Tertiary nutrient removal from wastewater by immobilised microalgae: impact of wastewater nutrient characteristics and hydraulic retention time (HRT)

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Abstract

Immobilising microalgal cells has been proposed as a process solution to overcome the barriers associated with the implementation of microalgae for wastewater remediation. This work evaluated the performance and remediation mechanisms of immobilised microalgae for continuous wastewater treatment under varying hydraulic retention times (HRT). Three domestic secondary wastewaters with differing concentrations of orthophosphate (PO₄-P), ammonium (NH₄-N) and nitrate (NO₃-N) were treated by Scenedesmus obliquus immobilised within 2% calcium alginate. Trials were run in continuous operation at HRTs of 3, 6, 12 and 20 h. Removal rates for PO₄-P improved with increasing HRT, with minimum residual concentrations of 0.3–3.1 mg·L⁻¹ observed at 3 h and 0.01–0.2 mg·L⁻¹ at 20 h. Ammonium remediation was not linked to HRT or NH₄⁺ concentration with minimum residual concentrations of <0.001 mg·L⁻¹. Reduction in NO₃-N improved with increasing HRT, with minimum residual concentrations of ≤19.3 at 3 h and ≤0.4 mg·L⁻¹ at 20 h. Remediation was achieved through a combination of mechanisms including biological uptake and precipitation as a by-product of photosynthesis and nutrient metabolism. As such, immobilised microalgae have been proven to be an effective alternative solution for PO₄⁻³ and NH₄⁺ remediation of wastewater effluents at HRTs of 6–12 h.

Key words: hydraulic retention time, immobilised microalgae, wastewater

INTRODUCTION

Microalgae are photosynthetic organisms that assimilate mainly inorganic nitrogen (N) and phosphate (P) during their growth when present in wastewater effluent. As such, they have been proposed as an alternative solution for the remediation of wastewater for both secondary and tertiary treatment (Lau et al. 1995; Martínez et al. 2000). For instance, the freshwater species Scenedesmus obliquus has been shown to achieve >98% remediation for total phosphate (TP) and ammonium...
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Both contaminants were reduced to <0.3 mg·L\(^{-1}\) over a 7 day period when treating a domestic secondary wastewater with an influent concentration of 27.4 mg·L\(^{-1}\) \(\text{NH}_4^+\) and 11.8 mg·L\(^{-1}\) \(\text{PO}_4^{3-}\) (Martínez et al. 2000). The application of algal-based technologies for nutrient removal offers a number of advantages over traditional nutrient removal options. For example, when using algal systems, the residual biomass has value as a bioenergy source following appropriate pre-treatment (Ometto et al. 2014b) and hence offers the possibility of meeting low discharge consents in an energy neutral, sustainable manner. Another advantage of algal treatment systems is the perception that they are more natural which helps to promote the public’s receptivity of such processes.

To date, the majority of research conducted on wastewater nutrient removal with microalgae has focused on the use of high rate algal ponds (Christenson & Sims 2011) containing a symbiotic suspended culture of microalgae and bacteria. Typical hydraulic retention times (HRT) from 4 to 10 days are required due to the dilute biomass concentration resulting in large footprints (Picot et al. 1992; Craggs et al. 2012). Furthermore, in suspended systems the algal biomass need to be removed (harvested) prior to discharge, requiring large coagulant demands or energy inputs (Ometto et al. 2014a). Such attributes restrict the attractiveness of suspended microalgal technologies, particularly in locations with limited land availability and/or concerns over energy/chemical use.

The immobilisation of microalgae by confining the living microalgal cells within a gel that is shaped into beads overcomes many of these barriers (Mallick 2002). These gels are hydrophilic in nature, with small pores to enable the diffusion of wastewater to the entrapped microalgal cells (de-Bashan & Bashan 2010). Immobilisation provides the opportunity to seed a bioreactor with a chosen biomass concentration (and species), with concentrations ranging from 0.9 g(DW)·L\(^{-1}\) to a reported hyper-concentration of 3.3 g(DW)·L\(^{-1}\) (Chevalier & De la Noue 1985). Furthermore, immobilisation provides a significant advantage when the biomass is harvested, as the algal beads can be easily settled from the water by gravity without the addition of coagulant chemicals. Immobilisation also assists in the prevention of biomass from washing out of the reactor (Travieso et al. 1992; Mallick & Rai 1994) in addition to protecting against predation and contamination of the microalgal culture (Park et al. 2010).

