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Rachel Whitton[a] [iD], Francesco Ometto[b] [iD], Marc Pidou[a] [iD], Peter Jarvis[a] [iD], Raffaella Villa[a] [iD] and Bruce Jefferson[a]* [iD]

[a]*Cranfield Water Sciences Institute, Cranfield University, Cranfield MK43 0AL, Bedfordshire, UK;* [b]*Scandinavian Biogas Fuels AB, Linköping University, SE-58 183 Linköping, Sweden*

This review explores the use of microalgae for nutrient removal in municipal wastewater treatment, considering recent improvements in the understanding of removal mechanisms and developments of both suspended and non-suspended systems. Nutrient removal is associated to both direct and indirect uptake, with the former associated to the biomass concentration and growth environment (reactor). Importantly, direct uptake is influenced by the Nitrogen:Phosphorus content in both the cells and the surrounding wastewater, with opposite trends observed for N and P. Comparison of suspended and non-suspended systems revealed that whilst all were capable of achieving high levels of nutrient removal, only non-suspended immobilized systems could do so with reduced hydraulic retention times of less than 1 day. As microalgae are photosynthetic organisms, the metabolic processes associated with nutrient assimilation are driven by light. Optimization of light delivery remains a key area of development with examples of improved mixing in suspended systems and the use of pulsating lights to enhance light utilization and reduce costs. Recent data provide increased confidence in the use of microalgae for nutrient removal in municipal wastewater treatment, enabling effluent discharges below 1 mg L^{-1} to be met whilst generating added value in terms of bioproducts for energy production or nutrient recovery. Ultimately, the review suggests that future research should focus on non-suspended systems and the determination of the added value potential. In so doing, it is predicted that microalgae systems will be significant in the delivery of the circular economy.

Keywords: nitrogen; phosphorus; bioreactor; suspended; non-suspended

Introduction

The potential for remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is well documented [1–9] and considered an environmental approach to nutrient polishing.[10,11] In addition to enabling low nutrient discharges, a number of added benefits have been described: (a) sequestering of CO_2 from the atmosphere during photosynthesis [12]; (b) oxygenating the treated effluent [13]; (c) unlike alternative biological treatment processes, a compulsory inorganic carbon source is unnecessary to optimize treatment [8,13] and (d) removal of trace organic micropollutants. Furthermore, following treatment, the algal biomass can be processed for the production of low-value products within human and animal nutrition, cosmetics and biofuels, including biomethane through anaerobic digestion of the residual biomass.[14,15]

Microalgae are ubiquitous to wastewater environments [16] albeit at dilute concentrations, confirming the nutrient characteristics of such environments are suitable for growth [17], with microalgae demonstrating the ability to remediate effluents at concentrations commonly encountered post-secondary treatment.[18] Microalgae are therefore considered as prospective candidates for tertiary wastewater treatment.[19–21]

The desired features for a microalgal solution for tertiary treatment include performance reaching the required level of remediation within a practical hydraulic retention time (HRT). The longest HRTs typically encountered in tertiary treatment are related to constructed wetlands extending up to 1 day.[22] Current operation of microalgae treatment is most commonly achieved in high rate algal ponds (HRAP) with HRTs of 4–10 days. Accordingly, uptake is predominately reported in locations where land availability is not restrictive (e.g. USA [23]) but represents a research challenge to broaden uptake.

The combination of microalgae being able to meet low nutrient discharges (e.g. sub 1.0 mg L^{-1} total phosphorus) and the generation of added value components (e.g. biomethane feedstock material) have resulted in refreshed consideration of the need and benefits of microalgae over traditional nutrient removal options (e.g. chemical dosing for phosphorus), which offer no added value. Illustration of this is seen in regard to phosphorus, where a

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*Corresponding author. Email: b.jefferson@cranfield.ac.uk

range of new technologies being implemented to meet sub 1.0 mg L^{-1} discharges are all based on chemical dosing and clarification (e.g. BluePro, CoMag), resulting in an increase in coagulant use and residual sludge production. Additional issues arise at sites that previously did not incorporate chemical dosing such as small rural works. In such cases, the chemical-dosing-based options generate additional challenges due to the need for better infrastructure around transport (roads) and health and safety, including supply of potable water safety showers and chemical storage facilities.[24] Accordingly, there is a need for microalgae treatment options that offer a real alternative to the chemical-dosing-based technologies beyond sites where use of HRAP is appropriate.[25,26] This has seen a growth in research around application to more nutrient-limited environments such as tertiary treatment [19] coupled with new insights and trials of alternative technologies.[27,28] This review aims to appraise the new insights and technologies to consider the impact on the future potential for microalgae systems for tertiary treatment.

Overview of microalgal nutrient remediation mechanisms

Direct (biological) remediation of nitrogen and phosphate and microalgal species' nitrogen:phosphorus composition

Nutrient remediation with microalgae occurs through one of two pathways (Figure 1). Direct remediation is the most commonly discussed mechanism of remediation and is achieved through interconnected biochemical pathways for the uptake of the target nutrients into the biomass for storage,[29,30] or assimilation into nucleic acids and proteins for biomass growth [23] (Figure 1).

