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Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review

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HIGHLIGHTS

- Microalgae are the most promising photoautotrophs to fix atmospheric carbon.
- Requirement of huge amounts of freshwater to culture microalgae is challenging.
- Alternatively, wastewater cultivation offers low-cost biomass production.
- Wastewater nutrient stress can manipulate the microalgal metabolite content.
- Mixed cultivation offers additional benefits of efficient wastewater treatment.

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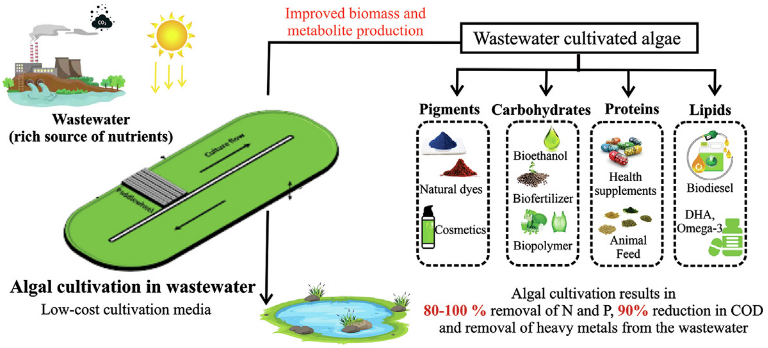
GRAPHICAL ABSTRACT



ABSTRACT

Water shortage is one of the leading global problems along with the depletion of energy resources and environmental deterioration. Recent industrialization, global mobility, and increasing population have adversely affected the freshwater resources. The wastewater sources are categorized as domestic, agricultural and industrial effluents and their disposal into water bodies poses a harmful impact on human and animal health due to the presence of higher amounts of nitrogen, phosphorus, sulfur, heavy metals and other organic/inorganic pollutants. Several conventional treatment methods have been employed, but none of those can be termed as a universal method due to their high cost, less efficiency, and non-environment friendly nature. Alternatively, wastewater treatment using microalgae (phycoremediation) offers several advantages over chemical-based treatment methods. Microalgae cultivation using wastewater offers the highest atmospheric carbon fixation rate (1.83 kg CO_2/kg of biomass) and fastest biomass productivity (40–50% higher than terrestrial crops) among all terrestrial bio-remediators with concomitant pollutant removal (80–100%). Moreover, the algal biomass may contain high-value metabolites including omega-3-fatty acids, pigments, amino acids, and high sugar content. Hence, after extraction of high-value compounds, residual biomass can be either directly converted to energy through thermochemical transformation or can be used to produce biofuels through biological fermentation or transesterification. This review highlights the recent advances in microalgal biotechnology to establish a

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biorefinery approach to treat wastewater. The articulation of wastewater treatment facilities with microalgal biorefinery, the use of microalgal consortia, the possible merits, and demerits of phycoremediation are also discussed. The impact of wastewater-derived nutrient stress and its exploitation to modify the algal metabolite content in view of future concerns of cost-benefit ratios of algal biorefineries is also highlighted.

1. Introduction

Dawn of the 21st century led the world towards increasing urbanization, industrialization and commercialization at the cost of increasing carbon emission, depleting energy and water resources, and contamination of water bodies with toxic pollutants (Khan et al., 2019). It is expected that the world's population will increase from 6.3 billion in 2015 to approximately 9 billion in 2050 (Ng et al., 2017). The increased population would require enough and cleaner energy supply as well as the clean water resources. To acquire and maintain a clean environment, it would be needed to reduce CO_2 content by 50–80% with an increase of 50% in water and energy resources respectively (Hightower and Pierce, 2008; Ng et al., 2017). According to 2014 statistics, estimated global freshwater consumption was 3700 billion m^3 (Diniz et al., 2017), where most of it is being converted into wastewater due to human activities. Based on its origin, wastewater produced by human activities is termed as domestic, aqua-cultural, agricultural or industrial sewage/effluents (Diniz et al., 2017). Various industries including paper and pulp industry, sugarcane industry, textile or tannery industry, and pharmaceutical industry (Lv et al., 2017; Ramlow et al., 2017) contribute to surface water pollution causing water scarcity. Polluted and untreated water contaminates freshwater resources by releasing excess amounts of nitrogen and phosphorus which enhances eutrophication and ecosystem destruction, making it unsuitable for human consumption (Godfray et al., 2010; Morée et al., 2013). Water pollution (acidification, eutrophication, sewage, heavy metals and other organic pollutants) (Godfray et al., 2010) have confirmed harmful effects on human health, including growth inhibition, feminization of male organisms, carcinogenicity, (Butkovskyi et al., 2017) development of waterborne diseases like diarrhea, typhoid, intestinal worms, gastroenteritis, *Cryptosporidium* infections, cardiovascular, renal failure and hypertension (Daud et al., 2017), both in developed and developing countries.

The main aim of wastewater treatment is to remove the excess amount of micropollutants (Grandclement et al., 2017), nutrients (nitrogen, sulfur, copper, phosphorus) (Wang et al., 2017a), heavy metals (copper, zinc, lead, mercury, chromium, nickel, cadmium) (Bilal et al., 2013) and organic pollutants (phenolic compounds, aromatic hydrocarbons, biocides, surfactants, antibiotics etc.) from wastewater (Salama et al., 2017). A variety of chemicals, physical and biological wastewater treatment methods have been employed (Wang et al., 2017a) while the biological approach being more common (Grandclement et al., 2017) to remove these pollutants. Depending on the effluent requirement, the wastewater treatment method is selected (Dvořák et al., 2014). However, there is no standalone method that can be applied to a variety of effluents because of the limitations associated with each method (Table 1). In general, conventional treatment methods have shortcomings such as the requirement of large land, intensive energy input, and extensive maintenance and operational costs (Udaiyappan et al., 2017). Hence, it is required to develop alternative technologies to recycle wastewater along with the fixation of atmospheric carbon (Hariz and Takriff, 2017). Microalgae-based bioremediation is the safest, promising, and the most efficient alternative replacing conventional treatment methods due to its vast availability, higher nutrient consumption ability and diverse applications of the algal biomass produced (Lam et al., 2012).

This review summarizes different wastewater treatment methods and focused on cost-effective and efficient microalgae-based treatment of wastewater (phycoremediation) along with the potential problems and opportunities (Fig. 1). It also highlights the possibilities of exploiting the wastewater as a low-cost growth media and as a natural stress-manipulative strategy for enhanced biomass productivity and algal metabolite content, presenting an algal-biorefinery concept.

2. Microalgae-based wastewater treatment

Algae are one of the most diverse groups of photosynthetic organisms starting from simple blue-green algae (cyanobacteria/prokaryotes) to complex sea-weeds and kelps. On average, more than 350,000 microalgal species have been discovered (Shahid et al., 2017). In general, they are believed to contain as high as 70% lipids, 60% carbohydrates and 65% of proteins and essential amino-acids respectively (Afzal et al., 2017). Microalgal biomass is an alternative of traditional feedstocks (Khan et al., 2018) because microalgae have a short growth cycle when compared to terrestrial plants or energy crops, higher biomass productivity, higher harvesting index, and the highest rate of carbon fixation. Additionally, microalgae don't require large arable land instead can be grown on marginal lands by using seawater or wastewater as growth media (Miranda et al., 2017). Microalgae play a vital role in environmental carbon mitigation and bioremediation due to their higher photosynthetic efficiency (40–50% higher than terrestrial plants) (Chen et al., 2015) and remarkable CO_2 sequestration (1 kg of microalgae consumes 1.83 kg of CO_2 and accounts for 40% of global CO_2 sequestration) (Chisti, 2007; Ng et al., 2017). Microalgae can also be used as bioindicators to detect the climate changes in aquatic environments (O'Neill et al., 2019) and can consume wastewater nutrients (80–100% uptake of nitrogen and phosphorus) for high productivities of biomass and value-added products (Grandclement et al., 2017; Miranda et al., 2017; Su et al., 2016). Depending upon the microalgal strains, they can be employed in various industries like cosmetics, poultry, biofertilizers, medicine and green-fuels namely bioalcohols, biogas and biodiesel (Afzal et al., 2017). However, it is required to develop cost-effective strategies to produce cost-competitive algal products, because at present the algal biofuels cannot compete the prices of the fossil fuels (Dasan et al., 2019). Hence, selection of appropriate strain (Gill et al., 2016), use of low cost media, optimization of conditions for higher biomass production, cell stoichiometry to divert the balance towards target product, suitable commercialization, and reducing the operational cost (mainly associated with cultivation and harvesting stages) are focused aspects of the algal research (Ng et al., 2017). Fig. 2 summarizes a roadmap of microalgal growth optimization and strain development for microalgal biorefinery.

Wastewater is the most suitable resource for algal biomass production because of several reasons such as; (i) cheaper growth

Table 1
Overview of the conventional and modern wastewater treatment methods.