The onset of the water framework directive (WFD) across Europe will require further reductions in wastewater P discharges to below the current 1–2 mg·L\(^{-1}\) specified within the Urban Wastewater Treatment Directive (UWWTD) (European Commission 1991), to 0.5 mg·L\(^{-1}\) with some sites expected to be as low as 0.1 mg·L\(^{-1}\) (Jarvie et al. 2006). While conventional coagulation strategies support satisfactory remediation of wastewater P, the treatment strategy aligns to current linear economy approaches where potential resources are unrecovered and ultimately disposed. As such, there is a desire to move towards technology options, such as microalgae, that offer wider environmental benefits through resource recovery and/or energy generation aligned to circular economy thinking.

With the majority of wastewater treatment works (WWTW) within the UK categorised as small (treating a population equivalence (PE) of <2,000 (Upton et al. 1995)), certain attributes are desirable in regards to the design and performance of a microalgal bioreactor before considering retrofitting to an existing WWTW. These attributes include: (1) low technology footprint due to potentially limited land availability around existing WWTW; (2) treatment time (<1 day) to coincide with upstream processes enabling constant output and flow as previously attained by the works; and (3) a residual P concentration of <0.5 mg·L\(^{-1}\) to satisfy the forthcoming requirements of the WFD.

To date, immobilised microalgae have demonstrated the ability to fulfil these requirements, with the majority of research undertaken using synthetic wastewater in batch or semi-continuous treatment (Mallick & Rai 1994; Tam & Wong 2000; Jiménez-Pérez et al. 2004) with limited studies on remediation performance within real wastewater effluents (Ruiz-Marín et al. 2010) and continuous operation simulating real-world conditions. One such study by Filippino et al. (2015) demonstrated the performance of immobilised microalgae for a continuous system treating a \(\text{PO}_4^{3-}\) with a maximum concentration of between 0.4 and 1.8 mg·L\(^{-1}\), with a 60–100% remediation at a 12 h HRT and...
10–40% at a 6.5 h HRT under differing lighting conditions and CO₂ addition. The current paper extends previous work to specifically investigate the role of HRT on the remediation performance when using immobilised algae for tertiary treatment of three real secondary wastewaters under continuous operation. Three different source waters have been used to represent the potential range of nutrient characteristics that are commonly encountered for tertiary treatment to provide an understanding of performance and the ability of the immobilised microalgae to satisfy forthcoming P consents in addition to N removal, and the mechanism of remediation.

METHODS

Wastewater

Secondary wastewater effluent was collected from three wastewater treatment sites located in the Midlands and south east of England. Sites A, B and C represent WWTW with a population equivalent (PE) of 200,000, 3,000 and 32,000, respectively, with effluent collected following secondary treatment by trickling filters (site A and B) and an oxidation ditch (site C). Average wastewater characteristics during the experimental period are summarised in Table 1. Effluent from site C was supplemented with NH₄Cl and KH₂PO₄ to increase residual concentrations. Supplementation for Site C was necessary, as the treated effluent is discharged into a catchment designated as a Special Area of Conservation (SAC) under the EC Habitats Directive (92/43/EEC) and a Site of Special Scientific Interest (SSSI). Effluent from this site therefore undergoes enhanced treatment (i.e., increased chemical dosing) to ensure the required residual concentrations are comfortably achieved and, as such, does not represent average residual nutrient concentrations observed following secondary treatment within the UK. Wastewater was used upon collection, with the remainder stored at 4 °C during the period of the trial until use.

<table>
<thead>
<tr>
<th>Parameter (mg·L⁻¹)</th>
<th>Site A (n = 4)</th>
<th>Site B (n = 5)</th>
<th>Site C* (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO₄-P</td>
<td>0.7 (0.2)</td>
<td>4.4 (0.6)</td>
<td>1.1 (0.01)</td>
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<tr>
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<td>2.6 (0.2)</td>
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<tr>
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<td>2.2 (1.0)</td>
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<td>10</td>
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<tr>
<td>pH</td>
<td>7.8</td>
<td>7.3</td>
<td>7.7</td>
</tr>
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</table>

*Average concentrations of 0.3 mg·L⁻¹ PO₄-P and 0.1 mg·L⁻¹ NH₄-N prior to supplementation.

Microalgae cultivation and immobilisation procedure

The freshwater species S. obliquus (276/3A) was obtained from the Culture Collection for Algae and Protozoa (CCAP) (Oban, UK) and cultured in 50 L of Jaworski medium. This medium was chosen as a result of the enhanced algal growth observed during preliminary experiments and following recommendations from the algal supplier. Algae were cultured in a temperature-controlled room at 20 °C under constant mixing by a circulation pump (900 L·h⁻¹) (Koralia Nano 900, Hydor). Cultures were illuminated at a light intensity of 100–150 μmol·m⁻²·s⁻¹ at the culture surface, under constant light to adapt the biomass for continual activity and the accompanying remediation necessary for continuous wastewater treatment. Microalgal biomass was harvested prior to the onset of stationary growth phase to enable maximum biomass recovery. The biomass was stored overnight at 4 °C prior to immobilisation.
Beads were prepared following the method of Tam & Wong (2000) and Ruiz-Marin et al. (2010) for a final 2% sodium alginate (Na-alg) concentration and solidified within 2% CaCl₂. The natural resin, sodium alginate, was selected over artificial resins which would enable greater light transparency, due to the ability to digest the Na-alg material alongside the algal biomass for energy recovery (San Juan et al. 2017), hence contributing to a circular economy approach.