Inorganic nitrogen – that is, nitrite (NO_2^-), nitrate (NO_3^-) and ammonium (NH_4^+) – are translocated across the cell membrane [23] in the preference of $NH_4^+ > NO_3^- >$ Org $-$ N.[31] These oxidized nitrogen species are subsequently reduced to NH_4^+ and assimilated into amino acids for the formation of proteins (Figure 1), with NH_4^+ uptake preferred due to the reduced energy requirement necessary for reduction and assimilation.[23] Accordingly, microalgae can be utilized for total nitrogen (TN) removal
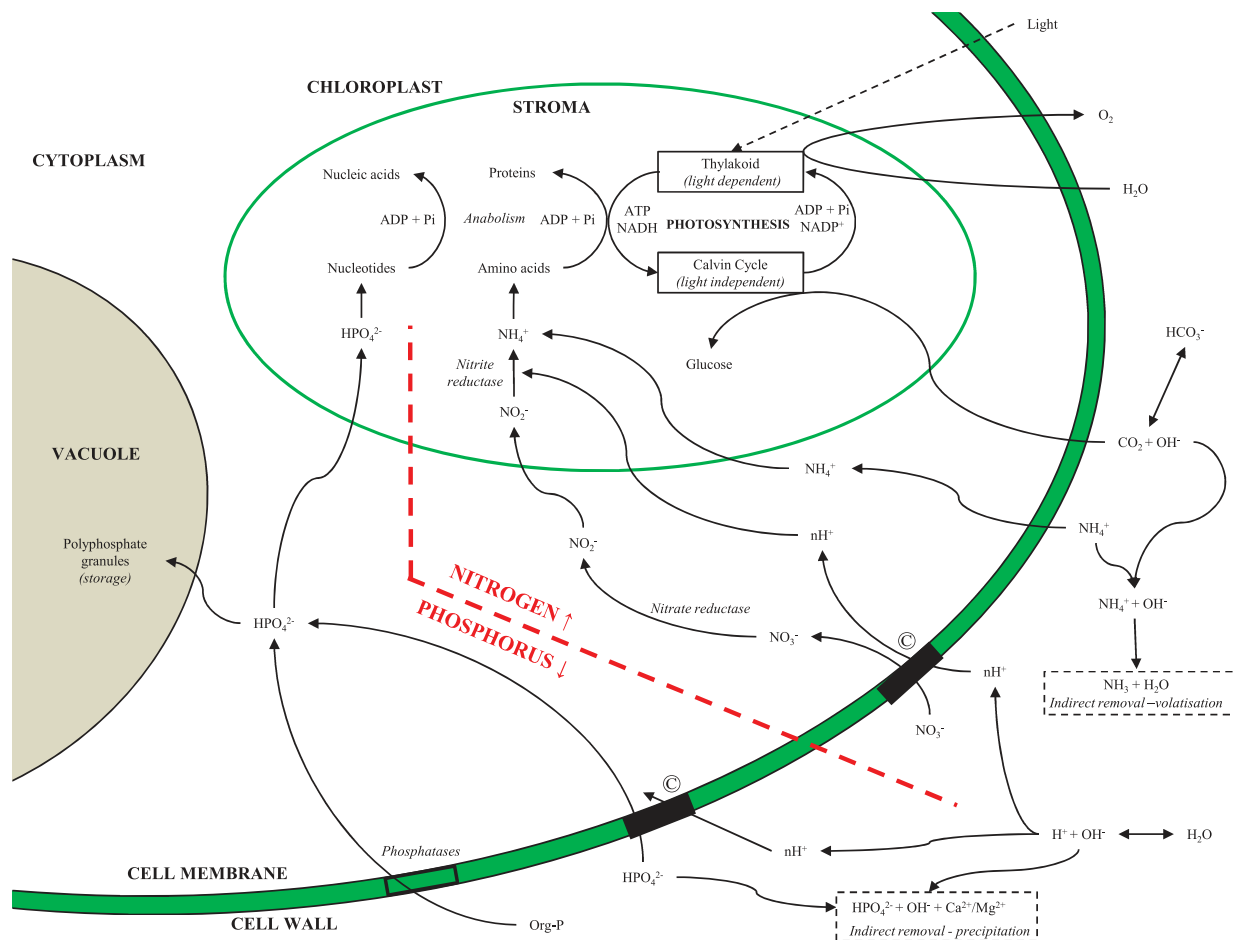

Figure 1. Schematic diagram of a microalgal cell summarising the biochemical pathways of nitrogen and phosphorus remediation, including indirect mechanisms (highlighted within a dashed box). © Represents co-transportation.

(nitrification and denitrification), with NO_3^- assimilation observed following the near-complete exhaustion of NH_4^+ [32] from the source wastewater.

Phosphate, in the preferred form of $H_2PO_4^-$ and HPO_4^{2-}, is transported across the cell membrane via energized transport [13,23] and assimilated into nucleotides following phosphorylation for the synthesis of ribosomal RNA [33] (Figure 1). A nitrogen source is therefore required for the synthesis of proteins to enable the assimilation of phosphorus, with a limitation of either nutrient resulting in a low cell-protein content and reduced biomass growth.[33] Furthermore, in high-phosphate environments, microalgae can consume excess phosphate through a luxury uptake pathway [29,34] for storage as an acid-insoluble polyphosphate granule [29] (Figure 1) for future use in times when the external phosphate concentration may become limiting.

Nutrient uptake by microalgae depends on the associated concentration in the microalgae biomass such that phosphorus removal is consistently lower than nitrogen (Table 1) due to a greater microalgal nitrogen biomass content.[42] Freshwater microalgae biomass nitrogen:phosphorus (N:P) molar concentrations range between 8:1 and 45:1, indicating the importance of species selection when optimizing treatment.[43] In addition, microalgae are known to adjust the N and P concentration in their biomass in relation to the levels in the surrounding medium.[33,44] For example, when cultured in mediums of varying N:P, internal N:P contents of 8.5–32 and 4.1–32 have been reported for the freshwater species *Chlorella vulgaris* and *Scenedesmus obliquus*.[33,45,46]

The remediation of nitrogen and phosphate is generally correlated to an increase in both the biomass volume [17] and the internal nutrient content within the biomass.[33,47] Biomass productivity (growth) decreases with an increasing external N:P concentration as illustrated in the case of *C. vulgaris*, with a maximum growth rate of 2.97 g L^{-1} d^{-1} at N:P (mg mg^{-1}) > 10 demonstrated through a transition from N to P limitation.[44] In relation to phosphorus, an inverse relationship between a species internal content and specific uptake rate is reported.[30,44] This results in reduced nutrient removal as illustrated with a culture of *C. vulgaris*, where phosphorus remediation decreased from > 80% with an increasing phosphorus cellular content when treating wastewater with an N:P up to 20 mg mg^{-1} down to only 20% when treating a wastewater with a nutrient ratio of > 50 N:P.[44] Similarly, Ruiz-Martínez [30] found an increased phosphate uptake rate of ∼3.3 $mgPO_4$-P $gTSS^{-1}$ h^{-1} in comparison to ∼0.7 $mgPO_4$-P $gTSS^{-1}$ h^{-1} for *Scenedesmus* sp. with internal P concentrations of ∼0.6% and 1% (w/w), respectively. An internal phosphate concentration of < 1% characterizes growth within phosphorus-limited environments,[48] whereas > 1% is indicative of microalga luxuriously consuming phosphate for growth and storage for future use

in conditions of limitation.[29] Observations from these studies suggest that an increased specific uptake rate for phosphorus is a result of either a limiting external concentration or an initially reduced internal content, with both conditions representing times of stress when phosphorus assimilation is necessary for the continued metabolic processes and growth of a culture. The opposite has been demonstrated for nitrogen assimilation, with a cellular content of ∼0.02 mgN $mgVSS^{-1}$ for *C. vulgaris* when remediating an influent profiled by an N:P (mg mg^{-1}) of 30 in comparison to 0.2 mgN $mgVSS^{-1}$ at an N:P of 80 [44] corresponding with phosphorus limitation. Similarly, the nitrogen removal efficiency of *Scenedesmus* sp. has been reported to decrease significantly when the N:P ratio exceeds 12:1.[17] Whilst no significant link has been observed between P removal and carbon concentrations, below a C:N of 10, nitrate uptake has been observed to be reduced in the case of *Chlorella* sp. [51].

