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WATER POLLUTION (G TOOR AND L NGHIEM, SECTION EDITORS)

Nutrient Removal by Algae-Based Wastewater Treatment

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Abstract

Algae cultivation complements wastewater treatment (WWT) principles as the process uptakes nutrients while assimilates CO_2 into biomass. Thus, the application of algae-based WWT is on the upward trajectory as more attention for recovery nutrients and CO_2 capture while reducing its economic challenge in the circular economy concept. However, the complexity of wastewater and algal ecological characteristics induces techno-economic challenges for industry implementation. Algae-based WWT relies totally on the ability of algae to uptake and store nutrients in the biomass. Therefore, the removal efficiency is proportional to biomass productivity. This removal mechanism limits algae applications to low nutrient concentration wastewater. The hydraulic retention time (HRT) of algae-based WWT is significantly long (i.e. > 10 days), compared to a few hours in bacteria-based process. Phototrophic algae are the most used process in algae-based WWT studies as well as in pilot-scale trials. Application of phototrophic algae in wastewater faces challenges to supply CO_2 and illumination. Collectively, significant landscape is required for illumination. Algae-based WWT has limited organic removals, which require pretreatment of wastewaters before flowing into the algal process. Algae-based WWT can be used in connection with the bacteria-based WWT to remove partial nutrients while capturing CO_2. Future research should strive to achieve fast and high growth rate, strong environmental tolerance species, and simple downstream processing and high-value biomass. There is also a clear and urgent need for more systematic analysis of biomass for both carbon credit assessment and economic values to facilitate identification and prioritisation of barriers to lower the cost algae-based WWT.

Keywords Algae-based wastewater treatment · Biomass application · Macroalgae · Microalgae · Nutrient removal · Wastewater treatment

Introduction

Algae are a diverse group of photosynthetic aquatic organisms. They can be either unicellular (microalgae) or multicellular (macroalgae). Unlike higher plants, algae do not have roots, stems, and leaves [1]. Microalgae are microscopic eukaryotes ranging from a few micrometres to a few hundred of micrometres. They can grow extremely fast, reproduce every few hours under favourable autotrophic or mixotrophic conditions, and efficiently uptake nutrients into the body cells [2••, 3••, 4•].

Macroalgae are also fast growing in aquatic ecosystems, and can size up to tens of meters in lengths. They are composed of thallus (leaf-like body) that have enclosed gas-filled structures (float) to help in buoyancy and a specialised basal structure to provide attachment to a surface (holdfast) [5•]. Because of their wide range of physiological and biochemical characteristics, microalgae and macroalgae are naturally

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capable of accumulating nutrients (N and P) from different aquatic environments to produce many different bioactive compounds (e.g. pigments, carbohydrates, proteins, and lipids) for commercial applications [5•, 6, 7].

Algae-based wastewater treatment emerged from the concept to reduce the production cost in algae cultivation (i.e. alternative nutrient and water sources) while finding a sustainable way of wastewater treatment (i.e. alternative to conventional activated sludge). As photosynthetic organisms, algae generate oxygen and assimilate CO_2, unlike bacteria-based WWT where significant amount of CO_2 is emitted. The cultivation of algae in wastewater can simultaneously provide the wastewater treatment service and generate valuable biomass [8]. Direct utilisation of wastewater also provides an inexpensive and effective source of nutrients that also reduces freshwater use [9].

Proof-of-concept studies have proven the feasibility of algae-based WWT process to remove nutrients using different wastewater sources (e.g. municipal, agricultural, aquaculture, and industrial wastewaters) [7, 10••, 11•, 12••]. However, further work is required to ensure the suitability of wastewater for subsequent algae cultivation. For example, wastewater is susceptible to indigenous bacteria and virus contamination, which can inhibit algae growth. Culture crush increases operating cost and system downtime, which cannot be compromised for wastewater treatment service.

There are considerable technical challenges to the commercialisation of algae-based WWT, associated with cost competitiveness, scalability, and process efficiency. This review aims to provide a comprehensive insight into the algae-based WWT process. It describes the nutrient removal difference between algae and bacteria and the ecological characteristics of algae in each cultivation methods. The paper reviews lessons learned from pilot and large-scale trials and emphasises the challenges of the current algae-based WWT. It is expected that this paper will provide a broad view for researchers, engineers, and relevant industry with interest in algae process.

Algae

Algae Mechanisms to Uptake Nutrients

Algae remove nutrients from the culture environment by nitrogen assimilation and phosphorylation. Nitrogen assimilation is the process of converting inorganic nitrogen (e.g. nitrate, nitrite, ammonium, and ammonia) to its organic form, which is the building block of peptides, proteins, enzymes, chlorophylls, and energy transfer molecules such as adenosine diphosphate (ADP) and adenosine triphosphate (ATP and genetic materials such as RNA and DNA [13]. During assimilation, nitrate and nitrite are reduced ultimately to ammonium with the help of nitrate and nitrite reductase, respectively [14•]. The incorporation of ammonium into the intracellular amino acid glutamine is then facilitated by glutamate (Glu) and ATP [4•, 15••]. This nitrogen uptake strategy by algae is different to that adopted by bacteria (Fig. 1). Nitrogen removal by bacteria can be achieved in two sequential steps of nitrification and then denitrification or by anaerobic ammonium oxidation, which is often called ANAMMOX [16•, 17].

In addition to nitrogen, phosphorous also plays a key role in microalgae metabolism and growth. Inorganic phosphorous ($H_2PO_4^-$ and HPO_4^{2-}) is incorporated into intracellular organic compounds (e.g. nucleic acids, lipids, and proteins) via phosphorylation [4•, 14•, 18•]. This process involves multiple phosphate transporters located at the plasma membrane of microalgae to uptake inorganic P for cellular phosphorous transformation. The transformation under light condition (i.e. photosynthesis) includes the generation of ATP from ADP and the synthesis of polyphosphate (e.g. acid-soluble and acid-insoluble polyphosphate) by polyphosphate kinase [4•].

