Renewable and Sustainable Energy Reviews 115 (2019) 109361

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

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

Insights into the microalgae cultivation technology and harvesting process for biofuel production: A review

Uganeeswary Suparmaniam[a,b], Man Kee Lam[a,b,*], Yoshimitsu Uemura[a,b], Jun Wei Lim[b,c], Keat Teong Lee[d], Siew Hoong Shuit[e]

[a] Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia
[b] Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia
[c] Fundamental and Applied Sciences Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia
[d] School of Chemical Engineering, Universiti Sains Malaysia, Engineering Campus, Seri Ampangan, Nibong Tebal, 14300, Pulau Pinang, Malaysia
[e] Department of Chemical Engineering, Lee Kong Chian Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, 43000, Selangor, Malaysia

ARTICLE INFO

Keywords:
Microalgae
Cultivation
Harvesting
Photobioreactor
Bioflocculant

ABSTRACT

Derivation of biofuel from microalgae biomass has been widely researched in the past few decades. Microalgae is capable of producing 58,700 litres oil per hectare that can generate 121,104 litres biodiesel per hectare, which seemingly a promising transition over conventional fossil fuels. Nevertheless, economic sustainability of commercial scale production of microalgae biomass is still in shadows of doubt, especially the cultivation and harvesting process. Apparently, the microalgae cultivation system has evolved from traditional open pond to various modern photobioreactor (PBR) designs. However, with regards to tubular and flat panel PBRs as the most ubiquitous systems for biofuel production at commercial level, extensive discussion on reactor configurations and design betterment was presented in this review, along with precise technical comparison on cost and energy requirements for the cultivation systems. This review intended to serve as guideline for long term adoption of these well-established cultivation technologies in biofuel plants given the numerous economic benefits. Besides that, in attempt to lower the harvesting cost, potential use of various waste biomass as bioflocculants to recover microalgae biomass was introduced in this review. This article also deliberates direction on potential policy interventions to produce microalgae biofuel in a more sustainable and cost-effective manners in near future.

1. Introduction

Algae belongs to a divergent group that encloses extensive number of known species that serve as raw material for biofuel production [1]. Algae typically can be grown in massive scale with inorganic compounds such as carbon dioxide (CO_2), light energy and nutrients like phosphorus (P) and nitrogen (N) [2]. Since ancient times, algae have been used for their role as food producer for human and animals. Besides, they also display excellent bioactivities and metabolite secretion which are largely explored for antibiotics and nutraceuticals application, fertilizer, wastewater treatment and acting as greenhouse gas mitigation agent [3–8]. Recent decade witnesses the conversion of microalgae biomass into variety of biofuel forms such as biodiesel, bioethanol, biohydrogen, and bio-oil that potentially to solve current energy crisis.

Microalgae cells portray important key features such as high growth rate, high biomass productivity, and less requirement for water and land for growth, which enables them to be an ideal feedstock over macroalgae for biofuel production [9]. Despite intensive research efforts on microalgae are being made, its commercial application is still not economical due to hurdles in selecting suitable cultivation system and harvesting methods for biomass processing [10]. Large scale industrial growth systems use open ponds to cultivate microalgae with a paddle wheel to circulate microalgae cells, nutrients and water around while constantly being exposed to environment [11]. However, closed systems such as PBRs of various designs are preferred to lower the contamination risks and ease the control of parameters for greater productivity [11]. Each designated PBRs have their own advantages which makes the system selection to grow microalgae at best seems harder.

On the other hand, the cost of microalgae harvesting can reach up to 20–30% of total biomass production cost [12]. Microalgae are usually

* Corresponding author. Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia.
E-mail address: lam.mankee@utp.edu.my (M.K. Lam).

https://doi.org/10.1016/j.rser.2019.109361
Received 30 January 2019; Received in revised form 16 August 2019; Accepted 27 August 2019
Available online 06 September 2019
1364-0321/ © 2019 Elsevier Ltd. All rights reserved.

Nomenclature

BSE	Bovine spongiform encephalopathy
C	Carbon
DAF	Dissolved air floatation
DCW	Dry cell weight
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DW	Dry weight
EISA	Energy Independence and Security Act
EF	Electro-flocculation
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization
MBM	Meat and bone meal
MW	Molecular weight
N	Nitrogen
NAS	Nitrifying enriched activated sludge
NER	Net energy ratio
ODW	Oven dry weight
P	Phosphorus
PAM	Polyacrylamide
PBR	Photobioreactor
RFS2	Second Renewable Fuel Standard
RPM	Rendered protein meals
RSM	Response Surface Methodology
R&D	Research and development
TAG	Tri-acyl-glyceride
TEA	Techno-economic analysis
TN	Total nitrogen
TP	Total phosphate
TS	Total solid
UK	United Kingdom
USD	United States Dollar
TMEN	True metabolizable energy

being harvested via energy-intensive methods such as floatation, centrifugation, filtration and electrical-based methods which accounted 90% of the total cost involved in producing microalgae biomass from open ponds [2]. As such, flocculation are being employed extensively due its simplicity and effectiveness [13]. Chemical flocculants have been highly acknowledged for its superiority in flocculating suspensions at low dosage and short period of time [13]. Nevertheless, toxicity and detrimental health risks that caused by chemical flocculants are the concerns of this harvesting method and substitutes to replace chemical flocculants are being the utmost important research topic [14]. Natural flocculants, on contrary to chemical flocculants, are non-toxic, safe-handling and eco-friendly. A variety of natural flocculants such as plant and fruit wastes have been explored lately in many futuristic applications as these wastes are abundantly available, cheap and show promising flocculability properties. *Moreinga oleifera*, *Stryconus potatorum*, Cactus species, *Phaseolus vulgaris*, surjana seed tannin, and gum Arabic are some of plant-based prominent flocculants that have been studied in the past [15]. Apart from that, waste seashells and eggshells that are being discarded to environment have been studied closely as adsorbent and biosorbent in dye and heavy metal removal even though limited information is available on their ability to function as bioflocculant.

Thus, current review aims to provide insights into the positive elements of existing cultivation technologies and sketch perspectives to new directions for technological improvements that will enable sustainable production of microalgae biofuels. This paper also highlights functionality of natural biomass as bioflocculant in reported literatures and draw attention towards their applicability in microalgae harvesting with respective to their composition and physicochemical properties. The findings from this review suggest possible policy framework to support microalgae biomass as feasible feedstock for biofuel production as well as to provide energy security in future.

2. Algae

2.1. Definition and biology of algae

Algae, can be grouped based on their morphological characteristics and size, which are referred as microalgae or phytoplankton ('phyto' = plant; 'planktos' = made to wander) at microscopic level [16] and macroalgae, that grow in aquatic environments [17]. In general, macroalgae or 'seaweeds' are multicellular primitive plants, which called thallophytes, range in size from 1/1000 of a mm to 2 mm floating in the upper 200 m of the ocean [16], that known to be lack of root, stem and leaf system [16–18]. Conversely, microalgae are unicellular microscopic organisms that have chlorophyll as their basic photosynthetic pigment for energy conversion. They have simple reproductive and cell growth system, allowing fast proliferation and long-term survival in various harsh environmental conditions [7,18], ranging from fresh water environments to salt, ice or hot springs [17]. They are amendable to genetic modification and able to inhabit variegated ecological habitats, which make microalgae known as one of the ancient life form and most prolific living organism on Earth [17]. Although microalgae can be defined as marine plants, scientists are facing real time complexity in distinguishing and estimating their vast population over one to ten million algae species that have been counted [17]. Algae, the collective name for eukaryotic macro and prokaryotic micro [1], able to capture solar energy and fix CO_2 rapidly for growth and living [10]. Algae are also defined as sunlight-driven, fast growing cell factories [19] that able to accumulate lipids, proteins, and other high value products like omega-3 polyunsaturated fatty acids, especially DHA and EPA [20] by taking up dissolved inorganic carbon (C), hence approximately 50% of their dry weight (DW) constitutes C.

A large and growing body of literature has reported, there are over 100,000 algae species are known at the present and in 2016, the estimated number of recognized species of marine algae that exist in worldwide was reported as shown in Fig. 1 [21]. The diverse population of algae species offer vast range of starting strains for their complete utilization and commercialization. Nevertheless, scientists and researchers have not escaped from challenges in identifying and characterizing them [1]. Classification of algae can be done in various ways by considering their differentiating characteristics such as structural features, membrane constitution, colors of the pigments and energy-storing molecules [22]. However, researches to date have not dealt in detail to provide definite taxonomical classification of algae which seems complex and draw limitations. To date, the widely used

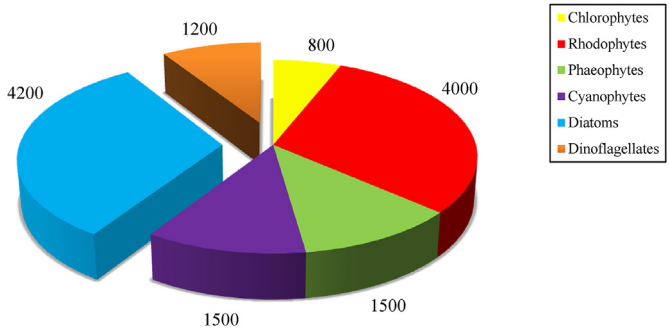

Fig. 1. Estimates of counted known species of marine algae found in worldwide [20].

microalgae can be divided into four main groups, which are Cyanophyceae (blue-green algae), Chlorophyceae (green algae), Bacillariophyceae (including the diatoms) and Chrysophyceae (including golden algae) and microalgae such as *Arthrospira (Spirulina)*, *Chaetoceros*, *Chaetoceros*, *Chlorella*, *Dunaliella* and *Isochyris* are the most commercially cultivated genera, preside over the rest [21].

2.2. Algae biomass as potential source of biofuel

Rapid economic development that has been witnessed in recent years are driven by ancient fossil fuels deposits. State-of-the-art findings revealed that 88% of energy used by human for domestic uses are derived from fossil fuels [23] such as gasoline, diesel and kerosene. This has resulted the economies of many countries all over the world are vulnerable to transitions in petroleum prices and supply. A considerable quantity of fossil fuel exists but gradually being exhausted due to over exploitation of fossil reserves. Apart from that, excessive burning fossil fuel has resulted in mounted greenhouse gas emissions [24] and therefore, contributed to global warming [25]. Till date, fossil fuels are responsible for 29 gigatons/year release of CO_2 with a total of 35.3 billion tons CO_2 [26]. Thus, global efforts are taking flight to move from fossil-fuel-dependent economy to a bio-dependent economy in which biomass replaces petroleum to satisfy the dramatic explosion of energy demands of developing countries.

Large-scale production of advanced bioenergy from biomass could result in sustainable benefits in environmental, social as well as economic sectors. In many parts of the world, bioethanol and biodiesel that derived from starch and oil yielding crops, respectively, are the most commercially available forms of renewable energies [27]. While ethanol production from corn and sugarcane is a well-established process, production from cellulosic material is yet to reach commercial value. On the other hand, biodiesel is derived from naturally occurring vegetable oils or animal fats that will undergo transesterification to run a diesel engine. However, it is not rational to rely on agricultural biomass production to meet the current growing demand for renewable energy by considering few factors. The urge to find new sustainable source of biomass that do not compete with agriculture has been a hot debate in recent years. Wide scale cultivation of crops yielding biofuel not only might trigger food scarcity but also consumes large area of plantation.

Hence, algae is gaining more interest as an alternative source of biofuel [27]. Microalgae have been considered as significant source of renewable biomass energy as it has promising amount of biomass as well as oil content for biodiesel production [28]. In comparison to plant crops and terrestrial plants, microalgae is found to capture solar energy and fix CO_2 at a rate 5–20 times higher and have greater biomass productivity since they take up dissolved inorganic C to grow, hence C constitutes 50% of their DW [10,29]. Microalgae are rapidly growing photosynthetic organisms having potential of transforming 9–10% of solar energy into biomass with a theoretical yield of about 77 g biomass/m^2/day which is about 280 ton/ha/year [26]. Furthermore, compared to microalgae cells, plants have complex structures that must be separated before extraction of fatty acids [30]. Microalgae have a very short life cycle and can grow almost in any conditions in presence of sunlight and other basic nutrients. Microalgae have no competition of valuable land with other food crops and provide much cleaner biodiesel than petroleum fuel [29]. Likewise, products with high value such as biogas can be synthesized from the residual biomass after lipid extraction [30]. The comparison of oil yield from various sources of biodiesel has been tabulated as in Table 1 [12,19]. Besides, macroalgae or seaweeds are also alternative sources of energy. They have biomass productivity with ten times higher than planktonic species and much higher than terrestrial biomass. General requirements of growth such as nutrients, salinity, temperature, light, are readily available as natural resource, and thus appear to be suited for large-scale cultivation [31].

There is a large volume of published studies emphasizing that, both macro- and microalgae are promising feedstocks to secrete premium metabolites with mixed bioactivities, ranging from carbohydrates, essential acids, pigments, nutraceuticals, fertilizer, and predominantly lipids by sequestering greenhouse gas [5,19,22,28,32,33] to environmental applications such as bio-indicators of heavy metal pollution and control of mosquito breeding [17,34] that are yet to be explored scholarly [17]. Selectively, production of one or more high-value products can be achieved, depending on the type of algae being cultivated and their residues too, being feasible for fuels (bio-oil, biochar, syngas) and energy conversion via thermal and combustion methods [22]. Lack of lignocellulose and abounding in lipids and proteins, algae biomass appears to be valuable source to meet demanding need for animal feed, food supplements and energy conversion [22,35]. Literature findings revealed that supply of inorganic nutrients through wastewater and CO_2 via flue gas makes coupling of algae biomass production to wastewater remediation and CO_2 removal possible and in the pipeline of implementation [35]. Algae biomass can be utilized in several sustainable ways to generate renewable biofuels for transportation and jet fuel, where production of methane through anaerobic digestion is labelled as the most primitive and well developed [19]. Apart from being the significant candidate for biodiesel production, algae biomass, especially *Chlamydomonas reinhardtii* has been gaining considerable attention in photobiological production of bio-hydrogen as emerging alternative source of green energy [19,33]. The relevance of algae biomass as a sustainable feedstock is a real optimism, but the algae biotechnology associated with energy sector has been disadvantaged by immoderate investment costs and the excessive need for auxiliary energy for biomass production and downstream processing [36] which draws focus of scientists worldwide.

2.3. Biochemical composition

Algae has noteworthy applicable characteristics which generated from its biochemical constituents [37]. Algae populate aquatic ecosystems and thus, lack of composite support structure like lignocellulosic biomass such as complex matrix of cellulose, hemicellulose and lignin [22,35]. Therefore, bioactive compounds such as proteins, lipids, sugars, and nucleic acids are the main constituents of algae [22] and their yields are selectively dependent on type of algae species and biomass composition [38], which in turn is highly influenced by nutritional and growth conditions [22,38]. Established literature reports that unicellular microalgae are potent source of proteins and lipids, while carbohydrates are dominant in macroalgae or seaweeds [8,22]. Therefore, it is pivotal important to explore both macro- and microalgae biomass. However, one major technological issue that has dominated algae field for many years concerns current algae cultivation methods with monitored growth environments, for example, open ponds or closed PBRs, which apply only to microalgae culture, causing macroalgae cultivation practice to count on the marine ecosystems [38].

2.3.1. Carbohydrate

Extensive approaches are being made for valorisation of algae components for production of biofuel and apart from lipid, carbohydrate is another commodity of interest. Sugars or carbohydrates are the

Table 1
Comparison of biodiesel productivity from various sources of biodiesel [11,18].

Crops yielding biodiesel	Biodiesel productivity (kg/ha/year)
Corn	152
Jatropha	656
Soybean	562
Oil palm	4, 747
Microalgae (70% oil in biomass)	121, 104
Microalgae (30% oil in biomass)	51, 927

main products of photosynthesis in algae and their accumulation varies with different algae species [22], for a selective strain, with cultivation medium [8]. Generally, macroalgae or seaweeds are richer in carbohydrates (up to 75%) [8] compared to microalgae due to the presence of extracellular carbohydrates for support and protection [22]. Meanwhile, distribution of carbohydrates in microalgae biomass are majorly in the forms of cellulose which present in the cell wall and starch in the plastids, which conserved upon intracellular secretion under stress conditions [8,39]. Comparison of annual production and polysaccharide composition of both macro- and microalgae species [8,22,39–41] are showed in Table 2.

Absence of lignin and scarce quantity of hemicellulose turn microalgae-based carbohydrates as feasible option for conversion into biobutanol, bioethanol and other forms of bioenergy through fermentation [42,43]. Extraction of stored carbohydrates from microalgae cells involve lysis which can be done via enzymatic, acidic or solvent extraction [26]. The extracted sugars undergo pre-treatment in which polymeric molecules are broken down into monomers before fermented to yield bioethanol [44]. However, yield of bioethanol highly dependent on method of extraction [44]. Bioethanol derived from microalgae carbohydrates has high prospect of being the permissive alternative fuel, so it is equally important to ensure its expansion is not obstructed by raw material constraints or production processes [21]. The main hurdles associated with up-scaled production and commercialization of bioethanol are the selection of suitable microalgae species, pre-treatment processes and optimized fermentation under aseptic conditions [45]. Continuous progress to overcome drawbacks regarding cultivation and harvesting steps are also necessary in producing carbohydrate-rich microalgae biomass and thus, maximizing bioethanol yield. Several carbohydrate-rich microalgae species [39,43,46–58] such as *Chlamydomonas reinhardtii* and *Chlorella vulgaris* (Table 3) are nominated as potential candidate for techno-economic analysis (TEA) of bioethanol production [44]. Therefore, significant attention should be given in utilization of microalgae carbohydrates for development of sustainable biofuel.

2.3.2. Lipid

The foremost reason microalgae are potential as biodiesel feedstock is because of their high lipid content as compared to macroalgae. Substantial amount of triglycerides, also known as triacylglyceride (TAGs) can be accumulated in certain microalgae species [19]. According to Farlex Partner Medical Dictionary, TAG is the glycerol that is esterified at each of its three hydroxyl groups by a fatty acid that is usually stored in the form of lipid in microalgae as energy source [59]. These TAGs are more favourable than other monoglycerides, such as glycolipids and phospholipids for biodiesel generation due to their higher fatty acids content and absence of phosphate, sulfur and N, which may be problematic in the end-product biofuel [60,61]. After being extracted from microalgae, the lipids can be easily converted into biodiesel via the common transesterification process [59]. Moreover, high conversion rate of TAGs to biodiesel turns TAGs the most feasible lipid fraction for biodiesel generation [61]. Thus, the content of TAGs in the algae is the best indicator for their suitability for biodiesel production [61]. On the other hand, macroalgae contain high concentration of metals which may have negative effect on the transesterification process [61]. High sulfur and N content in macroalgae, especially in green algae may cause drawbacks in the production of biogas due NH_3-toxicity and biodiesel as high concentrations of H_2S and NOx-compounds are generated [61]. Therefore, microalgae have proven to best material for conversion and biodiesel production over macroalgae. Table 4 presents the remarkably high lipid contents of several microalgae species [12] that been studied intensively over years for biodiesel generation.

