhibition, use of ammonia tolerant anaerobic inoculum, low N containing media and urban waste water were reported for improved COD solubilization and biomethane yield. To increase the biomethane production, pretreatment methods such as thermal, ultrasound, acid-alkali methods are generally used, which demands higher energy input and thus the use of low energy demanding pretreatments such as use of suitable enzymes or microorganisms to hydrolyze microalgae biomass is importantly considered (Magdalena et al., 2018). During prolonged acidogenesis, enzyme pretreated deoiled microalgal biomass can produce methane (He et al., 2016a). The pretreatment process enhanced the solubilization efficiency and methane production from microalgal biomass, in such a way, pretreatment of *C. vulgaris* with protease (Mahdy et al., 2015); combination of milling and enzymatic pretreatment for *Acutodesmus obliquus* biomass (Gruber-Brunhumer et al., 2015); application of thermal (< 100 °C, atmospheric temperature), steam explosion (> 100 °C) and hydrothermal pretreatment were employed for biomethane production (Passos et al., 2015). It is noteworthy to mention that the main advantage of using microalgal biomass for biogas production is that the CO_2 component of biogas could be utilized as a nutrient source for microalgal growth (Ward et al., 2014). The recent comprehensive review examined the biochemical and structural properties of algae produced as a part of wastewater treatment, and discussed the recent initiatives for producing enhanced biogas through anaerobic digestion (Zamorano-López et al., 2020). On the other hand, biogas production through anaerobic digestion with the microalgae biomass remnants (exhibiting low C: N ratio) need the integration of other energy-rich waste materials such as forestry residues, agricultural and industrial wastes for improving CH_4 yield (Ehimen et al., 2009). Production of methane from microalgae can be improved by anaerobic co-digestion with carbon-rich substrates which could alleviate inhibition accompanying with its low C:N ratio. Primary sludge addition had improved the microbial diversity of the system during the co-digestion for both *Chlorella* and *Scenedesmus* and promoted different microbial structures (Zamorano-López et al., 2020). Dewaterability of the digested sludge was amended due to the microalgal biomass addition in the anaerobic digestion process (Olsson et al., 2018).

On the other hand, biochar is the carbonaceous material produced by treating the algal biomass at moderate temperatures and algal biochar are reported to be superior to other feedstocks (Kumar et al., 2020b, 2020c; Kumar et al., 2020a). The lipid-recovered biomass remnants of *Chlorella vulgaris* was used as the feedstock for fast pyrolysis experiments using a fluidized bed reactor at 500 °C, had yielded bio-oil, biochar, and gas at 53, 31, and 10 wt.%, respectively. Biochar from biomass remnants had high inorganic content (potassium, phosphorous, and nitrogen) that suggested its suitability for supplying nutrients for crop production (Wang et al., 2013). The algal biomass residue after *in-situ* transesterification could be used for biogas (methane) production and bio-digestate can be used as nutrients. However, another study embraces the difficulty in using the microalgal sludge digestate as fertilizer, since the substrate biomass source used for anaerobic digestion for biogas production was comprised of the waste remediated microalgae. It was because flue gas was used as a CO_2 source during the microalgae cultivation and thus the high heavy metal content was observed in the microalgal nutrient digestate. Thus, cautious consideration is needed for CO_2 mitigation via algal cultivation especially in terms of the source of the CO_2-rich gas (Olsson et al., 2018). On the other hand, spent microalgal biomass can be employed for bio-adsorption of dyes and heavy metals from industrial waste waters (Rashid et al., 2013).