Nutrient availability can affect the patterns of nutrient uptake for algae [4•]. For example, slow-growing macroalgae develop large nutrient stored pools by N and P accumulation during nutrient-rich periods for growth during

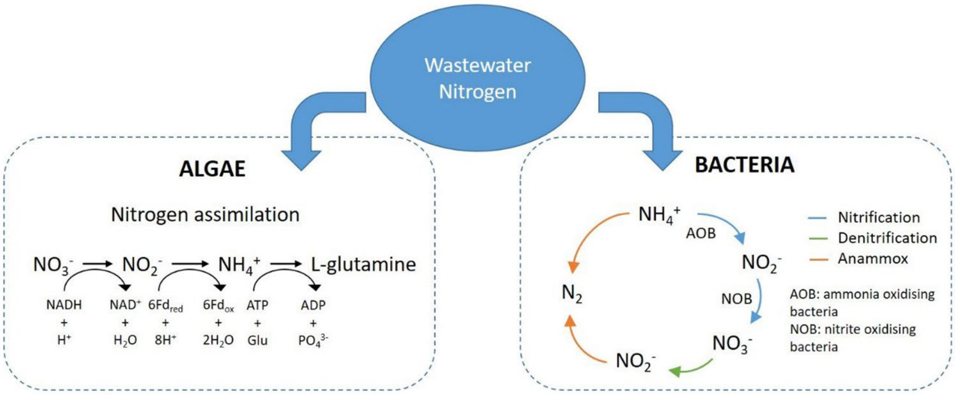

Fig. 1 Wastewater nitrogen removal strategy by algae vs. bacteria

nutrient-deplete time (i.e. luxury uptake) [19, 20••]. When the nutrients are limited, these slow-growing algae minimise their nutrient demand by reducing growth rates and optimise their carbon uptake capacity, profiting from sunlight abundance [21]. The ability to accumulate nutrients in excess of these species is useful for bioremediation. Freshwater *Oedogonium* was successfully cultured in situ for 12 months in secondary effluent at a municipal treatment plant and achieved biomass productivity of 9 to 15 g/m^2/day, 36 and 65% for TN and TP removal, respectively [20••]. On the other hand, opportunistic macroalgae with high nutrient demands for fast growth incline to high-affinity uptake systems instead of storing nutrient pools [19, 22]. The uptake and assimilation of nutrients in these species occur simultaneously to sustain high growth and the synthesis of amino acids, proteins, and other nutrient-rich organic compounds [19]. This strategy makes opportunistic algae such as *Cladophora glomerata*, *Enteromorpha ahlneriana*, and *Scytosiphon lomentaria* well suited for cultivation in culture with high nutrient loading such as wastewater. Increased growth with protein-rich biomass was observed for green, brown, and red macroalgae cultivated in wastewaters [23•], especially for green macroalgae which are often opportunistic species with high tolerance to environmental fluctuations [24].

Nitrogen has been identified as the limiting factor to the growth rate of both microalgae and macroalgae. It has also been observed that ammonium is often the preferred N source and more readily uptake by algae due to the lower energy requirement for cells to assimilate ammonium [15••]. High N concentrations increase the internal N content in the algal biomass, leading to improved assimilation of inorganic N into amino acids and proteins. Wastewater rich in ammonium is then a promising source for algal cultivation. Algae that grow in N-rich wastewater had 4 times higher crude protein content when compared to seawater controls [25]. N-replete culture can lead to algae accumulating N-containing photosynthetic pigments such as chlorophyll, thus obtaining a darker and more vibrant green colour at the end of growth. However, too high ammonia/ammonium concentration may be inhibitory to algal growth [28] by triggering intracellular oxidative stress and disturbing cell metabolisms [29]. Ammonium tolerance levels are reportedly at < 100 mg NH_4^+-N/L for microalgae and < 250 mg NH_4^+-N/L for macroalgae [30, 31•], but some species might possess superior ammonium tolerance than others [28].

Nutrient Uptakes Rate by Algae

Due to their diverse physiology and morphologies, algae species have shown varied effectiveness and efficiency in removing nitrogen and phosphorous from different wastewater streams (Table 1). This is partly due to the nitrogen to

Table 1 Nutrient removal and uptake rate by micro and macroalgae

Wastewater type	Source	Microalgae	Macroalgae	Duration (days)	Removal efficiency (%)			Nutrient uptake rate (mg/L/day)			Biomass productivity (g/L/day)	Reference
					NH_4^+	NO_3^-	TP	NH_4^+	NO_3^-	TP		
Aquaculture wastewater	Fishery	*Parachlorella kessleri* TY		5	96	94	95	3	0.14	0.7	0.015	[37]
	Shrimp farm pond		*Gracilaria birdiae*	28	34	100	94				0.018	[38]
Anaerobic digestate	Centrate 5% v/v	*Chlorophyceae* class		42	67		98	20		4	0.06	[39•]
			Chaetomorpha linum	12	87 (TN)		63	20 (TN)		0.3		[40•]
Municipal wastewater	Secondary effluent	*Scenedesmus obliquus*		25	96 (TN)		100	4.4 (TN)		0.9	0.08	[41]
			Oedogonium intermedium	90	59	49	65				0.008	[20••]
	Primary effluent		*Chaetomorpha linum*	12	98 (TN)		80	10 (TN)		0.59		[40•]
Agro-industrial wastewater	Science industrial park	*Chlamydomonas*sp. TAI-2		10	100	100	33	19.2	1.5	4.5	0.3	[42••]

phosphorous (N/P) ratio in wastewaters, which plays a critical role in ensuring an effective and simultaneous uptake of nutrients and biomass growth in algae species [15••]. The suitable ratio of N and P for macroalgae growth is between 10 N:1P and 80 N:1P [25] with optimal ratio of 30 N:1P, while it is 5–30 N:1P for microalgae [26, 27]. The optimal N/P ratio is strain-dependent; for example, it is reportedly 7 and 30 for *Chlorella* sp. and *Scenedesmus* sp., respectively [15••, 32••]. Wastewaters with too low N/P ratio (e.g. secondary effluent) or too high N/P ratio (e.g. centrate) might hinder algal growth; thus, strain selection is an important step when cultivating algae in wastewater. Because of this N/P ratio, faster uptake of nitrogen over phosphorous has been observed consistently for the growth of algae in wastewater (Table 1). This aligns with the empirical formula for microalgae ($C_{106}H_{263}O_{110}N_{16}P$), which indicates more nitrogen is needed for cell synthesis [33]. Strains within *Chlorella* sp. and *Scenedesmus* sp. have been widely used and found to be suitable for cultivation in wastewater due to their fast growth rate and high adaptability to different wastewater streams [8].