Table 2
Comparison of annual production and polysaccharide composition of algae [8,21,22,37,38].

Group	Seaweed/Macroalgae			Microalgae			
Divison	*Rdodophyta*	*Phaeophyta*	*Chlorophyta*	*Cyanophyta*	*Dinophyta*	*Crypto-phyta*	*Eugleno-phyta*	*Heterokonto-phyta*
Common Name	Red	Brown	Green	Blue-green/Cyanobacteria	Dinoflagelletes	Green/yellow-brown	–	–
Species	*Kappaphycus alvarezii*	*Laminaria japonica*	*Codium fragile*	*Dunaliella*				
	Eucheuma denticulatum	*Undaria pinnatifida*	*Caulerpa* sp.					
	Eucheuma sp.	*Sargassum fusiforme*	*Monostroma nitidum*	*Spirulina*				
	Gracilaria verrucosa	*Phaeophyceae*	*Enteromorpha clathrata*					
	Chondrus crispus	*Fucus vesiculosus*	*Ulva lactuca*	*Chlorella*				
	Gigartina papillata	*Macrocystis pyrifera*	*Ulva pertusa*					
Poly-saccharides	Carrageenan	Laminarin,	Starch	Lipopolysaccharides	Starch	Periplast	Paramylum/lipid	Leucosin/lipid
	Agar	Mannitol	Cellulose	Peptidoglycan	Absence or very	Starch		
	Cellulose	Alginate	Hemicellulose	Cyanophycean	few cellulose			
	Floridean	Fucoidan	Pectin	Starch				
	starch	Cellulose	Ulvan					
		Alginic acid						
Production (ton/year)	8,978,535[a]	6,784,93[a]	21,545[a]			10,000[a]		
						7,000[b]		

c Total world production of dry algae biomass that are mostly produced in open systems.
[a] Estimated in wet metric ton and adopted from FAO.
[b] Total world production of dry algae biomass.

Table 3
Carbohydrate or starch content in commonly studied algae species over years.

Group/division	Algae species	Carbohydrate/starch content, DW (%)	Reference
Microalgae	*Chlorella vulgaris* IAM C-534	37.0 (starch)	[20]
	C. vulgaris CCAP 211/11B	55.0	[20]
	C. vulgaris	55.0 (starch)	[20]
	Chlorella sp.	21–45	[40]
	Chlorella	16–50	[44]
	C. vulgaris P12	41.0 (starch)	[45]
	Chlorella vulgaris FSP-E	51.0	[45]
	Chlorella sorokiniana	18.0	[45]
	Chlorella sorokiniana SDEC-18	10.25 ± 0.82	[46]
	Chlorella sp.	60.9	[47]
	Chlorella vulgaris	6–12	[48]
Green algae *(Chlorophyta)*	*C. reinhardtii* IAM C-238	55.0 (starch)	[20]
	Chlamydomonas reinhardtii	9.2	[49]
	Chlamydomonas reinhardtii CC125	71.0	[50]
	Chlamydomonas reinhardtii UTEX 90	57.0 (starch)	[45]
Green algae *(Chlorophyta)*	*Chlorococum* sp.	32.5	[20]
	Chlorococcum sp. TISTR8583	26.0 (starch)	[20]
	Chlorococcum littorale	70.0	[45]
Green algae *(Chlorophyta)*	*Ulva fasciata*	46.73 ± 2.25	[51]
	Ulva fasciata	46.73 ± 2.25	[51]
	Ulva intestinalis	48.9[a]	[52]
	Ulva lactuca	48.7[a]	[52]
Green algae *(Chlorophyta)*	*Stigeoclonium* sp.	43.35 ± 0.05	[53]
Green algae *(Chlorophyta)*	*Oedogonium nodulosum*	64.37 ± 0.69	[53]
Green algae *(Chlorophyta)*	*Zygnema extenue*	46.00 ± 2.00	[53]
Red algae *(Rhodophyta)*	*Gracilaria* sp.	76.67	[54]
	Gracilaria sordida	12 (ODW)	[45]
	Gracilaria gracilis	28.6 ± 0.35	[55]
Red algae *(Rhodophyta)*	*Porphyridium cruentum*	40.0	[45]
Red algae (Rhodophyta)	*Chondrus crispus*	46.7[g]	[52]
Brown algae *(Phaeophyceae)*	*Laminaria digitata*	38.3[a]	[52]
	Laminaria hyperborea	17.4[a]	[52]
	Laminaria digitate	65.20 (TS)	[56]

[a] Calculated by difference; mass fraction quoted on dry basis.

Table 4
Lipid contents of Microalgae Species [11].

Microalgae species	Lipid content, DW (%)
Botryococcus braunii	25–75
Chlamydomonas pitschmannii	51
Chlorella	18–57
Chlorella emersonii	25–63
Chlorella vulgaris	5–58
Monoraphidium sp. FXY-10	56.8
Nannochloropsis sp.	12–53
Neochloris oleobundans	29–65

3. Microalgae cultivation systems for biodiesel production

Microalgae grow naturally in lakes, rivers and oceans but such ecosystems are unsatisfactory due to very low biomass concentrations for large scale harvesting [29]. To meet the commercial demand, 23.8 million wet tonnes of algae were cultivated in 2012 and China is one of the leading Asian country to actively involve in the farming of algae, accounting for 53.97% of the whole [62]. Till date, organic and inorganic chemicals have been on vast usage as feasible nutrient medium to cultivate microalgae at large scale [4]. However, its application is challenged by its high cost and environmental risks which may not sustain mass production of microalgae biomass. Therefore, organic fertilizers and wastewater from domestic and industry runoffs have been proposed as low-cost nutrient medium for effective cultivation. Following the trend, there are noteworthy approaches in turning microalgae cultivation as a potent tool for natural C assimilation and bioremediation since microalgae can be effectively grown in various wastewater through their ability to utilize abundant organic C, N and P in the systems [63]. For example, *Chlorella pyrenoidosa*, cultivated in soybean processing wastewater was capable to remove 78% of soluble organic C, 89% of total nitrogen (TN), and 70% of total phosphate (TP). Besides, it was also reported that *Chlorella vulgaris* was able to remove 90% and 80% of N and P content, respectively, from primary treated sewage [64].

Nevertheless, the nutrient compositions from wastewater are vary over time. Also, the requirement quantities of macronutrients such as the N and P may vary for different species of microalgae. Recent work had revealed that the growth of *Chlorella* sp. declined when the concentrations of N and P reduced to 31.5 and 10.5 mg/L, respectively [26]. It is worth to note that nutrient limitation such as N in the microalgae culture can reduce their growth and biomass productivity even though such technique increases the production of carbohydrates and lipid within their cells. For example, *Nannochloropsis* sp. was able to accumulate 60% of lipid under N-deprived conditions and demonstrated potential for an annual production of more than 30 tons per hectare of lipids when cultivated at tropical areas [65]. Elite strain selection, method adaptation in cultivation, and modification in the media compositions are some of the scholarly available and developing techniques to empower the utilization of nutrients in wastewater by microalgae. In wastewater containing low C/N ratio, the nitrifying enriched activated sludge (NAS) approach lowers fouling and enhances nitrification efficiency which seems promising for water reuse in microalgae cultivation [66].

Nevertheless, the nutrient compositions from wastewater are vary over time. Also, the requirement quantities of macronutrients such as the N and P may vary for different species of microalgae. Recent work had revealed that the growth of *Chlorella* sp. declined when the concentrations of N and P reduced to 31.5 and 10.5 mg/L, respectively [26]. It is worth to note that nutrient limitation such as N in the microalgae culture can reduce their growth and biomass productivity even though such technique increases the production of carbohydrates and

lipid within their cells. For example, *Nannochloropsis* sp. was able to accumulate 60% of lipid under N-deprived conditions and demonstrated potential for an annual production of more than 30 tons per hectare of lipids when cultivated at tropical areas [65]. Elite strain selection, method adaptation in cultivation, and modification in the media compositions are some of the scholarly available and developing techniques to empower the utilization of nutrients in wastewater by microalgae. In wastewater containing low C/N ratio, the nitrifying enriched activated sludge (NAS) approach lowers fouling and enhances nitrification efficiency which seems promising for water reuse in microalgae cultivation [66].

Apart from nutrients accessibility, other key factors such as light, pH, and temperature are found to be influencing robust growth and good cultivation of microalgae, resulting in predictable quantification of biomass for biofuel production [67]. Light is one of the major limiting concern in microalgae cultivation as light duration, intensity, spectral composition, and photoperiod are known to directly affect the photosynthesis rate in the green cell. In modelling of outdoor or indoor microalgae culture system, growth rate and biomass productivity are predicted as a function of light [26]. Temperature is another important factor in the growth of microalgae as it alters the biochemical processes in the microalgae cell factory. While most microalgae species have optimum temperature range of 20–30 °C, thermophile algaes such as *Anacystis nidulans* and *Chaetoceros* can withstand temperatures up to 40 °C and algae growing in harsh hot spring near temperature of 80 °C [26]. Cultivation of microalgae at area with non-optimal temperatures will result in high biomass losses, particularly in outdoor cultivation systems like open ponds [26]. The pH of the culture media is another important factor affecting the microalgae growth. Different sources media have different pH values and pH ranges from 6 to 8.76 is preferable by most microalgae species. Unlike *C. vulgaris*, which can grow in broad range of pH, most species are sensitive to minute changes in the values that creates unfavourable environment for cell growth [26].

Microalgae cultivation often associated with five modes of metabolic pathways: photoautotropic, heterotropic, mixotropic, amphitropic, and photoheterotropic [8]. Closely linked to biochemistry, metabolic pathways that occur in microalgae are highly selective based on environmental adversities and uncertainty favour cooperation [27]. From widely available studies, photoautotropic mode is the common primitive practice of cultivation to maximize photosynthesis rate of microalgae cells [29] and large-scale lipid production. In photoautotropic cultivation, microalgae cells sequester inorganic C, mainly CO_2 as C source and capture naturally available solar light as energy source to generate biomass and energy [33]. Employing photoautotropic method, fast-growing green cells are cultivated commercially in open ponds (low cost but easily contaminated) and enclosed PBR (clean but expensive) [20,29].

3.1. Open pond system

As a futuristic methodology, microalgae biomass is the best candidate for commercial biofuel generation and the diversity of ways to cultivate microalgae are of main interest. Cultivation of microalgae in ponds using energy driven from sunlight has been best regarded for more than past 60 years and can be carried out in open or covered and in natural waters (lakes and lagoons) or man-made shallow basins [18]. A few open pond systems are available and appear to be the favourites for most commercial practices [29], such as natural, circular, raceway and inclined systems mainly due to their simplicity and cost-effective construction features coupled with high production capacities [68,69] than closed systems. As for the nutrients supply, runoff water from land areas is common but also, the win-win strategy of integrating microalgae cultivation with sewage or wastewater treatment plants are now drawing possibilities in many industries [68,70,71] as a phytoremediation tool as well as to minimize upstream processing cost [4]. All in all, open pond system stands out in terms of economic perspectives and ease of scalability [63], albeit massive scale production of microalgae production is hindered by physical, chemical and biological factors that need to be addressed holistically whereupon eventually technology would be feasible.

Besides the need for plenty of space to assemble open pond system, variations in surrounding and culture conditions along with unmonitored solar light intensity and temperature trigger the intolerance of microalgae towards such cultivation method [68]. Poor light intensity and distribution affects the microalgae growth and cell concentration owing to the fact that sunlight can only reach the pond water to a certain limit of depths and thus, exposure of microalgae to sunlight is not even [68]. Another disadvantage of open pond system is the inability to uphold optically dark zone [18]. As cooling within the system is achieved by evaporation [19], difficulty to control the culture temperature causes excessive evaporation of water and consequently diffusion of CO_2 to the atmosphere [18]. Lack of CO_2 within the system

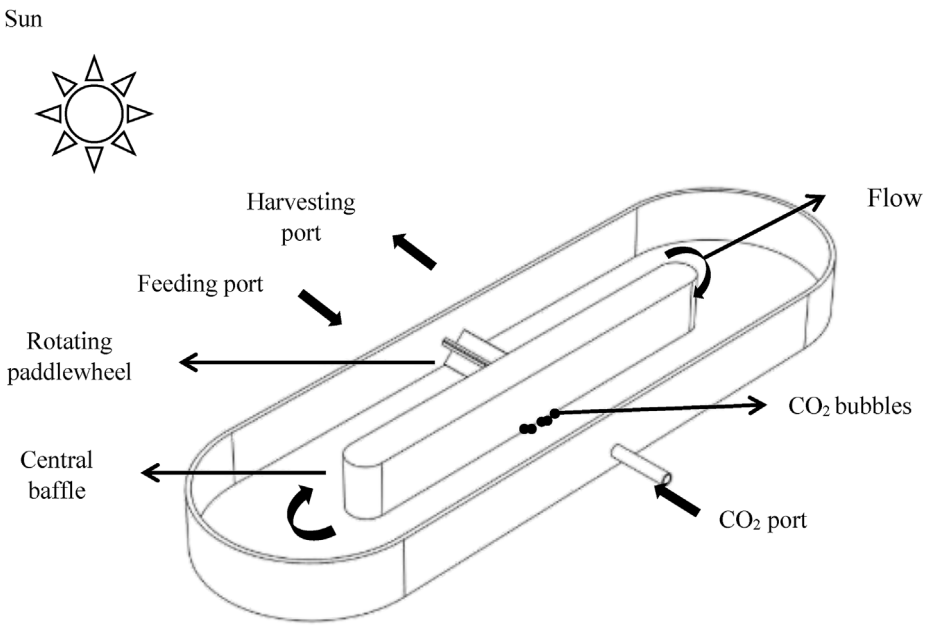

Fig. 2. Open Raceway Pond with modification [70].

restricts the utilization of C needed for cell proliferation and components synthesis which eventually results in low microalgae biomass yield. Another concerned problem associated with open pond cultivation method is the high susceptibility to contamination by foreign predators and flourish heterotrophs that feed on the algae, turning such system is only feasible for microalgae that can adapt to intense environmental conditions [18]. Even though a large and growing body of literature reports the success of open pond cultivation systems over decades, another drawback is the inefficient mixing mechanisms by paddlewheel (in open raceway ponds) and pivoted agitator (in open circular ponds) that lead to poor mass transfer and eventually reduced volumetric productivities [68].

Open ponds are typically built in circular or raceway configurations. In raceway ponds (Fig. 2), as the name indicates, mass microalgae culture is recirculated around a racetrack loop. Raceway ponds are designed to be shallow with a depth of 0.3 m in approximate [68] to ensure improved exposure to sunlight and subsequently good growth rate of microalgae cells [18]. It has a designed paddlewheel to provide mixing and recirculation as well as baffles to guide the flow at bends [18]. The construction feature of a raceway pond are either concrete or rammed earth walls with white plastic lining sometimes, which are highly claimable as inexpensive materials [68]. The system is operated continuously with the help of paddlewheel to avoid possibilities of sedimentation throughout the cultivation cycle, with constant feeding of microalgae broth and nutrients at the feeding port located in the front space of paddlewheel [68]. Upon completion of circulation through the loop, microalgae-containing water is removed at the harvesting port, positioned in the rearrmost of the paddlewheel [18]. Following the trending success stories of open raceway pond systems, the world's largest raceway-based microalgae biomass production plant is operating at Calipatria, CA (USA) that pioneered for production of Spirulina and Spirulina-based products, occupying an area of 444, 000 m^2 [68]. With renewable energy investment growing ever more politically contentious, a handful research studies has been conducted to evaluate the performances of raceway ponds and closed PBRs to grow microalgae with sponsorship from The United States Department of Energy [68]. Raceways are sought to be under cost-benefit microscope, regardless, low biomass productivity in comparison to closed PBRs remain the classical problem [68].

Another form of open pond system is the circular pond (Fig. 3), in which mixing and motion of the culture is aided by a central pivot rotating agitator [55]. However, the limitation in mixing offered by the rotating arm restricts the sizeability beyond 10, 000 m^2 [68]. Taking into account the cost factor involved in construction and operation of circular ponds, it is not recommendable to take on the risks in bringing this cultivation method to commercial scale [68].

Commercial production of high-quality microalgae biomass is now a challenge of scale and the prize is phenomenal. To provide a better control over the microalgae culture environment, closed pond systems are introduced recently as alternative to open ponds. The current concept of closed pond system is by covering with greenhouse to address contamination problems associated with open pond systems [18]. This technology allows mix culturing of several microalgae species and ease the possibility of desired species to stay dominant. Such system also increase the chance of trapped CO_2 by minimizing water and CO_2 loss through evaporation, thus again promoting robust cell growth [18]. While concrete and compressed earth are the building material for open ponds, closed ponds are constructed using plexiglass which cost more than open ponds, but relatively less than PBRs for same operation spaces [59]. Recently, closed ponds were used at Synthetic Genomics, Inc. in California for algae cultivation scale-up. The acquired dried algae biomass is then used as food, beverage and feed ingredient [72].