Treatment process	Type	Principle	Pollutant	Removal efficiency	Merits	Demerits	References
Filtration	Micro-filtration, Ultra-filtration, Reverse osmosis, Nano-filtration	Removal of solids (>5–20 mm) by passing liquid through porous membrane	domestic, industrial and pigment rich wastewater	99% (dyes and TC) 74% (TN) 77% (COD)	Easy operation, cost-effective, Remove suspended solid, alkalinity, organic and inorganic contaminants, high quality treated water	Filter may get clog, Poor micro-pollutant removal, membrane fouling, high operational cost	(Eyvaz et al., 2017; Udaiyappan et al., 2017; Wang et al., 2017)
Adsorption	Activated carbon or aluminum, zeolites, organic polymers,	Selective separation through binding of pollutant on absorbent surface	Industrial, agricultural, domestic and Heavy metal containing wastewater	96% (organic pollutants)	Easy operation, no additional chemicals, cost-effective, high metal binding capacity	Low selectivity, difficult maintenance, formation of waste products	(Crini et al., 2019; Guo et al., 2019)
Coagulation	Chemical, electro, natural- material based	Dissociation and hydrolysis of coagulant into positive ions; reactive to negative collides	Heavy metals, Textile, petroleum, cosmetics wastewater	>70% COD, 90–100% (Heavy metals)	Ecofriendly, lower operational cost, efficient pollutant removal, energy efficient	Identification of commercial scale-up parameters, electrode passivation, higher maintenance	(Mohd-Salleh et al., 2019; Sillanpää et al., 2018)
Advance oxidation	Electrochemical, Ultrasound-based, Plasma, ozonation, UV-radiation, photo-catalytic	Formation of organic pollutant removing reactive oxidizing species	Recalcitrant organic pollutants, industrial and pesticidal wastewater	53–96% (COD), 21–85% (TOC)	Degradation of organic pollutants, effective, vast applicability, oxidize odor compounds	Partial degradation, expensive, presence of toxic products	(Affam et al., 2018; Miklos et al., 2018)
Adsorption, membrane-filtration and photo-catalytic degradation (Hybrid)	Depends on membrane and nanoparticles being employed	Pollutant removal by serial treatment	Industrial wastewater, organic pollutants	88–92% (COD), 91–98% (TS), 85–91% (Detergents)	High efficiency, energy-saving, increased membrane performance, less fouling	Challenging scale-up	(Ejraei et al., 2019)
Filtration and coagulant-flocculation (Hybrid)	Chemical coagulants	Filtration of wastewater followed by treatment by coagulating flocculant	Phenolic compounds, organic pollutants, suspended solids,	92% (TS), 36% (COD), 81% (Fatty Matter)	High treatment efficiency, Energy saving	High maintenance cost,	(Enaime et al., 2019)
Nano-materials	Magnetic, carbonated, metal-oxide	Act as absorbent for the photolytic degradation of pollutants	Heavy metals, inorganic, organic & emerging pollutants, petrochemicals	90–100% (various metals)	High efficiency and adsorption capacity, compatible with other techniques	Eco-concern, high cost, poor recyclability, toxicity	(Gautam et al., 2019; Sadegh et al., 2017)
Biogenic Nano-particles	Bacteria, Fungi, Algae plant excreted bioactive molecules -based	Reduction or oxidation of metals by natural chemicals-based nanoparticles	Radio-active contamination, inorganic and organic pollutant	75–99% (Dyes), 66–85% (Heavy metals)	Eco friendly, non-toxic, cost-effective, sustainable, energy efficient	Instability, tricky recovery of intracellularly synthesized nanoparticles	(Ali et al., 2019; Gautam et al., 2019)
Microbial electro-chemical Technology	Microbial-fuel cell, Microbial-electrochemical cell, Bio-electrochemical treatment, Microbial electrolysis	Oxidation or reduction of pollutants by respiring microbes	Recalcitrant matter, industrial, domestic and food-processing wastewater	>25–63% (COD)	Wide applicability, production of electricity and other valuable commodities	Difficult scale-up with cost-effective & efficient performance	(Mohan et al., 2019)
Biochar	Biomass (algae, crop residues), industrial by-products, municipal waste	Act as catalysts or absorbent to degrade or remove the pollutant	Phenolic compounds, heavy metals, dyes, organic & inorganic contaminants	65–99% (dyes), >90% (phenols)	High efficiency, robust, economical, large specific area, high porosity, less energy consuming	Low removal efficiency of raw-biochar, some types may contain toxins or metals, non-ecofriendly production process	(Huang et al., 2019)
Biological method	Autotrophic & heterotrophic microbes (Bacteria, Fungi etc)	Assimilation and dissimilation of pollutants	Nitrates, phosphates, dairy waste	>90% (COD), 38–90% (Nitrogen)	Economical, high bio-degradability, efficient elimination of pollutants	Slow, requires constant maintenance, low applicability, compromised performance due to abiotic factors	(Ahmad et al., 2019; Crini and Lichtfouse, 2019)

(*continued on next page*)

Table 1 (*continued*)

Treatment process	Type	Principle	Pollutant	Removal efficiency	Merits	Demerits	References
Biomass	Algae, Cyanobacteria	Uptake of nutrients for biomass and metabolite production	Industrial, domestic wastewater, heavy metals, dyes, organic & inorganic pollutants	22–98% (TP), 20–100% (TN), >90% (COD)	High pollutant removal, energy & cost efficient, eco-friendly, biorefinery-based process	Compromised performance due to abiotic factors and wastewater characteristics, difficult harvesting, large area requires	(Molinuevo-Salces et al., 2019)

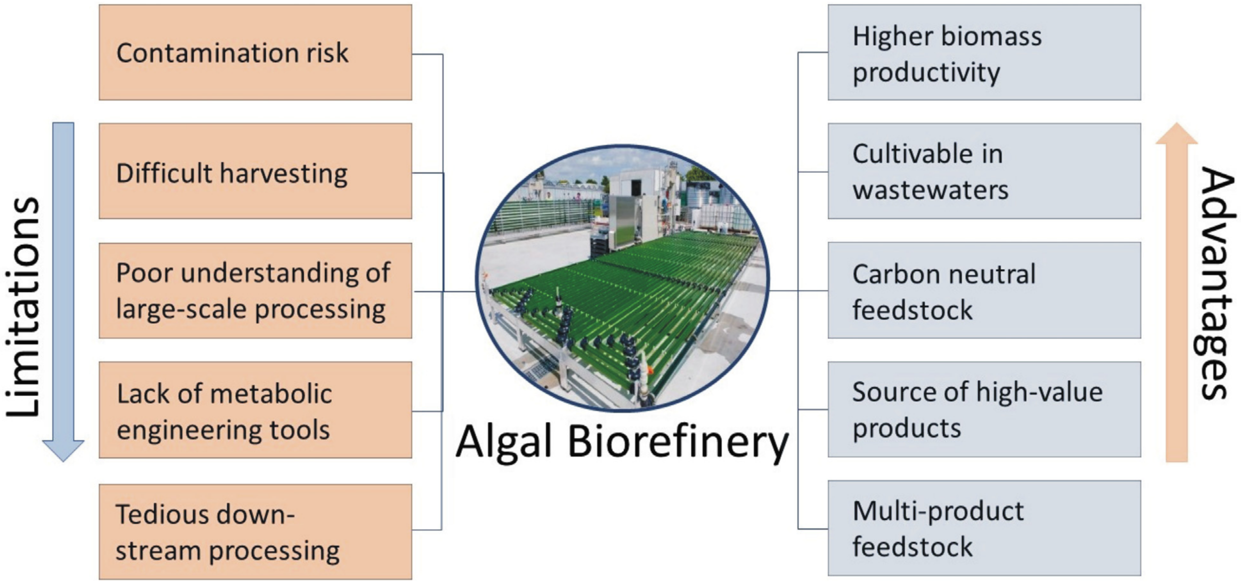

Fig. 1. Merits and demerits of algal biomass production and processing in algal biorefinery.

media, (ii) support bulk biomass and biofuel production, (iii) can supply ample nutrients, and (iv) offers the possibility of integrating algal cultivation with the existing infrastructure wastewater treatment (Roostaei and Zhang, 2016). Several studies have been conducted in last decades on microalgae-based phycoremediation of wastewater (Chen et al., 2015; Salama et al., 2017; Udaiyappan et al., 2017; Wang et al., 2017a) and biorefinery based approaches have been proposed (Gill et al., 2013). Microalgae have been studied for wastewater (industrial and domestic) treatment including brewery wastewater (Ferreira et al., 2017), domestic wastewater (Calicioglu and Demirer, 2017), textile wastewater (Wu et al., 2017a), pharmaceutical waste streams (Xie et al., 2019), slaughterhouse industry (Aziz et al., 2019), heavy metal-containing wastewater (Khan et al., 2017), palm oil mill effluents (Hariz and Takriff, 2017), starch-containing textile wastewater (Lin et al., 2017), and agro-industrial wastewater (Jayakumar et al., 2017). Though microalgae-based remediation has numerous benefits, however, there are several challenges that also need to be addressed.