The nutrient uptake rate of macroalgae is further determined by the morphological structure together with transfer processes across the thalli rather than phylogenetic affinity. Opportunistic, filamentous, delicately branched, or monostromatic macroalgae (e.g. *Cladophora glomerata*, *Enteromorpha ahlneriana*, and *Scytosiphon lomentaria*) possess better nutrient uptake rates due to a higher thallus surface area to volume ratio and larger numbers of hairs protruding from the thallus [34]. An increase in water flow in the culture system also increases the nutrient flux to the surface of the thalli, thus increasing the nutrient uptake rate [35]. However, excessive water flow rate can be counterproductive due to lower water and nutrient retention time near the thalli. Nitrate and phosphate removals of *Spirogyra* sp. cultivated in photobioreactors were fourfold and twofold higher under low air mixing (18 ± 2 cm^3/min) compared to high mixing (206 ± 14 cm^3/min) rate [36•].

Cultivation Methods for Algae-Based WWT

The application of algae-based WWT relies on the method of cultivation, which influences biomass growth, nutrient removal, and the downstream process. Cultivation methods also impact on the overall capital and operation costs. Algae including micro- and macroalgae are highly diverse groups of organisms that can be found in many habitats. Their ecological diversity includes phototrophic, mixotrophic, and even heterotrophic metabolisms. This section will focus on the three metabolisms and their effects on the cultivation of algae for nutrient removal from wastewater.

Phototrophic Cultivation of Algae

Phototrophic algae utilise visible light as a primary energy source, dissolved carbon dioxide (i.e. mainly bicarbonate), and nutrients for metabolism, a process known as photosynthesis. The application of phototrophic algae for wastewater treatment therefore relies on the ability to supply effectively dissolved carbon dioxide and light energy (photon) to algae cells to boost their metabolisms [2••, 4•].

Previous studies on phototrophic algae for nutrient removal from wastewater have adapted most of the techniques in algae culture. These include open ponds (e.g. raceways, high rate algal ponds) and closed photobioreactors (i.e. horizontal tube, vertical tube, flat plate, floating film bag, helical type). However, pilot and full-scale trials mainly used the open ponds or high rate algal ponds (the "Pilot Trials of Algae-Based WWT" section). The main benefits of open ponds over the closed photobioreactors are simple cultivation systems, low capital and operating costs, and low energy requirement. However, the open ponds require large land surface and often have low biomass productivity [2••, 43]. They are also sensitive to bacterial contaminations, high water evaporation rate, and challenging to maintain stable culture conditions.

One technical challenge for phototrophic algae cultivation is to supply light to algae cells. Light penetration is often poor, and the penetration further falls with increasing cell density and depth of culture due to self-shading effects. Nguyen et al. [2••] summarised three innovative methods to enhance light exposure to algae. These include a thin-layer cascade design, submerged illumination, and airlift-lop reactor. In the thin-layer cascade, the ratio of exposed surface over total culture volume could be above 100 [44, 45••]. In the thin-layer cascade system, microalgae culture is distributed evenly at less than 5 cm at a flow rate of 0.4 to 0.5 m/s. This configuration harnesses the benefits of open systems (i.e. direct light irradiance, easy heat diffusion, rapid light/dark cycle, simple cleaning, and efficient degassing) as well as those of the closed photobioreactors (i.e. high biomass densities and high volumetric productivity) [44]. Morillas-España et al. [46•] appeared to be the first group conducting a pilot scale thin-layer cascade for nutrient removals from primary effluent. The group achieved an average of 24.8 g/m^2/day of *Scenedesmus* sp. biomass (82 tone/ha per year) which is significantly higher than other culture setup. Thin-layer cascade also allowed a 1.4 times higher ammonium removal rate than that of the raceway pond [47]. However, it is noted that the treated volume per m^2 space by thin-layer cascade is lower than the raceway pond [47]. Submerged illumination is typically designed for indoor algae cultivation. The first concept was developed by Ogbonna et al. [48•]. Until recently, its application has achieved more attention over sideway or external illumination. The notable feature of the submerged illumination system is the lighting source that is placed vertically into the culture condition. Industrial Phycology (https://i-phyc.com/the-iphyc-solution/) currently adapted this cultivation for bioremediation of secondary effluent.

Another technical challenge of the phototrophic cultivation is the CO_2 availability. Dissolved CO_2 level has been considered the major limiting factor in mass cultivation of algae. Generally, higher CO_2 concentration to about 10 to 20 vol% is better than ambient CO_2 for algae growth. Utilisation of flue gas (10% CO_2) in *Scenedesmus* sp. cultivation with secondary municipal wastewater showed higher nutrient removal [49]. Therefore, an efficient method to supply CO_2 for fast growing biomass in phototrophic cultivation would increase the nutrient removal efficiency. Previously, sparging with air or flue gas, bicarbonate solution, or carbonation with membranes has been tried in algae cultivation. However, sparging is the most common method in algae-based WWT due to its simplicity [2••].

Heterotrophic Cultivation of Algae

Heterotrophic algae can grow in the dark using organic compounds for carbon and energy sources. Previously, heterotrophic algae are cultured in fermenter with periodical or continuous supply of carbon sources (e.g. glucose, volatile fatty acids) to generate high-lipid content biomass in for subsequent biofuel production. The heterotrophic cultivation of algae enables high-density biomass, consistent productivity, and quantities. Kim et al. [50] reported a biomass productivity rate of 5.4 g/L/day with a maximum concentration of 43 g/L in a culture with supplement of 72 and 8 g/L of glucose and $NaNO_3$, respectively. Heterotrophic production of algae has already been commercialised technology given the high lipid content of heterotrophically grown algae and the ability to manipulate the cell's biochemistry. However, heterotrophic cultivation requires inputs that are more expensive and the supply of oxygen in aerobic submerged cultivation.