The ability of a range of microalgae to remove nutrients from wastewater and synthetic wastewater has been analysed extensively within laboratory trials. The majority of research to date has been conducted on unicellular chlorophyceae,[50] in particular on the members from the *Chlorella* and *Scenedesmus* families (Table 1). Species from these families are largely used due to their dominance in freshwater environments,[51] the ease with which they are cultured and reproduce [20,36] and their ability to efficiently remove nutrients.[51] Recent comparisons of a range of algae have largely confirmed the suitability of *Scenedesmus* sp. for use in tertiary treatment applications.[20]

However, unicellular algae are difficult to harvest, resulting in recent research into non-planktonic algae, especially filamentous species such as *Oedogonium* sp. and *Tribonema* sp.[52,53] For instance, Liu and Vyverman [54] reported that for the filamentous algae trialled, *Cladophora* sp. was most efficient under low N:P ratio wastewater whilst *Pseudanabaena* sp. was better for removing nitrogen from high N:P ratio wastewater. In comparison to research on these identified species, fewer studies exist on other chlorophyceae as well as other taxa such as cyanobacteria and diatoms. The potential for nutrient removal by other chlorophyceae species in addition to diatoms and cyanobacteria are not as widely investigated, but are known to populate wastewater treatment works [55,56] and demonstrate beneficial characteristics. Cyanobacteria, for example, contain accessory pigments and an enhanced concentration of chlorophyll in comparison to chlorophyceae, enabling a more efficient use of available light.[57] In addition, species isolated from colder climates, for example, *Phormidium bohneri*, have shown acceptable growth and nutrient removal rates at cooler temperatures, demonstrating removal rates between 2.4 and 19.9 mg L^{-1} d^{-1} for NH_4^+ and 1.6 and 13.8 mg L^{-1} d^{-1} for total phosphorus (TP) within secondary

Table 1. Ammonium and phosphorus removal by microalgal cultures for varying waste streams.

Algae Concentration	HRT (d); Flow velocity; Scale (m³)	Aeration	Test waters; Temp. (°C); pH	Irradiance (μmol m⁻² s⁻¹)[a]; Day length (h)	NH₄⁺	TP	NH₄⁺	TP	Specific growth rate (d⁻¹); Effluent pH	References
Chlorophyceae										
Botryococcus braunii —	9 Batch 0.003	50 mL min⁻¹ CO_2	Secondary effluent — 7.6	— —	0.17	0.04	99.9[b]	75.0 —	—	[35]
Botryococcus braunii —	7 Batch 0.003	50 mL min⁻¹ CO_2	Secondary effluent — 7.7	— —	< 0.1	0.39	99.8[b]	97.4 —	—	[35]
C. vulgaris 253 mg L⁻¹	9 Batch —	—	Wastewater effluent — 7	100.8 16	7.7	0.9	55.8 —	— —	∼ 10	[36]
C. vulgaris —	2 Batch 0.0025	Air bubbling	Wastewater effluent 25 —	135 —	—	—	74.3 0.134 μg h⁻¹·10⁻⁶cells	70.2[d] 0.134 μg h⁻¹·10⁻⁶cells	0.186 9.0–9.5	[37]
C. vulgaris 2 × 10⁶ cells mL⁻¹	9 Batch 0.002	Air bubbling	Agro-industrial wastewater 20 —	60 —	—	—	—	55.0[d] —	—	[38]
C. vulgaris 5 × 10⁵ cells mL⁻¹	10 Batch —	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	74.1 —	63.8 —	0.274 —	[31]
C. vulgaris 1 × 10⁶ cells mL⁻¹	10 Batch —	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	97.8 —	87.0 —	0.277 —	[31]

(*Continued*).

Table 1. Continued.

C. vulgaris 5×10^6 cells mL^{-1}	10 Batch —	0.5 v.v.m filtered air	Primary settled sewage 24 7.1	58[a] 16	35.5	3.9	89.7 —	66.1 —	—	[31]
C. vulgaris 1×10^7 cells mL^{-1}	10 Batch —	0.5 v.v.m filtered air	Primary settled sewage 24 7.1 7.1	58[a] 16	35.5	3.9	99.9 —	78.7 —	—	[31]
Scenedesmus dimorphis 2×10^6 cells mL^{-1}	9 Batch 0.002	Air bubbling	Agro-industrial wastewater 20 —	60 24 24	—	—	— —	55.0[d] —	—	[38]
S. obliquus 14 mg L^{-1} (dm)	7.9 Batch 0.001	160 mL min^{-1}	Wastewater effluent 20 9.3	152[a] 24	27.4	11.8[a]	94 —	98.0 —	0.686 —	[39]
S. obliquus 14 mg L^{-1} (dm)	7.9 Batch 0.001	160 mL min^{-1}	Wastewater effluent 25 9.3	152[a] 24	27.4	11.8[a]	99 —	98.0 —	0.768 —	[39]
S. obliquus 14 mg L^{-1} (dm)	7.9 Batch 0.001	160 mL min^{-1}	Wastewater effluent 30 9.3	152[a] 24	27.4	11.8[a]	99 —	94.0 —	1.051 —	[39]

(*Continued*).

Table 1. Continued.