In addition, Lam and co-authors [63] have done an extensive review on best possible strategies to manage biological contaminants in open pond cultivation systems. The significant organisms that contribute to pond contamination have been grouped into 5, namely grazers, fungi, photosynthetic organisms, bacteria and viruses [71]. While grazers, pathogenic fungi and viruses are the common adulterants, weed algae species and bacteria draw special attention among researchers as they are detrimental and reduce the economic value of the produced biomass

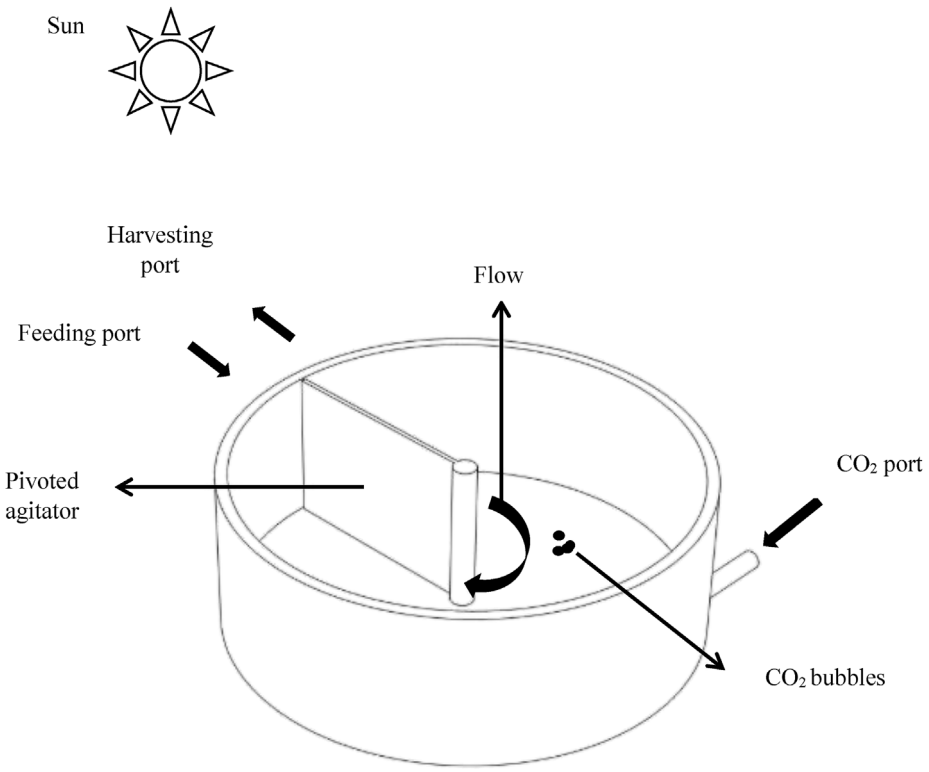

Fig. 3. Open Circular Pond with modification [70].

[71]. Based on the afformentioned authors [71], control of contaminants can be summarized as: i) installation of slow sand filter to taper off grazers present in incoming water coupled with acknowledged sets of wastewater treatment technologies (coagulation, filtration and etc.); ii) selection of algae species that free from history of fungal infection; iii) for macroalgae species, good maintenance of algae monoculture with help of suitable control agents such as good bacteria and viruses seemingly promising; iv) integration of probiotic bacteria in cultivation system to get rid of toxic bacteria that compete with microalgae culture for sunlight or nutrients.

Despite of the well establishment and extensive engineering experiences involved in open pond systems, continuous research are being done in designing and operation works to date, in strive to asses and optimize mass biomass collection for cheap and sustainable biofuel production [68]. The configurations and operation factors of open pond system in detail, in which these findings are aimed to provide the much-needed stimulus in improving the performances and at the same time, maintaining the low cost technologies over traditional ones had been discussed [73]. However, with the presence of research grants from plenty of government and private sectors, the much more expensive enclosed PBRs are of current trend for enormous reasons that will be highlighted in following sections.

3.2. Enclosed PBRs

Following the shortcomings concerned with pond systems, growers are typically desired to cultivate monocultures of algae and engineered strains in axenic enclosed PBRs for production of biochemical and high-value metabolites which offer low contamination possibilities. An enclosed PBR can be best defined as a man-made closed vessel that helps microalgae cells to carry out photosynthesis in the presence of light as energy source. However, economic risks are evident as construction and operating cost of a PBR is higher than pond system. Nevertheless, cultivation of microalgae in enclosed PBR require less or no agriculture land [70]. In fact, microalgae can also be cultivated on non-arable land in enclosed PBRs with nutrients supply from wastewater treatment plants [11]. Tubular and flat panel PBRs are the most well-regarded closed culture systems used in commercial scale for microalgae cultivation which will be presented extensively in next sub sections.

3.2.1. Tubular PBR

Academic researchers and commercial project developers have been working on designing enclosed PBRs over past 50 years [70] and tubular PBRs are the most common ones [74]. Typically, construction materials for tubular PBR consist of straight glass or plastic tubes that arrayed in horizontal, vertical, fence-like, inclined or helix configurations [70]. The tubular solar array is designated and oriented to collect maximum amount of sunlight [70]. They are arranged parallel to each other and positioned flat above the ground [11] as depicted in Fig. 4. These horizontal solar tubes can also be arranged fence-like (Fig. 5) so that more tubes can be housed in given space [19].

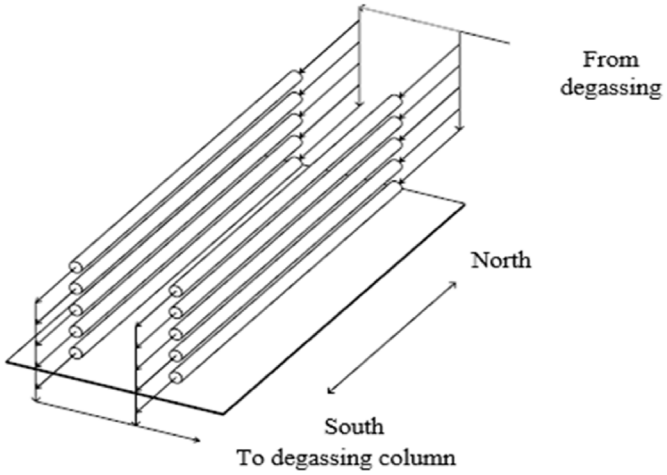

Fig. 5. A fence-like solar array with modification [18].

In fence-like solar collector, the tubes are positioned in North-South orientation on white-painted ground or sheets in attempts to maximize reflectance and so as received light by the tubes [19]. As optimal light penetration is the key aspect in ensuring high biomass productivity by means of photosynthesis, transparent glass and plastics are utilized [68]. Besides that, the solar array is made with limited diameter up to 0.1 m to enhance light penetration through the dense microalgae culture [19]. Microalgae culture is circulated from the degassing column to the solar array and reverted back to the degassing column in a continuous operation mode [19]. Interestingly, feasibility of tubular PBR for many existing operation modes such as batch, semi-batch, continuous and turbidostat, turning it as the most reliable type of PBR for scaling up due to ease of control [74]. In tubular PBR, mass transfer of CO_2 and photosynthetically-produced oxygen (O_2) is achieved with the aid of a mechanical or airlift pumps by continuous recycling of microalgae within the system [70]. Such arrangement helps to maintain the mixing process that commonly performed by sparger attached at the bottom of the reactor [68]. Discourse about tubular PBRs, the vertical ones can be further classified into bubble column and airlift bioreactors based on the mode of liquid flow [68]. A basic configuration of vertical tubular PBR is shown in Fig. 6.

Regardless of the well-experimented solar tube arrangements in tubular PBRs, helical-shaped reactors are the interest of new millennia, resulted from substantial researches in designing and viable technologies. Transparent and flexible tubes are coiled with or without degassing unit, giving the shape of helix as in Fig. 7 [59]. The microalgae

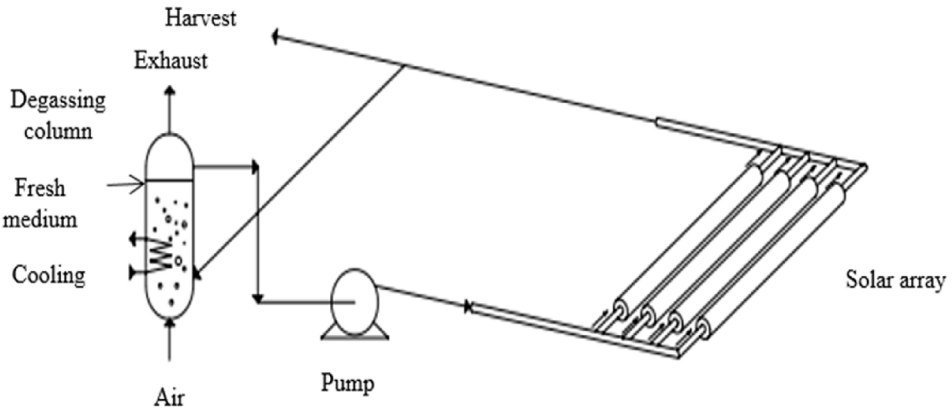

Fig. 4. A tubular PBR with paralleled horizontal solar tubes with modification [18].

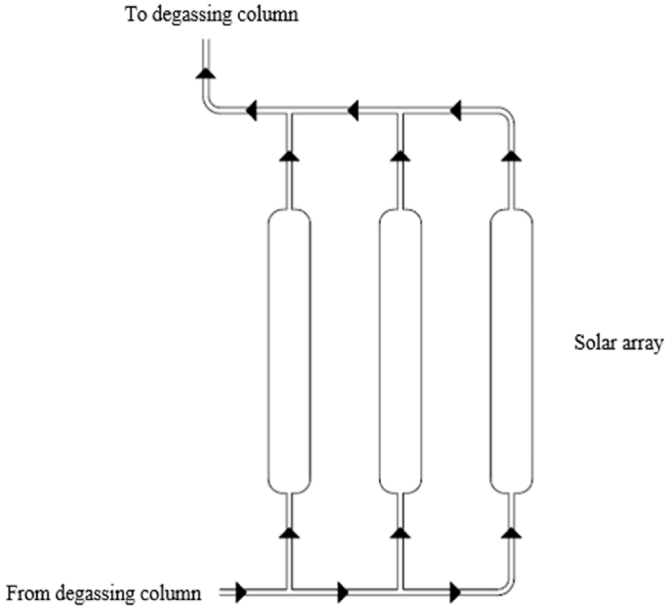

Fig. 6. Basic configuration of vertical solar tubes.

culture is driven to the degassing unit along the tube by a centrifugal pump [59]. A few case studies are available on the setups of helical type PBR, delivering its prevalence which includes small area requirement and better CO_2 transfer by applying bigger channels for absorption [59]. However, due to practical constraints associated with its complexity in design, scaling up is seemingly limited by the energy requirement for circulation of microalgae culture along with shear stress within the system [59]. Another setback of helical-shaped reactors is the fouling that reduces the efficiency of the affected tubes [59]. Instead of coiling the tubes into helix form, another way is to coil the tubes around a supporting material or frame [19], forming a helical tubular PBR which is hybrid between a horizontal and vertical tubular PBR (Fig. 8). According to previous designers, such design might offer supremacy to grow ampule inoculum to seed larger tubular PBRs for biofuel production [19]. Indeed, helical tubular PBRs are applied successfully in pilot plants named Biocoil at UK and Australia [75]. Albeit, this version of helical tubular PBR also, suffers from the previously mentioned fouling problem and maintenance of high turbulent flow.

Another improved brainchild derived from horizontal tubular PBR is the inclined or near-horizontal tubular PBR. In this type of reactor, series of tubes with small diameter are arranged and connected to a manifold at the bottom to provide compressed gas and to a degasser at the top [75]. The support system is inclined at a certain angle of 6–12% in attempts to increase the velocities of rising bubbles, gas hold-up and gas transfer coefficients [75]. Wang et al. [75] reviewed the feasibilities of closed PBRs for microalgae biomass production and documented that increased inclination angle increases the gas transfer coefficient and hold-up time and at meantime, notably decreases the mixing time needed. A design hypothesis was concluded, whereby an inclination angle of 45° was the finest and recommended. This is owing to the reason that structural framework to support column inclined beyond that angle costs money [75].

3.2.2. Flat panel PBR

Another type of reactor that dominates present decade production of microalgae is the flat panel PBR (Fig. 9) [65]. It has a cuboidal shape [59] and dense microalgae culture is passed through flat panels [68] made of transparent or semi-transparent materials like glass, plexiglass, polycarbonate and plastic bags [74]. Its high surface-area-to-volume ratio as well as cell densities makes it popular for pure algae cultivation or algae-wastewater cultivation [68]. Flat panel PBR can be classified as indoor type with exposure to artificial light or outdoor by sunlight penetration [74] with nominal path [59]. The agitation is provided by either mechanical rotation of a motor or air bubbling through perforated tube [68]. Flat panel PBR present diverse advantages for mass culture because the possibilities for the dissolved oxygen (DO) to be accumulated is low along with high photosynthetic efficiency in comparison with tubular-type PBRs [68]. Nevertheless, continuous research experiments and observations are being done to assess microalgae biomass scale-up and lipid productivity in flat panel PBR with reference to its configuration designs. Evaluation of the system for large scale cultivation mandates optimization studies including illumination, agitation, airflow, temperature, hydrodynamic properties, mass transfer and many more. Review by Faried et al. [68] implies that current design of flat panel PBRs are limited fluctuations in culture temperature, microalgae cell growth on the wall and the need for many compartments

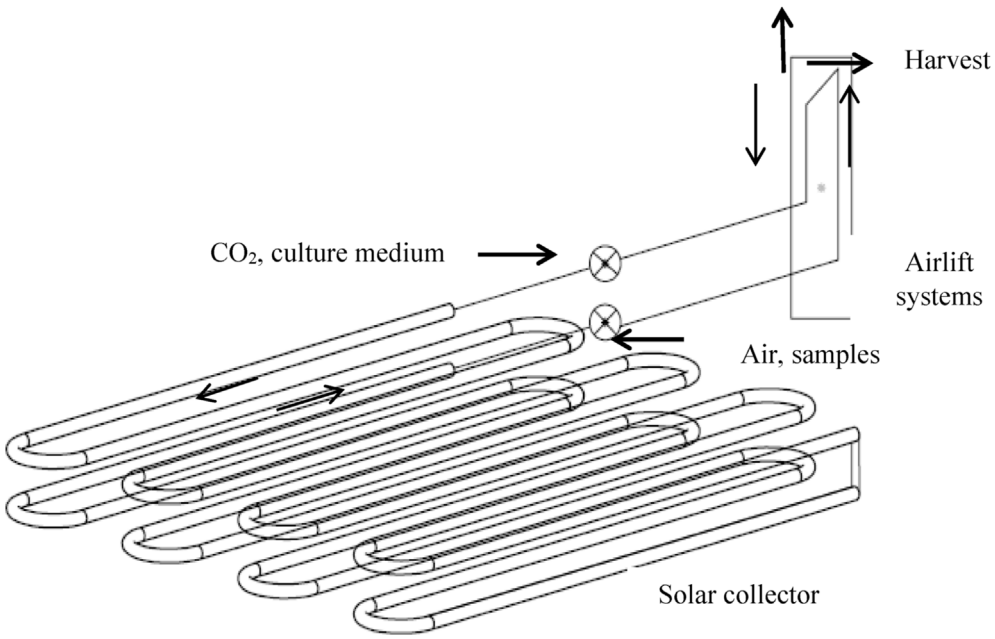

Fig. 7. Helical-shaped PBR [64].

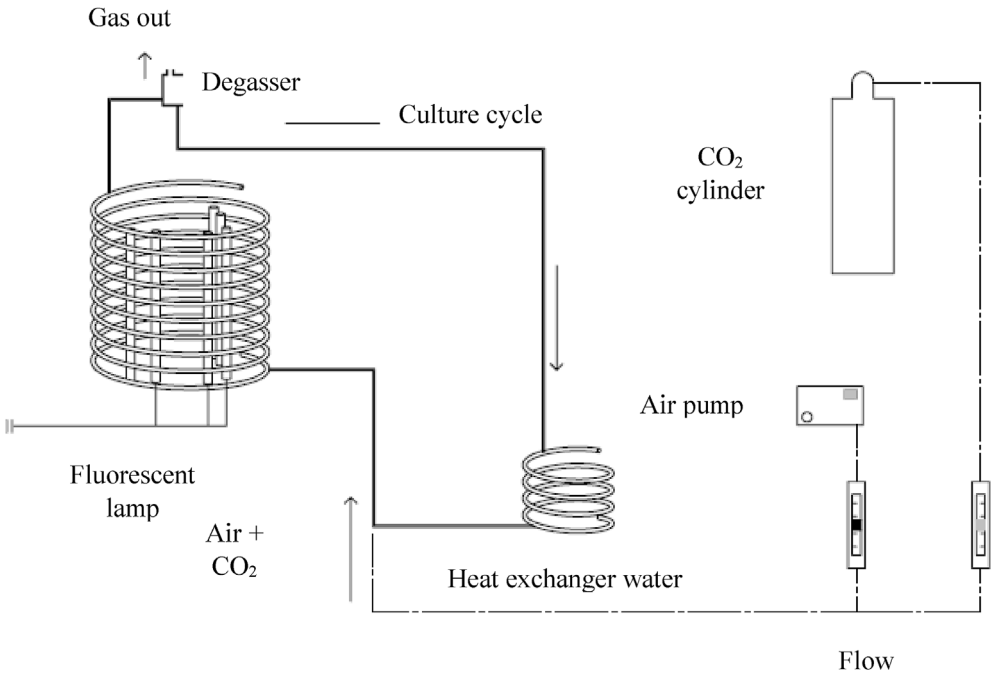

Fig. 8. A helical tubular PBR with modification [76].

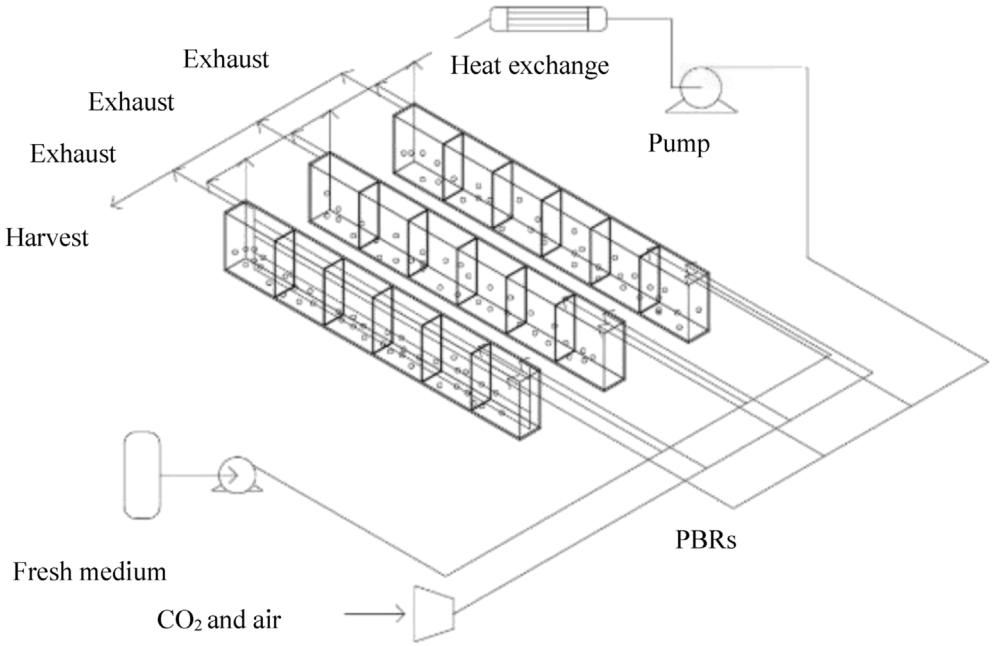

Fig. 9. A flat plate PBR [77].

for scaling-up purposes.