Heterotrophic cultivation of algae for nutrient removal leverages on the conditions that wastewater presents many types of organic compounds such as glucose, volatile fatty acids, glycerol, and ethanol. These organic carbon sources are typical for algae uptake [51]. However, there only limited number of algae species (e.g. *Chlorella vulgaris*, *Haematococcus pluvialis*, *Chlorella sorokiniana*, and *Botryococcus braunii*) with ability to use organic carbon as source of energy and carbon due to the lack of uptake mechanisms or transport pathway [4•, 52]. For example, glucose is metabolised via the pentose phosphate pathway and the Embden-Meyerhof Pathway without and with light conditions, respectively [51]. It is also noted that high nitrogen wastewater in the form of ammonium promotes the pentose phosphate pathway. Thus, heterotrophic cultivation may be selected for high nitrogen wastewater source. To date, there are only a few studies on heterotrophic cultivation of algae in wastewater. Guldhe et al. [53] utilised *Chlorella sorokiniana* culture for treatment of aquaculture wastewater. This heterotrophic culture achieved above 80% removal of ammonium, nitrates, phosphates, and chemical oxygen demand. Pretreatments of aquaculture wastewater by filtration and autoclave were required prior to microalgae culture. This study was only limited to 1 L culture volume, although the biomass production was 3 times of that phototrophic cultivation [53]. Likewise, Kim et al. [54•] observed a better removal of nutrient and biomass productivity with *Chlorella sorokiniana* under heterotrophic conditions.

Heterotrophic cultivation overcomes light and dissolved CO_2 limitations while achieves better organic carbon removal from wastewater compared to phototrophic algae. Therefore, wastewater compositions should be considered when selecting carbon metabolisms in algae culture. Another important notification is that heterotrophic algae culture often requires sterile conditions to avoid bacteria contamination. This limits its application in wastewater treatment process. Currently, there has been no pilot or large-scale trial of heterotrophic cultivation of algae for nutrient removal in wastewater (the "Pilot Trials of Algae-Based WWT" section).

Mixotrophic Cultivation of Algae

Mixotrophic cultivation of algae is a combination of phototrophic and heterotrophic metabolisms where energy and C source are light and organic and inorganic carbon [55]. In mixotrophic cultivation, both light limitation and inhibition are less effective, while dissolved CO_2 and organic carbon can be used for metabolisms. Thus, mixotrophic cultivation provides flexibility and higher efficiency in nutrient removal and biomass productivity.

Organic carbon sources have a great influence on nutrient removal in mixotrophic cultivation. Peng et al. [56] observed that nitrogen and phosphorus removal was better when supplemented with the mixotrophic culture of *Chlorella vulgaris* with glucose rather than protein or sodium acetate. Addition of glucose also enhanced the fixation of nitrogen and phosphorus in an artificial wastewater [57]. Higher glucose dose also generated lipid-rich biomass for biofuel production [57]. Mixotrophic cultivation often applies for high organic content wastewater. For example, Wang et al. [58] used green algae *Chlorella pyrenoidosa* for treatment of piggery wastewater with carbon content from 0.25 to 1 g/L and obtained an efficient ammonium removal of 90%. At 1 g/L carbon, the culture produced a maximum lipid of 6.3 mg/dL. The ability to use organic carbon in wastewater to produce high lipid content biomass is one advantage of mixotrophic cultivation. Nevertheless, pretreatment (e.g. dilution) to reduce the organic content, suspended solids, and nutrients to suitable level for algae growth is required [58]. Thus, it is envisioned that algae-based WWT would need to be strategically integrated into existing wastewater infrastructure.

Pilot Trials of Algae-Based WWT

Pilot and full-scale reports of algae-based WWT using microalgae and macroalgae are summarised in Table 2. These systems showed that nutrient removal efficiency varied significantly depending on species, initial nutrient concentrations, reactor sizes, and culture systems. It is also noticed that most of pilot and large-scale trials utilised phototrophic cultivation method.

A few lessons have been discussed from the pilot and large-scale trials. First, Sutherland et al. [59••] suggested that the size of high rate algal pond has significant impact on biomass growth and operational stability. In comparison of three reactor sizes (5 m^2, 330 m^2, and 1 ha), the 5 m^2 pond provided better nutrient removal and biomass productivity than that of 330 m^2 and 1 ha. The authors suggested that this observation might have implications for commercial scale development with respect to capital and operational cost.

Another lesson is the realisation of long hydraulic retention time (HRT) in algae-based WWT. This is directly related to the initial nutrient concentration and the nutrient uptake rate of selected algae. The HRT is also directly proportional to solar radiation. Thus, a dilution factor to shorten the HRT in summer and increase the HRT in wintertime can be applied when there are light limiting conditions and low temperatures. This strategy is important in regions with significant seasonal variations as Europe, California, and New Zealand.

The reported biomass productivity also varies significantly amongst studies (Table 2). Although a direct comparison cannot be made, some general observations can be highlighted. For example, a thin-layer cascade offered high biomass productivity [46•]. However, thin-layer cascade may be limited to a low treated volume. The obtained results from these experiments may not translate well to larger scale. It is also noticed that techno-economic analysis has been neglected in these studies. Thus, the validation of large-scale feasibility for algae-based WWT remains unclear.

Pilot and full-scale trials of macroalgae have recently received more attention because of its advantages over microalgae process. Macroalgae provides an easy harvesting process and greater resistance to bacteria and predation. The facilitation of biomass harvesting reduces the cost and complexity during the implementation into wastewater treatment. Macroalgae can also be retained easily in the reactor allows for a strong culture enrichment [60]. Future studies could focus on high-quality biomass and explore the chemical extraction from macroalgae derived from wastewater. This would potentially unlock the economic barriers in algae-based WWT.