Algae Concentration	Design parameters HRT (d); Flow velocity; Scale (m³)	Aeration	Test conditions Test waters; Temp. (°C); pH	Irradiance (μmol m^{-2} s^{-1})[a]; Day length (h)	Influent conc. (mg L^{-1}) NH$_4^+$	TP	Removal efficiency (%); Uptake rate NH$_4^+$	TP	Specific growth rate (d^{-1}); Effluent pH	References
S. obliquus	7.9 Batch	160 mL min^{-1}	Wastewater effluent	152[a]	27.4	11.8a	79	54.0	0.458	[39]
14 mg L^{-1} (dm)	0.001		35	24			—	—	—	
			9.3							
S. obliquus	2.1 Batch	Air bubbling	Wastewater effluent	135	32.5	2.5[a]	100[c]	60.0	0.285	[37]
—	0.0025		25	—			0.180 μg h^{-1}·10^{-6}cells	0.036 μg h^{-1}·10^{-6}cells	9.0–9.5	
					—	—				
Cyanobacteria *P. bohneri*	5 Batch	0.1 v.v.m	Secondary effluent	—	—	—	—	—	0.190–0.490	[40]
100 mg L^{-1} (dm)	0.02		—	—			2.4–19.9 mg L^{-1} d^{-1}	1.6–13.8 mg L^{-1} d^{-1}	8.5–11.1	
			—							

[a] Irradiance units converted to μmol m^{-2} s^{-1} using conversion guidelines within.[41]
[b] Total nitrogen.
[c] Nitrate.
[d] Orthophosphate
(dm) Dry mass.

effluent.[40] In comparison, optimum nitrogen removal of 77.5 mg L^{-1} d^{-1} was observed for *S. obliquus* at 31°C with no treatment predicted below 8.8°C based on fitting the observed data to the cordinal temperature model with inflexion.[30]

Mono-culture growth of a species is achieved commercially by operating at favourable loading rates, retention times and environmental parameters [57] (e.g. for the growth of *Chlorella*, *Spirulina* and *Dunaliella* [58]); or by the selective recycling of species.[59] However, species control within a wastewater environment is challenging as microalgae are opportunistic [60], and attempts to control the community have failed due to contamination from native algal species.[61]

Indirect nitrogen (volitisation) and phosphate (precipitation) remediation

A by-product of direct remediation and growth is the alkalization of the localized environment through: (1) production of hydroxyl radicals (OH$^-$) during photosynthetic consumption of inorganic carbon such as bicarbonate (HCO$_3^-$)[42,36,62] and (2) a net uptake of protons (H$^+$) from the dissociation of H$_2$O for co-transportation of NO$_3^-$ and PO$_4^-$ through the microalgal cell membrane [62,63] (Figure 1). The modification of the physiochemical environment through pH [42] facilitates the indirect removal method. In the case of NH$_4^+$, at pH values greater than 7, there is an equilibrium shift within the kinetic equilibria for NH$_4^+$ and ammonia (NH$_3$) towards the production of NH$_3$ (gas) [39], which is subsequently volatized and stripped from the solution. The mechanism of indirect removal of ammonium has been shown to contribute greatly to total NH$_4$-N remediation, with removal percentages of 38–100% reported for the cyanobacteria *P. bohneri* [64] and 53–82% for *S. obliquus* under varying temperatures and mixing regimes.[39]

Unlike ammonium, phosphate cannot exist in a gaseous state and precipitates with metal ions (e.g. Ca, Mg and Fe) within the effluent , at elevated pH and high dissolved oxygen concentrations [23,29], with a removal efficiency of 16–63% reported for *Monoraphidium* species upon treating sterile-filtered wastewater.[62] The indirect removal mechanisms are not specifically monitored in the operation of microalgal bioreactors in the vast majority of cases. However, increases from an influent pH ranging between 7 and 9.3 to a final effluent pH between 8.5 and 11.1 are documented during the operation of those bioreactors were the pH is uncontrolled (Supplementary information). Once pH increases beyond 10.5, phosphate precipitation decreases due to a switch towards calcium carbonate formation as a result of the relative change in precipitation kinetics between calcium and phosphate or carbonate.[65]

Microalgal bioreactor configurations for wastewater nutrient remediation

The assimilation of nutrients for growth is facilitated by the process of photosynthesis, which is driven by the supply of inorganic carbon, light and temperature (Figure 1). Inorganic carbon, however, is often regarded as non-limiting [57,64] within wastewater effluent and expressed indirectly as the chemical oxygen demand.[57] It is the external factors of light and temperature, as opposed to the concentration of target nutrients, which have the greatest influence on growth and productivity [64] and are considered the key design features in the operation of a microalgal bioreactor with studies focusing on light [66–68]; temperature [64,69] in addition to species selection [20,54,70] to optimize performance.

Various bioreactor designs are available to enhance growth and facilitate biomass removal following treatment, which include suspended and non-suspended systems [11,71], with sub-categories of either open to the environment or enclosed (Figure 2).

Suspended cultures enable the microscopic algal cells to move freely within a body of water in dilute concentrations [28] and are most commonly used in microalgal wastewater treatment.[11] The biomass concentrations of suspended systems are reasonably low (< 2 g L^{-1}) (Table 2) but can exceed 4.5 g L^{-1} in intensified systems when treating industrial sources.[81] If not efficiently removed (harvested) post-treatment, the biomass has been reported to contribute to an increase in suspended solids content and 60–90% of the effluent biological oxygen demand.[82]

Harvesting is challenging and expensive and described as the defining factor of overall affordability [28] representing 20–30% of the production cost.[54] The microalgal cell surface is negatively charged,[83] with cells repulsed from one another and maintained in suspension. Harvesting typically involves dosing a positively charged metal coagulant to neutralize the surface charge, allowing the cells to aggregate together, creating flocs.[83] Subsequent removal of the flocs is through filtration, sedimentation, centrifugation or dissolved air flotation (DAF),[84] with DAF taking advantage of the natural tendency of algae to float by raising the biomass to the surface, where it is skimmed off and recovered. Harvesting thorough centrifugation and DAF represent significant chemical and energy costs [85,15,86] and require additional assets within the process flow-sheet representing further capital and operational expenditure.
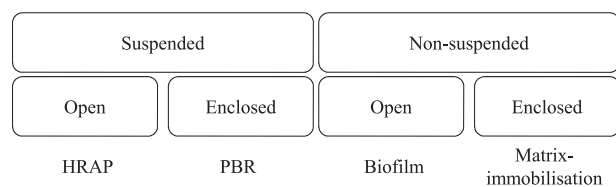

Figure 2.   Categories of microalgal bioreactors for wastewater remediation.

Table 2. Summary of microalgal bioreactor designs, operating parameters and performance.