For commercialization purpose, both open ponds and PBR systems can be used to produce microalgae biomass for conversion into biofuel products. PBRs demonstrate higher areal productivity than open ponds, with heighten capture of solar energy, and more optimal energy consumption for mixing between gas and liquid. It was reported that, power density of 55 and up to 2000–3000 W/m^3 is required for flat panel and tubular PBRs, respectively [65]. Meanwhile, open ponds only utilize 4 W/m^3 of power as they made from relatively cheap construction materials and also less energy requirement for mixing. However, open pond system is only favourable for certain types of microalgae strains i.e. *Scenedesmus* sp., *Chlorella* sp., *Dunaliella salina, Spirulina, Nannochloropsis* sp. whereas tubular and flat panel PBRs are dominated by *Porphyridium* sp. and *Chlorella* sp., respectively. The net energy ratio (NER) of a culture system can be defined as the ratio of the total energy generated (energy content of the oil and residual biomass) over the energy required for the operation of plants [65]. In this context, cultivation systems with NER > 1 is considered economically feasible for large scale biomass production intended for biofuel generation.

Burgess and Fernandez-Velasco [76] used tubular PBR for the photosynthetic production of hydrogen (H_2) by microalgae and an estimate of the NER (> 1) proved that energy consumption is largely dominated by costs related to reactor construction and materials. On the other hand, energy analysis by another researcher revealed that the use of open ponds i.e. raceway for microalgae cultivation is the only process with NER > 1 [65]. Based on the available literatures, Jorquera

et al. [65] later conducted comparative energy life-cycle analyses on microalgae biomass production in both open ponds i.e. raceway and PBRs i.e. tubular and flat panel. In the study, NER > 1 was obtained for raceway pond and flat panel PBR which showcase the economic realization of both culture systems. More recently, Dasan et al. [77] had reviewed life-cycle evaluation of microalgae biofuel production in three cultivation systems including, open ponds, bubble column PBR and tubular PBR. The results attained were adapted and compared with existing NER values for open ponds, tubular PBR and flat panel PBR as presented in Table 5. Apart from that, a precise technical comparison of open pond and PBRs in terms of culture area, productivity and capital costs involved for reactor design and production of biomass are summarized in Table 5. Additionally, technical viability and real case applications of open pond, tubular and flat panel PBRs at industrial level are tabulated in Table 6.

3.2.3. Biofilm PBR

In the case of wastewater treatment using microalgae, separation of biomass from the treated wastewater has been one of the major hurdles, till date. Therefore, fixed system such as biofilm PBR has been proposed as a more flexible design over traditional suspension system [74]. In the biofilm PBR, microalgae cells attach themselves to the supporting materials and wastewater is supplied through the biofilm, which decrease the nutrients concentration that reaches to the microalgae. Most of the biofilms constituted of a bacteria-microalgae colony whereby, when their activity is stable, a balance system between CO_2 and O_2 is realized that eliminates the need for additional CO_2 [78]. This type of PBR enhances the biomass removal efficiency and production of secondary valuable products that offer superiority over suspended PBRs [79]. Selection of supporting materials for microalgae attachment is the most crucial factor that determines the performance of biofilm PBR. The supporting material can be categorized into bio-material and non-bio-material in which the rougher the surface of the materials, the easier the microalgae attachment [74]. However, other criteria such as microalgae strain and characteristics of wastewater also need to be contemplated when selecting the supporting materials. There must be control steps to avoid reaction between the materials and the substances in certain types of wastewater [80].

4. Harvesting of microalgae biomass

Grim reality associated with fossil fuel diminution, sustainability, soaring fuel prices and CO_2 mitigation have directed policy makers and researchers to develop renewable energies and biodiesel from microalgae. Many emerging advancements in upstream and downstream processing of microalgae biomass have been witnessed lately to feed our imperative energy need. However, harvesting step, which is the separation of microalgae cells from broth remains as the major bottleneck in microalgae biofuel production besides critical studies on the yield of biomass composition and oil production [2]. Current harvesting technologies are challenged by the small size of microalgae cells, low density and colloidal stability, therefore requires large capital cost and energy input for operation [81]. Normally, biomass concentration in diluted nature of microalgae cultures are low, from 0.5 g/L in open raceway pond to 5 g/L in PBRs, due to mutual shading effect of microalgae cells [29]. In other words, a large volume of water has to be removed to recover the microalgae biomass, in which the existing conventional harvesting methods account for nearly one third of the total biomass production cost [81].

Therefore, a harvesting process that can achieve highly concentrated microalgae biomass recovery along with moderate operating costs including energy and maintenance costs would be ideal. Considering the properties of targeted end product, it is also worth to note that, microalgae screening such as strain structure, density and size, moisture level and salt concentration is one of the pivotal important upstream factor that should be assessed in selecting suitable harvesting method [6]. Some microalgae strains pose ease of recovery compared to others, such as *Spirulina* which can be easily harvested by sedimentation [70]. As mentioned earlier, residual microalgae biomass after lipid extraction are rich in proteins and other compounds of commercial interest. Instead of disposing, this residual biomass can be further processed to produce animal feed and wide array of high-value commodities such as cosmetics, nutraceutical and pharmaceutical products through biorefinery schemes. Therefore, the harvesting process should not be toxic as it will contaminate the microalgae biomass while separating them from culture broth, better still if the culture broth can be recycled for further usage to maximize the sustainability of the entire microalgae biomass production. It was reported that in order to produce 39 billion litres of microalgae biofuel, up to 15 million metric tonnes of N and about 2 million metric tonnes of P are required [82]. On the other hand, recycled medium can save approximately 84% of water and 55% of nitrate needed for microalgae growth [82]. Thus, the choice of harvesting method resent consequential effect on reusability and quality of the recycled medium after harvest [82].

Harvesting of microalgae biomass is often a two-stage process to achieve greater separation efficiency at a lower cost, involving thickening to increase the solid concentration of the microalgae culture and is accomplished by dewatering. Dewatering is the separation of concentrated slurry from broth, either by draining off the supernatant or skimming the cells off from the surface. Nevertheless, it is also practical to apply any one of the steps above depending on the amount of water footprint to be processed and the choice of harvesting method. Concentrating techniques comprise of gravitational sedimentation, floatation, electrical-based process and flocculation whereas filtration and centrifugation are the common dewatering steps applied to microalgae broth. All of the above-mentioned harvesting techniques can be categorized into natural, energy-intensive and with the aid of flocculants as depicted in Fig. 10.

4.1. Natural

4.1.1. Gravitational sedimentation

Gravitational sedimentation is best defined as settling of microalgae cells that driven by gravitational forces. It is commonly practised to harvest microalgae as gravitational sedimentation is widely recognized as simplest and most inexpensive method compared to others [83]. The

Table 5
Technical comparison of open ponds and PBRs [82,83].

Cultivation system	Favourable microalgae species	Culture area (ha)	Productivity (g m^{-2} day^{-1})	Reactor (per ha) and microalgae mass (per DCW) capital costs			NER
				Capital items included	$ ha^{-1}	$ kg^{-1} DCW	
Open pond	*Scenedesmus* sp., *Chlorella* sp., *Dunaliella salina, Spirulina, Nannochloropsis* sp.	0.2–4	12–30	Liner, paddlewheel, pond	10,111–76,132	0.12–1.39[a]	0.275–3.5
Tubular PBR	*Porphyridium* sp.	10	16	Liner, pump, tube, blower	189,606–303,461	1.03–3.19[a]	0.05–0.25
Flat panel PBR	*Chlorella* sp.	1	18	Panel, blower	422,759	0.92[b]	1.65

[a] Calculated from reported algae biomass productivities per ha and reactor cost per hectare.

Table 6
Microalgae commercial culture systems developed at industrial level [84].

Production system	Prospects	Limitation	Company	Scale	Application to wastewater treatment
Open ponds	• Easier to construct, operate, and clean up • Relatively economical after cultivation • Good for mass cultivation	• Poor light utilization • Evaporative losses • Diffusion of CO_2 to the atmosphere • Requirement of large areas of land • Limited to few strains of algae • Cultures easily contaminated	LiveFuels Kent BioEnergy Algaeventure Systems	Demonstration (18.2 ha site) Full (64.7 ha site) Pilot	Possible applications to wastewater treatment mentioned Demonstrated or specifically intended for wastewater treatment Possible applications to wastewater treatment mentioned
Tubular PBR	• Suitable for outdoor • Good biomass productivities • Relatively inexpensive	• High levels of DO • Adverse pH and CO_2 gradients • Fouling • Photoinhibition is very common in outdoor tubular PBRs	Solix Biofuels A2BE Carbon Capture Bionavitas	Pilot (0.8 ha site) Bench Bench	Possible applications to wastewater treatment mentioned Possible applications to wastewater treatment mentioned Possible applications to wastewater treatment mentioned
Flat panel PBR	• Modular design makes it easy to scale up production • Low accumulation of DO • High concentration of sunlight per square cm.	• Temperature control issues • Algae biofilm formation • Strain-specific hydrodynamic stress issues	Bionavitas	Bench	Possible applications to wastewater treatment mentioned

rate of sedimentation is highly selective based on density and radial size of targeted microalgae cells, in which denser and larger cells will settle faster than the ones with low density and small size [70]. However, this phenomenon serves to be the limiting factor of this method as it is time-consuming, low recovery of the microalgae biomass and could lead to chances of biomass deterioration. It is a well manifested fact that biomass is biologically and chemically active throughout the supply chain. This will affect the risks during handling and storing the biomass for prolonged duration as it will deteriorate due to its biological activity while at the same time allergenic spores may accumulate on the surface

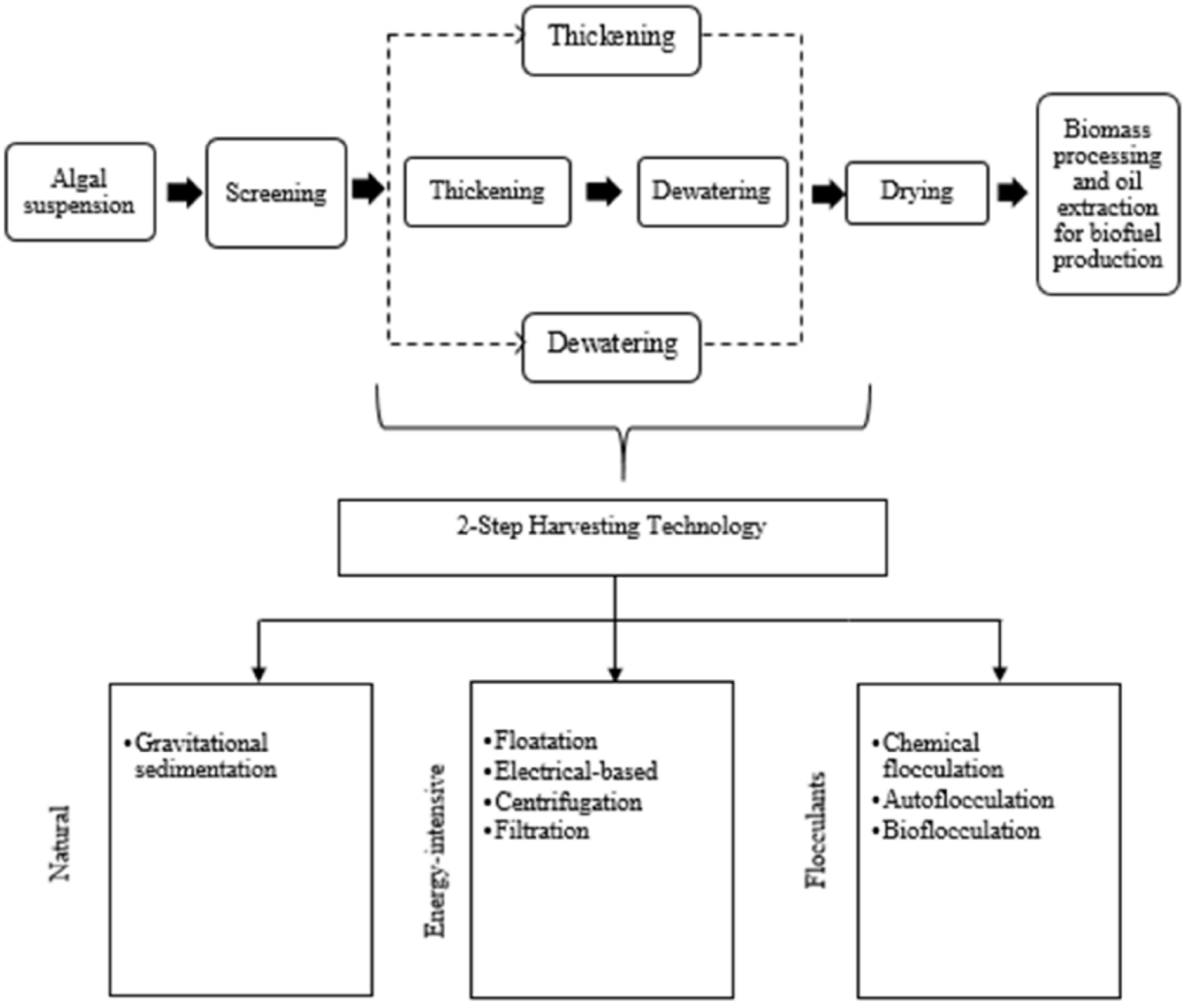

Fig. 10. Category of harvesting techniques.

of biomass suspension over time [18]. Besides that, the biological activity will alter the composition of the active microalgae biomass which is not favourable in the case of biofuel production. If the sedimentation process is carried out in a closed compartment, the biological activity may deplete the O_2 and lead to biomass degradation again. Nevertheless, the harvesting efficiency of this method can be improved with the use of lamella separator and sedimentation tank [18] and in the case of sewage-based processes, sedimentation tanks or settling ponds are generally used in biomass recovery.

4.2. Energy-intensive

4.2.1. Floatation

Floatation is found to be more effective and relatively fast method than sedimentation [70]. Microalgae cells with diameter from 10-30 μm–500 μm can be effectively removed through floatation and often used in combination with flocculation for large scale microalgae harvesting in wastewater due to reduced surface charges on microalgae cells [84]. It is a simple and low-cost method based on physiochemical gravity separation process in which gas bubbles pass through a liquid-solid suspension causing the microalgae to float to the surface by adhering to the gaseous bubbles [57]. Dissolved Air Floatation (DAF) technique separates microalgae from its culture using coupling of both floatation and flocculation. It uses alum to flocculate microalgae or air mixture, with fine bubbles supplied by an air compressor. The size of the bubbles created directly affects the efficiency of the biomass harvesting process induced by floatation [81]. Even though floatation appear to be potential harvesting technique, however the technical viability and upscaling are poorly understood and still at embryonic stage [81]. It has high operational costs that closely associated with the use of energy intensive compressor that functions at pressures of 390 kPa. The main parameter in determining the performance and efficiency of this is the suspended particles instability whereby, higher air-particle contact corresponds to a lower instability. In flotation technique, the size of the particle is the utmost importance, as the smaller the particle size (preferably less than 500 μm), the more likely it will be lifted to the top of the medium by the bubbles [57].

4.2.2. Electrical-based

In very recent years, electrical-based technology such as electrophoresis, electro-flocculation (EF) and electro-flocculation-floatation have been ascertained for lucrative microalgae harvesting [83]. It is rapid and applicable for wide spectrum of microalgae species, turning this method as highly successful in laboratory scale over others [83]. Electrolytic or electrophoresis entails two metallic electrodes, one as nonreactive anode and another one as (sacrificial) cathode [6]. Negatively charged microalgae cells will be attracted towards positively charged anode as a cause of electrophoretic motion [85]. Consequently, microalgae cells undergo charge neutralization (coagulation) and aggregate into flocs where the cells may settle down at bottom of vessel or float on the surface, depends on the density [85]. Being the opposite way, in electro-flocculation, reactive (sacrificial) electrodes are introduced in microalgae broth to produce metal flocculants that will trigger flocculation through following stages: (i) electrolytic oxidation of sacrificial anodes to release metallic flocculants, (ii) destabilization of microalgae cell suspension, and (iii) floc formation of the destabilized particulates as an act of coagulation [70].

Aluminium and iron electrodes are nominated as commonly used anodes in electrolytic flocculation studies [85], however, aluminium anode was found to perform better than iron anode [83]. Aluminium electrodes features high electricity and thus, produce more Al^{3+} ions upon dissociation for enhanced flocculation as compared to iron electrode [85]. Despite the unnecessity for addition of chemical flocculants, this method of microalgae harvesting is challenged by the need for electrodes [70] in which the reusability of electrodes are not favourable owing to internal resistance [85]. Apart from that, some electrodes such as ferric is not suitable for microalgae biomass collection as it tend to produce coloured cells after aggregation [85]. In a view to culminate environmental problems, electrical-based methods are sought after for being eco-friendly, inexpensive and energy-efficient [85] but its effectiveness for large-scale harvesting is yet to be explored [83]. In order to move this technology from lab scale to industrial scale, high energetic and electrophoretic equipment costs will need to be faced [85]. It is reported that 0.2 kWh m^{-3} of energy is needed for current density of 0.5 mA cm^{-2} while 2.28 kWh m^{-3} for 5.0 mA cm^{-2} [85].

4.2.3. Centrifugation

Centrifugation is a commonly applied method in microalgae biomass recovery in which centrifugal force is used to separate the broth [70]. This method is fast thus, often preferred over gravitational sedimentation and offers high biomass recovery rate up to 95% under optimized condition [27]. Furthermore, centrifugation is feasible for all microalgae strains, adding to the fact that the equipment is easy to clean and low risk of bacterial contamination of biomass [70]. Nonetheless, this technique of harvesting can be expensive as the equipment and its parts such as centrifugal pump requires high energy input for operation and maintenance costs. The use of centrifugation method to harvest microalgae cultures from 0.04% to 4% dry weight on average costs 1.3 kW h/m^3 of pond water [67]. As cost minimization is the core target of almost all industries, this method is therefore, not ideal for large scale microalgae harvesting. Owing to its hygienic operation, centrifugation is more satisfactory to be used for recovery of high-value products which will give high turn around with good profit. Another limitation of this method is the possibility of cell damage due to high shear forces that will cause release of microalgae intracellular materials into culture broth. It is important to note that additional downstream processing is required to accomplish biomass collection upon mixing of intracellular materials which in turn costs money. However, filtration can be contemplated as an optional technique to harvest fragile microalgae cells [27] as described in next section.