Benefits of Algae-Based WWT

Carbon Credit Assessment

The National Greenhouse and Energy Reporting Scheme estimated an emission factor of 4.9 tonnes CO_2-e per tonne of nitrogen removed [70]. Direct emissions at WWTPs are from biological carbon, nitrogen, and phosphate removal, sludge management, and off-gas from wastewater collection systems. In the conventional bacteria-based WWT process, Bao et al. [71] reported 0.97 kg of the direct emission of CO_2 for treatment of 1 kg chemical oxygen demand in wastewater. This was equivalent to 0.34 g CO_2 per m^3 of treated wastewater. There are also indirect emissions due to the large energy consumptions, chemical usages, and waste disposal [72].

Algae-based WWT utilises the ability of algae to lock CO_2 into their biomass, which contributes to the CO_2 emission reduction in two ways. First, it is about 1.83 kg of CO_2 for every 1 kg of algae biomass. Algae biomass production could consume 1.83, 0.05, and 0.01 ton of CO_2, nitrogen, and phosphorus, respectively [73••]. Second, the algae biomass can be used as renewable source for energy, biochemical, or long-term products (i.e. bioplastics, brick, and biochar) to lock away CO_2 [2••] (the "Biomass Applications" section). Preliminary estimates demonstrate that algae-based WWT may entirely offset the industry's greenhouse gas footprint and make it a globally significant contributor of negative carbon emissions. However, there has no system to report the carbon emissions for algae-based WWT. Future studies on life cycle assessment and carbon balance analysis are required to support the claim of algae-based WWT for carbon capture (Fig. 2).

Co-locations of wastewater treatment and algae cultivation for carbon capture and utilisation will bring opportunities for major CO_2-emitting industries. In this concept, algae cultivation will supplement the main wastewater treatment to capture and recycle the nutrients and CO_2 gas from anaerobic digester (Fig. 2). The harvested algae biomass can be recycled back into the digester for bioenergy production, thus closing the CO_2 loop at WWTP.

Biomass Applications

There are many products that can be made with the whole algal biomass without further processing (i.e. after harvesting and drying stages). These include food (e.g. *Spirulina* supplements) [74], energy (e.g. syngas or bio-oil), soil additives (e.g. biostimulants), or feedstock for anaerobic digestion [75]. In

Table 2 Pilot-scale microalgae and macroalgae-based wastewater treatment

Wastewater source	Volume (L)/strain/configuration	Initial concentration (mg/L)		Removal efficiency (%)		HRT (days)	Biomass productivity	Reference
		Nitrogen	Phosphorus	Nitrogen	Phosphorus			
Microalgae-based WWT								
Piggery farm	500 L/multi-culture of *Chlorellaceae*, Scenedesmaceae, Chlamydomonadaceae/HRAP*	195 ± 55	19 ± 14	90	90	208	10.7 g/m^2/day	[61]
Non-pre-treated agriculture digestate, Olsztyn, Poland	2000 L/*Chlorella vulgaris* UTEX2714/Raceway	86 ± 5.2	18 ± 3.9	21	82	12	720 kg/day	[62]
Sewerage of the University of Almería, Spain	250 L/*Scenedesmus* sp. CCAP 276/24/thin-layer cascade	210.6	11.3	99	86	330	19.1 g/m^2/day	[46•]
Swine wastewater China	3000 L/*Chlorella vulgaris* MBFJNU-1/HRAP* with 3% CO$_2$	292.7	35.4	85	30	10	1.4 kg/day	[63•]
Cambridge WWTP, New Zealand	2900 m^3/*Ankistrodesmus falcatus*; *Micractinium pusillum Fresenius*/HRAP*	38.2 ± 5.8	6.6 ± 1.1	34	34	8	5.7–30.0 g/m^2/day	[59••]
Cambridge WWTP, New Zealand	2900 m^3/unlined raceway HRAP*	43	6	86	50	9		[59••]
Agricultural runoff	11.7 m^3/*Chlorella* sp., *Stigeoclonium* sp., diatoms *Nitzschia* sp., and *Navicula* sp./tubular horizontal semi-closed photobioreactor	1.9 ± 0.8	1.84 ± 0.23	89	91	5	598 mg/m^2/day	[64•]
Macroalgae-based WWT								
Salinity amended wastewater India	20 L/*Ulva lactuca*/flat panel PBR	20.5	5	96	83.1	0.5	340 g/m^2/day	[65]
Primary settled sewage, Townsville	10 m^3/*Oedogonium*/a parabolic cultivation ponds, each with a surface area of 16 m^2	27.2	5.04	62	75	20	7–10 g/m^2/day	[66]
Primary settled sewage, Canada	70 L/*Chaetomorpha linum*/flat photobioreactor	39.5	1.55	97.6	79.1	12	10.1 g/m^2/day	[40•]
Rural Research Institute, Ansan-si, Gyeonggi-do, Korea	12 m^3/*Spirogyra* sp./HRAPs*	2.85	0.31	73.7	65.8	4	-	[67]
Te Puke municipal WWTP New Zealand	10 m^3/ *Oedogonium*, *Cladophora*, *Spirogyra*, and *Klebsormidium*/plastic bag	18.6	4.7	60–99	50–70	12	9.7 g/m^2/day	[68•]
The Cleveland Bay municipal WWTP north Queensland, Australia	80 m^3/*Oedogonium*/three large parabolic tanks (surface area 50 m^2)	3.18	0.92	36.1	64.6	91	9–15 g/m^2/day	[20••]

Table 2 (continued)

Microalgae-based WWT								
Wastewater source	Volume (L)/strain/configuration	Initial concentration (mg/L)		Removal efficiency (%)		HRT (days)	Biomass productivity	Reference
		Nitrogen	Phosphorus	Nitrogen	Phosphorus			
The Cleveland Bay Purification Plant, North Queensland, Australia	80 m^3/*Oedogonium*/ three large parabolic tanks (surface area 50 m^2)	-	-	80	99	49	3.57 t/ha	[69••]