Solution	Configuration	Scale	Algal biomass concentration dry mass (g L^{-1})	Algal community	HRT (d)	References
HRAP	Raceway pond	Full	0.3–1.0	Mixed	4–10	[11,42,72,73]
PBR	Tubular	Pilot	1.0–2.0	Mono	1–7	[11,74,75]
	Panel	Lab			2–5	
Biofilms	Floway	Full	130(g m^{-2})	Mixed	6–16	[6,60,76–78]
	Submerged	Pilot			6	
Matrix-immobilization	Packed bed	Lab	0.9–3.3	Mono or mixed	0.2–3	[50,79,80]

Recommendations to overcome the challenges associated with harvesting suspended cultures include the use of species known to self-flocculate, thereby aiding in removal by sedimentation or flotation,[5] or alternatively through the selection and growth of a non-suspended/filamentous culture.[54] Filamentous species naturally attach together in addition to other particles (i.e. suspended material and other biological entities), forming a biofilm layer on a surface interface.[56] This interface can be the surface of the reactor (i.e. floor, walls and baffles), or intentionally submerged substrates (e.g. polyurethane and polystyrene foam [79,87]) can be used to increase the available attachment surface area. Harvesting is achieved through physically scrapping the attachment surface to remove the biofilm, thereby eliminating the costs associated with harvesting suspended biomass.[88] Non-suspended systems can be further categorized into matrix-immobilization with microalgal biomass encapsulated in a hydrophilic polymer, whilst reducing the challenges associated with harvesting increases issues related to cellular access to CO_2, nutrients and photons.[89] Such systems are considerably less established than the suspended systems and questions remain around their suitability and affordability for municipal wastewater treatment.

The suspended and non-suspended bioreactors are then further categorized into open and closed systems. Open systems rely on the external environmental conditions to facilitate growth, characterized by the use of solar irradiance with sunlight hours and intensity affecting biomass productivity. Open systems are further influenced by external conditions, including temperature, rainfall (instigating culture dilution) and contamination by opportunistic species resulting in variability in annual performance. Alternatively, closed systems contain the biomass within the reactor, thereby minimising the opportunity for contamination [90] and supporting the culture of a mono-community through separation of the biomass from a potentially growth inhibiting environment.[91] An enclosed system offers greater control of the parameters to optimize growth [71,90] (e.g. irradiance, temperature, evapotranspiration, O_2, CO_2 and pH) and encourages increased specific growth, but requires greater infrastructure and operational costs, limiting their scalability.

The choice of bioreactor is evaluated upon performance at an economically accepted cost,[58] with examples including (but not limited to) HRAP, photobioreactors (PBRs), attached microalgal biofilms and matrix-immobilization (Figure 2).

High rate algal pond

A HRAP is a raceway-configured open pond mixed via a paddle wheel to circulate the algal culture and prevent settlement.[72,92] Sunlight is the primary method of irradiation and as such, culture depths of 20–60 cm are typical [58,72,73] to enable optimal light penetration and maximize growth. A HRAP supports a symbiotic community of microalgae and bacteria for the assimilation of nutrients and organic matter [59], supporting dilute microalgal biomass concentrations of up to 1 g(DW) L^{-1}.[11] Operational retention times of 4–10 days [73] are required to enable sufficient contact time with the biomass to achieve the required level of remediation (Table 2), resulting in large footprints.[27] Of the bioreactors available, HRAPs have received the most attention [11] and can be found operational at full scale with a demonstration plant located in New Zealand with individual pond footprints of 1.25 ha.[93,94] Further larger demonstration plants will be constructed in California, New Mexico, Hawaii and Florida with the primary focus on biomass production for biofuels.[23]

Photobioreactor

A PBR is an example of a closed, suspended system and are available in various configurations, including horizontal or vertical tubular photobioreactor (TPBR) or flat panel reactors.[58,90,95,96] A PBR encloses the culture in a series of narrow tubes (e.g. < 4 cm diameter [27]) or panels illuminated by sunlight and/or artificial sources.[90] Enclosing the culture enables a greater control of growth conditions (i.e. light, CO_2, O_2 and pH [11]) and permits the growth of a target species through optimized growth parameters, facilitating an increased biomass concentration in comparison to a HRAP of up to 2.0 g L^{-1} (Table 2) when cultured/remediating domestic wastewater effluents. The culture is circulated through the reactor by pumping

and degassing/bubbling processes (additionally releasing excess O_2 produced through photosynthesis). As a consequence of the sophistication of control, PBRs are expensive to install and operate [27] and are typically only employed in wastewater treatment as a plentiful source of low-cost culture medium for growth of a species for the return of a high-value product [27,97] to cover the cost of operation. High biomass production PBRs of up to 4000 L in capacity are operational and utilized for the cultivation of an inoculum species for HRAPs with the ultimate goal of biofuel production.[23]

Microalgal biofilms

Two types of microalgal biofilm processes exist; these include the use of an inclined floway (aka algal turf scrubber) with biofilm attachment to a surface [76,98] or submersion of a substrate to support biofilm growth and development [77,99,100] with practical examples including rotating algal biofilm reactors (RABR) [78] (Table 2). Biomass communities are heterogenous and multi-layered [56,101] and change seasonally [92] with reported biomass productivity of up to 60.9 g m^{-2} d^{-1} [76] and demonstrating enhanced metabolic activity.[102] Floway systems have been operated at full scale in Florida and California [60,103] with footprints up to 1012 m^2 treating a flow of 109–1336 m^3 d^{-1}.

Matrix-immobilisation

Matrix-immobilization is a variant of the attachment theme of reactors through the entrapment of living microalgae cells within a natural or artificial resin.[104] These resins are hydrophilic in nature with small pores to enable the diffusion of wastewater to the entrapped microalgal cells.[105] Immobilization enables intensification of a biomass concentration greater than a suspended bioreactor with concentrations up to 3.3 g L^{-1} reported (Table 2). The resin used to immobilize the microalgal biomass can provide additional remediation with the natural resin alginate found to contribute approximately 5% remediation efficiency of ammonium from a synthetic wastewater [37] through a chemical bond between the ammonium ions and the carboxyl groups of the resin.[37,106] This bond not only removes the ammonium from the source water but concentrates the nutrient for assimilation by the entrapped microalgae cells.[37] Further benefits of immobilising a microalgal culture includes the creation of a barrier around the selected species which prevents penetration by other organisms, which could inhibit productivity or outcompete the selected species [89,107]; and following treatment, the biomass can be harvested through the low-costing option of gravity settlement, eliminating chemical and energy costs associated with suspended systems. However, reduced space for mobility within the matrix leads to high shear stresses, with the matrix imposing additional hindrance

to photon accessibility being an area of concern along with the cost of the polymeric matrix when considered at full scale.[92,28] Of the microalgal reactors available, the immobilization technology for nutrient remediation is within its infancy with the majority of research and knowledge gained to date through lab-scale activities with bioreactors of up to 5 L in volume [106] (Table 2, see Table 6 at online supplemental data at doi:10.1080/21622515. 2015.1105308).

