Water Research 91 (2016) 371–378

Contents lists available at ScienceDirect

Water Research

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

Influence of microalgal N and P composition on wastewater nutrient remediation

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ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
21 December 2015
Accepted 31 December 2015
Available online 4 January 2016

Keywords:
Microalgae
Internal composition
Species
Biomass
Immobilisation

ABSTRACT

Microalgae have demonstrated the ability to remediate wastewater nutrients efficiently, with methods to further enhance performance through species selection and biomass concentration. This work evaluates a freshwater species remediation characteristics through analysis of internal biomass N:P (nitrogen:phosphorus) and presents a relationship between composition and nutrient uptake ability to assist in species selection. Findings are then translated to an optimal biomass concentration, achieved through immobilisation enabling biomass intensification by modifying bead concentration, for wastewaters of differing nutrient concentrations at hydraulic retention times (HRT) from 3 h to 10 d. A HRT <20 h was found suitable for the remediation of secondary effluent by immobilised *Scenedesmus obliquus* and *Chlorella vulgaris* at bead concentrations as low as 3.2 and 4.4 bead·mL^{-1}. Increasing bead concentrations were required for shorter HRTs with 3 h possible at influent concentrations <5 mgP L^{-1}.

1. Introduction

Microalgae are photosynthetic organisms that assimilate nitrogen (N) and phosphorus (P) during their growth. The subsequent biomass generated can be converted into energy or further raw materials following appropriate processing (Ometto et al., 2014), offering benefits in its use and renewing interest in a microalgae based technology for wastewater nutrient remediation.

Nutrient remediation characteristics for N and P have been shown to positively correlate to growth rate (Xin et al., 2010) with growth a function of internal rather than external nutrient concentration (Portielje and Lijklema, 1994). The internal composition of marine phytoplankton has been established as 106:16:1 as a molar ratio for C:N:P, known as the Redfield Ratio (Redfield, 1934). However, in the case of freshwater microalgae, the Redfield Ratio is an exception rather than a rule with N:P molar ratios ranging between 8:1 and 45:1 (Hecky et al., 1993) through a species' specific cellular quota for structural components and storage for growth (Droop, 1968). More importantly, freshwater microalgae have been shown to be able to adjust the N and P concentration in their biomass in relation to the surrounding concentration in the water (Beuckels et al., 2015; Choi and Lee, 2015) with biomass P accumulation influenced by the external P and N supply whereas N accumulation is independent of P (Beuckels et al., 2015). This behaviour is due to the predominate use of nitrogen for protein synthesis with P incorporated into ribosomal RNA. Accordingly, under limited nutrient conditions cell growth is reduced whilst carbon uptake continues (through photosynthesis) resulting in enrichment of carbohydrates or lipids. This is often exploited prior to bioenergy recovery to maximise yield for the microalgae biomass (Craggs et al., 2013). In high nutrient environments, microalgae can also accumulate excess nutrients through luxury uptake pathways (Eixler et al., 2006) enabling adaptation across a wide range of environmental situations. Such flexibility in nutrient compositions enables microalgae to successfully adapt to the local environment and influences the biochemical composition of the resultant biomass (Loladze and Elser, 2011; Choi and Lee, 2015).

Furthermore, the nutrient remediation characteristics of microalgal species have been correlated to the internal elemental concentration, with P remediation inversely correlated to biochemical composition (Choi and Lee, 2015; Ruiz-Martínez et al., 2015). With the nutrient concentration in microalgal biomass

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http://dx.doi.org/10.1016/j.watres.2015.12.054

shown to vary significantly from 0.03 to 3% of dry mass for P and between 3 and 12% for N (Reynolds, 2006), the design of microalgae reactors for wastewater treatment based on fixed stoichiometry (Redfield Ratio) are not likely to be reliable. Studies to date have analysed the impact of varying N:P mediums on cell composition, or evaluated a suitable wastewater nutrient balance for microalgal treatment in relation to internal composition for a specific species (Choi and Lee, 2015). It is posited however, that the efficacy of nutrient remediation can be further enhanced through a targeted selection of a species with a suitable composition following adaptation to a balance of nutrients in a wastewater to be processed, thereby achieving an enhanced level of remediation.

Furthermore, the majority of the work to date on microalgal wastewater nutrient remediation has considered suspended microalgal biomass operated in relatively passive technologies such as high rate algae ponds (HRAP). HRAPs are typically configured as raceways ponds with shallow depths (20–60 cm) containing dilute biomass concentrations around 0.3 g(DW) L^{-1}. Biomass concentration is relatively low through the variability of the light source (solar radiation) and associated poor light efficiency, in addition to other external factors related to open systems including temperature, predation and contamination (Park et al., 2011). Consideration and uptake of microalgae based technology for wastewater treatment is restricted in many countries due to the large footprints associated with the required long HRTs and shallow depths (Lundquist et al., 2010). Intensification of the algae biomass (and reduction in footprint) can be achieved through immobilisation where the biomass is encapsulated within an alginate gel affording biomass concentrations of up to 3.3 g(DW) L^{-1} (Chevalier and De la Noue, 1985). Whilst the technology is within its infancy, remediation of PO_4–P and NH_4–N from secondary wastewater effluents from 1.1 mgP L^{-1} and 2.6 mgN L^{-1} to 0.07 mgP L^{-1} and 0.02 mgN L^{-1} have been demonstrated for immobilised *S. obliquus* within hydraulic retention times of 6 h (Whitton et al., 2014). In addition to a concentrated biomass and reduced HRT, immobilisation facilitates the removal of biomass post-treatment through gravity settlement; eliminating costs associated with harvesting technologies which require coagulation and intensive energy requirements i.e. centrifugation. Following these positive attributes, the immobilised technology warrants further research to determine whether the solution can be optimised for adequate treatment within suitable HRTs prior to further development to improve its suitability for application within the wastewater treatment industry e.g. operational costs related to bead longevity and resin material.