**HRAP* High Rate Algal Pond

the algae-based wastewater treatment, the generated biomass can be used for production of biocrude, biofuel [76•, 77], bioplastics [78••], and animal feed [2••, 79]. These applications could offset or reduce carbon emissions at wastewater treatment plant by replacing items currently derived from carbon-intensive practices using fossil fuel. For example, Naaz et al. [80•] demonstrated the ability of two algae species *Chlorella pyrenoidosa* and *Phormidium* cultivation in municipal wastewater at 100 L outdoor attached biofilm system. While the nutrient removal was 53 to 87% after 6 days HRT, the generated biomass was stable at 3.5 g/m^2day with high lipid content (35% of total solid). Biomethane (i.e. anaerobic digestion of the generated biomass) and biocrude (hydrothermal liquefaction of the generated biomass) analysis showed the better net energy ratio of biocrude production [80•]. Although biocrude production has high-energy ratio, biomethane production from algae-based WWT could have multi-fold benefits. For example, the biogas generated from the anaerobic digester can be used to provide CO_2 for algae cultivation. The digestate can be used to supply nutrients. This configuration was first introduced by Converti et al. [81], who combined a mixed sludge anaerobic digester with a photobioreactor leading to the production of a biogas with a CH_4 content above 70%. This approach also allows direct utilisation of algae biomass.

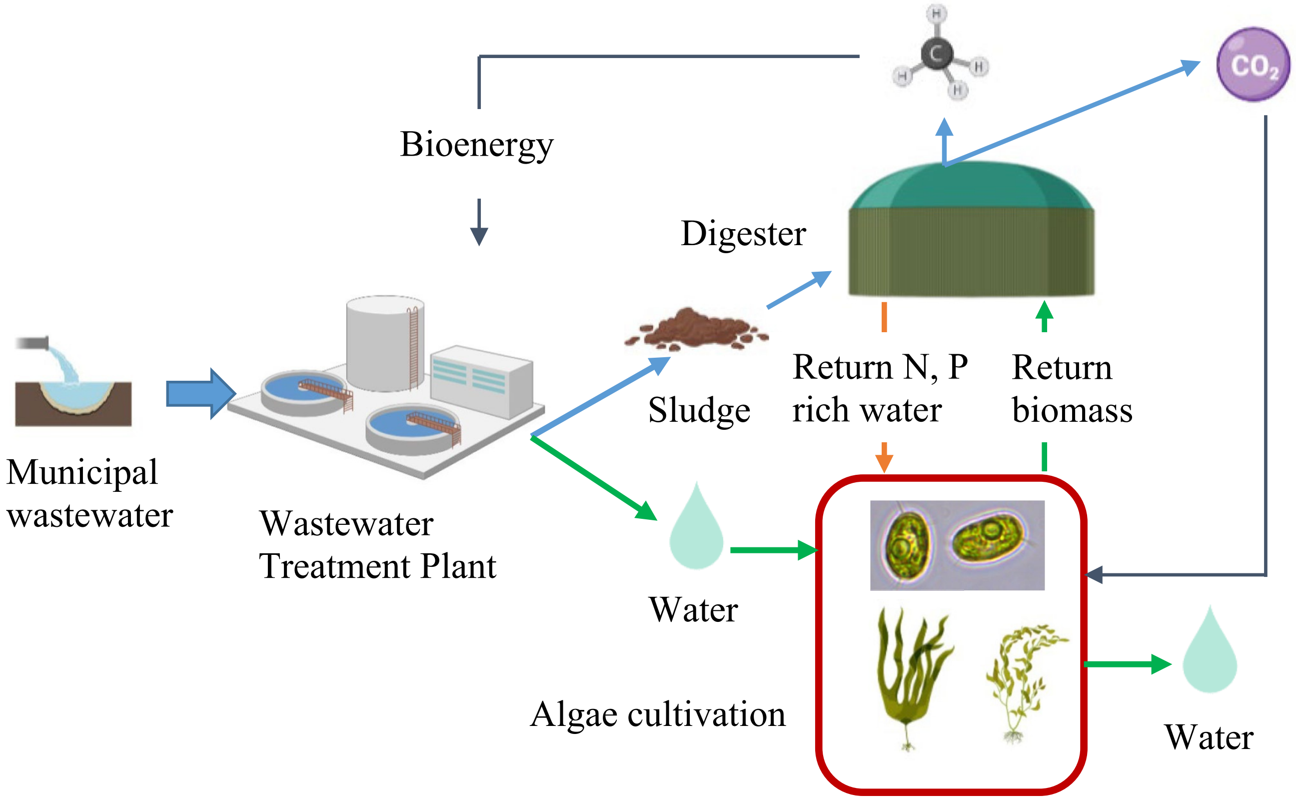

Fig. 2 Algae cultivation enables carbon capture and utilisation wastewater treatment

Algae biomass from wastewater treatment process can potentially be used in several long-term products for carbon storage such as in cement and bioplastic production [78••, 82]. Unlike plant-based biomass (i.e. lignocellulosic biomass), algae biomass has low percentage of lignin and rich in long-chain hydrocarbons (i.e. high purity of cellulose) which can be extracted to make bioplastics [78••, 83]. Another potential usage of algae is the use of algal biochar, which can be added to the soil for more long-term carbon storage [84] and moving towards sustainable agriculture [74]. Algae could also be used as biostimulants to improve crop production, thereby reducing the need for fossil-based fertilisers [85].

The benefits of algae biomass have been well discussed in the literature review [2••, 72]. In general, the application of algae biomass product is technically feasible; their economic feasibility is still under discussion [73••, 86••]. The production cost includes cultivation, harvesting, pretreatment, and chemical extraction at scale that is still significant. Unfortunately, commercial techno-economic assessment models are not available. Currently, commercial algae biomass is mainly used to produce high-value ingredients for the cosmetic and nutraceutical industries.