4.2.4. Filtration

Recent advancement in biomass recovery employs the use of membrane filtration to separate microalgae cells with smaller cell dimensions like *Scenedesmus*, *Dunaliella* and *Chlorella* species. Conventional filtration is aided by microstrainers, with size normally more than 70 mm due to its simplicity and ease of availability, but great deal of studies had reported to carry out flocculation prior to microstraining to flocculate smaller sized cells into bigger flocs [18]. Nonetheless, microstraining is efficient for large microalgae cells such as *Coelastrum proboscideum*, *Spirulina* and *S. Platensis* [27]. There are two types of membrane filtrations namely microfiltration (pore diameter of 100 nm–10,000 nm) and ultrafiltration (pore diameter of 1 nm–100 nm) [27]. Different materials are explored to produce membranes of different geometries like compressed, tubular, multi-channelled, hollow, capillary or spiral based on their field of application [27]. For instance, polymer membranes were found to be effective to harvest marine microalgae species such as *Haslea ostrearia* and *Skeletonema costatum*, but is challenged by the hydrodynamic conditions along with microalgae characteristics and cell concentration [70]. On the other hand, studies by microalgae experts proved that freshwater microalgae species such as *Stephanodiscus hantzschaii*, *Cyclotella* sp., *Rhodomonas minuta*, and *S. Astraea* can be harvested with 70–89% by using tangential flow filtration, that incorporates high rate filtration for harvesting cells [18].

It is widely established that, protozoans and viruses are eliminated via this technique which allows reusability of culture broth [27]. Even though membrane filtration enables high recovery of shear-sensitive species, possibility of membrane fouling has been well documented as one of its drawback [70]. Regular cleaning and replacement of expensive membranes present significant challenge in biomass processing area and thus, the focus on designing feasible and cost-effective harvesting strategy has been shifted to use the of flocculants. However,

emerging membrane technology with cheap and sustained production of membranes could elevate current filtration technique a step above in the near future. Nurra and colleagues have tested commonly used membrane materials (ceramic, polysulfone and polyacrylonitrile) and other new ones such as (acrylonitrile butadiene styrene, glycol-modified polyethylene terephthalate and polylactide [86]. Their experiment results revealed that polysulfonePluronic®F127 blended membranes and polyacrylonitrile membranes showed high permeability but relatively expensive. Polylactide membranes are cheap, possesses good mechanical properties and biodegradability, but low permeability. On the other hand, glycol-modified polyethylene terephthalate was cost-effective and highly permeable but poor mechanical properties. Acrylonitrile butadiene styrene was regarded as best membrane material with permeability value up to 19 ± 0.9 L/h/m^2/bar. Significantly, novel introduction of cheap biodegradable polylactide polymer was made to harvest microalgae cells [86].

4.3. Flocculants

Due to energy-intensive and costly harvesting techniques, addition of flocculants to promote microalgae cell aggregation for high-density floc formation started to hit the dewatering trends three decades back. Microalgae cells are predominantly carry negative charges due to the ionized functional groups that present on their surface and also adsorption of ions from organic matter, causing cell-cell repulsion [70,85]. Hence, stable microalgae cell suspension must be upset through addition of flocculants to enable aggregation or floc formation via coagulation [81]. In line to face paramount challenges in microalgae harvesting process, myriad of stratagem have been mapped in flocculation studies involving chemical flocculation or by cheap and toxic-free methods using bioflocculants or natural biomass-derived flocculants or by altering culture conditions (autoflocculation) [85].

4.3.1. Chemical flocculation

Chemical flocculants can be classified into inorganic and organic flocculants, depending on the C nature [85]. Multivalent or polyvalent metal salts, such as $Fe_2(SO_4)_3$ and $FeCl_3$ are examples of inorganic flocculants that frequently used wastewater treatment and microalgae harvesting [81]. Apart from that, alum is also being used commonly, which is the name given for several trivalent sulfates of metal such as aluminium, chromium, or iron and univalent metal such as potassium or sodium. Despite being widely used, inorganic flocculants are toxic and generate huge volume of sludge which in turn needs further dewatering steps [2]. Microalgae flocculation can also be induced through addition of organic flocculants or cationic polymers such as chitosan and starch [81]. However, their feasibilities as ideal flocculants are challenged by high cost (Table 7) [81] and the microalgae culture condition itself [81]. Low pH is highly preferred for effective microalgae flocculation but affected by the growth phase of the microalgae, in which culture harvested in the late log and early declining growth phases are able to flocculate well upon addition of cationic flocculants [81]. Therefore, in order for the chemical flocculation to be ideally applicable for large scale biomass processing, cheap, safe and easily produced chemical flocculants need to be explored.

To address problems associated with large water footprint needed for mass microalgae cultivation, Farooq et al. [82] investigated the possibilities of medium recycling for cultivation of *Chlorella vulgaris* using chemical flocculation with $FeCl_3$ and alum owing to cost and energy factor, whereas centrifugation was used as reference harvesting method. Also, harvesting efficiencies of each method were also analysed with respective to quality of medium, biomass and lipid productivity together with standard of biodiesel produced. Based on the results, the biomass recovery by ferric chloride was comparable to centrifugation but lower than alum. However, an increase in biomass concentration demands higher concentration of flocculants than the biomass concentration itself which seemed unacceptable [82].

As for growth of *C. vulgaris* in recycled medium, only mediums obtained from centrifugation and ferric chloride harvest supported the growth of *C. vulgaris* as compared to control after nutrients adjustment while medium from alum harvest retarded the growth rate of microalgae. Additionally, medium obtained from harvesting by centrifugation and ferric chloride enhanced the biomass and lipid productivity of the microalgae cultivated. Therefore, it was clearly demonstrated that ferric chloride was better chemical flocculant over alum in case of reusability of culture medium and subsequently sustainable microalgae-based refinery. Nevertheless, the major controversy lied in the removal of metal ions from the biomass and biodiesel produced. In the study conducted, the amount of ferric ions were lowered from 58% to 1.5% by lowering the pH values and the recovered metal ions were reused for subsequent microalgae harvesting. On the other hand, majority of residual ferric ions that present in the FAMEs were removed by washing with water. Even though residual metal ions were effectively removed through cost-cutting methods, complete removal is necessary for oxidative stability of the produced biodiesel and positive engine performance. Table 8 presents the comparison of microalgae harvesting efficiencies by various chemical flocculants [6,32,85].

4.3.2. Autoflocculation

Autoflocculation is a substitutive concept over chemical flocculation which mediated by increase in pH due to CO_2 depletion [83]. It is cheap, safe, low energy requirement with zero use of flocculants which enables reusability of medium [6]. At basic pH, precipitates of calcium and magnesium are formed automatically which induces flocculation of microalgae cells [87]. In previous studies reported by Kim et al. [84] flocculation performances of Botryococcus braunni with auto-, inorganic and polymer flocculation were evaluated and autoflocculation displayed the highest harvesting efficiency for 3 weeks culture. Vandamme et al. [35] have drawn attention towards investigation of different techniques to promote autoflocculation of *C. vulgaris* and it was found that the addition of calcium hydroxide increased the biomass concentration *C. vulgaris* culture up to 50 folds, which was low cost and environmental friendly. Microalgae cultivation is coupled with wastewater treatment for excess phosphate removal through interaction of positively charged calcium ions and negatively charged microalgae cells which results reserves for phosphate sources, turning the algae cells surface active [87]. When the pH increases, flocculation is induced due to formation of inorganic precipitates which later present in the harvested biomass through addition of metallic salts, an alkaline compound, or polyelectrolyte [87]. Apart from commonly used calcium hydroxide, other alkaline compounds such as sodium hydroxide, potassium hydroxide or magnesium hydroxide can be used to promote autoflocculation [87]. Magnesium hydroxide was used to flocculate

Table 7
Price estimates of flocculants [78].

Type of chemical flocculant	Flocculant name	Price/kg (in US$)	Cost involved in flocculation for 1000 L (in US$)
Inorganic	$FeCl_3$	14.1	0.7
	$Al_2(SO_4)_3$	5.6	0.3
	$CaCl_2$	60.7	3.7
Organic	Chitosan	207.2	31.1
	Carboxymethyl cellulose	18.3	2.2
	Rice starch Maize starch Oxidized starch Tapioca starch Yellow dextrin Potato starch Pregelatinized starch Cationic starch	5.9 (1 kg of each, total 8 kg) US$ 0.7 approximately per kg	0.1

Table 8
Comparison of microalgae harvesting efficiencies by various chemical flocculants [6,30,82].

Microalgae strain	Flocculant	Dose (mg/L)	Harvesting efficiency (%)
Anabaena sp.	$Fe_2(SO_4)_3$	0.25 mM/L × 100	≤78
Anabaena sp.	Polyferric sulphate	0.25 mM/L × 100	≤95
Anabaena sp.	Aluminium sulphate	0.25 mM/L × 100	≤95
Asterionella sp.	$Fe_2(SO_4)_3$	0.25 mM/L × 100	≤70
Asterionella sp.	Polyferric sulphate	0.25 mM/L × 100	≤93
Asterionella sp.	Aluminium sulphate	0.25 mM/L × 100	≤95
Chlorella vulgaris UTEX-265	Nano-aminoclays (Mg^{2+} or Fe^{3+} with 3-aminopropyl triethoxysilane [APTES])	1000 g/L	98
Chlorella consortium	$Fe_2(SO_4)_3$	250	90
Chlorella consortium	$FeCl_3$	250	98
Chlorella minutissima	$AlCl_3$	750	80
Chlorella minutissima	$Fe_2(SO_4)_3$	750	80
Chlorella minutissima	$AlCl_3$	500	90
Chlorella sorokiniana	$Fe_2(SO_4)_3$	250	98
Chlorella sorokiniana	$FeCl_3$	250	66
Chlorella sorokiniana MIC-G5	Aluminium sulphate	50	~70
Chlorella sorokiniana MIC-G5	Calcium chloride	90	~20
Chlorella sorokiniana MIC-G5	Ferric chloride	200 μM	> 80
Chlorella stigmatophora	$FeCl_3$	25	90
Chlorella sp. MCC29	Aluminium sulphate	50	> 50
Chlorella sp. MCC29	Calcium chloride	150	> 70
Chlorella sp. MCC29	Ferric chloride	1000 μM	> 80
Chlorella sp. MCC6	Aluminium sulphate	50	~20
Chlorella sp. MCC6	Calcium chloride	150	> 40
Chlorella sp. MCC6	Ferric chloride	100 μM	~50
Chlorococcum sp.	$Fe_2(SO_4)_3$	150	87
Chlorococcum sp.	$FeCl_3$	150	90
Dunaliella salina	$FeCl_3$	8.0×10^{-4} mol/L	85
Muriellopsis sp.	$Al_2(SO_4)_3$	1.42×10^{-4} mol/L	10
Microcystis aeruginosa	$AlCl_3$ + Chitosan	15 + 7	71.55
Phaeodactylum tricornutum	$Al_2(SO_4)_3$	0.27 kg/DCW algae	82.6
Phaeodactylum tricornutum	PAC (Polyaluminium chloride)	0.27 kg/DCW algae	66.6
Scenedesmus obliquus	Ferric sulphate	100	458 mg algae/mg flocculant
Scenedesmus obliquus	Aluminium sulphate	200	189 mg algae/mg flocculant
Scenedesmus obliquus	$Fe_2(SO_4)3$	100	96
Scenedesmus obliquus	$FeCl_3$	100	95

three microalgae species namely, *Chlorella vulgaris*, Scenedesmus sp., *Chlorococcum* sp. and achieved flocculation efficiency up to 90% as documented in studies reported by Shuba and Kifle [81]. However, consideration need to be made to label autoflocculation as a potential harvesting method as in order for it to happen, the amount of calcium, magnesium and P ions should be sufficient in the culture broth. In such case, seawater and wastewater that are abundant in ions are suitable medium for autoflocculation [84]. Apart from that, the effects associated with addition of base to induce flocculation and acid to neutralize the pH should be treated in detail in terms of economic feasibility and environmental impact [84].

4.3.3. Bioflocculation

Naturally, bioflocculation interaction occurs in lakes or ponds which referred to flocculation induced by secreted extracellular polymer substances [87] by flocculating microalgae strain, bacteria or fungus [6]. Bioflocculation may be considered as a cost-effective alternative method to harvest microalgae over autoflocculation or chemical flocculation without alteration of culture condition or use of expensive and toxic flocculants, respectively [6]. This presents incredible opportunity for economical processing of microalgae biomass. Nonetheless, usability of these harvested biomass for food and feed purposes remain largely speculative as co-cultivation of microalgae with bacteria, fungi or flocculating microalgae lead to microbiological contamination [6]. Contradictorily, interaction between microalgae and added bacteria, fungi or flocculating microalgae found to increase the lipid secretion, creating tremendous impetus on biofuel research [6]. Additionally, effective reusability of the culture broth for subsequent microalgae cultivation attracted mounting interest among growers as upstream costs are greatly reduced [6]. However, to move bioflocculation into successful applied pipeline, secretion of concentrated flocculant in high amount along with attachability of microalgae towards floc formation must occur, which reserves enormous demand for biotechnology and bioengineering input [6]. Also, bioflocculation method has been widely established in wastewater treatment plants only due to cost and energy efficiency. Thus, pivotal importance need to be given on understanding the reaction mechanism [87].

4.3.3.1. Fungus-derived bioflocculant. Fungus aided microalgae flocculation is a natural occurrence in lichens through symbiosis, where CO_2 fixation takes place in microalgae to produce organic compounds that promote fungal growth and forms microalgae entrapment by hyphae production [6]. Most investigations reported that fungal-based bioflocculation is applicable for both heterotrophic and autotrophic microalgae species [6]. Furthermore, a number of filamentous fungi like *Rhizopus oryzae*, *Penicillium expansum* and *Mucor circinelloides* are capable of forming pellets up to 2–5 mm of diameter which have been widely implemented in wastewater treatments plants to remove sludge solids via entrapment [6]. Apart from that, some fungi such as Trichoderma viride NRC 314 was found to secrete lipid up to 30% of the total biomass, making them interesting feedstock candidate for biodiesel generation along with microalgae biomass [6,88]. In fact, there is no requirement for alteration of culture condition for this harvesting technique and permits medium recycling [6].

Co-culture of microalgae, mostly *Chlorella vulgaris* strains, with filamentous fungi to achieve assisted bio-flocculation has been recently reported by various authors [89–92] and extensively reviewed by Gultom and Hu [93]. Present studies on effects of ions on harvesting of *C. vulgaris* via *A. niger* co-pelletization reveals that pH values and the ionic strength of the medium are the significant parameters that affect the co-pelletization process and the surface charges of cells were examined through zeta potential measurement [94]. It was found that,

Table 9
Nutrient composition of animal by-product proteins [94].

Item	Meat and bone meal (MBM)	Blood meal[a]	Feather meal	Poultry meal
Crude protein, %	50.4	88.9	81.0	60.0
Fat, %	10.0	1.0	7.0	13.0
Calcium, %	10.3	0.4	0.3	3.0
Phosphorus, %	5.1	0.3	0.5	1.7
TMEN, kcal/kg	2666	3625	3276	3120
Amino acids				
Methionine, %	0.7	0.6	0.6	1.0
Cystine, %	0.7	0.5	4.3	1.0
Lysine, %	2.6	7.1	2.3	3.1
Threonine, %	1.7	3.2	3.8	2.2
Isoleucine, %	1.5	1.0	3.9	2.2
Valine, %	2.4	7.3	5.9	2.9
Tryptophan, %	0.3	1.3	0.6	0.4
Arginine, %	3.3	3.6	5.6	3.9
Histidine, %	1.0	3.5	0.9	1.1
Leucine, %	3.3	10.5	6.9	4.0
Phenylalanine, %	1.8	5.7	3.9	2.3
Tyrosine, %	1.2	2.1	2.5	1.7
Glycine, %	6.7	4.6	6.1	6.2
Serine, %	2.2	4.3	8.5	2.7

[a] Ring or flash dry.

surface charges of the fungi *A. niger* and microalgae *C. vulgaris* cells are not the only factor influencing the co-pelletization process but also the zeta potential. Analysis on zeta potential values suggests that it is possible that the degree of repulsion and dispersion between these microorganisms is low which facilitates the attraction between them. In addition, it was observed that Ca^{2+} and Mg^{2+} affected the concentration of microalgae in the pellet. However, without Ca^{2+} and Mg^{2+}, the harvesting efficiency was still high (> 90%). It was also observed that the fungus could survive and form pellets at high salinity levels (30 g/L of NaCl), which suggested that the fungus could possibly be used to harvest marine microalgae through co-pelletization. In order to fully understand the mechanism of microalge/fungi co-pelletization, other factors and mechanisms that assist in fungal-microalgae cell attraction need to be studied, such as internal pH of fungi or hydrophobic-hydrophilic properties of the cells involved or protein-carbohydrate interactions between the cells.

4.3.3.2. Waste biomass-derived bioflocculant: A novel introduction

4.3.3.2.1. Animal protein waste. By-product proteins sourced from agriculture waste are widely used in animal feeding for decades. However, the supply of protein waste seemed to be unparallel with the market absorption resulting from dramatic utilization of agricultural fats and carbohydrates for generation of biodiesel and bioethanol, respectively [95]. Animal by-product proteins like heads, feet, offal, excess fat, blood, feathers, and bones [96] are often processed into rendered protein meals (RPMs) that includes items such as meat and bone meal (MBM), blood meal, and feather meal [97] to be channelled to animal feeding industries. The production is in concomitant excess of its demand for feed purposes and further aggravated by governal feeding stipulations in several countries [95]. In Japan, MBM was used as components in animal feed before the outbreak of Bovine Spongiform Encephalopathy (BSE) in 2001 [96], commonly known as mad cow disease [97]. Apart from that, there is limited commercial value for some animal protein sources such as chicken blood and its under-utilization leads to environmental issues and debates on disposal cost is still evident [3].

Therefore, to solve the avalanche of protein wastes, especially animal by-product proteins, various diversified approaches to incorporate these proteins into non-food industrial applications are being sought. Present decade witnesses the successful application of protein wastes in manufacturing of wood products as an adhesive agent and intensive researches are ongoing for other applications such as control agent for plant pathogens, valuable N capital for fermentation, significant ingredient in plastic production and more interestingly in conversion of protein into fuel as mentioned in previous sections. In line to seek inexpensive renewable flocculant over the years, meat and bone meal (MBM) were discovered for flocculability by Piazza and Garcia from United States Department of Agriculture on 2010 [95]. Following the discovery, a series of novel experiments have been conducted by the researchers till date for premium extraction of renewable bioflocculants from several products of RPMs.