To arrive at a zero-waste concept for microalgal biorefinery, the efficient utilization of glycerol by-products from biodiesel production has to be ensured. Heterogeneous catalyst mediated bioconversion methods have been reported to be an eco-friendly pathway for glycerol conversion to commercial chemicals (Okoye et al., 2017). Besides, glycerol acetylation pathways have attracted significant researchers since glycerol derivates (acetates and acetins) have huge commercial applications (Banu et al., 2020; Okoye et al., 2017). In addition to these biochemical glycerol conversion pathways, the crude glycerol from biodiesel production has been widely reported as an alternate organic carbon nutrient for microalgae. Recently, waste glycerol was shown to enhance the dry cell weight, FAME recoveries, biomass and lipid productivities of *Scenedesmus obliquus* (Abomohra et al., 2018; Xu et al., 2019). Also, one-step co-pyrolysis of biodiesel derived glycerol and *Chlorella vulgaris* biomass yielded enhanced combustible oxygenated organics including esters and alcohols in the bio-oil (Wang et al., 2019). A pilot-scale photobioreactor study using *Chlorella vulgaris* in the presence of 1 g L^{-1} of crude glycerol showed maximal biomass/lipid production and nutrient recovery (Ren et al., 2017). Thus, integrated approaches employing crude glycerol in various biochemical conversion processes are feasible to attain a comprehensive and successful microalgal biorefinery process. It is noteworthy to mention that, coproduction of biomaterials and biochemicals will deliver a new dimension to the biorefinery business model for revenue generation. Bio-materials are used for their chemical or physical properties (Kircher, 2015). In such a way, a novel and circular biorefinery process was initiated by waste water bioremediation by *Spirulina* biomass growth, followed by bioethanol production, pellet production, and mineralization of persistent organic pollutants (POPs) from wastewater. The results indicated efficient heavy metals removal (>99%) during microalgal growth. Simultaneous saccharification and fermentation of rich-heavy metal lead to bioethanol (0.4 L per kg of dried microalgal biomass). After bioethanol production, the residual biomass was dried and compressed into pellets which was used as a fuel in biomass boilers. The iron-rich ashes formed via combustion are then used as heterogeneous re-usable Fenton-like catalysts for the photo-Fenton degradation (mineralization >99%) of POPs. Whereas the low-activity ashes are added into an ash-based medium for the efficient cultivation of microalgae (Serrà et al., 2020). Interestingly, in yet another study of integrated algal biorefinary approach, *in-situ* dimethyl ether (DME) production along with biodiesel synthesis via *in-situ* transesterification was emphasised. This would lead to the reduction in the volume of unreacted methanol thereby reducing the operating cost (Salam et al., 2016). In another study, hydrogen, ethanol and volatile fatty acids were produced from the dilute acid pre-treated biomass of macro- and microalgae rich in fermentable carbohydrate monomers. An optimal specific hydrogen yield of 85.0 mL/g VS (volatile solids) was obtained at an algal C/N ratio of 26.2. The energy conversion efficiency was improved from 31.3% to 54.5% with decreasing algal concentration from 40 to 5 VS g/L (Xia et al., 2016).