Immobilisation affords the ability to seed and maintain a chosen species or community with known nutrient removal capacities such that it is posited that appropriate bead concentrations can be tailored to the required loading rates. To date, the work completed to optimise biomass density through bead concentration have preselected a bead mL^{-1} concentration and evaluated remediation performance regardless of the chosen species nutrient uptake characteristics. For example, Abdel Hameed (2007) evaluated the remediation performance of *Chlorella vulgaris* using bead concentrations of 10.66, 16, 32 and 64 bead mL^{-1} (1:3 to 2:1 bead:wastewater v/v) at 10^6 cells·$bead^{-1}$. Concentrations of 10.66 and 16 beads·mL^{-1} both achieved 100% NH_4^+ and 95% PO_4^{3-} removal efficiency, suggesting a concentration of 10 beads·mL^{-1} and associated biomass concentration to be suitable for optimal treatment under the conditions tested, with the possibility of a lower concentration performing similarly.

With the onset of the water framework directive (WFD) across Europe, the discharge consent for wastewater P will reduce from the current 1–2 mgP L^{-1} outlined within the Urban Wastewater Treatment Directive (UWWTD) to <0.5 mgP L^{-1}, with some sites expected to be as low as 0.1 mgP L^{-1} (Jarvie et al., 2006; Mainstone and Parr, 2002). Microalgae can be considered an alternative solution to meet these new stringent targets, providing the solution represents a practical alternative in terms of treatment time (HRT) and footprint, which can be achieved through immobilisation.

As such, the objectives of this study are to investigate how the internal composition of microalgae, through their ability to adapt to the external nutrient concentrations, relate to their nutrient uptake. Remediation performance of two of the characterised species, *C. vulgaris* and *S. obliquus*, are further analysed within real wastewater effluent. The findings are then translated into the impact on the design of an immobilised reactor for the improved remediation of wastewater nutrients; through the selection of a species for immobilisation and manipulation of biomass concentration through bead concentration to enable a suitable HRT for the integration of a microalgal reactor into a wastewater flow sheet for nutrient polishing.

2. Materials and methods

2.1. Microalgal biomass culture and immobilisation

The freshwater species *C. vulgaris* (211/11B), *Chlorella sorokiniana* (211/8K), *Microcystis aeruginosa* (1450/3), *Scenesdesmus obliquus* (276/3A) and *Stigeoclonium* sp (477/24) were obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK). Mono cultures were cultivated in 100 L reactor containing 50 L of medium as recommended by CCAP for optimal growth with an N:P molar ratio of approximately 2:1 for *M. aeruginosa* and 6:1 for the remaining species. Cultures were illuminated under a 24 h light regime with a light intensity of approximately 100–150 μmol m^{-2} s^{-1}. Constant mixing was through a circulation pump (900 L h^{-1}) (Hydor Koralia Nano 900), with no external supply of CO_2 provided and the temperature maintained at 18 °C. Microalgal biomass was harvested prior to the onset of stationary growth phase determined through previous growth experiments to characterise growth under the stated operational conditions and monitored through; cell counts for single celled species using a haemocytometer and light microscope (Olympus, BH Series), or dry weight following standard methods for total suspended solids (TSS) (APHA, 2005) for filamentous species. Knowledge of the chosen species' growth profile enabled biomass harvesting at the latter stages of exponential growth.

Microalgal immobilisation and encapsulation within calcium-alginate beads were completed following the method of Ruiz-Marin et al. (2010), with the adsorption capacity of the calcium-alginate resin determined through the method of Gotoh et al. (2004) (see supplementary information, Appendix A).

2.1.1. Freshwater species characterisation – nutrient remediation and internal N and P composition

Nutrient removal batch trials were completed in 100 L reactors using 50 L modified BG11 medium under the same operational conditions as those for cultivation and supplemented with NH_4Cl and KH_2PO_4 for an N:P molar concentration of 2:1, selected as a N:P < 10:1 is associated with enhanced biomass productivity (Choi and Lee, 2015). Biomass was seeded at an approximate concentration of 40 mg(DW) L^{-1}. Reactors were mixed on a daily basis and pH monitored and corrected to pH 7 using 1 M NaOH and HCl to prevent alkalisation of the medium and nutrient remediation through the indirect processes of precipitation and volatilisation. Batch trials were run over a period of 10 days, with analysis on day 0, 3 5, 7 and 10.

Nutrient remediation was determined by measuring the residual concentration of NH_4–N and total phosphorus (TP) within the

medium in triplicate using Spectroquant test kits1.14752.0001 (NH_4–N) and 1.14543.0001 (PO_4–P) (Merck Millipore), following the manufacturer's instructions and read via a Spectroquant Nova 60 spectrophotometer. Biomass growth was monitored as previously described and specific growth rate calculated using Equation (1), where μ = specific growth rate (d^{-1}), *x*1 and *x*2 the biomass concentration in cells mL^{-1} or mg(DW) L^{-1} at time t1 (d) and t2 (d).

$$\mu = \frac{\ln\left(\frac{x1}{x2}\right)}{t2 - t1} \quad (1)$$

Characterisation of the internal total nitrogen (TN) and C content of the microalgal biomass was analysed following freeze drying (ModulyoD Freeze Dryer, USA) and analysis using a TCN Vario III Elemental Analyser (Isoprime, DE) according to standard method ISO 10694:1995. Phosphorus content of the digested biomass sample was measured by UV/VIS spectrophotometry, following calibration with P standards of 0–7 mg L^{-1} with a ±0.005 accuracy, according to standard methods (USEPA, 1995).