The first research paper on successful clay flocculation by MBM was produced by Piazza and Garcia on 2010 in which flocculation in clay (kaolin) suspension was induced through addition of polyacrylamide (PAM), an anionic polymeric flocculant together with few types of protein extracts such as soy proteins (PF891 and PF974), protein fraction from MBM, porcine skin gelatin, and whey protein Provon [95] in a laboratory scale. Sedimentation-flocculation studies were conducted at neutral pH 7.0 to verify the flocculability of each agents in the absence and presence of buffer solutions and calcium ions, respectively, and the kaolin sedimentation efficiencies were compared [95]. Calcium ions in the form of calcium chloride was added to allow charge bridging between the clay particles and flocculants and subsequently to form flocs for some cases [95]. The two soybean proteins showed poor flocculation activity whereas whey protein was unable to flocculate clay. However, soy proteins were able to function effectively upon addition of acidic buffer but not as effective as PAM, gelatin and MBM.

Calcium ions were not required for clay flocculation by gelatin and MBM as they able to promote flocculation in both absence and presence of calcium chloride and acidic buffer. Addition of calcium chloride did not alter the performance of MBM but contributed minorly to the flocculability of gelatin as its effective concentration was lowered to 0.202 g/L. This observations suggested that protein fractioned from MBM might carries positive charge in overall at tested pH and interacting with clay particles through positively-charged amino groups [95] by coagulation [97]. Net charge of a protein is determined by pH value of the medium in which the change in charge is termed as protein isoelectric point (pI) and is selectively different for each protein's unique amino acid composition [97]. The pI of MBM proteins are yet to be found but amino acid analyses of broad range of MBM samples display 14.8% and 21% of basic and acidic amino acids content in MBM [95]. Table 9 shows the nutrient composition of animal by-product proteins that obtained from Ref. [98].

However, findings by Piazza and Garcia [95] is limited by the cost of producing flocculant from MBM. Nevertheless, the cost could be reduced tremendously by carry on flocculation-sedimentation at pH 5.5. Additionally, as the flocculation is accelerated by supply of calcium ions, the production cost for flocculant can be keep at minimum [95]. The major hurdles in producing cost-effective flocculant from protein source is the extraction process, as only less than 10% of protein could be extracted using aqueous reagents from MBM [95]. Moreover, lower folds of molecular weight (MW) of extracted protein molecules is another drawback that need to be tackled by means of simple chemical procedures to reduce the production cost too [95].

Research on flocculants developed from animal protein waste by Piazza and Garcia was further expanded by his team by conducting clay flocculation using hydrolysates of blood meal, feather meal and MBM [97]. Previous findings limited by the cost factor and unefficient extraction of protein molecules which later on improved through alkaline hydrolysis and by using enzymes such as proteases, Versazyme, Flavourzyme, and Alcalase together with peptone and hydrolysed fish and bovine collage (gelatin) [97]. With these documented informations on functionality of bioflocculants extracted from animal protein waste, further research experiments to flocculate microalgae cells should be conducted as these bioflocculants contain various positively-charged functional groups that able to neutralize negatively-charged microalgae cells and subsequently induces floc formation. Although there are technical similarities between clay flocculation and microalgae harvest,

it is necessary to refine the overall approach and the technical needs that are unique to microalgae recovery.

4.3.3.2.2. Plants and fruit-based bioflocculant. Recently, plants and fruits-based bioflocculants are found to be attractive alternatives over polymeric flocculants for microalgae harvesting [99]. Natural polymeric flocculants or biopolymers like chitosan and grafted starch possesses satisfactory harvesting efficiency for microalgae biomass at lower dosage requirement with reduced environmental risks compared to metallic flocculants [84]. On the other hand, polymeric flocculants that derived from biomass feedstocks, marine resources, and microorganisms undergo various pre-treatments and chemical modifications to improve their production quality and performances [100]. Nevertheless, the application of natural biopolymeric flocculants is limited by their expensive production cost compared to conventional synthetic flocculants. In order to address the drawbacks associated with costs, myriad of initiatives have been devoted to develop natural flocculants or biopolymers from waste biomass [100].

Over the past few years, researches have been conducted on different types of natural flocculants from waste plant and fruit parts, and prominent bioflocculants include *Moringa oleifera*, *Stryconus potatorum*, Cactus species, surjana seed, maize seed, tannin and gum arabic [15]. *Moringa oleifera* showed excellent flocculating properties for dye wastewater treatment, while *Cicer arietinum* was reported as effective coagulant for turbidity reduction [15]. *Acanthocereus tetragonus* for dye wastewater treatment was also reported in recent papers [15]. Vijayaraghavan et al. [101] have reviewed traditionally used plant-based coagulants in wastewater treatment applications which includes, Nirmali seeds (*Strychnos potatorum*), *Moreinga oleifera*, tannin and cactus. Another review focusing on use of 16 types of plants' seed in removing turbidity and heavy metals from wastewater locally was done by Edogbanya et al. [102], including *Moringa oleifera*, *Prosopis juliflora*, *Cicer arietinum*, *Dolichos lablab*, *Phaseolus vulgaris*, *Parkinsonia aculeate* and etc. The authors highlighted the methodologies involved in the preparations of bioflocculant from the plants' seed that are extremely simple and without use of any chemicals.

In 2013, Choy and his colleagues had extensively reviewed commonly studied vegetables and legumes for water clarification purposes [103]. Research summaries on their preparation, experimental conditions and optimization studies were presented collectively for 14 types of vegetables and legumes in which the preparation steps involve both simple and vigorous chemical extractions [103]. Following that, another review have been documented by Choy and his colleagues on 21 types of plant-based coagulants that categorized into fruit waste and others (cactus, cereals, fungus, nuts, shrubs and spice) and key findings on previous flocculation studies conducted on each waste has been described [13]. In spite of the many published literatures on outstanding flocculability of these bioflocculants from waste biomass, most of their preparation processes involve purification after the proteins have been extracted and use of waste plant and fruit parts with minimal modifications or pre-treatment process were still scarce [15].

Narrowing down the focus in microalgae harvesting, one of the most established bioflocculant in wastewater treatment studies, *Moringa oleifera* was investigated for its flocculation activity in harvesting *Chlorella vulgaris* [104]. Preliminary studies proved the effectiveness of milled seeds of *Moringa oleifera* in flocculating *Chlorella vulgaris* and *Scenedesmus* sp., in which 1 g/L of bioflocculant concentration with 240 min of sedimentation time able to yield biomass recovery up to 84% and 72%, respectively. Thus, a series of experiments involving parameter studies like pH, sedimentation time and bioflocculant concentration were conducted to explore the full potential of *Moringa oleifera* as flocculating agent to harvest *Chlorella vulgaris*. The dry pods of *Moringa oleifera* were shelled and grounded, then sequentially sieved through both 860-and 420-μm pore sieves in order to obtain the seed flour used in the flocculation assays [104]. At the end of experiments, flocculation efficiency of about 80% could be attained using 0.6 g/L in a sedimentation time of 120 min which is comparable to alum which yields 72% of flocculation efficiency at 2 mg/L dosage with a settling time of 10 min [104]. It was reported that, the economic feasibility of *Moringa* seeds as bioflocculant is highly dependent on parametric studies, with pH being prioritized, as a tool to investigate the mechanism and optimization of their flocculation activity. Moreover, suggestions to lower the cost while maintaining the efficiency were given as such different extraction conditions should be explored like saline extracts instead of aqueous solutions [104]. It is also of utmost importance to identify the active compounds that responsible for the flocculating activity of *Moringa* seeds.

To further validate the findings, recovery of *Chlorella vulgaris* biomass through flocculation using *Moringa* seed cake was conducted as an initiative to lower the cost since seed cake is a residue from biodiesel production using oil from *Moringa* seed [105]. The use of seed cake extracts generated the best cost-effective ratio (flocculation efficiency from 78 to 97% with a saving in mass of seed of 75%). The highest efficiency was reached with extracts prepared with seawater and saline NaCl solutions [105]. Therefore, it can be said that high flocculation efficiency of *Moringa*-derived bioflocculant can be improved with mild extraction processes involving simple chemicals. However, extraction process involving chemicals is still debatable when it comes to cost factor and thus, on 2018 Ogbonna and Edeh had investigated the ability of *Moringa oleifera* seed powder, filtrate from cold aqueous suspension of seed powder and autoclaved filtrate were compared for their ability to flocculate *Chlorella variabilis* cells without any pH adjustment [106]. It was claimed that previous research activities using *Moringa oleifera* as bioflocculant demand rigorous extraction steps with pH of the media was adjusted to either highly alkaline or acidic level and these adds to the cost of harvesting the cells [107]. Their findings revealed that *Moringa oleifera* seed able to harvest the cells without any pH adjustment and use of filtered cold water extract of *Moringa* seed resulted in decreased sedimentation of *Chlorella variabilis* cells but high flocculation efficiency could be attained by increasing the dosage and sedimentation time. However, researchers have not dealt in detail with studies to identify its active ingredients which are the fundamentals for optimized extraction processes. The debate has gained fresh prominence with some arguing that the active flocculating agents are proteins while a few researchers noted that they are organic polyelectrolytes [107,108]. In a nutshell, in a view of the limited information on potential bioflocculant derived from crude *Moringa oleifera* extract, it is worthwhile to explore other waste plant and fruit parts in search for new bioflocculants, without using complicated purification or extraction steps that involve chemicals.

4.3.3.2.3. Shell waste. Food and seafood industry produce mounting amount of eggshell and other seashell wastes annually, which leads to irreversible environmental and health risks upon dumping. In United States, approximately 50, 000 tons of eggshell wastes are generated per year, in which the landfill costs ranging from $20 to 70 per tonne, depending on the location [109]. On the other hand, egg production marked about 10 billion in 2011 in Poland which generated equal amount of eggshell wastes to be dumped into landfill [110]. In China, development of oyster cultivation result in large amount of oyster shell residues, where for every 1 kg of oyster shells, about 370–700 g of waste shells are produced [111]. The Port Said coast, Egypt is the famous dumping port for bivalve shells in and big amount of money are spent for its enormous disposal [112]. At global landscape, about 1.5 million tons of crabs are consumed yearly, generating about 0.5 million tons of waste shells [113]. Until now, most seashell waste processing approaches are focused on extracting chitosan and in recent years, these wastes were researched for different purposes, such as heavy metal removal and incorporation into polymer composites. With a view to explore the potential utilization of these wastes, the following section summarizes and reviews a few previous findings on the use of seashells and eggshell as adsorbent and bioflocculant which share the common removal properties with simple preparation steps.

The novel introduction of eggshells as bioflocculant to harvest

Chlorella vulgaris was made in 2015 [114]. Parameters such as pH, dosage, mixing time, mixing rate and settling time were evaluated and it was found that, bioflocculant prepared through simple acid extraction from eggshells able to achieve over 99% of harvesting efficiency at optimal conditions: 80 mg/L flocculant dosage, 150 r/min mixing rate, 20 min mixing time, 20 min settling time and pH 6. The use of eggshells as bio-coagulating material with great properties such zero-toxic, non-corrosive, safe, biocompatible, biodegradable and able to act as adsorbent and floocculant was proved [114]. It is hypothesized that eggshells poses high cationic charge density and therefore, able to absorb and destabilize negatively charged particles such as microalgae cells [114]. It is widely documented that, eggshells and seashells contain 95% of calcium carbonate and the remaining include calcium phosphate, magnesium carbonate, soluble and insoluble proteins [114]. Biogenic calcium carbonate is believed to be a good candidate for inorganic or metal ions removal over geologic carbonates, which are rich in shell wastes [111]. Many types of shell wastes also have been applied in heavy metal and dye removal studies, including shrimp, oyster, crab, fish bone, bivalve mollusc shells which have high potential to be further explored as bioflocculant to harvest microalgae cells.

Rahman et al. [115] studied untreated shrimp shells as adsorbent to remove arsenic from ground water in columns and investigated the effects of various parameters like particle size, adsorbent amount, flow rate, initial concentration, adsorbate volume, and pH. Current adsorption technologies recognizes chitin as superior sorbent for removal of arsenic and other heavy metal ions [115]. Nevertheless, the production process of chitin involve complex chemical extraction with high requirement for acid and base [115]. Thus, work by Rahman et al. [115] investigated untreated or waste shrimp shells with zero pre-treatment as adsorbent to substitute chemically prepared chitin, as raw shrimp shells constitutes 16–20% of chitin which can be utilized for adsorption purposes coupled with optimization studies. At optimized conditions, almost 95% of removal efficiencies could be achieved for all the above-mentioned parameters and it was found that, desorption of arsenic is possible and adsorbate is recoverable. Approximately 80% of arsenic was desorbed from 120.28 μg/L of total arsenic in the tested samples with 100 mL of 4 M NaOH solution [115].

On the other side, Peinemann et al. [116] made state-of-the-art discovery on using seashell powder as adsorbent to recover phosphate from fermentation broth. Commonly, phosphate recovery from solution is being done by adsorption on bases or precipitation by metal ions like magnesium, calcium, iron and aluminium ions [116]. However, precipitation by metal ions are less favourable because of problems associated with detaching of phosphate from metal ions besides the effect on fermentations when recycled water streams are utilized [116]. Therefore, the above mentioned author used seashell powder for phosphate removal studies as seashells contain major amount of calcium ions in the form of calcium carbonate [116]. This can be further applied in microalgae harvesting as the calcium carbonate could be served as bioflocculation agent as reported by Choi [114] in previous research. Table 10 shows application of waste biomass in microalgae harvesting till date [104,105,107,114].

5. Flocculation mechanisms in microalgae culture

Presence of proton-active carboxylic, hydroxyl, phosphodiester, phosphoric and amine functional groups causes microalgae cells to carry negative charge at neutral pH condition. As a result, opposite charged ions will be attracted to the surface of microalgae cells and form a double layer known as Stern layer. Therefore, the electrical double layer, which is the total system of cell surface charge and associated counter ions in the surrounding solution is formed [29]. As for interaction between microalgae cells, the electrostatic repulsion prevents them from sticking together due to the van der Waals forces [73], making the cells stabilized in colloidal dispersion form [29]. But, when counter ions (simple metal salts) are introduced in high concentrations into that stabilized colloidal suspension, the added salt ions will penetrate into the Stern layer. Such phenomenon causes compression and repulsion between colloids, enabling aggregation of microalgae cells which then termed as flocculation [117]. According to the Schulze-Hardy rule, the higher the charge density of the counter ions, the stronger the flocs formed [117]. The formation of aggregates or flocs can be attributed to four mechanisms namely coagulation, sweeping, bridging, and electrostatic patch as illustrated in Fig. 11. Flocculation mechanisms in microalgae suspension work based on the properties of flocculant used and one or more mechanisms could be employed for more efficient floc formation [103]. Table 11 presents flocculation mechanisms reported for few flocculants used to harvest microalgae species [100,114].

5.1. Coagulation

Coagulation is effective or applicable when oppositely charged bioflocculants are added into the colloidal suspension [99]. Positively charged counter ions or polymers strongly adsorb on the surface of the colloids, ultimately cancel the negative charge of the microalgae cells at neutral pH [29,117]. As a result, the electrostatic repulsion between microalgae cells disappears due to thinning of double layer and therefore, cells get closer and forms flocs which will eventually settle down due as flocs get denser [117]. This mechanism is sought to be effective for polysaccharide-based flocculants of low molecular weight [100]. Characterization studies such as quantification of zeta potential is helpful in identifying optimum flocculant dosage needed to achieve effective flocculation [103]. An ideal flocculant dosage sufficient to completely neutralize charge on microalgae cells can be indicated by near-zero net charge [103]. Therefore, optimization of this mechanism is highly dependent on added flocculant dosage and exceeding the ideal dosage can cause restabilization of suspension [103].

Moreinga oleifera, a natural plant-based bioflocculant that been used vastly to treat wastewater, clarify drinking water and recently to harvest microalgae, was indicated to contain cationic proteins of molecular weight ranging from 6.5 to 14 kDa [101]. Owing to its tremendous high charge density, the mechanism of *Moreinga oleifera* was proposed to be coagulation in the case of clay flocculation [108]. The cationic protein fraction from *Jatropha curcas* was also reported to flocculate particles of interest through this mechanism [103].

Table 10
Literatures on application of waste biomass in microalgae harvesting.

Waste biomass	Microalgae species	Preparation/conditions	Flocculation efficiency (%)	Reference
Moreinga oleifera	*Chlorella vulgaris*	Milled seeds, 1 g/L dosage with 240 min flocculation time	84	[110]
	Scenedesmus sp.		72	
	Chlorella vulgaris	Seed flour, 0.6 g/L dosage with 120 min of flocculation time	80	
	Chlorella vulgaris	Seed cake prepared with seawater and saline sodium hydroxide (alkaline extract)	97	[101]
	Chlorella variabilis	Cold aqueous filtrate of seed power, 10 g/L dosage, 180 min of incubation time, 30 min sedimentation time	74	[103]
Eggshell	*Chlorella vulgaris*	Acid extract, 80 mg/L dosage, 150 r/min mixing rate, 20 min mixing time, 20 min settling time at pH 6.	99	[110]

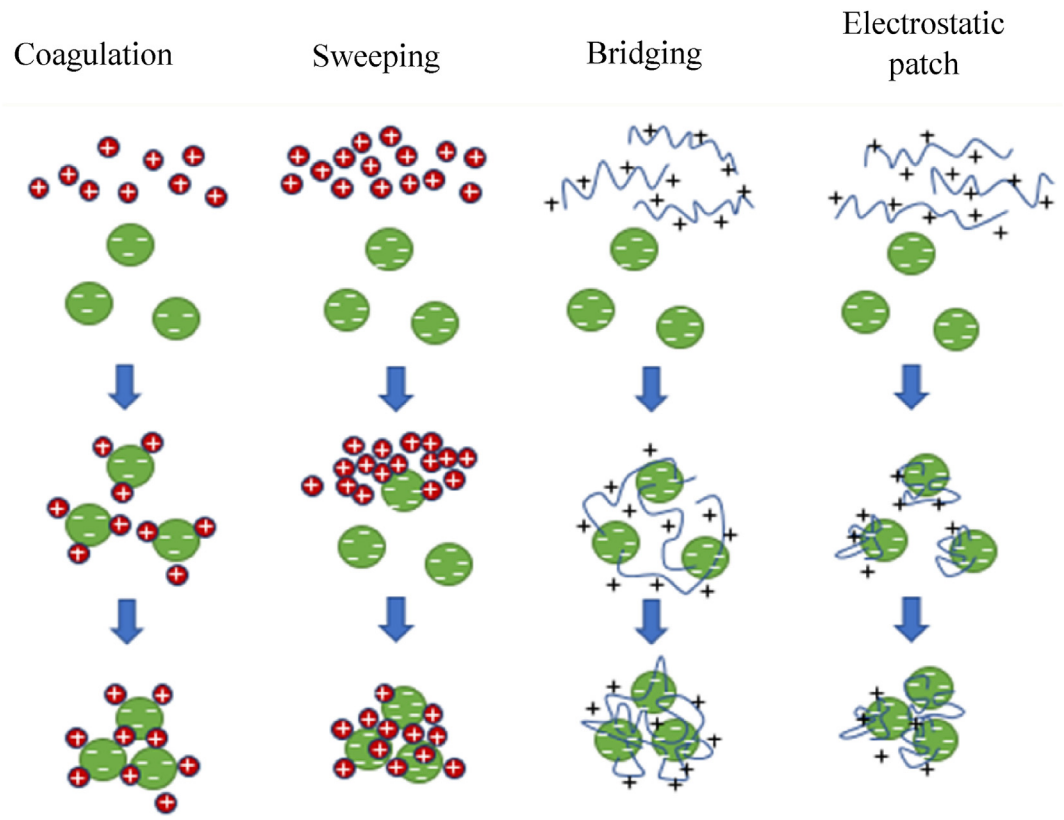

Fig. 11. Illustrations on different types of flocculation mechanisms.