5. Techno-economic analysis (TEA) and Life Cycle Assessment (LCA)

TEA is performed to govern the potential of economic feasibility of process technologies at the stage of research and development. This determines the real-time potential of the process (Thomassen et al., 2018; Van Dael et al., 2014). TEA of an algal biofuel production can evaluate the production costs starting from the biomass production to the specific downstream process flow on the basis of existing procedure and established method advancements. The operation cost of algae production is affecting the total biofuel production cost significantly and the main influencing process are algae growth, harvesting, and dewatering (Özçimen et al., 2018). For an instance, in a TEA study of CO_2 capture from a thermoelectric plant, upstream processing steps such as biomass cultivation, harvesting and drying were performed by evaluating 24 scenarios using process simulation tools. The scenarios were created from the combinations of raceway pond cultivations, primary harvest with three types of flocculants, secondary harvest by centrifugation, three filtering technologies, and the drying was evaluated with Spray and Drum Dryers. It was found that the operating costs range from US $ 4.75–6.55/kg of dry biomass (Valdovinos-García et al., 2020). Algal biomass (and hence biofuel) economics are highly dependent on achievable cultivation productivity, for an instance biomass selling price could decrease from $1,000/ton to $430/ton by improving productivity from 10 g/m^2/day up to 35 g/m^2 /day while maintaining the cost of cultivation pond at $40–$45k/acre (Davis et al., 2016). In another study, the cost per kg of fuel or fuel precursor for different cultivation process were assessed in which the open pond produced TAG at $7.50/kg; LED-lit photobioreactor process produced TAG at $33/kg. The open pond scenarios were nearer to the $1/kg price point which was found to be the most feasible economic options (Amer et al., 2011). With regard to biodiesel production process, a case study on TEA of direct transesterification (DT) process of *Botryococcus braunii* showed decreased biodiesel production cost than conventional extractive transesterification process. This analysis had showcased that, out of 500,000 kg biomass/year, 4,50,000 kg remnants/year (remnants comprise of lipid-extracted biomass along with unpurified glycerol waste) could be produced after DT process (Lee et al., 2019). Lipid extracted biomass could be readily employed for co-product production. Particularly, sales of co-product such as lipid-extracted microalgae, naphtha would pave the ways for decreased minimum fuel selling price- MFSP (Batan et al., 2016). Algal biorefinery greatly reduces the production cost on obtaining multiple products with maximum efficiency. Techno-economic analysis indicates that the combined fuel (renewable diesel and bioethanol) production improved the yield potential (reduction in MFSP) by 18% when compared to a lipids-only process (Laurens et al., 2015). The sustainability and value stream of algal biofuel production could be improved through new market inclusions such as waste bioremediation by carbon sequestration, soil amendments, absorbents and fertilizers (Allen et al., 2018; Chen, 2017). It is noteworthy to mention that algal bioenergy-based biorefinery can also have focus towards biobutanol (potent biofuel) production from microalgae biomass. The microalgal biomass is introduced as nutrient source to *Clostridium acetobutylicum* bacterium for anaerobic digestion and biobutanol is produced by the acetone-butanol-ethanol (ABE) fermentation process. TEA analysis indicated increased ABE yields with the addition of carbon source and enzymes, showing 160% (7.27 g L^{-1}) and 250% (9.74 g L^{-1}) increase, respectively. Biobutanol cum lipid and methane gas manufacturing from microalgae improves the process economics (Yeong et al., 2018).

Life cycle Assessment (LCA) is a tool employed to evaluate the environmental impacts and sustainability of the production process (Nezammahalleh et al., 2018). LCA could estimate the greenhouse gas (GHG) emission balances (Maranduba et al., 2015). With regard to algal biodiesel production, lipid extraction process contributes to GHG emissions and fossil energy consumption significantly. Enhancing the solvent recovery during the process can play a foremost role in reducing total GHG emission (Dutta et al., 2016). To make zero GHG emissions, algal biorefinery model was proposed to sequester CO_2 from upstream coal-fired power plants for algal biomass cultivation and convert it into value added products. With this model, the optimal unit CO_2 sequestration was obtained with reduced utilization cost from $33.65/ton of CO_2 to $9.52/ton of CO_2 considering the power plant size of 300–2400 MW (Gong and You, 2014). Integrating waste remediation with algae has demonstrated improved process economics. A case study on TEA and LCA from 'pond to pump' with *Botryococcous braunii* had shown that the use of wastewater nutrients had improved (reduction) the minimum lipid selling price (MLSP) and emissions to respectively about $1.8 L^{-1} and 4.2 kg CO_2 kg^{-1} respectively (Nezammahalleh et al., 2018). Waste utilization, CO_2 mitigation and biochar production along with fuel production decrease the overall production costs (De Bhowmick et al., 2019). Refinements of algal biorefinery models based on resource availability (e.g., inclusion of nutrients from the sources of wastewater and incorporation of CO_2 sources from anaerobic digesters) and economic feasibility (e.g., land costs, opportunity costs with other economic activities and multiple microalgal production technologies) are required to decrease the uncertainty of microalgal production (Allen et al., 2018). In yet another study, TEA of integrated algae-based biorefinery with palm oil mill were performed. The economic assessments of the processing route alternatives viz. (i) Combustion of residual algae, (ii) Production of biogas from the palm oil mill effluents (iii) Production of biochar and bio-oil (iv) Production of biogas and bio-oil (v) Production of green diesel were compared with the baseline scenario, which was the processing of algae into biodiesel and glycerol without the palm oil mill integration. The results showed that all alternatives were not economically feasible with palm oil mill integrated algae-based biorefinery for biogas production was the alternative with least loss (Abdul Hamid and Lim, 2019). Therefore, detailed TEA analysis is required at variable scale corresponding to the bioprocessing method. Table 4 represents the cost assessment for some of the case studies of microalgal biofuel production employing definite process route starting from raw material production to final product. These cost-assessment reports clearly indicate that reduction in MFSP (there by improved process economics) is possible with combined biofuel production.