2.1.2. Wastewater nutrient remediation trials for S. obliquus and C. vulgaris

Secondary wastewater effluent was delivered weekly from a wastewater treatment works located in the Midlands, UK and stored at 4 °C until use. The 32,000 population equivalence (PE) wastewater treatment plant (WWTP) comprises of an oxidation ditch operated for biological nutrient removal in addition to iron salt precipitation prior to the secondary clarifier. Effluent was selected from this site as the WWTP is located in a catchment designated as a Site of Special Scientific Interest (SSSI) which will be required to meet the stricter P consents prescribed within the WFD. As such, the WWTP has been selected as a trial site to evaluate the performance of multiple alternative technologies for the purpose of P polishing to meet the upcoming change in consent.

The average characteristics of the effluent collected were 0.3 mg L^{-1} PO_4-P and 0.1 mg L^{-1} NH_4-N. Effluent was supplemented with NH_4Cl and KH_2PO_4 to compensate for the current dosing strategy (which ensures appropriate nutrient discharge to the SSSI) and maintain a set NH_4-N concentration of 5 mg L^{-1} and a range of PO_4-P concentrations between 0.5 and 10 mg L^{-1}. These concentrations represent a possible range of secondary effluent characteristics that could be encountered by a tertiary microalgal system without advanced upstream treatment (A. Brookes & P. Vale, 2011, pers. Comms., 20 October), with an N:P molar ratio between approximately 22.1 to 1.1 suitable for microalgal activity with ratios <22 indicating sufficient phosphorus (Hecky et al., 1993). The wastewater also contained a non-supplemented and variable NO_3-N concentration of a maximum of 2 mgN L^{-1}, lower than the concentration of NH_4-N, which was not analysed through the preference of microalgae to assimilate NH_4-N over NO_3-N (Lau et al., 1995); and results from previous trials which found no accumulation of NO_3-N associated with the nitrification of NH_4 but rather a decrease of NO_3-N in parallel to NH_4-N remediation.

Conical flasks with 250 mL modified effluent were seeded with 10^4 and 10^5 cells.mL^{-1} of *S. obliquus* and *C. vulgaris* respectively to ensure a sufficient initial biomass concentration for growth. Batch trials were run over a period of 7 days under the same operational conditions as those for cultivation with sample analysis on days 3, 5 and 7. Analysis included pH, NH_4-N, PO_4-P and cell concentration. Residual nutrient concentrations and cell concentration were analysed as previously described.

The NH_4-N and PO_4-P cell uptake rate for *S. obliquus* and *C. vulgaris* was estimated through the analysis of the residual concentration according to Equation 2, where V is the cell uptake rate (mg $cell^{-1}$ d^{-1}), N the cell concentration (cells mL^{-1}) at time t (d^{-1}) and C_i and C_f the initial and final residual concentrations (mg L^{-1}) respectively.

$$V = \frac{C_i - C_f}{N \times t} \quad (2)$$

2.2. Calculations for optimal biomass concentration for wastewater treatment by S. obliquus and C. vulgaris – suspended and immobilised cultures

Following determination of the cell uptake rate for *S. obliquus* and *C. vulgaris*, calculations were completed to determine initial biomass concentrations required for 'optimal remediation' of phosphorus (residual <0.1 mgP L^{-1}) in line with changes to P discharge consent within the forthcoming WFD. Results were translated to an immobilised culture assuming a fixed cell stocking of 10^6 cells·$bead^{-1}$ as recommended by Abdel Hameed (2007). Calculations estimated the biomass concentration required for the remediation of phosphate at concentrations of 1, 5, and 10 mgP L^{-1} operating at a range of HRTs varying from 3 h to 10 days. The range of HRTs chosen complement the work by Whitton et al. (2014) which characterised remediation of an immobilised system at HRTs of up to 20 h and compared to the typical retention time of a HRAP (4–10 days).

3. Results

3.1. Freshwater species characterisation – nutrient remediation and internal N and P composition

3.1.1. N:P composition changes with change in external N:P

Following cultivation, the internal molar N:P composition of the

Table 1
Specific growth rate and internal N:P composition throughout the nutrient remediation trials, mean (±standard error).

Species	Specific growth rate (d^{-1})	Start (Day 0)		Day 3–10	End (Day 10)	
		N (μg·μg^{-1})	P (μg·μg^{-1})	N:P (molar)	N (μg·μg^{-1})	P (μg·μg^{-1})
		N:P (molar)			N:P (molar)	
Stigeoclonium sp.	0.19 (0.03)	66.0 (0.6) 18.9 (0.2)	7.72 (0.1)	13.5 (0.6)	46.8 (0.3) 13.9 (1.3)	7.3 (0.1)
C. vulgaris	0.17 (0.04)	69.8 (0.4) 16.8 (1.3)	9.2 (1.2)	10.5 (0.9)	75.6 (2.9) 12.0 (1.2)	24.0 (11.8)
S. obliquus	0.10 (0.03)	79.5 (2.1) 7.8 (0.8)	22.6 (2.8)	9.6 (0.5)	81.5 (7.8) 11.3 (2.2)	16.0 (3.0)
C. sorokiniana	0.12 (0.05)	82.4 (17.8) 20.3 (1.1)	9.8 (0.8)	15.0 (1.0)	75.4 (2.7) 16.3 (0.8)	24.5 (11.0)
M. aeruginosa	0.06 (0.03)	87.0 (nd) 16.6 (nd)	11.6 (nd)	16.6 (1.0)	91.0 (nd) 15.1 (nd)	13.3 (nd)

nd = undetermined.

tested algal species ranged from 7.8 to 20.3 (Table 1) despite the similar N:P molar concentration (6:1) of the growth medium (excluding the medium for *M. aeruginosa,* (2:1)), demonstrating the potential significance of algal selection.