Influence of operational parameters and bioreactor design on remediation performance

Influent nutrient concentration and treatment period

The final effluent concentration and treatment period are key criteria when assessing the performance of microalgal bioreactors for wastewater nutrient remediation. European regulations within the Urban Wastewater Treatment Directive require a final effluent concentration prior to discharge of 15 or 10 mg L^{-1} TN and 2 or 1 mg L^{-1} TP for works treating a population equivalence of either 10–100 k or > 100 k, respectively,[108] with further site specific reductions in phosphorus to ~ 0.1 mg L^{-1} proposed with the onset of the water framework directive in 2015. Furthermore, the inclusion of an additional asset within a flow sheet to satisfy the required discharge concentrations must complement upstream processes to enable a constant output and flow. Microalgal bioreactors have been analysed for the treatment of a variety of wastewater streams, including primary and secondary domestic effluent, dairy manure wastewater, agricultural run-off and centrate (Supplementary information) with remediation data available for a wide range of influent concentration from 3.3 to 309 mg L^{-1} for ammonium and 0.04 to 770 mg L^{-1} for phosphorus (Figure 3).

When comparing microalgal bioreactor options for ammonium remediation, the influent and effluent concentrations show no clear relationship (Figure 3(a)) with effluent concentrations ranging from 0.11 to 140.9 mg L^{-1}. Those showing enhanced remediation performance include a TPBR with a 99.7% removal efficiency and effluent concentration of 0.11 mg L^{-1},[74] in addition to a polystyrene submersion system achieving 99.9% removal and an effluent concentration of 0.3 mg L^{-1} [99] (Figure 3(a)). These systems were either inoculated or naturally dominated by species of chlorophyceae well known for their nutrient remediation abilities, namely *Chlorella* sp. (TPBR) and *Scenedesmus* sp. (biofilm). Both systems demonstrated high biomass yields and productivities with a specific growth rate of 0.39 d^{-1} for *Scenedesmus* sp. and an increase in biomass concentration from 0.4 to 2 g L^{-1} (approximate maximum biomass concentration reported for a TPBR configuration [11]) and *Chlorella* sp. with a biomass concentration of 30–35 g(dry mass) m^{-2} equivalent to a productivity of 2–4 g m^{-2} d^{-1}.[99]
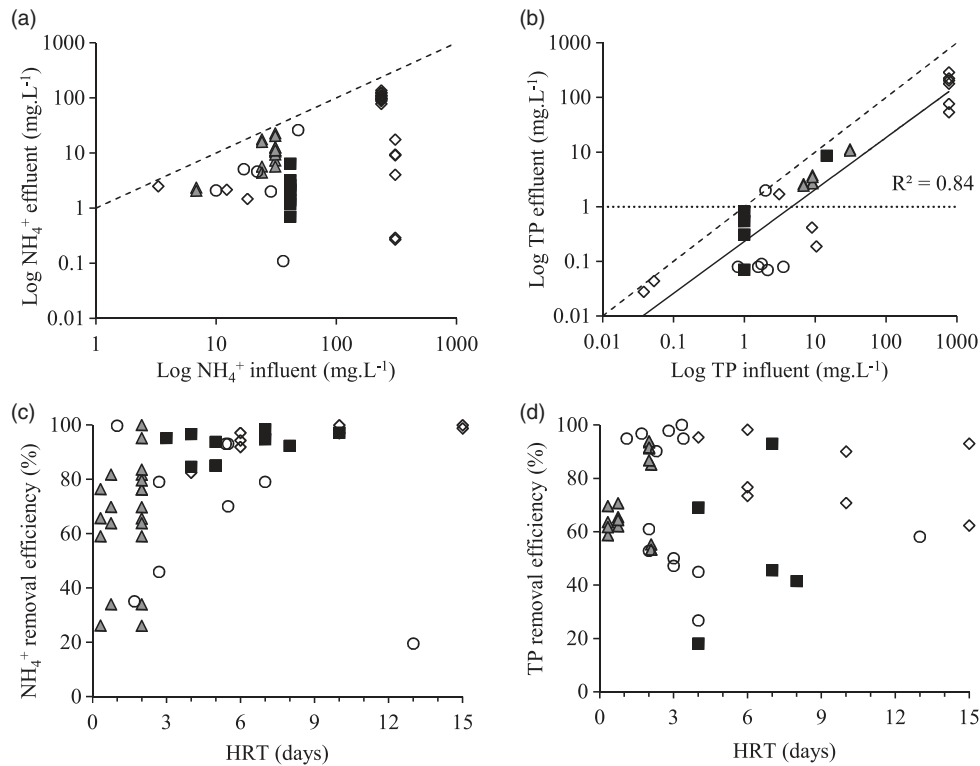
Figure 3. Influent concentration versus effluent concentration for (a) ammonium and (b) phosphate; and treatment period versus remediation efficiency for (c) ammonium and (d) phosphate for all bioreactors. HRAP (■), biofilms (◊), matrix-immobilization (▲), PBR (○) and parity line (- -).

Those bioreactors which did not perform as well belonged to the biofilm category of reactors with an algal turf scrubber demonstrating a 24.2% removal and effluent concentration of 2.5 mg L^{-1} [98] and a PBR containing rough surfaces to facilitate biofilm attachment with a removal efficiency of 45.8% and an effluent concentration of 26.0 mg L^{-1}.[109] Although the algal turf scrubber reported an extremely high yearly biomass productivity of 35 g m^{-2} d^{-1}, the biofilm contained a significant proportion of bacterial matter, particulates and cyanobacteria with the chlorophyceae *Chlorella* sp. and *Scenedesmus* sp. reported as only 'present' or 'few'.[98] Findings from these studies demonstrate the importance of species selection in addition to biomass concentration for the enhanced remediation of ammonium. Performance could therefore be improved by an increased biomass concentration or longer contact time with the available biomass to facilitate remediation through the direct mechanisms.