6. Challenges, technological breakthroughs and future perspectives of algal biorefinery

The versatility of the microalgal biorefinery includes the three crucial algal bioproducts which makes this bioprocess more robust. This includes a. microalgal proteins which are alternative food and health product as they are essential nutrients; b. microalgal carbohydrates such as glucose, cellulose, exopolysaccharides and starch which are amenable feedstocks for value added chemicals; c. microalgal lipids such as glycolipids, triglycerides, and free fatty acids which are suitable feedstock for high quality biodiesel production (Fan et al., 2020). However, the microalgal unit operations offer several bottlenecks to translate the pilot scale algal cultivation to commercial level. This includes algal biomass cultivation/harvesting, dewatering, biofuel conversion and nutrient cycling (Pal et al., 2019). Microalgal harvesting is an energy intensive process and attributes to increased production cost due to their smaller cell size and lower concentration in the cultivation medium (Prajapati et al., 2013). To overcome this, cheaper solid substrate like pine bark are used to develop microalgal biofilm that yielded dual benefit of efficient harvesting as well as significant enhancement in wastewater quality (Garbowski et al., 2020). Research direction towards utilizing such readily available raw materials for microalgal biorefinery approaches are warranted. Besides, the lipid extracted microalgal biomass (grown in wastewater medium) were reported to contain significant levels of proteins, carbohydrates, nitrates, glucose and xylose that favours zero-waste microalgae biorefinery approach (Mishra and Mohanty, 2019). At the same time, biorefinery concept producing both biofuels and antioxidants seems to be a difficult concept due to the disparate market dimensions and scope (Thomassen et al., 2018). Co-accumulation of marketable products, an intensified

Table 4
Cost assessment for microalgal biofuel production.

S. No.	Microalgae	Bioprocessing method	Cost assessment	References
1.	*Scenedemus acutus*	Parallel Algal processing	Total biofuel yield (biodiesel and bioethanol) of 114 gasoline gallon equivalent (GGE) per dry ton biomass (on an assumed algal feedstock cost of $1092/dry ton) at MFSP of $10.86/GGE (2011-dollars).	(Dong et al., 2016)
2.	*Scenedemus acutus*	Combined Algal processing. (dilute acid biomass pretreatment, fermentation, lipid extraction, hydrotreating to RDB – Renewable diesel blendstock and finally anaerobic digestion of spent biomass for biogas production).	Total biofuel yield (biodiesel and bioethanol) of 126 GGE/ dry ton biomass at MFSP of $9.91/GGE (2011-dollars) (on an assumed algal feedstock cost of $1092/dry ton).	Dong et al. (2016)
3.	*Scenedesmus* sp.	Combined processing- (dilute acid biomass pretreatment, fermentation, lipid extraction, hydrotreating to RDB and finally anaerobic digestion of spent biomass for biogas production).	Total biofuel yield (biodiesel and bioethanol) of 141.1 GGE/ dry ton biomass at MFSP of $4.35/ GGE (2011-dollars) (on an assumed algal feedstock cost of $430/dry ton).	Davis et al. (2014)
4.	*Scenedesmus almeriensis*	Lipid extraction and purification. Then conversion to biodiesel and crude glycerol via transesterification. Products purification by multiple hot water washes and crude glycerol recovery from water washes by distillation.	MFSP of $10.55/ GGE (2011-dollars) (on algal feedstock cost of $1279/dry ton).	Acién et al. (2012); Dutta et al. (2016)
5.	*Botryococcus braunii*	Direct transesterification (DT) [Mass ratio of biomass (g), methanol (mL) and hexane (mL) were 1:10:10] by homogenous alkaline (NaOH) catalysis.	Biodiesel production costs were 12.5 $ kg^{-1} (DT process) and 18.2 $ kg^{-1} (conventional extractive process) [on assuming 90% recycling of solvents in both the process]. MFSP data – NR.	Lee et al. (2019)