Several studies have demonstrated species of *Chlamydomonas*, *Chlorella* and *Scenedesmus* can be employed for efficient nutrient uptake (N, P) (Gao et al., 2016) and removal of toxic pollutants and heavy metals (Khan et al., 2017; Matamoros et al., 2015) from wastewater. *Ankistrodesmus* along with *Scenedesmus* and *Chlorella* has shown to degrade organic pollutants present in paper and oil mill industry wastewater (Bhattacharya et al., 2017). Similarly, *Scenedesmus* sp. represented higher utilization efficiency of 98.2%, 97.1% and 95.2% for butyrate, propionate, and acetate respectively with a growth rate of 0.53 gd^{-1} when cultivated in textile desizing wastewater (Lin et al., 2017). *Chlorella variabilis* showed to consume nutrients from textile effluents and with 100% remediation ability for nickel, aluminum, and iron with biomass productivity of 74 gm^{-2} d^{-1} with lipid yield of 20% (Bhattacharya et al., 2017). Similarly, high nitrogen (99.6%) and phosphorus (91.2%) removal efficiency with the concomitant production of biomethane (523 mL) was reported by *C. vulgaris* grown in municipal wastewater (Calicioglu and Demirer, 2017). Freshwater microalgae including *Cladophora glomerata* and *Oedogonium westii* can remove heavy metals from industrial wastewater and showed 80% of cadmium and 66% of nickel accumulation, respectively (Khan et al., 2017). Additionally, *Neochloris aquatica* CL-M1 was employed to produce butanol along with wastewater treatment (Wang et al., 2017c), where biobutanol yield of 0.89 g L^{-1} h^{-1} with 96.2% of NH_3-N removal efficiency was observed. Moreover, lipid-rich *Botryococcus* sp. removed 59.9% nitrogen, 36.8% phosphate and 54.5% organic carbon along with the production of 72.5% of crude oil content, when cultivated in domestic wastewater (Gani et al., 2017).

Biotic factors (microbial load / competing pathogens) and abiotic factors (nutrients, pH, and CO_2) play a pivotal role in microalgal metabolism. Manipulation of stress factors including biotic and/or abiotic through wastewater significantly affects the productivities of both biomass and product of interest (Table 2). Unravelling the underlying processes and reorienting the related pathways can greatly contribute to achieving robustness, enhanced productivities in energy and cost-efficient manner (Chen et al., 2017). Utilization of wastewater as an alternative media offers additional benefits including wastewater cleaning, recycling, reducing the

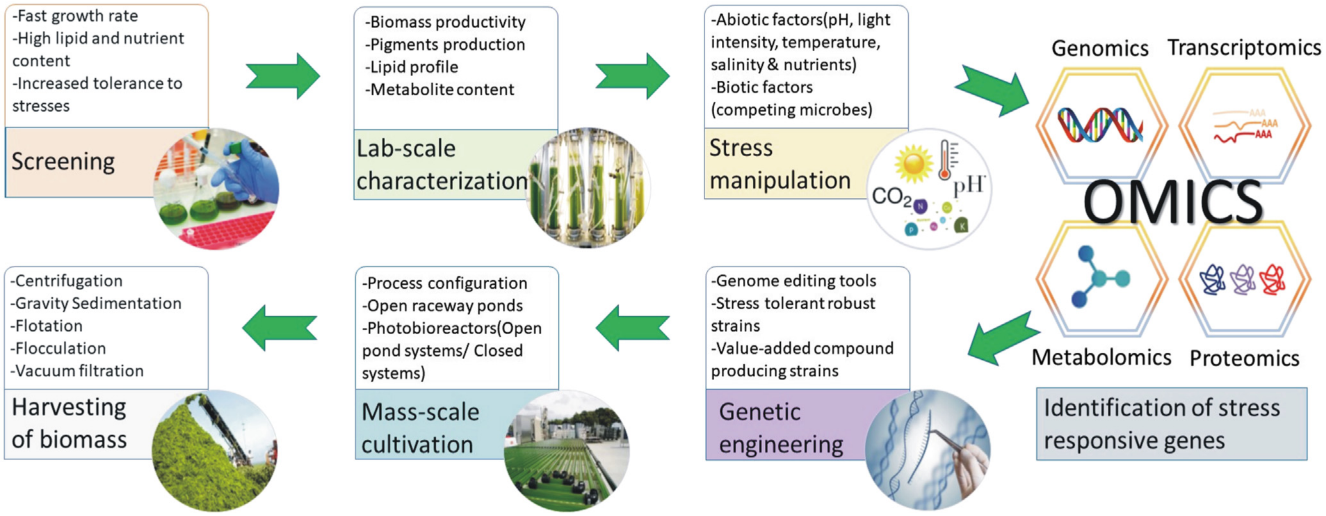

Fig. 2. Road map of steps involved in strain development and process optimization.

environmental pollutions, and provision of low-cost growth media (Yadav et al., 2019). However, it requires detailed studies of using wastewater as low-cost growth media and studying the impact of biotic and abiotic factors to attain the full potential of microalgae for the biorefinery.

2.1. Removal of macro and micro-nutrients

Microalgae store lipids as polar (polyunsaturated fatty acids) and non-polar forms (saturated fatty acids, mainly in the form of triacylglycerides) which are important to maintain membrane functions or to perform other cellular activities. These lipids can be transesterified to produce biodiesel (Paliwal et al., 2017). The stress of various macro and micronutrients, mainly nitrogen and phosphorus, has been studied widely to enhance algal metabolite content mainly lipids.

2.1.1. Nitrogen

Nitrogen is one of the principal nutrients for the synthesis of nucleic acid, protein, energy-carrying molecules (ATP), and enzymes (Juneja et al., 2013). Naturally, nitrogen is available in the form of nitrate, urea, ammonium, and peptones (Minhas et al., 2016). In microalgae, nitrogen accounts for 1–20% of dry cell matter and is part of the essential functional and structural proteins of algae (Juneja et al., 2013). Nitrogen starvation has been considered as the most effective induction strategy (Shi et al., 2017) that shifts the organism's metabolic pathway towards enhanced lipid synthesis, triglyceride accumulation, protein content reduction and increased carotenogenesis, at the expense of biomass production (Juneja et al., 2013; Minhas et al., 2016).

An increase of 2.27-fold in lipid content was observed in *Scenedesmus quadricauda* when grown under nitrogen-starved conditions (226 mgL^{-1}) (Anand and Arumugam, 2015). *Acutodesmus dimorphus* accumulated 75% neutral lipids of total lipids under nitrogen-starvation conditions (Chokshi et al., 2017). Cultivation of *Pseudokirchneriella subcapitata* under nitrate stress conditions resulted in 160 $mgL^{-1}d^{-1}$ fatty acid accumulation (Del Río et al., 2017). *Scenedesmus obliquus* showed 4.4 $mgL^{-1}day^{-1}$ of total nitrogen removal efficiency with 1.4 gL^{-1} of biomass and 29.8 mgL^{-1}-day^{-1} of lipid productivity when grown on urban wastewater (Álvarez-Díaz et al., 2017). Similarly, removal of 84.51% of nitrate and 75.56% of ammonium along with the production of 172.9 $mgL^{-1}day^{-1}$ of carbohydrate, 150.2 $mgL^{-1}day^{-1}$ of lipid and 141.5 $mgL^{-1}day^{-1}$ of protein productivities were reported *C. sorokiniana* cultured in wastewater (Guldhe et al., 2017). Cultivating *C. vulgaris* in mixed piggery and brewery wastewater reduced nitrogen and ammonia by 96% and 100%, respectively producing 2.85 gL^{-1} biomass (Zheng et al., 2018). Similarly, COD and nitrogen removal efficiencies of 76% and 98%, respectively, were reported for wastewater treatment using *S. obliquus* (Gupta et al., 2016).

These studies have clearly indicated that nitrogen stress has a global impact on various microalgae to enhance lipid content, but it cannot be applied on a commercial scale using synthetic growth media, which will raise the cost. Alternatively, nitrogen stress can be applied using wastewater, which often has higher nitrogen content (municipal wastewater), however, wastewater from industrial sources may have lower nitrogen content. Consequently, water from these sources can be used to exert nitrogen stress on the microalgae. Removal of nitrogen by microalgae does not only treat wastewater but also trigger the accumulation of value-added products. However, aore detailed studies are required in the future to elucidate the role of wastewater derived N-stress on growth and biomass productivity of microalgae.

2.1.2. Phosphorus

Phosphate is another important (second only to nitrogen) macronutrient of a living system which is part of RNA, DNA backbone, ATP, phospholipids (Juneja et al., 2013), phosphoproteins, polyphosphates and in the form of NADPH. Phosphate limiting conditions lead to organic carbon accumulation (TAG) and decreased cell division. Phosphorus deficiency is also known to affect the energy-requiring processes like protein synthesis, transcription, and carbon cycle (Mühlroth et al., 2017). Generally, it constitutes <1–1% of the total dry mass of microalgae (Minhas et al., 2016). The *C. protothecoides* has shown to accumulate 32.8% of lipid content under mixotrophic conditions using wastewater as growth media which was deficient in phosphorus (Li et al., 2014). Similarly, under nutrient stress, the *C. vulgaris* was shown to accumulate lipids by 17.41% producing 9.81 gL^{-1} of biomass, which is comparatively low than nitrogen and sulfur stress (Sakarika and Kornaros, 2017). Similarly, 53% of lipid content and 23.45 mgL^{-1} of lipid productivity was reported for *Chlorella* under phosphorus deprivation conditions (Wong et al., 2017).