Whereas for phosphorus, a reasonable log–log relationship ($r^2 = 0.84$) is observed for all bioreactors (Figure 3(b)) with a lower influent concentration resulting in lower effluent concentration despite vast differences in operating parameters, including biomass concentration, treatment time and irradiance. This relationship suggests that unlike ammonium, mechanisms other than direct remediation are primarily responsible for the remediation of phosphate with treated effluent concentrations of < 1 mg L^{-1} possible at influent concentrations < 10 mg

L^{-1} (Figure 3(b)) providing there is an adequate supply of nitrogen.[33]

In terms of treatment period, performance data for NH_4-N removal exhibit a more defined relationship with performance efficiency and treatment period, in comparison to those for PO_4-P (Figure 3(c),(d)). The enclosed and more-intensive reactors remediate $> 80\%$ NH_4-N within less than 2 days (Figure 3(c)) with HRTs > 3 days required by HRAPs through the necessary increased contact period with the reduced biomass concentration. The biofilm solutions are characterized by a high biomass concentration but require a HRT > 10 days for a $> 90\%$ NH_4-N removal[99]; however, this is a necessary design feature as increasing the flow velocity (hence reducing the HRT) creates increased shear stress, reducing biofilm colonization [110] with impacts on remediation performance and increased suspended solids within the treated effluent.

In comparison, PBRs and matrix-immobilization can achieve $> 99\%$ NH_4-N remediation efficiency within HRT < 2 days through an increased biomass concentration and protection from biomass washout with increased flows. For example, a matrix-immobilized system with an algal concentration of 10^6 cells $bead^{-1}$ and 11.7 beads mL^{-1} was shown to remediate 100% NH_4-N within 24 h,[106] in addition to a TPBR with a 2 g L^{-1} biomass concentrations achieving a 99.7% NH_4-N remediation.[74] The remediation of ammonium is a function of biomass concentration and contact time, with those

bioreactors with a dilute biomass concentration requiring a greater treatment period in comparison to bioreactors with high biomass concentrations, with biofilms an exception through operational limits.

A relationship between HRT and TP removal efficiency is not as clear as NH_4-N (Figure 3(d)) further supporting previous assumptions that an alternative mechanism other than direct remediation significantly contributes to the removal of phosphate. Phosphorus removal efficiencies are generally $< 69\%$ for HRAPs with removal of approximately 40% seen in the majority of studies (see Table 3 at online supplemental data at doi:10.1080/21622515. 2015.1105308). The cases where $> 90\%$ removal are observed involve the modification of parameters to enhance the performance of a HRAP for phosphorus removal. For example, a 99% PO_4^{3-} removal efficiency and a residual of 0.07 mg L^{-1} (see Table 3 at online supplemental data at doi:10.1080/21622515.2015.1105308) are achieved through the addition of lime (CaO) to promote autoflocculation and the precipitation of phosphorus (equivalent to the indirect mechanism),[42] with a consensus that the role of indirect removal plays a more significant role than that of direct in HRAPs systems.[72,111]

Light, temperature and biomass productivity

As microalgae are photosynthetic organisms, metabolic processes associated with nutrient assimilation through growth are driven by light, [91,112] with light described as a key parameter of microalgal reactors.[90,91,112] The required light intensity for optimal growth is species specific with an example range of 150–400 μmol m^{-2} s^{-1} reported for *Scenedesmus* sp.[113,114] Light intensities below a species threshold range are associated with a reduction in biomass productivity [113] and can be generated through light limitation as a result of high-density microalgal cultures creating self-shading [115] and/or light attenuation and reduction with an increasing transmittance pathway.[115] Intensities beyond a species' preferred range results in oxidative damage through photoinhibition associated with a reduction in biomass productivity with the dissipation of the excess photons into heat.[116] To illustrate, Di Termini et al. [74] observed a specific growth rate of 0.39 d^{-1} and a remediation efficiency of $> 98\%$ for NH_4-N and PO_4-P for an autochthonous culture of *Scenedesmus* sp. when grown within an indoor TPBR with a constant light intensity of 200 μmol m^{-2} s^{-1} in comparison to a reduced growth rate of 0.02 d^{-1} and $< 80\%$ NH_4-N and PO_4-P removal efficiency within an outdoor TPBR with a variable light intensity reaching a daylight maximum of 1300 μmol m^{-2} s^{-1}.

As a consequence of biomass concentration, incident light intensity and culture depth (hence light transmittance depth), multiple 'light zones' are simultaneously evident within microalgal bioreactors.[117] These zones can be described as light inhibited, saturated, limited and no light

with the zones determined by the increasing depth from the culture surface, with a changing profile as a consequence of the incident light intensity and biomass concentration [118] estimated through the Beer–Lambert law.[91,119] The challenge of microalgal bioreactors is to maintain the culture within the light saturating zone to enable optimal productivity and the associated direct remediation of the target nutrients. This can be achieved through (1) reducing the light transmittance pathway (short depths),[27] (2) increasing culture circulation through mixing to ensure the microalgal cells move within the saturation zone [94] and (3) maximising the surface to volume ratio [11,112,115] to ensure sufficient light reaches the culture surface.

Outdoor systems are typically exposed to variable levels of light intensities through seasonal (and daily) changes in the available solar radiation. Intensities during the summer months of > 1200 μmol m^{-2} s^{-1} in comparison to 170–685 μmol m^{-2} s^{-1} are reported in the winter months (see Table 3 at online supplemental data at doi:10.1080/21622515.2015.1105308). However, only 50% of the radiation provided by sunlight is available to the microalgae for use as photosynthetically active radiation (PAR) (400–700 nm) [120] with open systems demonstrating poor photosynthetic efficiency in the conversion of solar energy into chemical energy of approximately 1.5%.[121] The variabilities in light intensities are reflected in fluctuating biomass productivities with ranges between 4.4 and 11.5 g m^{-2} d^{-1} observed in a 5 ha demonstration HRAP plant with removal efficiencies for NH_4-N and dissolved reactive phosphorus (DRP) between approximately 40–80% and 10–50%, respectively, mirroring the pattern in seasonal biomass productivity.[93]

Artificially lit reactors are employed to overcome the variability in biomass productivities and the associated treatment profiles, with generally lower intensities in the range of ~ 200–400 μmol m^{-2} s^{-1} reported for commercial PBRs.[116] Exposure to lower intensities are possible through design optimization (e.g. surface to volume ratio) to enable effective use of the provided light with increased photosynthetic efficiencies of 3–5% for PBRs [121] and/or the selection of a light source with a specific wavelength within the PAR to enable a more efficient use of the provided light, particularly as light is supplied at an operational cost. For instance, within PAR the microalgal chlorophyll molecules absorb light more efficiently within the blue (~ 400 nm) and red (~ 600–700 nm) region of the spectrum, with exposure to these wavelengths improving the photosynthetic efficiency and enhancing biochemical processes aligned to nitrogen and phosphorus remediation. For example, growth under a blue light regime is associated with increased phosphorus remediation through the activation of protein synthesis,[122] demonstrated by a culture of *Scenedesmus* sp. with a 45% increase in removal rate under a blue light regime of 1.8 mg L^{-1} d^{-1} in comparison to 1 mg L^{-1} d^{-1} when grown under white light (400–700 nm).[66] Furthermore, red light is known

to enhance microalgal growth rate, with a 38% increase in the specific growth rate of a culture of *Spirulina platensis* in comparison to growth of the same species within white light.[123]