culture system, and recovery of multiple products by a cascade extraction approach are the strategies to be considered for effective algal biorefinery (Gifuni et al., 2019). Thus a comprehensive phycoremediation and microalgal biorefinery processes using large-scale open raceway pond or photobioreactors are essential for cutting down the production cost to make it economically feasible (Sharma et al., 2018; Weise et al., 2020). Genome editing approaches in microalgae for bioproduct production are the emerging technological breakthrough that tunes the commercial level production of renewable biofuels. In spite of lower technology readiness level (TRL) that makes microalgae a less suitable feedstock for fuel, the development of alternate/novel pathways using genome editing are crucial for future research, development and politics (Varela Villarreal et al., 2020). The major advantage of this biotechnological inventions include strain manipulations for increased biomass/lipid accumulation, blocking competent pathways, gene pyramiding, controlling several upstream regulators, microRNAs and transcription factors (Sharma et al., 2018). Even though, there are several sequenced oleaginous microalgal genome available in database, the explicit and manipulable genome editing methods were reported for few microalgae including *Nannochloropsis*, *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum* (Wang et al., 2016). It is estimated that the genome editing by molecular biology tools could subsidize 15-20% reduction in the overall production cost (Chung et al., 2017). Thus, this breakthrough technology could promote microalgal biorefinery from the current technology-driven status to market-based commercialization. With the advent of expeditious stride in the next generation sequencing, genome editing tools and transcriptome mapping of oleaginous model microalgae, genome biology have assured a means to tune the metabolic pathways involved in efficient biomass production, photosynthetic conversion rates and adaptation of stress environments (Sharma et al., 2018). And thus, future research directions are warranted for genetically modified microalgae to mitigate greenhouse gases and bioproduct accumulation.

7. Conclusion

From this overview of cascade approach of bioenergy production from microalgae, the following conclusions may be drawn:

- In spite of the recent developments in the field of bio-energy based biorefineries, the fundamental process flow and reaction parameters for cascade biofuel production for improved as well as combined biofuel yield are still to be analysed for effective biomass utilization. In order to enhance the potential and sustainability of the process, fundamental and applied research is indispensable at various steps.
- Integrated combined biomass processing of pre-treated microalgae, starting with FAME production by direct transesterification route, and then using the remnant biomass residue for bioethanol production is one of the feasible approaches. Because it is important that plentiful lipid-free microalgal biomass residues would be generated with industrial scale production of microalgae-based biodiesel.
- The protein rich spent biomass remnants of microalgae after biodiesel and bioethanol production could be effectively used for biogas production through appropriate pre-treatment strategies.
- Studies on techno-economics and life cycle assessment clearly indicated the necessity of integrating waste remediation with bioenergy based algal biorefinery models for profitable economics and sustainable bioenergy.
- Though genetically modified microalgae and open pond cultivation can serve the higher biofuel yields, the environmental risks and health are the primary concerns when exposing engineered microalgae to the natural ecosystems. Standard operating procedures, standard biosafety regulations, potential risk assessment and rigid monitoring are indispensable to overcome this problem.

Declaration of competing interest

Authors declare no conflict of interest.

Acknowledgement

Authors thankfully acknowledge Department of Science and Technology, New Delhi, India for the financial support to R. Karpagam [DST/INSPIRE/04/2018/001484] (File No. DST/INSPIRE Faculty Award/ Batch-14/2018 dated 24.1.2019). We also acknowledge Centre for Plant Molecular Biology and Biotechnology (CPMB & B), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for hosting and facilitating the project.

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