5.2. Sweeping

In this mechanism, microalgae cells are entrapped in massive precipitation of amorphous metal hydroxide ($M(OH)_3$) in the medium when metal salt flocculants are added in sufficiently high amount [117]. These salts act as nucleation sites to promote formation of precipitates and thus could be removed from suspension [103]. Growing literatures on flocculation studies report that sweeping mechanism is favoured for greater aggregation performance compared to coagulation [13]. However, high amount of sludge is being generated at the end of flocculation process is not favourable as great dosage of flocculant is needed then as for coagulation [103]. Divalent and multivalent metal ions such as Mg^{2+}, Ca^{2+} and so on plays important role in the case of microalgae flocculation by pH variation where these ions in the growth medium undergo hydrolysis to form positive precipitates which flocculate negatively charged microalgae cells by sweeping mechanism apart from coagulation [114]. Unlike metal flocculants such as alum, plant-based flocculants may not work under sweeping mechanism as the flocculation efficiency decline for flocculant dosage above optimum values [103].

5.3. Bridging

Bridging mechanism occurs when polymer chains bind to microalgae cells to link and form bridges between them [29]. These bridges bring the microalgae cells together to form particle-polymer-particle complex [99]. However, in order for the polymer chains to link more microalgae cells together, there should be sufficient unoccupied space on the cell surface for attachment of the polymer segments [117]. It was reported that, the net charges of the microalgae colloids are more negative and polymer chains are extended at pH above isoelectric point, which promotes flocculation through bridging mechanism [117]. In contrast to coagulation, flocculation experiments where bridging mechanism predominates, high molecular weight of flocculants plays role in improving aggregation irrespective to charge density [117]. Flocculants with higher molecular weight form stronger bridge onto the particle surface than lower molecular weight flocculants [118]. Apart from that, bio-based polymers as effective flocculants possess chemical

Table 11
Flocculation mechanisms of flocculants used to harvest microalgae [96,110].

Type of flocculant	Microalgae species	Flocculation mechanism
Chitosan	*Chlorella sorokiniana*	Coagulation and electrostatic patch
Starch (green Floc 120)	Freshwater microalgaes: *Parachlorella kessleri*, *Scenedesmus Obliquus* and *Parachlorela*	Bridging and electrostatic patch
STC-g-MAPTAC (starch-g-3-methacryloyl amino propyl trimethyl ammonium chloride)	Microalgae suspension (*S. obliquus*)	Coagulation
CHPTAC-g-Cassia (N-3-chloro-2-hydroxyprophyl trimethyl ammonium chloride (CHPTAC)-g-cassia)	*Chlamydomonas* sp. CRP7 and *Chlorella* sp. CB4)	Bridging and electrostatic patch
Inulin-g-CHPTAC (inulin-g-3-chloro-2-hydroxypropyl) trimethylammonium chloride)	*Botryococcus* sp.	Bridging
Eggshell	*Chlorella vulgaris*	Coagulation

groups such as free hydroxyl groups for good flocculation activities [99]. Likewise, carboxyl groups in polysaccharide-based flocculants provide more adsorption sites for colloidal particles and the bridging between flocculants and particles are strengthen and extended [118]. When the flocs formed are stronger, bigger and denser, they will possess good settling characteristics which in return increases the harvesting efficiency [99].

As for polysaccharide-based flocculants, bridging mechanism could be due to van der Waals force, static, hydrogen bonds or chemical interaction between radial groups of the polysaccharide and the particle of interest [100]. As documented in previous research cases for non-microalgae flocculation, the charge for most studied plant-based bioflocculants are anionic for Okra, Psyllium and Isabgol, neutral for Funegreek and unknown for Mallow and Tamarind [99]. Therefore, discounting coagulation, these bioflocculants are assumed to work under bridging mechanism where the biopolymers serve as bridge based on particle-polymer-particle complex formation [99]. Bridging has also been proposed as responsible flocculation mechanism in clay for mucilage of cactus *Opuntia ficus indica* which is an anionic polysaccharide [103,108]. Apart from that, flocculation mechanism of *Plantago psyllium* mucilage and *Tamarindus indica* mucilage in treatment of textile wastewater was identified to be bridging [99]. Likewise, effectiveness of *M. subcordata* in removing turbidity was reported to work under this mechanism. Presence of polysaccharides mainly amylopectin could serve as bridges to form linkages between colloidal particles, increasing the floc size and eventually enhance the precipitation process [13].

5.4. Electrostatic patch

In electrostatic patch mechanism, a charged polymer or flocculant adsorbs onto microalgae cell with opposite charge and locally reverse the surface charge of microalgae cells, resulting in patches of opposite charge on the cells [29]. Microalgae cells connect with each other through patches of opposite charge, leading flocculation of the suspension [99]. If the added flocculant is a polyelectrolyte with high charge density, it will adsorbed onto surface of particle of interest in a flat configuration and overall ionic concentration is altered, causing patches of localized excesses of polymer charge [103,117]. Direct electrostatic interaction between oppositely charged patches induces aggregation. It has been reported that particles with strong negative zeta potential such as silica will work under electrostatic patch mechanism in response to polyelectrolytes with high cationic charge density (> 0.15) and as for low cationic charge density (< 0.15), bridging mechanism is favoured [117].

6. Techno-economical and policy analysis with future perspectives

It comes to the limelight that, energy policies established around the globe are promoting the utilization and healthy development of renewable energies [119]. The progress of first-and second-generation biofuels has largely benefited from variegated policy arbitrations. These include direct acts such as tax compromises, controlled fuel use, and subsidies for production as well as infrastructure or indirect acts like biofuel blending mandates which were estimated to cost about US$11 billion in 2006 and US$25 billion in 2017 [119]. United States is the largest dominant country in producing industrial commodities and supplying agricultural products and has been acknowledged moderator for evolution of biofuels. In 2022, production of 79.5 billion litres of advanced biofuel is targeted by The US Energy Independence and Security Act (EISA) as a part of Second Renewable Fuel Standard (RFS2) [120]. Even though microalgae biofuels have been touted as excellent substitute for petroleum fuel, the vital challenge is the production economic feasibility while scholarly innovations and technological refinements are still at scarce. The cost of microalgae biofuel production using the existing conventional approaches are too high as compared to fossil fuel [120]. In order to turn microalgae biofuel as a marketable commodity, costs associated with two major processing elements, the microalgae cultivation and harvesting techniques for large biomass production need to be tackled with brand new blueprints which can be amalgamated into the existing approaches reviewed in this paper. Raeisossadati et al. [120] have reported on the estimation of impacts of biomass productivity on the production cost of microalgae biofuel. The importance of high biomass productivity to reduce overall production costs while improving the oil yields were highlighted in the findings and the approaches is believed to support production of sustainable microalgae biofuel at less than USD 0.7 per litre [120].

As for microalgae cultivation, it is highly challenged by the system design and cost for medium or nutrients. Despite of many emerging improvement and technologies in designing appropriate cultivation systems, such systems still unlikely to be economically feasible at large scale. Selection of PBR that allow high penetration of sunlight and nutrients removal at low cost still at ongoing debate among researchers. One of the solutions to lower the cultivation costs is to use wastewater as nutrient source through coupling of microalgae cultivation with wastewater treatment. Furthermore, there are tonnes of by-product wastes that being generated by various industries and these wastes should be explored as nutrients, replacing the conventional ones to cultivate microalgae. Such innovations are not new as there are considerable amount of published literatures on potential utilization of wastewater and other wastes to grow microalgae. But, a very few real applications have been documented by researchers. Perhaps, such approaches should be able portray potential to be incorporated into established upstream steps so that a continuous biodiesel production can be developed.

Availability of various microalgae harvesting technologies promise vast choice of application to researchers, yet, the supreme way of harvesting regardless of microalgae species and end product specificity, has not been identified. As emphasized in this review, bioflocculants extracted form waste biomass could offer superiority over other harvesting technologies in terms of cost with similar recovery efficiencies but baseline understanding on these biomaterials functionality need to be established. To our best knowledge, only a few waste biomass have been explored to produce bioflocculants for microalgae harvesting as they are still newfangled in the field. Therefore, more waste biomass should be investigated for their ability as bioflocculants other new extraction methods while being sufficiently simple, inexpensive and non-toxic. Characterization analysis such as zeta-potential reading would be helpful in identifying functional groups and flocculation mechanisms of the extracted bioflocculant which in turn aids in selection of appropriate extraction techniques. Kinetic and thermodynamic studies can be done to evaluate surface binding of bioflocculant and microalgae cells that can be used to further optimize the flocculation conditions, besides other optimization tools such as Response Surface Methodology (RSM).

Even though bioflocculation is seemingly cost-effective compared to other methods of microalgae harvesting as emphasized in this review, mixing factor plays an important role in this technique. Effective mixing is crucial to enhance the dispersion of bioflocculant within the microalgae suspension in short frame of time. An uniform mixture is achieved through even distribution of bioflocculant, permitting alleviated and effective bioflocculation process. Therefore, bioflocculation studies equipped with mixer is the key for excellent mixing and harvesting of microalgae cells. Further studies are needed in designing mixer at low lost and energy consumption, in line with reducing overall harvesting cost and positioning bioflocculation att the forefont of the cheapest microalgae harvesting method. In addition, life cycle analysis for bioflocculation studies involving bioflocculants at both lab and large scale is essential to gain data on energy consumption and process cost. This will further drive development and commercialization of bioflocculants extracted from waste biomass.

Apart from that, most of the bioflocculation experiments consider the concentration of bioflocculants, neglecting the influence of

microalgae cell concentration on the flocculation efficiency. Too low count of microalgae cells leads to formation of small flocs due to low probability of contacts and reduced removal performance. Vice versa, high number of cells induces too large flocs which may cause settling hindrance as the flocs might interact together. Thus, microalgae harvesting aided by bioflocculation technique should takes into account the optimum microalgae cell concentration for maximum separation efficiency. This can be done by modelling the concentration of bioflocculants needed to completely harvest microalgae cells at varied initial microalgae cell densities using a polynomial equation. However, it should be taken into consideration that the reviewed harvesting technique for biomass production is still at very early stages and further research works are required to find prospective harvesting technique at commercial scale.

All in all, upstream processing of microalgae biomass should compensate the following technical needs to elevate both economics and efficiency of the overall process towards sustainable biofuel production. The cultivation and harvesting method should be able to applicable for all species of microalgae regardless of the morphological characteristics, without altering the biocomposition of the microalgae cells and secreted end products. As such, the subsequent lipid extraction and biodiesel conversion should be affected by the ways of microalgae cells are being cultivated and harvested in the earlier part. Additionally, the developed upstream strategies must be able to allow recycling of water and nutrient after biomass separation so that the total downstream cost can be further minimized. Last but not the least, the chosen harvesting approach must be scalable, in way that, it can be used to recover large amount of microalgae biomass without significant changes in effectiveness to meet the commercial biofuel production. Succinctly, the upstream processing of microalgae biomass must be cheap while being environmental-friendly to make microalgae biofuel as competitive energy product that reachable to community. In order to secure continuous development in biofuel field, the United States through EISA2007 has offered financial supports and loan guarantees worth $550 million for R&D works and establishment of advanced biofuel plants [120]. The execution of such relevant policy mechanisms was able to improve the economic feasibility and production viability as a long-term replacement over fossil fuel. For example, the world bioethanol production has expanded from 6.4 to 23.4 billion gallons from 2003 to 2013 and in the last decade, the production of biofuels has experienced tremendous growth [119]. Therefore, with the support and implementation of appropriate government policies in attempt to provide incentives for third generation biofuel, it is justifiable that challenges associated with high production cost, immature technology and poor facility can be overcome in near future.

7. Conclusion

Microalgae biomass has been identified as promising feedstock for the third generation of biofuel production. Although they can be easily grown under lab-scale, cultivation in commercial scale required several crucial considerations, such as design, cost, risk of contamination and cleaning. Apparently, raceway pond is still a preferred option to cultivate microalgae for commercial biofuel production meanwhile other PBR designs, e.g. tubular, is more suitable to be used to produce higher market value products like protein, astaxanthin and omega-3 fatty acid. On the other hand, harvesting of microalgae posed an extreme challenge in commercial scale due to the diluted biomass concentration in water. It is identified that flocculants could be an immediate solution to overcome this problem rather than energy-intensive methods. Flocculants derived from waste biomass have gained enormous attention from researchers in present days owing to the facts that they are cheap, toxic-free, safe and biodegradable. Plant and fruit wastes, besides animal proteins, show ability to flocculate various types of suspensions and have been highlighted as potential flocculants to harvest microalgae cells as presented in the paper. Nonetheless, detailed and continuous research activities are essential to identify the fundamental properties of biomass-based flocculants in order to establish their role in microalgae harvesting. Policy implementation was sought to have credited the vast inauguration of conventional biofuels and likewise, systematic policy support is the much needed agenda to warrant sustainable microalgae biofuel production.

Acknowledgements

The authors would like to acknowledge the financial support received from Universiti Teknologi PETRONAS (YUTP-FRG with cost centre 0153AA-H46) and Ministry of Higher Education (MOHE) Malaysia (Fundamental Research Grant Scheme (FRGS) – Malaysia's Rising Star Award (MRSA) 2016 with account number 203/PJKIMIA/6071362). Support from Ministry of Education Malaysia through HICoE award to Centre for Biofuel and Biochemical Research (CBBR) is duly acknowledged.

References

[1] Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. Biofuels 2010;1:763–84.
[2] Singh G, Patidar SK. Microalgae harvesting techniques: a review. J Environ Manag 2018;217:499–508.
[3] Article O. Utilization of byproducts and waste materials from meat, poultry and fish processing industries : a review vol. 49. 2012. p. 278–93.
[4] Baldev E, Mubarakali D, Saravanakumar K, Arutselvan C, Alharbi NS, Alharbi SA, et al. Unveiling algal cultivation using raceway ponds for biodiesel production and its quality assessment. Renew Energy 2018;123:486–98.
[5] Banerjee C, Ghosh S, Sen G, Mishra S, Shukla P, Bandopadhyay R. Study of algal biomass harvesting through cationic cassia gum, a natural plant based biopolymer. Bioresour Technol 2014;151:6–11.
[6] Barros AI, Gonçalves AL, Simões M, Pires JCM. Harvesting techniques applied to microalgae: a review. Renew Sustain Energy Rev 2015;41:1489–500.
[7] Brennan L, Owende P. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–77.
[8] Cesário MT, da Fonseca MMR, Marques MM, de Almeida MCMD. Marine algal carbohydrates as carbon sources for the production of biochemicals and biomaterials. Biotechnol Adv 2018;36(3):798–817.
[9] Han B, Goh H, Chyuan H, Yee M, Chen W, Ling K. Sustainability of direct biodiesel synthesis from microalgae biomass : a critical review. Renew Sustain Energy Rev 2019;107:59–74.
[10] Salama ES, Jeon BH, Kurade MB, Abou-Shanab RAI, Govindwar SP, Lee SH, et al. Harvesting of freshwater microalgae Scenedesmus obliquus and Chlorella vulgaris using acid mine drainage as a cost effective flocculant for biofuel production. Energy Convers Manag 2016;121:105–12.
[11] Fulbright SP, Robbins-Pianka A, Berg-Lyons D, Knight R, Reardon KF, Chisholm ST. Bacterial community changes in an industrial algae production system. Algal Res 2018;31:147–56.
[12] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14:217–32.
[13] Choy SY, Prasad KMN, Wu TY, Raghunandan ME, Ramanan RN. Utilization of plant-based natural coagulants as future alternatives towards sustainable water clarification. J Environ Sci (China) 2014;26:2178–89.
[14] Kothari R, Pathak VV, Pandey A, Ahmad S, Srivastava C, Tyagi VV. A novel method to harvest Chlorella. Bioresour Technol 2017;225:84–9. 2017.
[15] Chethana M, Sorokhaibam LG, Bhandari VM, Raja S, Ranade VV. Green approach to dye wastewater treatment using biocoagulants. ACS Sustainable Chem Eng 2016;4:2495–507.
[16] Queensland Environmental. Microalgae and fisheries production Microalgae Information sheet. Microalgae Inf Sheet 2008:1–6.
[17] Rajkumar R, Takriff MS. Prospects of algae and their environmental applications in Malaysia: a case study. J. Biorem. Biodegrad. 2016;7:321.
[18] Tan X, Uemura Y, Lim JW, Wong CY, Lee T. Cultivation of microalgae for biodiesel production: a review on upstream and downstream processing. Chin J Chem Eng 2017;26(1):17–30.
[19] Chisti Y. Biodiesel from microalgae 2007;25:294–306.
[20] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy 2010;87:38–46.
[21] Rajkumar R, Yaakob Z, Takriff MS. Potential of the micro and macro algae for biofuel production: a brief review. BioResour 2014;9:1606–33.
[22] Debiagi PEA, Trinchera M, Frassoldati A, Faravelli T, Vinu R, Ranzi E. Algae characterization and multistep pyrolysis mechanism. J Anal Appl Pyrolysis 2017;128:423–36.
[23] Guo Y, Yeh T, Song W, Xu D, Wang S. A review of bio-oil production from hydrothermal liquefaction of algae. Renew Sustain Energy Rev 2015;48:776–90.
[24] Silitonga AS, Masjuki HH, Mahlia TMI, Ong HC, Chong WT, Boosroh MH. Overview properties of biodiesel diesel blends from edible and non-edible feedstock. Renew Sustain Energy Rev 2013;22:346–60.