Transferring the algal species from the growth medium to a more N limited test medium (N:P 2:1) for the nutrient remediation trials, reduced the difference in the biomass nutrient molar ratio to between 11.3 and 16.3 (average N:P 13.7) (Table 1). The molar N:P composition of *M. aeruginosa* was found to change the least, with a decrease of 1.5 from a molar N:P of 16.6 to 15:1 due to the similarities in the N:P characteristics of the cyanobacteria growth medium and test medium (both N:P 2:1) (Table 1). The difference observed with the change in N:P medium is congruent with the microalgae adapting the nutrient concentration within its biomass to the new environment (Beuckels et al., 2015), with the nitrogen content varying between 6.5 and 9.0% by weight (0.065 and 0.09 mgN·mg biomass^{-1}) with comparable variations in biochemical N composition of *Chlorella* sp of 3.6–10% previously demonstrated (Åkerström et al., 2014).

All microalgae were found to adjust their internal N:P content within the first 3 days of the trial and remained at the their new N:P composition for the remainder of the trial (Table 1). For example, *Stigeoclonium* sp. demonstrated a change from an initial N:P of 18.9 (±0.2) to an average of 13.5 (±0.6) for days 3–10 of the experiment, concluding with an N:P of 13.9 (±1.3) by day 10 with mass balances estimating 94.0 and 93.9% of N and P removal through microalgal growth. The final internal N:P values exhibited by all the microalgae analysed generally decreased (excluding *S. obliquus*) through the adjustment of the microalgae.

Whereas species with higher initial N:P composition (>16) reduced to between 12.0 and 16.3, *S. obliquus* with the lowest initial N:P of 7.9 increased to 11.6 (Table 1) suggesting *S. obliquus* can tolerate a greater N concentration than supplied by the growth medium (N limited) for incorporation and conversion into new biomass, supporting previous work of tailoring growth mediums to species' biochemical composition for growth optimisation (Mandalam and Palsson, 1998).

An average phosphorus content of 0.01 mgP mg biomass^{-1} (ranging 0.8–2.1% by weight) (Table 1) was found for all species, characterising growth in non-limiting P conditions (Hessen et al., 2002). The narrow variation in P within the biomass of the different algae is consistent with previous work that has shown that P level vary less when the N content within the biomass is relatively low (Beuckels et al., 2015). The N content therefore dictated the overall N:P composition of the species (varying from 0.07 to 0.09 mgN mg biomass^{-1}), and remained within the N:P ranges of 8.5–42 and 4.1–32 previously reported for *C. vulgaris* and *S. obliquus* (Rhee, 1974; Oh-Hama and Miyachi, 1988; Beuckels et al., 2015) when grown within varying N:P concentrations of differing retention times and growth conditions.

3.1.2. Nutrient remediation performance and internal N:P composition

Analysis of the remediation through a reduction in liquid phase nutrient concentration revealed >99% removal of NH_4–N during the experimental period with species with an initial N:P > 18 compared to between 24.7 and 60.8% for species with an N:P < 18. The increase in uptake by species characterised with a greater N composition is associated with the greater nitrogen content required per cell supporting previous work demonstrating a relationship between cell growth rate (Droop, 1974) and N remediation characteristics (Choi and Lee, 2015) in relation to internal concentration of the algal cell. The remediation efficiency of phosphate was lower than ammonium, at between 12.5 and 19.6% and unlike N, species characterised as more P limited were found to remediate at the higher end of this range supporting the inverse relationship demonstrated by Ruiz-Martínez et al. (2015) for P uptake and biomass composition.

Mass balances considering biomass growth and N:P composition estimates between 82.6 – 94.5% and 83.7–98.1% of N and P remediation is attributed to incorporation into new biomass for all species analysed. Remediation performance attributed to abiotic processes such as precipitation and volatilisation were considered negligible through the attainment of average pH values (prior to pH adjustment) during the trials of 6.9 (±0.09) for *C. vulgaris*, 6.2 (±0.32) for *C. sorokiniana,* 7.7 (±0.30) for *M. aeruginosa*, 7.7 (±0.38) for *S. obliquus* and 7.7 (±0.15) for *Stiegoclonium* sp respectively. As such, when considering the pKa value for ammonium at 20 °C an estimated 2–4% (equivalent to 0.2–0.4 mg L^{-1})of removal can be contributed to volatised free ammonia at the peak pH value of 7.7 (±0.38); and minimal P precipitation through pH values lower than the required 8–9 required for precipitation with metal ions such as calcium (Ca) (Montastruc et al., 2003).

Species with a lower N composition were found to have an enhanced specific growth rate (Fig. 1) through the reduced N requirements for growth. This is illustrated by a 1.5× increase in the final biomass concentration of 2.0 g(DW) L^{-1} compared to 1.3 g(DW) L^{-1} at the end of the trials by species with the lowest (0.06 mgN mg(DW)$^{-1}$) compared to the highest (0.09 mgN mg(DW)$^{-1}$) N composition respectively. However, the increase in biomass concentration of those species with a lower N:P could not outcompete the remediation performance of those species with a greater N:P composition at a lower biomass volume. Overall, species with a greater N:P composition (high N and low P), where found to remediate ammonium and phosphate more efficiently than those species with a lower N:P under the specified conditions even when considering total biomass concentration.