Challenges for Adaptation of Algae-Based WWT

Scalability and Efficiency to Meet the Wastewater Treatment Process

Although there have been a number of pilot and full-scale trials around the world, the adaptation of algae-based WWT by industry is still very low. The main reason is that algae-based WWT currently faces significant challenges to scale up and maintain its treatment efficiency. The intrinsic physical limitation is that the light transmissivity is around 5 cm. Therefore, the ratio of effluent-treated water over surface area needed is significantly larger than that of other treatment methods. Nguyen et al. [87] provided an initial calculation of the required surface and volume of an algae membrane reactor to treat membrane bioreactor effluent at a hydraulic retention time of 1 days. With the assumption that nutrients are removed at 100% efficiency, the volume of an algae membrane reactor would be 37 times that of the membrane bioreactor. Likewise, Neveux et al. [66] used a nitrogen removal rate of 1 g/m^2/day and estimated that a 3.5 ha of macroalgae cultivation area is required to remove 90% of nitrogen at 40 mg/L concentration from 1 ML/d treatment capacity. In the field study, Neveux et al. [66] reported that a 94-ha land surface is needed to treat 29 ML/d using *Oedogonium* culture. The large footprint will hinder the adaptation of algae-based WWT into the existing plants situated in urban and other space-deficient locations.

Nutrient removal by algae is mainly by cell assimilation. Thus, the removal rate is proportional to the biomass production rate (the “Cultivation Methods for Algae-Based WWT” section and the “Pilot Trials of Algae-Based WWT” section). This limitation of nutrient uptake (i.e. slow and low level) by algae requires a long HRT. The HRT of algae-based WWT is often above 10 days, compared to a few hours in bacteria-based WWT process. The long HRT future intensifies space requirements for algae-based WWT. Therefore, algae-based WWT has to be strategically integrated with existing wastewater infrastructure. One approach is to integrate algae-based WWT for tertiary wastewater treatment for polishing step (i.e. to remove the residual nutrients). Cole et al. [20••] utilised freshwater macroalgae as an in-line tertiary treatment of treated effluent from a municipal wastewater treatment in Australia. They used *Oedogonium* in open ponds to reduce 36 and 65% total nitrogen and total phosphorous in treated effluent. Another approach is to utilise submerged LED, which can reduce land requirement [2••]. The algal biological reactor developed by Industrial Phycology from the UK adapted to use internal illumination and treatment of tertiary effluent. In this process, the algae solution is mixed in a series of tanks. LED lamps are installed vertically into the tanks. This concept significantly improves the treated volume and reduces space requirement. Future research to improve the areal productivity and nutrient uptake rates are required to reduce the land requirements.

Resilience and Consistency in the Performance

The traditional objective of wastewater treatment is to remove carbon, nitrogen, phosphorus, and other contaminants so the effluent meets environmental quality regulation. In this context, algae-based WWT must be realised without compromising treatment efficacy given that protection of the local aquatic environment and public health will remain paramount for the wastewater industry. However, algae-based WWT still have uncertain performance reliability [7, 72].

Algae-based WWT faces seasonal performance due to mainly sunlight availability and temperature variation. In the pilot scale study, Cole et al. [20••] observed a maximum variation of 180% in biomass productivity (8.9 vs 15.8 g/m^2/day) between winter and summer time during the cultivation of freshwater macroalgae *Oedogonium* in secondary treated water. Xu et al. [88] also observed that nutrient removal and biomass yield varied with seasonal conditions. In summer and autumn conditions, the highest removal of total nitrogen, total suspended solids, and specific growth rate were achieved. Morillas-España et al. [46•] reported a significant variation of nitrogen (695.4–2383.4) and phosphorus removal rates (70.4–111.8 mg/m^2/day), respectively, from winter to summer. One approach to alleviate the seasonal variation is to rotate between high- and low-temperature tolerant strains. Culture mixing and reactor design (i.e. depth selection) could also improve performance when there is less sunlight availability. However, this approach needs to consider other broad factors such as space requirement and treatment capacity.

Algae-based WWT has limited organic removal. In fact, the presence of organic matter in wastewater can cause significant problem such as bacteria contaminations and light penetration limitation due to high turbidity. Therefore, most of algae-based WWT studies mainly utilised tertiary effluent, which has low organic carbon. Heterotrophic and mixotrophic cultivation can utilise the organic carbon present in wastewater. However, algae will be in competition with bacteria for this carbon. In this competition, bacteria often outcompete the algae populations [88]. Bacterial contamination and zooplankton grazing also reduce biomass productivity and reliability of nutrient removal efficiency and increase downtime [89]. The algae-bacteria consortium system is an alternative method for preventing contamination [90]. However, this system is difficult to implement in practice, and it takes at least 9 days for both microorganisms to adapt [91•]. Selection of algae species and operating conditions that support simultaneously the proliferation of algae and bacteria are also needed in this concept [3••].

The resilience of algae-based WWT process remains questionable. Algae-based process does not fit all types of wastewaters [8]. This is unlike bacteria-based process in which there are multiple of bacteria species to perform organic carbon and nutrients removal. Algae-based process largely relies on single species. Besides, wastewaters have inconsistent component event at daily timescale (i.e. various flow, peak hour, wet and dry weather). The component variation will impact algae performance significantly [4•, 8, 55].