Phycoremediation of municipal wastewater by *Micractinium* sp. IC-76 resulted in 77% of PO_4-P removal efficiency with 37.18 mg $L^{-1}day^{-1}$ of biomass productivity and 36% of lipid content

Table 2

Impact of wastewater derived nutrients on the production of biomass and metabolite contents with the focus on microalgal phycoremediation and biorefinery ability.

Microalgae	Wastewater	Wastewater-derived stress	Nutrient removal (%)			Biomass productivity (mgL^{-1}d^{-1})	Metabolites (%)			References
			Total Nitrogen	Total Phosphorus	Chemical Oxygen demand		Lipid	Protein	Carbs/ others	
Scenedesmus obliquus	Brewery effluent	Low pH, high nutrient and sugars	88	30	71		64 (bio-oil)	31.4	0.2–1 mgmL^{-1} (Phenol)	(Ferreira et al., 2019)
Tetraselmis suecica	Dairy	High Nutrient, varying pH	83	100	–	42.5	–	–	11.7 mgL^{-1} (Chl-a)	(Daneshvar et al., 2019)
Tribonema minus	Tofu-whey	High nutrients and COD	92.8	72	86.7	431.6	37	15.5	31	(Wang et al., 2019a,b)
Ecuadorian *Chlorella* sp.	Synthetic (Secondary effluent)	High nutrients, alkaline	52–93	67	–	0.6–1.8	28	–	–	(Benítez et al., 2019)
C. pyrenoidosa	Olive-oil mill	High organic matter	–	97	96.2	1.25	23	11	65	(Malvis et al., 2019)
C. sorokiniana	Cooking cocoon	Organic pollutant, high protein	29.4	46	89	85.7	23.3	39.3	29.4	(Li et al., 2019a,b)
Scenedesmus sp.	Meat market	High N and P	90	85	–	98.5	23.2	41.2	–	(Apandi et al., 2019)
Chlorella sp.	Soybean-processing (diluted)	Moderate nitrates	85	97	70.5	–	7.22 mgL^{-1}d^{-1}	–	2.86 mgL^{-1}d^{-1}	(Qiu et al., 2019)
Ascochloris sp.	Raw-Dairy	High N and P	80	97	96	94–98	33.4	–	–	(Kumar et al., 2019)
Chlorella & *Oocystis* sp.	Power plant	Sulfate rich	22–32 (Sulfates)			50–25.24 (Respectively)	Reported biodiesel potential			(Mohammadi et al., 2018)
Dunaliella salina	Tertiary treated municipal	Moderate Nitrate and P	57.5	69	52	28.25	–	–	–	(Liu and Yildiz, 2018)
C. sorokiniana	Diluted municipal & industrial	High nitrates and phosphate	84.2	47	–	1524	62.4 mg/g (FAME)	388.2 mg/g	11.82 mg/g (chlorophyll)	(De Francisci et al., 2018)
Chlorella sp. MM3	Diluted winery & piggery (80:20)	Low pH, high organic matter & nutrients	89.3	56.5	–	4.4x10^6 cells/mL	51	–	–	(Ganeshkumar et al., 2018)
Desmodesmus sp.	Synthetic industrial effluent	High P and heavy metals	85 (Ni)	>90	94 (Cu)	0.05	17.6	–	–	(Rugnini et al., 2018)
C. vulgaris	Aqua-culture and pulp	High carbon & nutrient	76.5	92.7	75.5	187	9.07	47.5	19.09	(Daneshvar et al., 2018)
Chalymondomonas sp.	Palm oil mill effluent (POME)	High nutrient and CO_2	65	34	56	–	90 mgg^{-1}	–	–	(Hazman et al., 2018)
Ascochloris sp. (ADW007)	Raw-dairy	High nutrients	78	98	95	102–207	34	–	–	(Kumar et al., 2018)
Dunaliella tertiolecta	Diluted food leachate	High nutrients	>80	80	–	200	37 (SFA)	–	–	(Wu et al., 2018)
Coelastrum sp.	Cattle farm	High nutrients	80	100	42	281	50.7	–	–	(Mousavi et al., 2018)
Chlorella sp. (ISTLA)	Domestic-treatment plant	High nutrients	82 (Zn), 80 (Mn), 56 (Cu)			50	43.8	–	–	(Mishra et al., 2018)
Tetraselmis chuii	Aqua-culture	High nutrients	54	–	–	3.25x10^5 cells/mL	17.6	33	12	(Khatoon et al., 2018)
Dunaliella sp.	Synthetic	High amount of heavy metals	93.6 (Ni)			–	65 pgcell^{-}	–	0.97 mgg^{-1}	(Moussa et al., 2018)
Tetraselmis sp	Synthetic mariculture	High nutrients and solids	90	–	80	124.5	29.48 mg^{-1}d^{-1}	–	–	(de Alva et al., 2018)
Parachlorella kessleri	Agro-waste	High nutrients	>98	59	39	62	23.8 (FA)	–	–	(Koutra et al., 2018)
Asterarcys quadricellulare	Municipal wastewater treatment plant	Nutrients	48	50	–	65	21.9	–	50	(Odjadjare et al., 2018)

Table 2 (*continued*)

Microalgae	Wastewater	Wastewater-derived stress	Nutrient removal (%)			Biomass productivity ($mgL^{-1}d^{-1}$)	Metabolites (%)			References
			Total Nitrogen	Total Phosphorus	Chemical Oxygen demand		Lipid	Protein	Carbs/ others	
S. obliquus	Secondary brewery effluent	Low pH and high nutrient	94	–	74	200	–	50	–	(Marchão et al., 2018)
S. acuminatus	Paper and pulp	High pH and nutrients	100	>97	–	685	19.9	24.3	60.5	(Tao et al., 2017)
C. vulgaris	Swine manure	High solids and ammonium	71	54	78.7	157	17.4	58.8	9.1	(Deng et al., 2017)
Desmodesmus sp.	Oil-refinery (Dilute)	Acidic, high COD & phosphate, greases	–	53	82	270	22	–	–	(Mar et al., 2016)
Chlorella	Sea-food processing	High nutrients	94.5	68.4	–	77.7	27	–	39 mgL^{-1} Chlorophyll	(Gao et al., 2018)

(Piligaev et al., 2017). Cultivation of *Desmodesmus abundans* on synthetic wastewater showed 16% of lipid content and 87.52% of phosphate removal rate (Prasad et al., 2017). Treatment of sewage water by phosphate starved *Scenedesmus* (Yewalkar-Kulkarni et al., 2017) resulted in an enhanced carbohydrate ratio with 87% of phosphate removal. Hence, it is suggested to expose the microalgae to the combined stress of nitrogen and phosphorus to achieve higher biomass and lipid productivities (Chen et al., 2017). A suitable cost-effective alternative is the use of wastewater. Cultivation of microalgae in wastewater will consume phosphorus (act as stress factor) and thus help in wastewater treatment.

2.1.3. Sulfur

Sulfur is also one of the most important macronutrients. Commonly, sulfate is consumed as a sulfur source by plants and algae. Cellular processes namely assimilation, secondary metabolic pathways, oxidative stress responses, flavonoid, and nitrogen metabolism are affected by sulfur. In algae, enhanced assimilation of Sulfur is believed to downregulate the photosynthesis, and its stress induces expression of stress-associated proteins (Giordano and Raven, 2014). Naturally, sulfate is present in excessive amounts in the wastewater from paper and pulp industry, pharmaceutical industry, mining, and food processing (Lv et al., 2017). An increasing trend in neutral lipids (123–172%), oligosaccharide, TAG and polysaccharide levels was observed for wild-type *Chlorella* strain cultivated under sulfur-deprived conditions (Cakmak et al., 2012). Moreover, the sulfur starvation showed to increase the β-carotene level from 6.753 mgL^{-1} to 14.616 mgL^{-1} with decreasing biomass production in *Dunaliella salina* (Shaker et al., 2017). Unlike Nitrogen, Sulfur starvation has proven to be more efficient for starch accumulation when compared to Nitrogen starvation which causes lipid accumulation (Vitova et al., 2015). An increase of 18-fold in starch content was observed in *C. reinhardtii* when cultured under S-deprived conditions. It is believed that sulfur starvation inhibits energy-consuming pathways like cell growth and division while, shifting the pathways towards starch accumulation (Antal et al., 2014). Interestingly, Sulfur deprivation altered the fatty acid composition of *C. lobophora* (Takeshita et al., 2014). On the other hand, sulfate stress negatively affected the pollutant removal ability, growth and self-flocculation efficiency of *Chlorococcum* sp. GD when grown in synthetic municipal wastewater (Lv et al., 2017). It indicates that the impact of sulfate may not be global, hence we need to conduct carefully designed detailed studies to evaluate the potential of pollutant removal, biomass and metabolite productivities under sulfur stress alone, and/or the combined stress of other nutrients, which may lead towards the selection of suitable strains. While the selection of strain will be influenced by the type and source of the wastewater too.