The algal biomass was mixed within the Na-alg gel then passed through a peristaltic pump and dripped into a magnetically stirred CaCl₂ solution from a height of 30 cm. Approximately 4,000 beads per 100 mL gel with an approximate biomass concentration of 10⁵ cells·bead⁻¹ were formed. Beads with an average diameter of 3 mm were left to solidify within the CaCl₂ solution overnight and stored in the dark at 4 °C for a period of 24–48 hours prior to use. Preliminary experiments showed that this had no effect on cell viability once placed in the light again. Beads were rinsed several times with DI water to remove any surplus CaCl₂ with no cell lysis observed during this procedure. The beads themselves played no significant role in nutrient removal as confirmed by a negligible change in N and P from batch adsorption tests when beads with no algae were added to wastewater (data not shown).

The cell·bead⁻¹ concentration was confirmed by removing a sample of ten beads and dissolving in a known volume of 2% sodium citrate. The cell concentration was recorded in triplicate using a haemocytometer and light microscope (Olympus, BH Series) and back-calculated to confirm approximately 10⁵ cells·bead⁻¹.

**Experimental setup for continuous treatment**

Trials were run in continuous operation within an Algenuity Labscale Photobioreactor (Algenuity, Stewardby, UK) (Figure 1) at 20 °C under constant light at a photon irradiance of 200 μmol·m⁻²·s⁻¹ provided to the base of the photobioreactor over a surface area of 133 cm². An intensity of 200 μmol·m⁻²·s⁻¹ was selected following preliminary experiments carried out to observe growth and nutrient remediation performance of immobilised *S. obliquus* under varying light intensities.

Continuous flows were achieved using a peristaltic pump simulating a reactor HRT of 3, 6, 12 and 20 h. Influent was fed to the top of the reactor and extracted from the base of the reactor below the bed of beads. Reactors were mixed via a gimbal system (Figure 1) at 120 rpm with fluidisation of the bead bed limited to the lower third of the vessel.

The reactor conical flasks of 1 L within the photobioreactor (Figure 1) were filled with 600 mL of wastewater effluent with a bead concentration of 10 beads·mL⁻¹, as suggested by Abdel Hameed (2007), with an approximate initial dry weight concentration of 0.5 g·L⁻¹ of solely algal biomass. The beads were retained within the reactor throughout the period of the trials. Trials were terminated when: (1) the residual concentration of the target nutrients returned to that of the feed (while the beads remained intact) due to the high nutrient loading and insufficient biomass concentration for nutrient assimilation, defined as performance breakthrough; or, (2) when a substantial release of microalgal cells were observed within the reactor following bead deterioration.

**Sample analysis and biomass growth**

Samples of the treated effluent (excluding the microalgal beads) were taken twice daily during the start-up period and once a day when performance stabilised. Daily analysis included pH, NH₄-N, NO₃-N and PO₄-P with analysis of the total and dissolved fraction of phosphorus completed. Insoluble phosphorus was determined by subtracting the dissolved fraction from the total concentration. Dissolved PO₄-P was analysed daily following syringe filtration at 0.45 μm (Millipore, DE), whereas total phosphate (TP) was analysed every 2–3 days for unfiltered samples.
Remediation performance was quantified as the difference in the influent and effluent concentration of the continuous flow for the individual reactors, with removal associated with direct uptake and precipitation. The contribution of P removal through the conversion of dissolved P to a precipitate was calculated through Equation (1), with the influent TP and dissolved P concentration found to be equal for each wastewater. Residual concentrations were analysed in duplicate using Spectroquant test kits (Merck Millipore) and read via a Nova 60 spectrophotometer (Spectroquant) and reported as the mean ± standard error when possible. Elemental analysis of precipitation was investigated through a scanning electron microscope attached with an energy dispersive electron probe X-ray analyser (SEM–EDS, FEI XL30, Philips).

\[
\text{Precipitation} \% = \left( \frac{\text{Effluent total } \text{PO}_4^{3-} - \text{Effluent dissolved } \text{PO}_4^{3-}}{\text{Influent } \text{PO}_4^{3-}} \right) \times 100
\]  

Figure 1 | Schematic of the Algem™ Labscale Photobioreactor and auxiliary equipment.