3.2. Wastewater nutrient remediation by S. obliquus and C. vulgaris

Two commonly used singled celled species initially characterised by a low and high N:P composition during cultivation; *S. obliquus* and *C. vulgaris*, were selected for trials with secondary effluent from a municipal wastewater treatment works. Ammonium concentration was fixed at 5 mgN·L^{-1} and the phosphorus concentration varied between 0.5 and 10 mgP L^{-1}, varying the medium N:P molar ratio between approximately 22.1 to 1.1 and encompassing a range of concentrations possible within secondary wastewater effluent (A. Brookes & P. Vale, 2011, pers. Comms., 20 October). Increasing the P concentration and hence reducing the N:P ratio resulted in an increase in cell uptake for both species in terms of P (Fig. 2a) and a decrease in cell uptake for N (Fig. 2b)

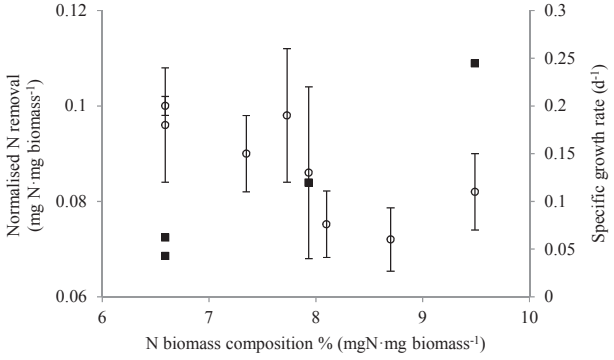

Fig. 1. N remediation and specific growth in relation to species' internal N composition. N remediation (■) and specific growth rate (○). Uptake rates calculated using TSS data when available (mean ± standard error).

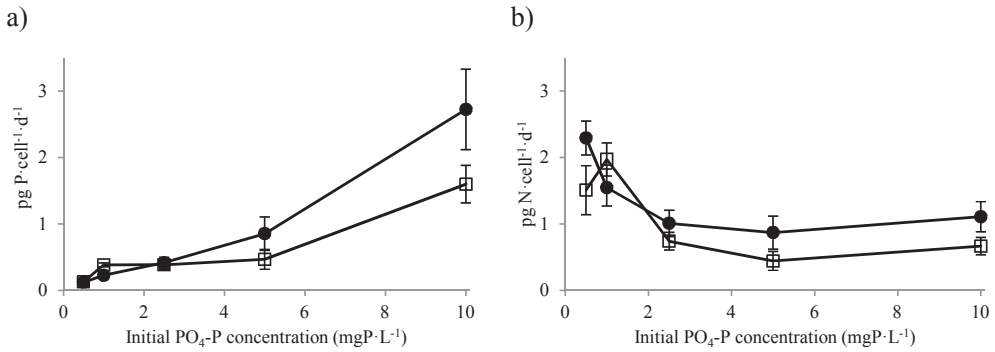

Fig. 2. Cell uptake rate for a) PO_4–P and b) NH_4–N (mean ± standard error) by suspended *S. obliquus* (□) and *C. vulgaris* (●) in secondary wastewater effluent with varying initial PO_4–P concentration.

reflecting nutrient availability within the supplemented effluent.

The phosphorus removal rate increased from 0.1 to 1.6 pgP cell^{-1} d^{-1} and 0.2 to 2.7 pgP cell^{-1} d^{-1} for *S. obliquus* and *C. vulgaris* respectively as the initial concentration increased from 0.5 to 10 mgP L^{-1}. A minimum P remediation efficiency through the incorporation into new biomass of 47–82% and 18–77% for *S. obliquus* and *C. vulgaris* is estimated, prior to an increase in the effluent pH to values indicative of remediation through abiotic processes, with the initial biomass incorporation rates decreasing with increasing P concentration.

The removal of phosphate is associated with N removal through their respective roles in cellular metabolism (Loladze and Elser, 2011). In microalgae, N is mainly integrated into proteins that in turn links to the production of ribosomes and ribosomal RNA. Phosphate uptake is predominately associated with storage into the ribosomal RNA such that the observed function between P concentration and uptake rate requires sufficient N to ensure no restriction of protein synthesis. Previous work has shown that in low N environments, the uptake of P into the biomass remains low irrespective of the P concentration in the solution (Beuckels et al., 2015). In such cases the uptake rate also relates to the ability of microalgae to store available phosphate in time of surplus, through a luxurious uptake pathway where polyphosphate accumulates within the cells (Wang et al., 2010).

The pattern of remediation for NH_4–N were similar for both species and decreased as the concentration approached 2 mgP L^{-1} prior to stabilising. Remediation rates of 0.7–1.5 pgN cell^{-1} d^{-1} and 1.1–2.3 pgN cell^{-1} d^{-1} for *S. obliquus* and *C. vulgaris* were demonstrated (Fig. 2b), with remediation through incorporation into new biomass estimated at a minimum of 54–99% and 38–93% for *S. obliquus* and *C. vulgaris* respectively, prior to an increase in the effluent pH and the contribution of volatilisation to total remediation.