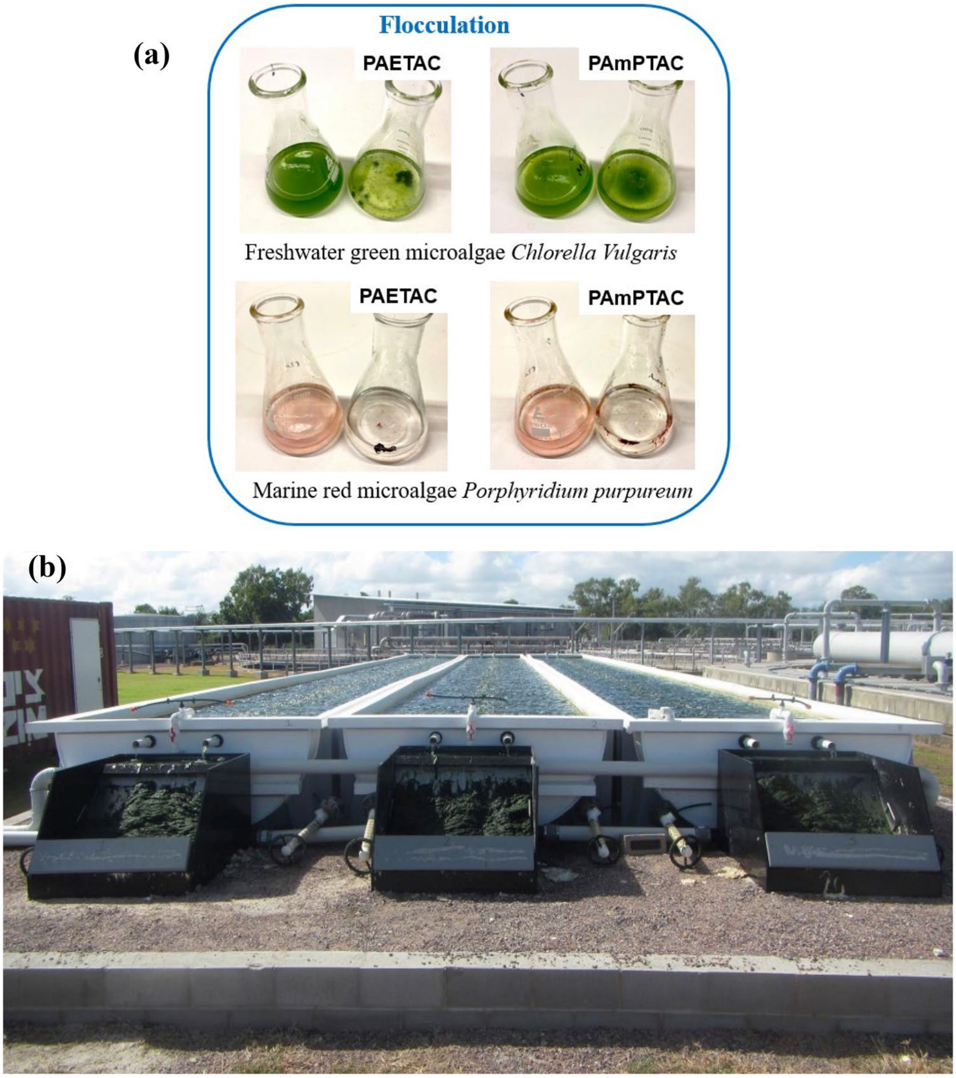

Fig. 3 **a** Flocculated microalgae biomass by cationic polymers PAETAC and PAmPTAC and **b** screen filter to harvest macroalgae in algae-based wastewater treatment.

Efficient Downstream Processes

The application of algae-based WWT also includes separation of algal biomass from treated water and downstream processing (i.e. chemical extraction or direct application). Currently, harvesting and chemical extraction are still expensive. Harvesting and extraction account for over 60% of the total operating cost. Due to the high cost of downstream processing, the full-scale algae-based WWT still have limited application.

The algae-based WWTs utilised microalgae have significant operation cost compared to that of macroalgae. Microalgae have small cell size (2 to 10 um), negative surface charge, and neutral buoyancy. To date, common harvesting methods include centrifugation, membrane filtration, flocculation, and flotation which are used for microalgae [92•]. Amongst these methods, flocculation and flotation appears to be more suitable due to their low operating cost and readily infrastructure at the wastewater treatment. Nguyen et al. [93•] demonstrated an efficient flocculation of freshwater *Chlorella vulgaris* using a cationic polyacrylamide flocculant which is commonly used for sludge dewatering at full-scale wastewater treatment plant (Fig. 3). Over 98% of algae biomass was recovered.

The utilisation of macroalgae species could overcome the harvesting challenge with microalgae due to its sizes [7]. Macroalgae can be easily separated from the treated water by filter screen. The pore size of the filter screen can be selected and changed based on the size of each macroalgae species [7, 20••]. Cole et al. [20••] developed a passive harvesting method for *Oedogonium* culture (Fig. 2). The algae biomass was filtered into a 750-µm filter screen twice a week for 18 h per time. The flow rate of harvesting was equivalent to the rate of the incoming water. They estimated that 50 to 75% of produced biomass was harvested. The remaining (0.18 to 0.37 g/L dry weight) continues the treatment until next harvesting. This passive harvesting required no energy input. The harvested biomass is dried or used freshly on a dairy farm.

Perspectives and Conclusions

Algae-based technology has been proposed as a sustainable solution for removal and recycle of nutrients in wastewater. Naturally, algae (both microalgae and macroalgae) can uptake nutrients from water for growth. Thus, laboratory studies have successfully demonstrated nutrient removal via algae cultivation. There have been also significant number of studies devoted to algae species selection, designing cultivation systems, assessing different wastewater sources, and evaluating the performance at pilot and large-scale systems. However, the pathway to implement algae-based WWT process still has several barriers.

There is limited full-scale adaptation of algae-based WWT both due to its technical and economic challenges. The technical challenge includes maintenance of viable algae culture for stable and long-time performance. Techniques to intensify algae cultivation for large-scale wastewater treatment are needed. Currently, the most suitable way is to co-locate algae cultivation with exiting wastewater treatment plant, where algae cultivation is mainly used to supplement part of nutrient removal. This approach could ensure minimal disturbance to the existing process. Co-location of algae cultivation also benefits to use CO_2 from digester biomass as a way to intensify algae growth.

Life cycle analysis, carbon balance, and economic analysis have been neglected in the literature, particularly in pilot and large-scale trials. It is suggested that these analyses should be conducted parallel or early stage of the projects. The economic aspect would improve at the species selection process. For example, the selections should focus on the fast growth rate, high photosynthetic rate, and strong environmental tolerance species with simple downstream processing (e.g. harvesting) and high-value biomass. Algae are a highly diverse group of microorganisms (0.2–1 million recognised species), providing a significant biobank for selection. In addition, the development of algae biotechnology (e.g. genetic modifying organisms) can be applied to enhance its capacity.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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