2.1.4. Heavy metals

Metals are the natural constituents of soil and earth crust. However, in ecological terms, any metalloid or metal which has bioaccumulation ability and causes environmental pollution is termed as heavy metal. Some of the metals are micronutrients (Cu, Zn, Ni, Mn, and Co) and are essential for growth (Kumar et al., 2015), and some others like Mn, Cu, I, Zn, Fe, Pb, etc. are proven to be beneficial for nutritional quality improvement and other important functions of living system (Wells et al., 2017). Some heavy metals including Pb, Cd, Cr, and Hg have unidentified biological functions and are toxic in nature (Afshan et al., 2015; Kumar et al., 2015) as their excessive uptake may affect metabolic processes, the physical structure of algae and can cause toxicity, mutagenesis and allergenicity (Mikulewicz et al., 2017).

Heavy metals stimulate the ROS (reactive oxygen species) formation resulting in oxidative damage. Decreased metabolite content (pigments, proteins, and monosaccharides), size and number of cells observed to be related to lead accumulation in *Acutodesmus obliquus* (Piotrowska-Niczyporuk et al., 2015). Increased protein and carbohydrate ratios were observed when *C.* sarokiniana cultivated in mixotrophic and photoautotrophic conditions under the influence of titanium-dioxide (TiO_2) nanoparticles (Marchello et al., 2018). Exposure of copper sulfate ($CuSO_4$) induced lipid peroxidation due to the formation of ROS and reduced the levels of carotenoids, chlorophyll-a, and b in Chlorella, which reflects highly toxic nature of $CuSO_4$ (Wan et al., 2018). Cheng and coworkers (Cheng et al., 2016) reported 71%, 93% and 74% reduction in carotenoids, chlorophyll-a, and chlorophyll-b level, respectively, in C. vulgaris as a result of 7 mgL^{-1} cadmium treatment while ~96% increase in soluble protein accumulation was observed.

Modern agronomic practices (metal-based pesticides), intense industrialization (metal, textile, mining etc.), enhanced anthropogenic activities (rubber, paint, paper, and metal alloys production), and unauthorized waste disposal have increased the concentration of these pollutants in environment (Das and Osborne, 2018; Kumar and Gunasundari, 2018). Biosorption of heavy metals from wastewater by microalgae is a promising alternative as it is a cost-effective, ecologically safe and efficient method (Ummalyma et al., 2018) when compared to conventionally employed methods. *C. vulgaris*, *Chlamydomonas reinhardtii*, *Chlorococcum spp*, *Phaeodactylum tricornotum*, *Scenedesmus quadricauda*, *Spirogyra* spp. and many other algal species have been reported for heavy metal biosorption from wastewater (Brinza et al., 2007; Ummalyma et al., 2018). Biosorption studies of *Scenedesmus* sp. showed the potential of said species to remove heavy metals namely Cu (73–98%), Zn (65–98%), Cr (81–96%) and Pb (75–98%) from tannery wastewater under laboratory conditions (Ajayan et al., 2015). Similarly, the biomass of *Chara aculeolate* removed Cd, Pb, and Zn via biosorption at the rate of 23 mgg^{-1}, 105.3 mgg^{-1} and 15.2 mgg^{-1} respectively from municipal wastewater (Sooksawat et al., 2016). Moreover, *S. armatus* and *C. vulgaris* showed bioaccumulation efficiency for Cd^{2+} and Pb^{2+} by 89.96% and 88.98%, respectively (Zabochnicka-Świątek and Rygała, 2017). Similarly, *Cladophora glomerate* and *Oedogonium westii* showed the removal of Cd and Ni by 80% and 66.3%, respectively from industrial wastewater (Khan et al., 2017). Interesting results were obtained for self-flocculating microalga *C. vulgaris* JSC-7 which was able to remove Cd and Zn (60–80% respectively) more efficiently as compared to the non-flocculating similar strain. Moreover, an increase in the photosynthetic pigments and growth was observed under heavy metal stress, showing its excellent heavy metal tolerance ability (Alam et al., 2015).

Heavy metal accumulation is associated with toxicity and ROS production where Pb, Cu, Cd, and Ti greatly reduce the growth and pigment content of microalgae. However, in some cases, an increase in the protein and lipid content was also observed. Heavy metal-containing wastewater poses many adverse effects and is not suitable for human consumption. Phycoremediation of heavy metal-containing wastewater is an eco-friendly and cost-effective approach owing to the remarkable potential of microalgae to remove high levels of heavy metals from wastewater.

2.2. Impact of pH

Invasion of pathogenic microbes (predators or competitors) in the open pond cultivation of microalgae causes contamination of microalgae and poses a major challenge during cultivation. There are several factors that can influence the load of invading organisms. For instance, microalgae may produce some extracellular compounds to inhibit the competing organisms resultantly dominating the environment. Some secretory metabolites can even help the microalgae to modify the pH of the media (Shahid et al., 2019) which helps to outcompete the invading organisms. Hence, pH is one of the most important parameters which can be employed to inhibit or outcompete the invading organisms. Alteration in pH has been reported to improve culture densities by decreasing the microalgal contaminants (Bartley et al., 2014). Additionally, pH plays a vital role in algal cultivation as it is responsible for nutrient and CO_2 availability and solubility. Moreover, it is known to affect the activity of various enzymes to enhance the triglyceride accumulation (Juneja et al., 2013; Ying et al., 2014). Optimum pH for microalgae cultivation usually ranges from 7.0 to 7.6 (neutral pH) but, pH tolerance is species-specific and maximum biomass productivities for *S. obliquus* and *Ettlia* sp. were observed at pHs 7.0 and 8.5, respectively (Chen et al., 2017). The pH of the growth media influences the microbial enzyme activity and solubility of environmental micro-pollutants. Therefore, pH variation determines the fate of pollutants during bioreactor treatments. Furthermore, acidic to neutral pH values are significant for improved pharmaceutical degradation (Grandclement et al., 2017).

Cultivation of *C. vulgaris* at pH ranging from 3 to 11 represented its ability to act differently under specific pH as highest biomass productivity (0.541 days^{-1}) and lipid content of 53% was observed at pH 7.5 while, pH 9.5 found to be suitable for cell aggregation (Sakarika and Kornaros, 2016). Interestingly, in the case of *N. oleoabundans*, pH 9.5 supported maximum cell growth (1.04 gL^{-1}), lipid content (151.2 mgg^{-1}) and lipid productivity (19.1 mgL^{-1}day^{-1}) under oxygen stress (Peng et al., 2017). *S. abundans* was able to tolerate a large range of pH (5–8) however, pH 8 was optimum to obtain the highest growth rate of 769 mgL^{-1} and pH 6 favored lipid concentration to 179 mgL^{-1} (Mandotra et al., 2016). Similarly, pH alteration enhanced the self-flocculation ability of *Dunaliella* sp. when 2 or 6 N NaOH was added in the culture media (Byrd and Burkholder, 2017). These studies indicated that pH manipulation can be employed to enhance the algal metabolite content and biomass productivity by outcompeting the pathogens. However, more detailed studies are required to elucidate the impact of wastewater-derived pH on biomass productivity and metabolite content.

3. Binary culture to enhance phycoremediation of wastewater

It is difficult to maintain pure cultures under field conditions, which is even more difficult when using ponds receiving wastewater. Hence, recently researchers focused on the cultivation of mixed cultures. Binary cultures in the form of consortia (microalgae-microalgae or microalgae-bacteria) have been reported extensively for enhanced wastewater treatment due to their higher nutrient removal ability with enhanced biomass production (Table 3). Polycultures allow us to develop robust biological systems for wastewater treatment as they can be combined with various metabolic processes to enable themselves to survive under environmental stress conditions. Moreover, integrated consortia can uptake nutrients at a higher rate (Johnson and Admassu, 2013; Rawat et al., 2011; Renuka et al., 2013) because one strain can remove nitrogen and another can remove heavy metals specifically. These consortia have several advantages including (i) contamination and predator resistances due to production of allelochemicals (ii) enhanced nutrient consumption; ensuring sufficient nutrient supply during whole process, (iii) development of settleable system for flocculation thus, eliminating limitations of harvesting, and (iv) enhanced viability of phycoremediation; as loss of one microbe is compensated by other species. However, it faces some constraints as it's difficult to develop robust consortia as a variety of combinations are possible. Moreover, maintenance of consortia for longer periods especially in an open-pond system proven to be challenging (Gonçalves et al., 2017).

3.1. Microalgae-bacteria (MB) consortia

The symbiotic relationship of microalgae and bacteria may be in the form of commensalism, mutualism or parasitism. In general, bacteria heterotrophically produce CO_2 and other important nutrients that are consumed by microalgae for their growth (Zhu et al., 2019). In return, oxygen produced by microalgae during photosynthesis is valuable for bacteria (Rashid et al., 2018). Moreover, bacteria provide growth-promoting hormones and vitamin B to microalgae which are necessary for growth (Fuentes et al., 2016). Additionally, this symbiotic relationship protects the microalgae from other invading species. On the other hand, bacteria can damage the microalgal cell wall to utilize intracellular nutrient (Magdouli et al., 2016). This property is of special interest during harvesting stages where cell rupturing is required to obtain the desired product thus, reducing cost and time of downstream processing in the biorefinery. The MB-consortia also consume the dead algal cells as a nutrient source (Ramanan et al., 2016). Excretion of special chemicals by bacteria and microalgae may also suppress the growth of each other (López-Serna et al., 2019). This property

Table 3
Exploitation of binary cultures for the wastewater treatment and production of value-added products.