Remediation performance was furthermore reflected in the cell uptake rate for both species remediating the equivalent of 18.5–82.1 and 36.3–95.6 fmolN·cell^{-1} h^{-1} for *S. obliquus* and *C. vulgaris* respectively; greater than that reported for ammonia-oxidising bacteria (AOB) in wastewater of 0.03–53 fmolN cell^{-1} h^{-1} (Lydmark, 2006). When considering the difference in mass of AOB and microalgae, AOB concentrations of 10^{10} cells·gVSS^{-1} (Hallin et al., 2005) in comparison to approximately 5×10^9 cells g^{-1}. VSS for *Chlorella* (considering 7.7×10^9 cells gCOD^{-1} and 1.43 gCOD·gVSS^{-1} (Ras et al., 2011)) are reported. The associated mass uptake rates based on the higher ranges of 5.3×10^{-9} and 1.8×10^{-8} fmolN gVSS^{-1} h^{-1} for AOB and *Chlorella* respectively further demonstrate the effectiveness of microalgal cells for ammonium remediation.

Comparison of the two microalgal species in terms of cell uptake revealed similar levels of 0.4 (±0.07) pgP cell^{-1} d^{-1} in low phosphate wastewater (<2.5 mg L^{-1}) (Fig. 2a). In contrast, at higher initial phosphate concentrations of 5 mgP·L^{-1}, uptakes rates of 0.5 (±0.2) pgP cell^{-1} d^{-1} and 0.9 (±0.3) pgP cell^{-1} d^{-1} were observed for *S. obliquus* and *C. vulgaris* respectively (Fig. 2a). Nitrogen uptake was also slightly greater for *C. vulgaris* at higher P concentration consistent with *C. vulgaris'* higher N:P cell content but in contrast to previous work that showed that N concentration in biomass was independent of P supply (Beuckels et al., 2015) indicating other mechanisms. Notable differences were observed between species in terms of cell growth and associated alkalisation of the surrounding medium. The growth rate of *C. vulgaris* was lower and more consistent across all concentrations with a range of specific growth rates between 0.16 and 0.29 d^{-1} (in comparison to 0.51 and 0.71 d^{-1} for *S. obliquus*) suggesting the greater P and N uptake observed at higher P concentration was not due to cell growth. Luxury phosphate uptake has been demonstrated to take effect for *Scenedesmus* beyond a critical growth concentration of 1.5 mgP·L^{-1} (Azad and Borchardt, 1970). At concentrations beyond this level uptake through luxury consumption has been observed, with no impact on growth (Azad and Borchardt, 1970). Similar observations were found within this study, and supports the improved remediation performance at the higher concentrations despite the narrow range of growth rates observed for the different PO_4–P concentrations. The lower growth rate observed for *C. vulgaris* resulted in a reduced degree of alkalinisation as evidenced by an average final pH of 10.9 for *S. obliquus* in comparison to 9.7 for *C. vulgaris*.

4. Discussion: implications for an immobilised microalgal reactor for tertiary wastewater nutrient remediation

The aim of the study was to examine the impact of variation in nutrient content of algal biomass on the associated nutrient uptake rates and understand the importance of algal species selection when operating a tertiary treatment system. Overall, the nutrient concentration in algal biomass is not fixed and so does not map to predictions based around the Redfield ratio (Hecky et al., 1993). Furthermore microalgae display flexibility in the nutrient concentrations in the biomass enabling adaptation to the local environment with nutrient uptake limited by the species' specific cellular quota for structural components and storage for future growth (Droop, 1968). Accordingly, design of microalgae reactors for wastewater treatment need to consider species selection and nutrient concentrations in the biomass and ability to adapt to external concentrations as it impacts on the maximum treatable loading rate and associated footprint.

Experimental results characterising nutrient uptake for the species *S. obliquus* and *C. vulgaris* were used to calculate cell

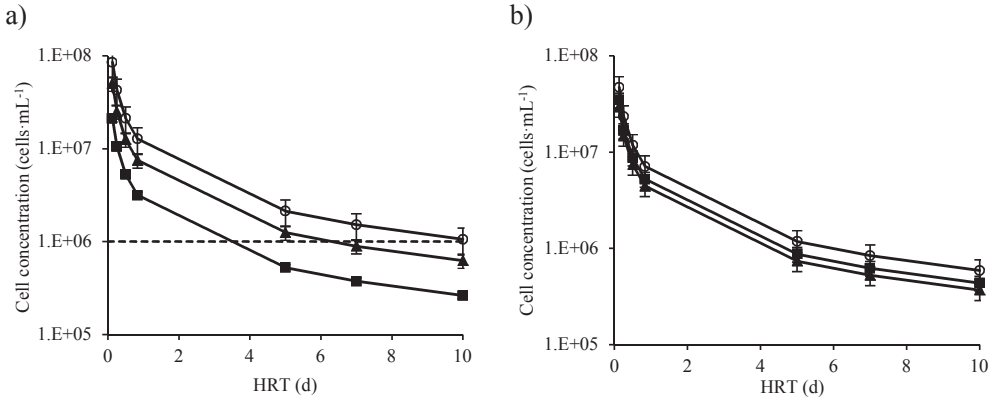

Fig. 3. Optimal cell concentration for a) *S. obliquus* and b) *C. vulgaris* with HRT for influent PO_4–P concentrations of (■) 1, (○) 5 and (▲) 10 mgP·L^{-1} (mean ± standard error), (--) denotes approximate equivalent biomass concentration for a HRAP.