[25] Lam MK, Lee KT. Microalgae biofuels : a critical review of issues , problems and the way forward. Biotechnol Adv 2012;30:673–90.
[26] Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Factories 2018;17:1–21.
[27] Kotasthane T. Potential of microalgae for sustainable biofuel production. J Mar Sci Res Dev 2017;07.
[28] Ummalyma SB, Gnansounou E, Sukumaran RK, Sindhu R, Pandey A, Sahoo D. Bioflocculation: an alternative strategy for harvesting of microalgae – an overview. Bioresour Technol 2017;242:227–35.
[29] Vandamme D, Foubert I, Muylaert K. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol 2013;31:233–9.
[30] Lemos JS, Vargas JVC, Mariano AB, Kava V, Ordonez JC. A flocculation strategy for harvesting high lipid content microalgae biomass. IEEE conf technol sustain SusTech 2016 2017. 2016. p. 240–5.
[31] Ganesh Saratale R, Kumar G, Banu R, Xia A, Periyasamy S, Dattatraya Saratale G. A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. Bioresour Technol 2018;262:319–32.
[32] Rakesh S, Saxena S, Dhar DW, Prasanna R, Saxena AK. Comparative evaluation of inorganic and organic amendments for their flocculation efficiency of selected microalgae. J Appl Phycol 2014;26:399–406.
[33] Zhan J, Rong J, Wang Q. Mixotrophic cultivation, a preferable microalgae cultivation mode for biomass/bioenergy production, and bioremediation, advances and prospect. Int J Hydrogen Energy 2017;42:8505–17.
[34] Rahul R, Kumar S, Jha U, Sen G. International Journal of Biological Macromolecules Cationic inulin : a plant based natural biopolymer for algal biomass harvesting. Int J Biol Macromol 2015;72:868–74.
[35] Vandamme D, Foubert I, Fraeye I, Meesschaert B, Muylaert K. Flocculation of Chlorella vulgaris induced by high pH: role of magnesium and calcium and practical implications. Bioresour Technol 2012;105:114–9.
[36] Park KY, Lee K, Kim D. Characterized hydrochar of algal biomass for producing solid fuel through hydrothermal carbonization. Bioresour Technol 2018;258:119–24.
[37] Vo Hoang Nhat P, Ngo HH, Guo WS, Chang SW, Nguyen DD, Nguyen PD, et al. Can algae-based technologies be an affordable green process for biofuel production and wastewater remediation? Bioresour Technol 2018;256:491–501.
[38] Ometto F, Steinhovden KB, Kuci H, Lunnbäck J, Berg A, Karlsson A, et al. Seasonal variation of elements composition and biomethane in brown macroalgae. Biomass Bioenergy 2018;109:31–8.
[39] Chen CY, Zhao XQ, Yen HW, Ho SH, Cheng CL, Lee DJ, et al. Microalgae-based carbohydrates for biofuel production. Biochem Eng J 2013;78:1–10.
[40] El Gamal AA. Biological importance of marine algae. Saudi Pharm J 2010;18:1–25.
[41] Lee OK, Lee EY. Sustainable production of bioethanol from renewable brown algae biomass. Biomass Bioenergy 2016;92:70–5.
[42] Chen CY, Zhao XQ, Yen HW, Ho SH, Cheng CL, Lee DJ, et al. Microalgae-based carbohydrates for biofuel production. Biochem Eng J 2013;78:1–10.
[43] Chia SR, Ong HC, Chew KW, Show PL, Phang SM, Ling TC, et al. Sustainable approaches for algae utilisation in bioenergy production. Renew Energy 2017;129:838–52.
[44] Khan MI, Shin JH, Kim JD. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microb Cell Factories 2018;17:1–21.
[45] Trung TS, Thi N, Huyen K, Minh NC, Thi T, Trang L, et al. Optimization of harvesting of microalgal thalassiosira pseudonana biomass using chitosan prepared from shrimp shell waste [n.d]..
[46] Raikova S, Le CD, Beacham TA, Jenkins RW, Allen MJ, Chuck CJ. Towards a marine biorefinery through the hydrothermal liquefaction of macroalgae native to the United Kingdom. Biomass Bioenergy 2017;107:244–53.
[47] Zhang W, Zhao Y, Cui B, Wang H, Liu T. Evaluation of filamentous green algae as feedstocks for biofuel production. Bioresour Technol 2016;220:407–13.
[48] Wu FC, Wu JY, Liao YJ, Wang MY, Shih IL. Sequential acid and enzymatic hydrolysis in situ and bioethanol production from Gracilaria biomass. Bioresour Technol 2014;156:123–31.
[49] Parsa M, Jalilzadeh H, Pazoki M, Ghasemzadeh R, Abduli MA. Hydrothermal liquefaction of Gracilaria gracilis and Cladophora glomerata macro-algae for biocrude production. Bioresour Technol 2018;250:26–34.
[50] undefined Ding L, Gutierrez E, Cheng J, Xia A, Energy RO. Assessment of continuous fermentative hydrogen and methane co-production using macro-and micro-algae with increasing organic loading rate. Elsevier; 2018. p. 151. 2018.
[51] Zhu S, Huang W, Xu J, Wang Z, Xu J, Yuan Z. Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga Chlorella zofingiensis. Bioresour Technol 2013;152:292–8.
[52] Shakya R, Adhikari S, Mahadevan R, Shanmugam SR, Nam H, Hassan EB, et al. Influence of biochemical composition during hydrothermal liquefaction of algae on product yields and fuel properties. Bioresour Technol 2017;243:1112–20.
[53] Zhang L, Pei H, Chen S, Jiang L, Hou Q, Yang Z, et al. Salinity-induced cellular cross-talk in carbon partitioning reveals starch-to-lipid biosynthesis switching in low-starch freshwater algae. Bioresour Technol 2018;250:449–56.
[54] Samiee-Zafarghandi R, Karimi-Sabet J, Abdoli MA, Karbassi A. Increasing microalgal carbohydrate content for hydrothermal gasification purposes. Renew Energy 2018;116:710–9.
[55] Lee J, Lee K, Sohn D, Mo Y, Young K. Hydrothermal carbonization of lipid extracted algae for hydrochar production and feasibility of using hydrochar as a solid fuel vol. 153. 2018.
[56] Kightlinger W, Chen K, Pourmir A, Crunkleton DW, Price GL, Johannes TW. Production and characterization of algae extract from Chlamydomonas reinhardtii. Electron J Biotechnol 2014;17:14–8.
[57] Wang X, Ruan Z, Sheridan P, Boileau D, Liu Y, Liao W. Two-stage photo-autotrophic cultivation to improve carbohydrate production in Chlamydomonas reinhardtii. Biomass Bioenergy 2015;74:280–7.
[58] Singh R, Balagurumurthy B, Bhaskar T. Hydrothermal liquefaction of macro algae: effect of feedstock composition. Fuel 2015;146:69–74.
[59] Singh RN, Sharma S. Development of suitable photobioreactor for algae production - a review. Renew Sustain Energy Rev 2012;16:2347–53.
[60] Mondal M, Goswami S, Ghosh A, Oinam G, Tiwari ON, Das P, et al. Production of biodiesel from microalgae through biological carbon capture: a review. 3 Biotech 2017;7:1–21.
[61] Suutari M, Leskinen E, Fagerstedt K, Kuparinen J, Kuuppo P, Blomster J. Macroalgae in biofuel production. Phycol Res 2015;63:1–18.
[62] Kawai S, Murata K. Biofuel production based on carbohydrates from both brown and red macroalgae: recent developments in key biotechnologies. Int J Mol Sci 2016;17.
[63] Lam TP, Lee TM, Chen CY, Chang JS. Strategies to control biological contaminants during microalgal cultivation in open ponds. Bioresour Technol 2018;252:180–7.
[64] Ali R, Celik S. Effect of cooling on solar panel performance rehan. Int Proc Chem Biol Environ Eng 2017;100:118–23.
[65] Jorquera O, Kiperstok A, Sales EA, Embiruçu M, Ghirardi ML. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresour Technol 2010;101:1406–13.
[66] Sepehri A, Sarrafzadeh MH. Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor. Chem Eng Process - Process Intensif 2018;128:10–8.
[67] Dassey AJ, Theegala CS. Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresour Technol 2013;128:241–5.
[68] Faried M, Samer M, Abdelsalam E, Yousef RS, Attia YA, Ali AS. Biodiesel production from microalgae: processes, technologies and recent advancements. Renew Sustain Energy Rev 2017;79:893–913.
[69] Ranganathan P, Amal JC, Savithri S, Haridas A. Experimental and modelling of Arthrospira platensis cultivation in open raceway ponds. Bioresour Technol 2017;242:197–205.
[70] Tan XB, Lam MK, Uemura Y, Lim JW, Wong CY, Lee KT. Cultivation of microalgae for biodiesel production: a review on upstream and downstream processing. Chin J Chem Eng 2018;26:17–30.
[71] Lam TP, Lee TM, Chen CY, Chang JS. Strategies to control biological contaminants during microalgal cultivation in open ponds. Bioresour Technol 2018;252:180–7.
[72] Lundguist TJ, Woertz IC, Quinn NWT, Benemann JR. A realistic technology and engineering assessment of algae biofuel production. Energy Biosci Inst 2010;3–9.
[73] Chatsungnoen T, Chisti Y. Continuous flocculation-sedimentation for harvesting Nannochloropsis salina biomass. J Biotechnol 2016;222:94–103.
[74] Ting H, Haifeng L, Shanshan M, Zhang Y, Zhidan L, Na D. Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: a review. Int J Agric Biol Eng 2017;10:1–29.
[75] Wang B, Lan CQ, Horsman M. Closed photobioreactors for production of microalgal biomasses. Biotechnol Adv 2012;30:904–12.
[76] Burgess G, Fernández-Velasco JG. Materials, operational energy inputs, and net energy ratio for photobiological hydrogen production. Int J Hydrogen Energy 2007;32:1225–34.
[77] Dasan YK, Lam MK, Yusup S, Lim JW, Lee KT. Life cycle evaluation of microalgae biofuels production: effect of cultivation system on energy, carbon emission and cost balance analysis. Sci Total Environ 2019;688:112–28.
[78] Li T, Strous M, Melkonian M. Biofilm-based photobioreactors: their design and improving productivity through efficient supply of dissolved inorganic carbon. FEMS Microbiol Lett 2017;364:1–9.
[79] Moreno Osorio JH, Pinto G, Pollio A, Frunzo L, Lens PNL, Esposito G. Start-up of a nutrient removal system using Scenedesmus vacuolatus and Chlorella vulgaris biofilms. Bioresour Bioprocess 2019;6:27.
[80] Nawar A, Khoja AH, Akbar N, Ansari AA, Qayyum M, Ali E. Physical abrasion method using submerged spike balls to remove algal biofilm from photobioreactors. BMC Res Notes 2017;10:1–5.
[81] Shuba ES, Kifle D. Microaglae to biofuels: “Promising” alternative and renewable energy, review vol. 81. Elsevier; 2018. p. 743–55.
[82] Farooq W, Moon M, Ryu B gon, Suh WI, Shrivastav A, Park MS, et al. Effect of harvesting methods on the reusability of water for cultivation of chlorella vulgaris, its lipid productivity and biodiesel quality. Algal Res 2015;8:1–7.
[83] Mallick N, Bagchi SK, Koley S, Singh AK. Progress and challenges in microalgal biodiesel production. Front Microbiol 2016;7:1–11.
[84] Kim J, Yoo G, Lee H, Lim J, Kim K, Kim CW, et al. Methods of downstream processing for the production of biodiesel from microalgae. Biotechnol Adv 2013;31:862–76.
[85] Mathimani T, Mallick N. A comprehensive review on harvesting of microalgae for biodiesel – key challenges and future directions. Renew Sustain Energy Rev 2018;91:1103–20.
[86] Nurra C, Franco EA, Maspoch ML, Salvadó J, Torras C. Cheaper membrane materials for microalgae dewatering. J Mater Sci 2014;49:7031–9.
[87] Enamala MK, Enamala S, Chavali M, Donepudi J, Yadavalli R, Kolapalli B, et al. Production of biofuels from microalgae - a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renew Sustain Energy Rev 2018;94:49–68.
[88] Ali TH, El-Ghonemy DH. Optimization of culture conditions for the highest lipid

production from some oleaginous fungi for biodiesel preparation. Asian J Appl Sci 2014;02:2321–893.
[89] Zhang J, Hu B. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. Bioresour Technol 2012;114:529–35.
[90] Zhou W, Cheng Y, Li Y, Wan Y, Liu Y, Lin X, et al. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. Appl Biochem Biotechnol 2012;167:214–28.
[91] Xie S, Sun S, Dai SY, Yuan J S. Efficient coagulation of microalgae in cultures with filamentous fungi. Algal Res 2013;2:28–33.
[92] Zhou W, Min M, Hu B, Ma X, Liu Y, Wang Q, et al. Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. Separ Purif Technol 2013;107:158–65.
[93] Gultom SO, Hu B. Review of microalgae harvesting via co-pelletization with filamentous fungus. Energies 2013;6:5921–39.
[94] Zamalloa C, Gultom SO, Rajendran A, Hu B. Ionic effects on microalgae harvest via microalgae-fungi co-pelletization. Biocatal Agric Biotechnol 2017;9:145–55.
[95] Piazza GJ, Garcia RA. Meat & bone meal extract and gelatin as renewable flocculants. Bioresour Technol 2010;101:781–7.
[96] Andriamanohiarisoamanana FJ, Saikawa A, Kan T, Qi G, Pan Z, Yamashiro T, et al. Semi-continuous anaerobic co-digestion of dairy manure, meat and bone meal and crude glycerol: process performance and digestate valorization. Renew Energy 2018;128:1–8.
[97] Piazza GJ, Garcia RA. Biobased flocculants derived from animal processing protein by-products. Elsevier Inc.; 2016.
[98] Kerr BJ, Jha R, Urriola PE, Shurson GC. Nutrient composition, digestible and metabolizable energy content, and prediction of energy for animal protein by-products in finishing pig diets. J Anim Sci 2017;95:2614–26.
[99] Lee CS, Chong MF, Robinson J, Binner E. A review on development and application of plant-based bioflocculants and grafted bioflocculants. Ind Eng Chem Res 2014;53:18357–69.
[100] Salehizadeh H, Yan N. Recent advances in extracellular biopolymer flocculants. Biotechnol Adv 2014;32:1506–22.
[101] Vijayaraghavan G, Sivakumar T, Kumar AV. Application of plant based coagulants for waste water treatment. Int J Adv Eng Res Stud 2011;1:88–92.
[102] Edogbanya, et al. A review on the use of plants' seeds as biocoagulants in the purification of water. Cont J Biol Sci 2013;6:26–32.
[103] Choy SY, Prasad KMN, Wu TY, Ramanan RN. A review on common vegetables and legumes as promising plant-based natural coagulants in water clarification. Int J Environ Sci Technol 2015;12:367–90.
[104] Teixeira CMLL, Kirsten FV, Teixeira PCN. Evaluation of Moringa oleifera seed flour as a flocculating agent for potential biodiesel producer microalgae. J Appl Phycol 2012;24:557–63.
[105] Lapa Teixeira CML, Teixeira PCN. Evaluation of the flocculation efficiency of Chlorella vulgaris mediated by Moringa oleifera seed under different forms: flour, seed cake and extracts of flour and cake. Braz J Chem Eng 2017;34:65–74.
[106] Ogbonna CN, Edeh I. Harvesting chlorella variabilis biomass using Moringa oleifera seed-induced sedimentation. J Adv Biol Biotechnol 2018;18:1–11.
[107] Ogbonna CN, Edeh I. Harvesting chlorella variabilis biomass using Moringa oleifera seed-induced sedimentation. J Adv Biol Biotechnol 2018;18:1–11.
[108] Megersa M, Beyene A, Ambelu A, Woldeab B. The use of indigenous plant species for drinking water treatment in developing countries: a review. J Biodivers Environ Sci (JBES) 2014:269–81. 2014.
[109] Hassen AA, Dizbay-onat M, Bansal D, Bayush T. Utilization of chicken eggshell waste as a bio-filler for thermoplastic Polymers : thermal and mechanical characterization of polypropylene filled with naturally. Derived CaCO 3 2015;23:653–62.
[110] Podstawczyk D, Witek-Krowiak A, Chojnacka K, Sadowski Z. Biosorption of malachite green by eggshells: mechanism identification and process optimization. Bioresour Technol 2014;160:161–5.
[111] Wu Q, Chen J, Clark M, Yu Y. Adsorption of copper to different biogenic oyster shell structures. Appl Surf Sci 2014;311:264–72.
[112] Elwakeel KZ, Elgarahy AM, Mohammad SH. Use of beach bivalve shells located at Port Said coast (Egypt) as a green approach for methylene blue removal. J Environ Chem Eng 2017;5:578–87.
[113] Zhou C, Gong X, Han J, Guo R. Removal of Pb(II) and Zn(II) from aqueous solutions by raw crab shell: a comparative study. Water Environ Res 2016;88:374–83.
[114] Choi HJ. Effect of eggshells for the harvesting of microalgae species. Biotechnol Biotechnol Equip 2015;2818:1–7.
[115] Rahman MA, Hossain MM, Samad A, Alam AMS. Removal of arsenic from ground water with shrimp. Shell 2012;60:175–80.
[116] Peinemann JC, Krenz LMM, Pleissner D. Is seashell powder suitable for phosphate recovery from fermentation broth? N Biotech 2018;0–1.
[117] Suopajarvi T. Functionalized nanocelluloses in wastewater treatment applications. 2015.
[118] Salehizadeh H, Yan N, Farnood R. Recent advances in polysaccharide bio-based flocculants. Biotechnol Adv 2018;36:92–119.
[119] Su Y, Zhang P, Su Y. An overview of biofuels policies and industrialization in the major biofuel producing countries. Renew Sustain Energy Rev 2015;50:991–1003.
[120] Raeisossadati M, Moheimani NR, Parlevliet D. Luminescent solar concentrator panels for increasing the efficiency of mass microalgal production. Renew Sustain Energy Rev 2019;101:47–59.