Type of consortia	Consortia	Cultivation mode	Wastewater source	Nutrient removal (%)	Biomass production (gL^{-1})	Impact on metabolites (mgL^{-1})	References
Algal-bacteria	*Chlorella, Klebsiella* & *Acinetobacter*	Co-cultivation in automated bioreactor	Dairy Farm	84.7 (Nitrates) 90 (COD)	2.87	–	(Makut et al., 2019)
Algal-bacteria	*Chlorella, Klebsiella* & *Acinetobacter*	Co-cultivation in automated bioreactor	Synthetic	93.5 (nitrates) 82 (COD)	2.84	–	(Makut et al., 2019)
Algal-bacteria	*C. vulgaris* & activated sludge	Co-cultivation	Municipal	55–64 (COD)	1.1–1.0.42	17.4–22% (lipid)	(Leong et al., 2019)
Algal-bacteria	*Chlorella, Scenedesmus* & activated sludge	Symbiotic system	Synthetic municipal sewage	100 (COD & PO_4^{-3}-P) 98 (NH_4^+-N)	0.76	15.24–16.67% (lipid)	(Chen et al., 2019)
Algal-bacteria	*C. sarokiniana, Nitrosomonas & Dechloromonas*	Light-limiting heterotrophic	Synthetic municipal	98 (N) 88 (COD) 96 (P)	2.5	–	(Fan et al., 2020)
Algal-bacteria	*Desmodesmus* sp. & nitrifying-bacteria	Algae-based co-cultivation	Piggery	52 (NH_4^+-N) 100 (TP)	–	4.74 chlorophylls	(Wang et al., 2019a,b)
Algal-bacteria	*C. vulgaris* & *Exiguobacterium profundum*	Aerobic-illuminiation	Synthetic (metal-rich)	80 (Ni) 79 (Cu) 56.4 (Cr)	–	–	(Batool et al., 2019)
Algal-bacteria	*Chlorella, Klebsiella* & *Acinetobacter*	Batch mode Photo-bioreactor	Paper industry	99.9 (TN) 95 (COD)	3.17	15% crude oil	(Goswami et al., 2019)
Algal-bacteria	*Spongiochloris* & *Hydrocabonoclastic*	Airlift bioreactor	Petroleum	97 (COD) 99 (hydrocarbon)	8.51	338 chlorphyll-a, 2.92 $gL^{-1}day^{-1}$ CO_2 fixation	(Abid et al., 2017)
Algal-bacteria	Scenedesmus & aerobic-heterotroph	Photo illumination	Coke (Petroleum)	100 (TN) 90 (phenol)	–	32–40 FAME	(Ryu et al., 2017)
Algal-bacteria	Scenedesmus *Flavobacteria* & *Sphingobacteria*	Two-phase photoperiodic	Municipal	98 (TP) 96 (TN) 92 (COD)	1.8	22.6% lipid	(Lee et al., 2016)
Algal-fungal	*Scenedesmus obliques* & wild yeast	Non-sterile heterotrophic	Municipal	96 (nitrates) 100 (TAN)	2.74	2200 bio-ethanol	(Walls et al., 2019)
Algal-fungal	*C. vulgaris* & *Aspergillus niger*	–	Synthetic pharmaceutical	47.4 (TN)	0.65	59% lipids	(Hultberg et al., 2019)
Algal-fungal	*C. pyrenoidosa* & *Rhodotorula glutinis*	Pilot-scale bioreactor	Piggery	83 (TN) 53 (TP) 85 (COD)	1.0	60% protein	(Li et al., 2019a,b)
Algal-fungal	*Chlorella vulgaris* & *Rhodotorula glutinis*	Co-culture fermenter	Starch	80 (COD) 85 (organic acids)	9.8	12.34 carotenoids	(Zhang et al., 2019)
Algal-fungal	*Scenedesmus* & *Trichoderma ressei*	Non-sterile	Seafood processing	74 (COD) 93 (TP) 44 (TN)	2.17–6.64	600–1700 lipids	(Srinuanpan et al., 2018)
Algal-fungal	C. vulgaris & Yarrowia lipolytica	Photo-bioreactor	Yeast industry	80 (NH_3-N & COD)	1.23–1.56	183 lipids	(Qin et al., 2018)
Algal-fungal	*C. vulgaris* & *Ganoderma lucidu*	CO_2 supplementation (Photo-bioreactor)	Biogas slurry	68 (COD) 61.7 (TN) 64 (TP)	644.3	–	(Zhou et al., 2018)
Algal-fungal	C. vulgaris, Ganoderma lucidum	Photobioreactor	Biogas slurry	72–73 (TN & TP)	0.41	Improve biogas by 89% CO_2	(Wang et al., 2017b)
Algal-algal	*Chlorella* & *Scenedesmus*	Symbiotic system	Synthetic municipal sewage	100 (COD & Nitrate) 95 (PO_4^{-3}-P)	0.05–0.7	15–18% lipid	(Chen et al., 2019)
Algal-algal	Wild-algae & *Scenedesmus*	–	Simulated municipal	87 (Nitrate) 19 (P)	0.278	11.5% lipid	(Qu et al., 2019)
Algal-algal	*Chlorella* & *Scenedesmus*	Thin-layer reactor	Anaerobically digested Piggery	98 (Ammonia) 44 (COD)	2.96	5.4 $mgL^{-1}d^{-1}$ lipid	(Raeisossadati et al., 2019)
Algal-algal	*Leptolyngbya* & *Ochromonas*	Non-aseptic	Cheese whey	70 (nitrate) 93 (COD) 84 (P)	0.9	124 algal oil	(Tsolcha et al., 2018)
Algal-algal	Mixed algal phylum	Heterotrophic	Whey processing	–	14.32	1910 lipid	(Jordaan et al., 2018)
Algal-algal	Mixed algal phylum	Sterile-heterotrophic	Fish cannery	–	14.02	1240 lipid	(Jordaan et al., 2018)
Algal-algal	*Leptolyngbya* & *Ochromonas*	Mixotrophic	Winery & raisin	93 (COD) 78 (TN) 99 (P)	1.3	13% lipids	(Tsolcha et al., 2017)
Algal-algal	Native algal consortia	Pilot-scale (raceway pond)	Greywater	99.7 (nitrate) 99 (TP)	0.7	45.8% lipid, 28% protein, 10% carbs	(Kumar et al., 2017)
Algal-algal	*C. zofingiensis, Scenedesmus & Chlorella*	Photo-autotrophic	Dairy	91–95 (TP) 57–62 (COD)	5.1–5.4	143–150 $mgL^{-1}d^{-1}$ lipids	(Qin et al., 2016

(*continued on next page*)

Table 3 (*continued*)

Type of consortia	Consortia	Cultivation mode	Wastewater source	Nutrient removal (%)	Biomass production (gL^{-1})	Impact on metabolites (mgL^{-1})	References
Algal-algal	Native microalgae consortia	Photo-autotrophic	Livestock	80–100 (all nutrients)	1.93	54% proteins, 31 lipids, 10% carbs	(Choudhary et al., 2016)
Algal-algal	*Chlorella, Scenedesmus, Chlamydomonas*	High-rate algal pond (HARP)	Dairy farm	98 (COD)	153.54 Ton $ha^{-1}yr^{-1}$	29,470 $Lha^{-1}yr^{-1}$ Algal oil	(Hena et al., 2015)

can be useful to reduce sterilization cost as it eliminates the contamination chances (Kouzuma and Watanabe, 2015).

The MB-consortia have shown to be effective for removal of phosphorus (35–88%), nitrogen (43–89%) and carbon (59–80%) respectively from municipal wastewater in a lab-scale photobioreactor (Lee et al., 2015). Moreover, a marine algal-microbial (Picochlorum sp, Pseudomonas sp., and *Chitrinomycetes*) consortium was developed for the treatment of marine aquaculture effluents which showed up to 95% of removal efficiency in 4–5 h (Babatsouli et al., 2015). Improvement in total phosphorus and total nitrogen removal by 46% and 12% from domestic wastewater was observed in batch reactors cultivated with the MB-consortia (Tang et al., 2016). The MB-consortium based on B. licheniformis + C. vulgaris showed excellent efficiency to remove COD (86.5%), TN (88.9%) and TP (80.2%) from synthetic wastewater (Ji et al., 2018). Similarly, the symbiotic relationship of Chlorella and Proteobacteria removed 72% TN in an outdoor cultivation system while 100% TP and 83% Zn removal efficiency were observed in an indoor cultivation system using piggery wastewater (García et al., 2017).

3.2. Microalgae-microalgae consortia

In order to minimize the issues and challenges regarding the microalgal cultivation, one promising alternative is to cultivate multiple algal species which have a synergistic impact on one another to enhance the production and productivities as diverse communities may enhance biomass specific lipid production when compared to corresponding monocultures (Stockenreiter et al., 2016). Additionally, diversity correlates with the biomass stability which is an important requirement of mass cultivation (Nalley et al., 2014). Moreover, a prodigious variety of traits by primary producer communities relates to higher diversity (Stockenreiter et al., 2016).