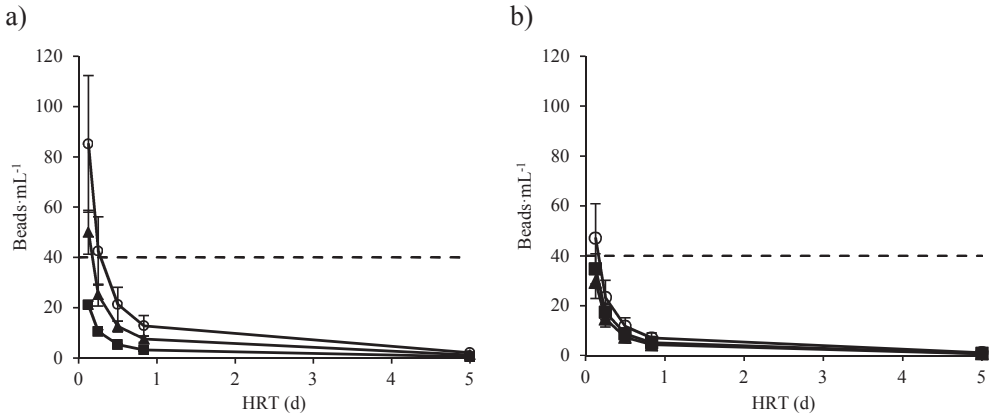

Fig. 4. Corresponding bead mL^{-1} concentration for a) *S. obliquus* and b) *C. vulgaris* with HRT up to 5 days for influent PO_4–P concentrations of (■) 1, (○) 5 and (▲) 10 mgP·L^{-1} (mean ± standard error), (--) denotes 1:1 (bead:wastewater v/v) and maximum bead·mL^{-1} concentration possible.

concentrations required for 'optimal remediation' (<0.1 mgP·L^{-1}) from initial concentrations similar to those found within tertiary effluent. Required concentrations were predominately calculated through PO_4^{3-} remediation as phosphate is targeted for further reductions in consent through the WFD. Required cell concentrations for both *S. obliquus* and *C. vulgaris* were found to increase to >3 × 10^6 cells mL^{-1} for HRTs less than 24 h, with maximum cell concentrations of 8.5 × 10^7 and 4.7 × 10^7 cells mL^{-1} at a treatment time of 3 h for *S. obliquus* and *C. vulgaris* respectively for the higher P concentrations (Fig. 3).

The required cell concentration for *C. vulgaris* was found to be less variable than *S. obliquus* with similar biomass concentrations necessary for the remediation of 1, 5 and 10 mg L^{-1}. *C. vulgaris* is therefore considered a better option for the treatment of wastewater with a varying influent concentration. However, for both species, greater biomass concentrations were necessary for treatment at 5 mgP L^{-1} than 10 mgP L^{-1}due to a cell uptake rate three times greater when treating 10 mgP L^{-1} highlighting the ability of microalgae to adjust their performance to suit a changing environment, with enhanced remediation through luxury uptake within nutrient rich environments (Eixler et al., 2006) thus the reduced biomass concentration at the higher P concentration.

Open pond systems (i.e. HRAPs) are reported to maintain an approximate biomass concentration of 0.4 g(DW) L^{-1}, equivalent to a cell concentration of approximately 10^6 cells mL^{-1} for *S. obliquus* (based on laboratory growth data). This concentration is sustained through the variation in light, temperature and biotic factors including zooplankton grazers and pathogens (Park et al., 2011). As such, HRTs >4 days are necessary to achieve an optimal level of treatment when using a HRAP through the biomass concentration achievable. To illustrate the consequence of this, a wastewater treatment works with population equivalence (PE) of 2000 treating the standard 0.18 m^3 pe^{-1} d^{-1} of effluent would require a HRAP with a surface footprint of 7200 m^2 at a depth of 0.2 m and minimum HRT of 4 days. This footprint is considerably larger than that of conventional tertiary treatments such as rotating biological contactor (RBC) unit or trickling filter with land footprints of 40–50 m^2 (Butterworth et al., 2013). As such, a HRAP would be an unlikely solution for retrofitting to a site of 2000 PE. To overcome limitations around treatable load and footprint an intensification of the algal biomass is required. Immobilisation enables biomass concentrations beyond 10^7 cells·mL^{-1} through either an increase in the cells per bead or the number of beads per unit volume, with example levels of up to 10^8 cells·mL^{-1} (Abdel Hameed, 2007) and biomass concentration of up to 3.3 g(DW) L^{-1} (Chevalier and De la Noue, 1985) reported, equivalent to the typical biomass concentration found within the activated sludge (AS) process (Metcalf et al., 2003).

To illustrate the impact of immobilisation on intensification of microalgae based wastewater treatment, the cell concentration required for each influent concentration (Fig. 3) was used to determine the required bead concentration (based on an initial internal bead concentration of 10^6 cells·$bead^{-1}$) (Fig. 4). Calculations for PO_4–P removal considering 10^6 cells·$bead^{-1}$ found bead

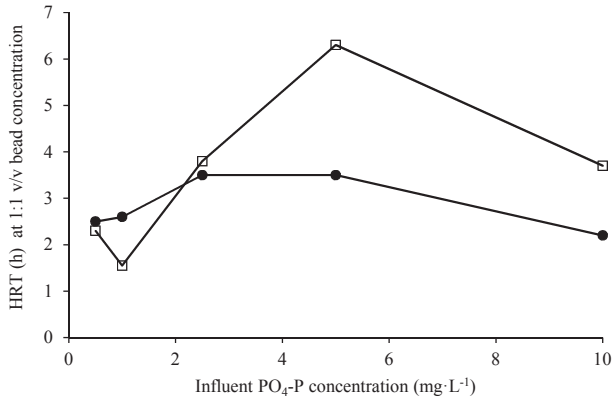

Fig. 5. Theoretical minimum HRT at 1:1 (v/v) bead concentration for PO_4–P remediation by *S. obliquus* (□) and *C. vulgaris* (●).

concentrations of 3.2–85.1 beads·mL^{-1} (1:12.5 to 2.1:1 bead:wastewater v/v) for *S. obliquus* and 4.4 to 47.1 beads·mL^{-1} (1:9 to 1.2:1) for *C. vulgaris* (Fig. 4) for remediation of influent concentrations of 1, 5 and 10 mgP·L^{-1}.