The use of constant artificial light can represent a significant proportion of the total operational costs. Strategies are employed to improve the efficiency of artificial light which can be reflected within these costs and include (as discussed) the selection of an appropriate intensity to minimize wasted photons and the application of a light source with a suitable wavelength (i.e. LEDs) to eliminate energy use on unutilized wavelengths.[124] However, the antenna structure of the microalgal light-harvesting complex is unable to absorb all the photons provided under constant light [125], offering a further option of cost reduction and increased photosynthetic efficiency through reduced photoperiods and flashing/pulsating light regimes. For instance, under a flashing light regime of 37 kHz, the cell concentration of a culture of *C. vulgaris* was 20% greater than that of the same species grown under a constant light regime.[125]

Overall, artificial lighting offers a variety of options for increasing biomass productivity and associated remediation of nutrients through lighting regimes, which cannot be benefitted from within open systems. Advances made within LED industry resulting in increased bulb life, associated energy savings and predicted reduction in unit cost over time [126] make the use of artificial lighting a more attractive option for intensifying the remediation performance of microalgal reactors.

Microalgae exhibit a similar relationship to temperature as light, profiled by an increase in biomass productivity (and associated nutrient remediation) with increasing temperature [127] until reaching a critical temperature, beyond which has a negative effect on growth. For instance, Martinez et al. [69] documented an increase in specific growth rate of $0.69\,\text{d}^{-1}$–$1.10\,\text{d}^{-1}$ for *Scenedesmus* sp. grown within secondary wastewater effluent with an increasing temperature from 20°C to 30°C coupled with $>90\%$ remediation of nitrogen and phosphorus. At 35°C, the specific growth rate decreased to $0.46\,\text{d}^{-1}$ with a remediation efficiency of 79% and 54% for nitrogen and phosphorus, respectively. A temperature range between 15 and 30°C [71,127] are believed optimal for microalgal bioreactors, with maximum critical temperatures being species-specific, providing the external nutrient concentration and light provision are not limiting.[127]

Maintenance of a constant temperature is challenging within open reactors [127] with seasonal variations from 7.2°C to 25°C documented (see Table 3 at online supplemental data at doi:10.1080/21622515.2015.1105308) and extreme lows of 5°C and highs $>30\text{°C}$ reported for HRAPs.[42] Application of open systems are therefore favoured within locations with suitable annual climates to facilitate biomass productivity and achieve the required level of remediation throughout the year; for example, a floway periphyton scrubber located in the Florida Everglades with an daily mean air temperature of 19°C corresponding to a water temperature ranging from 18.1°C to 27.2°C.[60] Furthermore, microalgae within low-temperature environments are more susceptible to photoinhibition, which can constrain the use of open reactors in countries with a cold climate,[71] particularly with winter light intensities of up to $600\,\text{μmol}\,\text{m}^{-2}\,\text{s}^{-1}$ reported.[72] Temperature maintenance in closed reactors (and systems located indoors) is easier to control with documented temperatures of 20°C–30°C (see Tables 4–6 at online supplemental data at doi:10.1080/21622515.2015.1105308), corresponding to the range of temperatures associated with enhanced growth and nutrient remediation and more suited to locations with a cooler annual climate.

Conclusions and key remaining challenges

Algal treatment of wastewater, realized through a combination of direct uptake and indirect removal associated with elevated pH, provides a potential alternative to traditional tertiary treatment options for nutrient removal. Recent advancement in the understanding of both the mechanisms by which algae remediate nutrients in wastewater and specifically non-suspended algae treatment systems attests to the suggestions outlined by Hoffmann [92]. Accordingly, the ability of algal-based wastewater treatment to meet future challenges can be viewed with greater confidence. The most pressing illustration of which is associated with compliance to emerging sub $1\,\text{mg}\,\text{L}^{-1}$ phosphorus discharge standards. Further, developments in non-suspended systems have significantly reduced the required HRT of such systems to mirror existing passive tertiary treatment technologies. Consequently, consideration of the use of microalgal treatment can more reliably extend to sites where previously the lack of availability of sufficient inexpensive land was seen as a barrier to uptake of HRAP.

Future research will likely focus on the remaining challenges that require resolution before widespread use of algae can be realized. The costs associated with either harvesting of suspended systems, irradiance of closed systems and/or the chemicals associated with either harvesting and matrix- immobilization require better understanding and optimization to truly established the relative merit of microalgal systems compared to alternative tertiary treatment systems. As part of that better refinement is required with respect to the understanding of the added value algae systems can offer (in terms of associated bioenergy production, biofuels and bioproducts). This will become increasingly important in positioning microalgae treatment options as part of the delivery of the circular economy, which is expected to increasingly shape future investment consideration. Furthermore, the associated removal of hazardous chemicals and the ability for total nutrient removal need exploring in detail so that they can be properly valued. In addition, technical challenges remain associated

with intensification and seasonal stability (HRAP), scalability and light utilization (PBRs and non-suspended) and selection of better strains/mixtures to match the target wastewater and maximize biomass growth and by-product yields (all systems). Whilst development and increased uptake of all reactors types should be expected, it is perhaps in relation to non-suspended systems that the greatest advancements can be anticipated. Increasing demonstration of non-suspended systems will better enable appropriate comparison to be made with HRAP and PBRs and practical optimization achieved. Ultimately this will enable the potential for such systems to be considered in places where HRAPs are either not practical or desirable such as small wastewater treatment works with limited land availability.

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ORCID

Rachel Whitton http://orcid.org/0000-0001-9378-0859
Francesco Ometto http://orcid.org/0000-0002-6989-6197
Marc Pidou http://orcid.org/0000-0003-3031-9584
Peter Jarvis http://orcid.org/0000-0003-2740-6740
Raffaella Villa http://orcid.org/0000-0001-6646-894X
Bruce Jefferson http://orcid.org/0000-0003-4320-628X

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