More than 96% of nutrient removal efficiency along-with 6.82% lipid content and 9.2–17.8 tonnes $year^{-1}ha^{-1}$ of biomass productivity was achieved using polyculture of 15 native-algal strains (Chinnasamy et al., 2010). Mixed microalgal cultures predominantly consisting of *Chlorella* with small amounts of *Scenedesmus* could remove 31 mgL^{-1} of phosphorus and 481 mgL^{-1} of nitrogen from textile wastewater when cultivated under photoautotrophic conditions (Huy et al., 2018). Polyculture of *Chlorella* sp. displayed the ability to removal 100% nitrates from swine wastewater and accumulated high lipid and protein content in the ratio of 59% and 34% respectively. However, lipid content only accounts for 3% of total biomass content (Michelon et al., 2016).

3.3. Myco-algal consortia

Naturally, microalgae live in association with fungi in the form of lichens. This strategy benefits both partners; fungi obtain essential nutrients and sugars from algae and in return provide protection to microalgae from abiotic stress. It is proposed that this association can act as a self-sufficient organization to improve the overall performance and economics of the integrated microalgal industry at large scale (Ummalyma et al., 2017). Additionally, fungal pellets may act as natural coagulants and ultimately helping the microalgal flocculation, hence making harvesting easier. These pellets also have the potential of treating wastewater by entrapping sludge solids (Ummalyma et al., 2017). Furthermore, 30% of the total fungal biomass consists of lipids, making them suitable biodiesel producer candidates (Zhou et al., 2013).

The mixed culture of *C. vulgaris* and *Mucor indicus* reduced the phosphate and total ammonia and nitrogen (TAN) to almost undetectable levels from synthetic aquaculture wastewater. This process also enabled the flocculation of 860 mg DW of myco-algal biomass (Barnharst et al., 2018). Biogas up-gradation and domestic sewage wastewater treatment were simultaneously performed by algal-fungal co-cultivation which removed TP and COD by 81% (Xu et al., 2017). Similarly, co-cultivation of *C. vulgaris* with Ganoderma lucidum reduced TP, TN and COD concentrations by 84%, 74%, and 79% respectively from swine wastewater treatment. Moreover, an association of *P. subcapitata* and *G. lucidum* successfully removed CO_2 with 84% efficiency for biogas up-gradation (Guo et al., 2017). These studies have clearly demonstrated that myco-algal associations have the potential to enhance the wastewater treatment potential and fungal pellets can contribute towards easier harvesting of the algal biomass. However, detailed studies are required to evaluate the impact of myco-algal associations on the metabolite content of the microalgae. Because the presence of fungi in the culture media can substantially modify the extracellular and intracellular algal metabolites which may improve or even lower the content of any desired molecule. Moreover, the fungal pellets may also interfere with the post-harvesting processing of the algal biomass for the extraction of any desired organic compounds.

4. Carbon dioxide fixation

According to 2014 statistics, the total emission of CO_2 was 6870 MMT (Million metric tons), equivalent to 81% of the world's GHG emission (Wilbanks and Fernandez, 2014) and major global warming contributor. Industrial processes contributed to 21% of GHG emissions (Cheah et al., 2015). Microalgae are believed to have the potential to fix the atmospheric carbon at the highest rate when compared with any other photosynthetic system (Gill et al., 2016, 2013). Algae have been extensively studied for GHG mitigation by reducing the CO_2 content in biosphere through photosynthetic fixation (Abid et al., 2017). Their CO_2 sequestration efficiency is 10–50 times higher than terrestrial plants (Abid et al., 2017; Cheng et al., 2013). Microalgae can convert CO_2 from industrial gas and atmosphere into high chemical energy-containing organic biomass like carotenoid, bioethanol, acetone and lipids (Zhu et al., 2017). Different microalgae namely *S. obliquus*, *C. pyrenoidosa*, *C. reinhardtii*, *Chlorococcum littorale*, and *D. terteolacta* have shown the ability of HCO_3^{-1} and CO_2 utilization because they harbor external carbonic anhydrase (Zhou et al., 2017). Immobilized *Nannochloropsis* sp. Mitigated >99% CO_2 in secondary POME (Palm Oil Mill Effluent) with concomitant lipid production of 0.35 gL^{-1} lipid production which was 1.41-fold higher when compared to the control (Cheirsilp et al., 2017). Cultivation of *S. obliquus* in beer wastewater supplemented with 5% CO_2 promoted cell growth and lipid accumulation (Wu et al., 2017b). Microalgae-based sludge treatment reduced CO_2 emission by 22–54% and 43–103% during the whole year and in summer, respectively (Nordlander et al., 2017). Phycoremediation of palm oil mill secondary effluents by immobilized *Nannochloropsis* sp. showed > 99% CO_2 mitigation efficiency (Cheirsilp et al., 2017).

The CO_2 content may manipulate the algal metabolite content. For instance, higher fatty acid content was achieved in *C. reinhardtii*, *S. obliquus*, *C. minutissima*, and *D. tertiolecta* by exposing them to enhanced CO_2 concentrations (Zhu et al., 2017). In another study, involving eight locally isolated microalgal strains, cultivation was conducted under 20% CO_2 feeding (Hussain et al., 2017), and maximum biomass production of 1.4 gL^{-1} for strain UMN268 was observed and it was correlated with nutrient uptake. Exposure of *Asterarcys quadricellulare* and *C. sarokiniana* to high levels of CO_2 resulted in an increase in carbohydrate content of total dry cell mass by 55–71% (Varshney et al., 2018). Similarly, *S. bajacalifornicus* supplemented with 25% CO_2 accumulated lipid and carbohydrate by 25.81% and 26.19%, respectively (Patil and Kaliwal, 2017). These studies have clearly indicated that CO_2 concentration can manipulate the content and the composition of the algal metabolites and this manipulation may be employed to achieve the desired metabolite content in selected microalgal strains. However, the impact of CO_2 content derived from wastewater (the soluble form of CO_2) needs to be carefully evaluated in future through wastewater-oriented studies because wastewater from different sources may have different levels of soluble CO_2 depending upon nature, geographic position and origin of the wastewater. Fig. 3 shows a schematic diagram of microalgal cultivation and processing as a carbon neutral process.

5. Wastewater integrated algal-biorefinery for bioproducts

Circular bio-economy is an emerging concept, focusing on the sustainable production, conversion, and utilization of renewable resources into value-added products. Photosynthetic organisms such as microalgae are the focal point in developing closed-loop systems due to their eco-friendly and versatile properties. Wastewater integrated closed-biorefinery contributes to process economy and sustainability by resource recovery and by reducing the ecological footprint (Javed et al., 2019; Mohan et al., 2019). Wastewater cultivated algae are rich source of primary (carbohydrates, proteins, lipids) and secondary (pigments, anti-oxidants) metabolites that could be exploited to produce biofuels, biopolymers, biofertilizers, nutraceuticals, food/health grade compounds, enzymes, feed supplements etc. and the treated wastewater can be reutilized for the agricultural or industrial purposes (Mohan et al., 2019).

5.1. Algae as fuel source

Algae are known to accumulate 30–80% of lipids which normally consist of 90–95% of triacylglycerides. Wastewater cultivation is a cost-effective method to enhance biomass production and to modify lipid content and fatty acid (FA) composition. Most of the algal strains such as *Chlorella* produce FA ranging from C_{16}-C_{18} which are suitable for biodiesel production; similar in properties to traditional fossil-based diesel. Ethanol is another important fuel and a chemical source. It can be produced from syngas or directly by fermenting high carbohydrate (>40%) containing algal strains. Algal species like *Chlamydomonas*, *Spirulina*, *Euglena*, *Chlorella*, *Scenedesmus*, and *Dunaliella* have been extensively studied for bioethanol production. Various studies indicated the potential of wastewater grown algae to accumulate carbohydrates (Mehar et al., 2019). Residual biomass (lipid-free, carbohydrate rich) can also be utilized for bioethanol fermentation (Shahid et al., 2019) and doesn't require laborious pretreatments as the case with plant biomass. Carbohydrate content of algae can also be used to produce bio-butanol, bio-methane, biogas, and syngas.

5.2. Algae as high-value nutraceutical/health supplement

Some algal strains tend to produce health related FA like omega-3, omega-6 and docosahexaenoic acid (DHA) as major algal-based FA. They are non-toxic and more stable as compared to fish DHA (Kumar and Singh, 2019). Cyanobacteria and algae are the natural sources for carotenoids and diverse carotenoids including astaxanthin and lutein. Production of these compounds can be enhanced through abiotic factor manipulation. Wastewater grown algae can be utilized for this purpose and provides a cost-effective sustainable alternative. Astaxanthin has vast applications in medical sciences due to its anti-oxidant, anti-inflammatory, anti-cancer, and anti-aging properties. Moreover, it also improves the nervous system, respiratory system, fertility, and digestive system. Wastewater integrated algal-growth has been suggested for improved astaxanthin production. This impact has been reviewed in detail by Shah et al., (Shah, 2019). Similarly, other carotenoids have shown various nutraceutical properties. Cyanobacteria (group of algae) produce large amounts of industrially and nutraceutically important compounds called phycobilins (Pancha et al., 2019) which are colored protein-pigment complexes having antimicrobial, anticancer, and antioxidant properties. They can also be used to produce protein-rich energy drinks and can be applied has food colors.