The required bead·mL^{-1} concentration was found to increase with the reduction in HRT due to the increased loading rate and required increase in biomass concentration. Overall, a lower bead·mL^{-1} concentration was found for *S. obliquus* at the lower PO_4–P influent concentrations (1 mgP·L^{-1}) and *C. vulgaris* for the higher concentrations (>5mgP·L^{-1}) (Fig. 4) due to the increased cell uptake rate demonstrated by *C. vulgaris* during batch trial characterisation (0.13–1.6 and 0.12–2.7 pgP·cell^{-1}·d^{-1} for 0.5–10 mgP·L^{-1} for *S. obliquus* and *C. vulgaris* respectively) (Fig. 2a) and the corresponding performance associated to the characterised internal N:P composition.

These calculated bead concentrations can be compared to observed experimental performance of an immobilised algae reactor of *S. obliquus* treating a PO_4–P concentration of 0.7 mg L^{-1} at fixed bead concentration of 10 beads·mL^{-1} and variable HRTs of 3, 6, 12 and 20 h (Whitton et al., 2014). Similar residual concentrations, following the initial start-up period of 0.10, 0.17 and 0.11 mgP·L^{-1} were observed for 6, 12 and 20 h respectively confirming a suitable bead/biomass concentration. However, a reduction in residual performance at a 3 h HRT of 0.43 mgP·L^{-1} was observed suggesting the biomass concentration to be inadequate. Based on the calculated bead concentrations presented (Fig. 4a), a concentration of approximately 13 beads·mL^{-1} (equivalent of an additional 10^6 cell·L^{-1} and approx. 0.2 g(DW)·L^{-1}) would have provided the additional biomass necessary to remediate within the shortened retention time and as such, the predicted biomass concentrations presented in Fig. 4 can be used to inform cell concentration through bead volume for *S. obliquus* and *C. vulgaris*.

Extending this to higher loading rates needs to consider other practical aspects which limit the applicable bead concentration to 1:1 v/v in order to minimise practical issues. These include sinking and crushing of beads under their own weight (Abdel Hameed, 2007) and self-shading restricting light penetration (Lau et al., 1995) which can contribute to a significant reduction in NH_4^+ remediation performance (Abdel Hameed, 2007) as well as improving irradiation efficiency.

When applying the maximum bead concentration (1:1 v/v) to the range of influent concentrations, a treatment period of 1.5–2.5 h for an effluent with a concentration < 1mgP·L^{-1} is achievable by immobilised *S. obliquus* and *C. vulgaris* (Fig. 5). Treatment periods >3 h are then necessary for the remediation of effluents >2.5 mgP L^{-1} by both *S. obliquus* and *C. vulgaris* with required HRTs of 6.3 h and 3.5 h for *S. obliquus* and *C. vulgaris* at 5mgP·L^{-1} respectively (Fig. 5). In situations where immobilised algae are used as a tertiary treatment the solution is unlikely to encounter influent concentrations greater than 5 mgP·L^{-1}. As such the required HRT is less than 3 h indicating the potential for effective use of microalgae without the need for large footprint technology.

Immobilisation also introduces an additional component in the form of the calcium-alginate beads that contain the microalgae and offers an additional uptake pathway. Adsorption trials with blank Ca-alginate beads found PO_4–P uptake by the resin material to be negligible across the tested PO_4–P concentrations (see Appendices, Figure B.1), confirming previous trials with blank alginate beads in sterile conditions (Cruz et al., 2013). However, within non-sterile wastewater Cruz et al. (2013) demonstrated a capacity of >15 μgP·g^{-1} over a 48 h period with removal contributed to the formation of a concentrated biofilm layer supported by the bead's surface area and not directly through the adsorption capacity of the resin material. In contrast, uptake of NH_4–N resulted in removal efficiencies of 9.1, 20.6, 25.4 and 23.4% for NH_4–N at starting concentrations of 0.5, 2.5, 5 and 10 mgN·L^{-1} respectively, with an adsorption capacity of 6 μgN·g^{-1} determined through fitting the data to a Freundlich isotherm model (see Appendices, Figure B.2) and providing an additional pathway for nutrient removal when using immobilised systems. As such, this study provides a conservative estimate on the ability of immobilised microalgae to remediate wastewater nutrient through species selection and biomass concentration as additional mechanisms, including the Ca-alginate resin and indirect methods of volatilisation and precipitation, would further enhance the overall remediation performance.

5. Conclusions

- A relationship between internal N:P composition and nutrient remediation is evident and can be considered when selecting a species for remediation. Species with a high N and low P internal composition remediate ammonium and phosphate more efficiently.
- Required biomass concentrations varied with wastewater characteristics and nutrient uptake abilities. When translated into immobilised beads, concentrations as low as 3.2 beads·mL^{-1} is possible for *S. obliquus* at HRT of 20 h.
- A HRT <3 h is impractical for an immobilised microalgal solution for concentration >5 mgP·L^{-1}, due to the volume of beads required to achieve maximum remediation.

Acknowledgements

The authors gratefully acknowledge financial support from the Engineering and Physical Sciences Research Council (EPSRC) through their funding of the STREAM Industrial Doctorate Centre (Grant no. EP/L015412/1), and from the project sponsors Anglian Water, Severn Trent Water and Scottish Water. Also a special thank you to Maria Biskupska and Jan Bingham for assisting with the internal biomass composition analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.12.054.

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