Wastewater-cultivated algae could be used as animal feed, biofertilizer, and cosmetic agents. Biochar produced by algal pyrolysis is rich source of nutrients and can be utilized as biofertilizer in agriculture. *Spirulina*, *Chlorella*, *Euglena*, *Tetraselmis*, *Synechococcus*, *Nannochloropsis* have been utilized for human consumption as well as aquaculture and animal feed supplement due to their high protein content and nutritional value (Shahid et al., 2020). Algal extracts have been widely used in cosmetic industry as skin and hair protectants due to their anti-oxidant, anti-irritant, anti-aging, sun-protecting and tissue regenerating abilities. *Chondrus, Chlorella, Dunaliella, Nannochloropsis, Spirulina* have been widely utilized for commercial cosmetic products (Javed et al., 2019; Shahid et al., 2020). Fig. 4 shows production and processing of algal biomass to produce diverse products in an algal biorefinery.

6. Conclusion, prospects and recommendations

Microalgae are a remarkable biological resource to produce biofuels and value-added products at industrial scale in an eco-friendly manner. However, the cost associated with algal biomass production and processing questions the commercial viability of the process. Fortunately, microalgal cultivation in wastewater offers a promising alternative to deal with the higher operational cost. Because wastewater does not only provide a low-cost growth media for algal cultivation but also provides cost-benefits associated with the wastewater treatment. Moreover, the unbalanced ratios of nutrients on the microalgae may exert abiotic stresses on algal biomass diverting the metabolic pathways to produce either more protein or more lipids and pigments which may add additional value. In this regard, a biorefinery approach is feasible

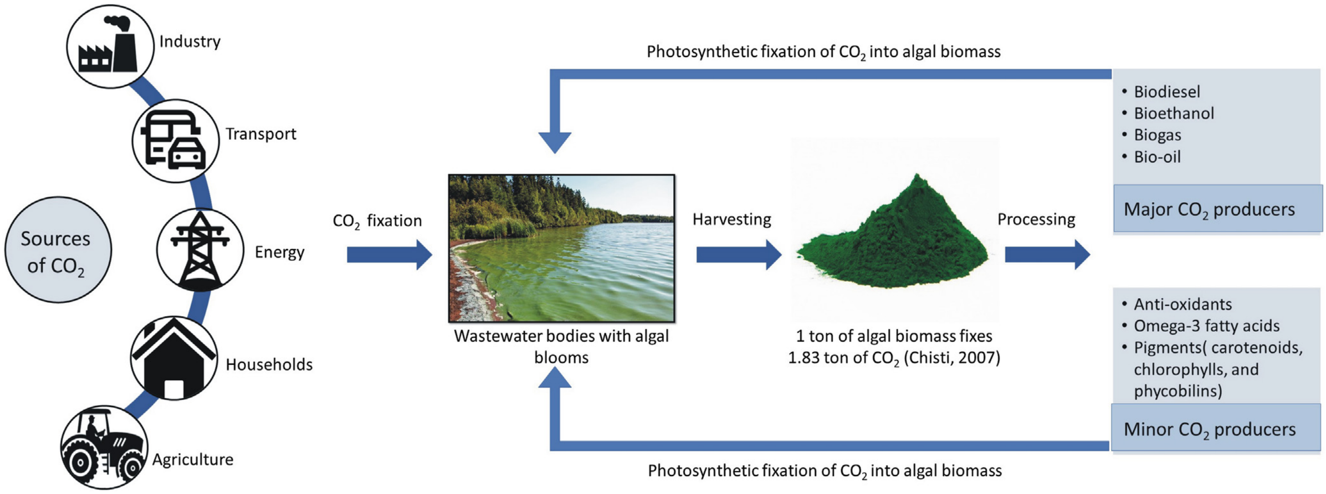

Fig. 3. Schematic representation of carbon neutral nature of microalgal cultivation and processing.

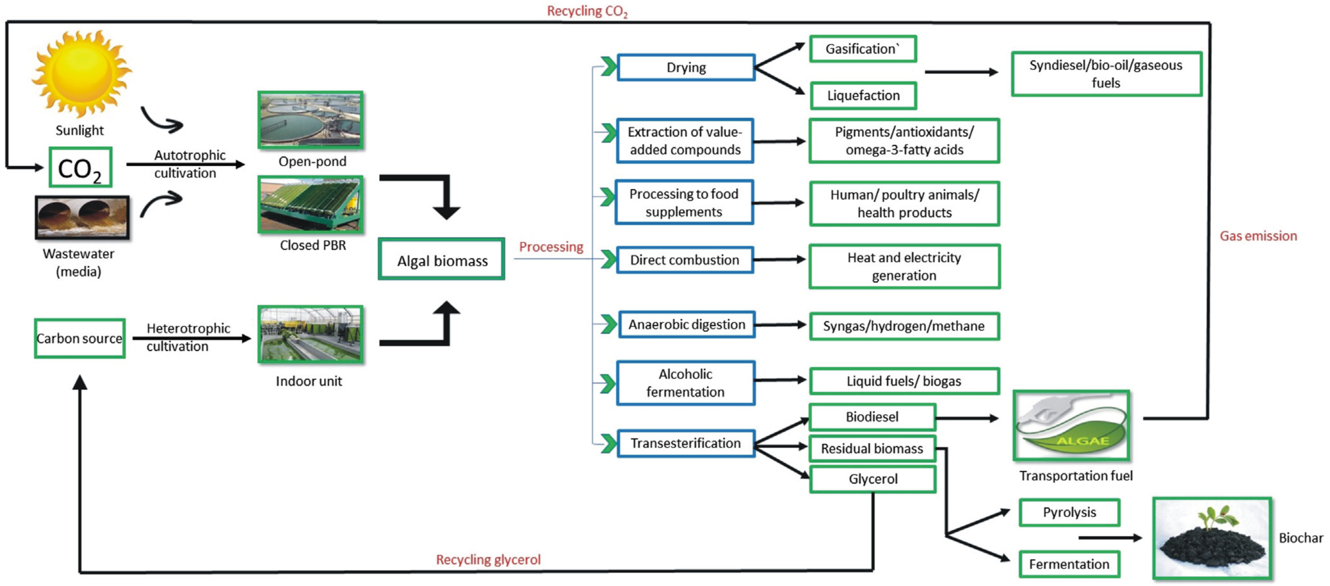

Fig. 4. Overview of microalgal biomass production and processing in an integrated microalgal biorefinery to produce a variety of products in a multi-product algal biorefinery.

where a spectrum of marketable products may be obtained from the single process. Wastewater is a major global problem especially in heavily populated areas and must be recycled or treated before disposal. On the other hand, wastewater is a rich source of nutrients and consumption of these nutrients by microalgae (phycoremediation) not only enhance productivities of biomass but other value-added products (lipids, pigments, proteins, carbohydrates, etc.). Moreover, the nutrient removal efficiency of microalgae can be enhanced by applying binary cultures that may consist of microalgae-microalgae, microalgae-bacteria or microalgae-fungi associations. These associations also improve the flocculation ability while avoiding the contamination chances and may improvise the natural metabolite content of microalgae. However, detailed studies are required in the future to study the impact of wastewater-derived stress on the biomass productivity of consortia and the post-harvesting processing of the biomass along with the metabolite content. Moreover, stress-responsive genes should be identified in the future which would be later targeted to engineer the selected microalgal strains to achieving industrial robustness in algal biorefineries. Keeping in view the progress made in algal research, recent scenario and prospects, the following recommendations are made to achieve commercial robustness.

- **Cultivation**: the microalgal cultivation systems need to be improved by developing low-cost growth media or using wastewaters as growth media to reduce the cost. Bioprospecting is required to isolate indigenously successfully adapted strains for regional or territorial applications. The strains adapted to grow at odd pH values (low or high) may be selected to outcompete the contaminating microbes at large-scale cultivation.
- **Harvesting**: harvesting involves 20–30% cost in the algal biomass production and processing which can be lowered by developing robust harvesting techniques. Bio-flocculation and self-flocculation seem attractive harvesting approaches. Hence, isolation of self-flocculating strains and robust bio-flocculation would be required to develop in the future.

- **Strain development**: unlike *Saccharomyces cerevisiae* and *Escherichia coli*, the metabolic engineering systems are not fully established to develop engineered robust algal strains. Hence, it is recommended that multi-OMICs, synthetic and system biology-based research should be focused to elucidate and engineer the metabolic pathways of the selected algal strains.
- **Downstream processing**: the processing of microalgal biomass into products is tedious and expensive. It would be better if a multi-product approach is adopted for the algal biorefinery. It would be required to conduct detailed studies to elucidate the technical and economical impact of using residual algal biomass for traditional applications in energy, environment and agriculture sectors after extracting the high-value products.
- **Cost-energy-environment input–output balance**: more detailed studies are required to study the balance ratios of the cost-energy-environment triangle especially when it comes to a large-scale multi-product algal biorefinery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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