Growth of the immobilised biomass was analysed by dissolving a sample of ten beads from the experimental reactors at time points throughout the experimental period as previously described. The specific growth rate was then calculated using Equation (2), where \( \mu = \) specific growth rate.
\( \mu = \frac{\ln(x_2/x_1)}{t_2 - t_1} \)  

Determination of the dry weight biomass concentration within the reactor \((g(DW) \cdot L^{-1})\) was based on previous growth experiments under the same growth conditions (e.g., light and temperature) to correlate dry weight of \(S.\ obliquus\) to cell number (data not shown). Suspended biomass released by the beads into the reactor was found to contribute to approximately 0.40 mg L\(^{-1}\) (equivalent to 0.07\% of the total biomass concentration) from preliminary 24 h batch trials. The contribution of the suspended biomass was therefore considered negligible within performance calculations through the selection of HRTs ≤20 h for the continuous trials. Contribution of bacteria to removal of contaminants was also found to be negligible following controlled experiments containing no algal biomass (data not shown).

RESULTS AND DISCUSSION

Phosphate remediation

A consistent treatment profile was observed across each of the wastewater samples analysed from all sites and HRTs with an initial period where the effluent PO\(_4\)-P decreased before reaching a plateau. For example, site A samples demonstrated more than 80\% removal at HRTs of 3, 6, 12 and 20 h over a period of approximately 2, 1, 3 and 5 days (Figure 2). Treatment efficiencies are similar to those observed by Filippino et al. (2015) for continuous treatment by immobilised \(C.\ vulgaris\) treating an average influent concentration of 0.2–0.5 mg L\(^{-1}\) PO\(_4\)-P (similar to the characteristics of Site A) at a contact time of 6.5 h, however this study demonstrates a similar level of performance at a further reduced reactor HRT of ≤6 h.

Figure 2 | Phosphate remediation for site A for (a) 3 h, (b) 6 h, (c) 12 h and (d) 20 h HRT. PO\(_4\)-P (●), TP (♦) and pH (○).
The residual concentration of PO$_4$-P averaged 0.10, 0.001, 0.12 and 0.03 mg·L$^{-1}$ until day 8, 19, 19 and 19 when a breakthrough in concentration (HRTs 3 and 6 h) or deterioration of the beads (HRTs 12 and 20 h) was observed. Breakthrough in concentration is believed to have occurred at the lower HRTs due to a higher nutrient loading of 3.4 and 1.7 mg P·d$^{-1}$ for the 3 and 6 h HRT, respectively, in comparison to a lower loading of approximately 0.8 and 0.5 mg P·d$^{-1}$ for the 12 and 20 h HRT. While biomass growth remained similar for all HRTs, the biomass concentration is believed to be insufficient for enhanced nutrient assimilation.

Comparison of samples across the sites revealed that following the initial start-up period, but prior to breakthrough, the minimum PO$_4$-P residual concentration for site A and C samples achieved the required <0.5 mg·L$^{-1}$ for all HRTs (Table 2). The similar levels of performance observed between site A and C samples are due to the similar PO$_4$-P influent concentration. The samples from site B were not reduced to such low levels, achieving <0.5 mg·L$^{-1}$ at ≥12 h HRT. The reduced performance was attributed to the higher PO$_4$-P influent concentration which was up to six and four times greater than site A and site C samples, respectively, suggesting load limiting conditions had been reached (Whitton et al. 2016). Mass balances estimate PO$_4$-P uptake by the algal biomass equivalent to ≤0.01 mgP·mg biomass$^{-1}$ similar to that observed for S. obliquus within non-growth limiting environments (Whitton et al. 2016).

The site effluents were characterised by a molar N:P ratio of 78, 15 and 10 for site A, B and C, respectively. These ratios differ to the recommended N:P of 16 for marine microalgae (Redfield 1954), but are more suited to the range of N:P ratios deemed suitable for freshwater species of 8–45 (Hecky et al. 1993). The growth rate of the immobilised biomass (for trials terminated through performance breakthrough) ranged between 0.22 and 0.42 d$^{-1}$ with an increase in cell concentration from $10^5$ to approximately $10^6$ cells·bead$^{-1}$ suggesting phosphorus was not limiting for sites A, B and C. These findings are supported by previous work demonstrating effective N and P removal by immobilised microalgae within growth mediums characterised by N:P ratios between 4 and 230 (Filippino et al. 2015).

A change in the removal pathway was observed during the operating cycle for all HRTs and site samples. To illustrate for the site A sample, the TP residual concentration initially followed the

| Table 2 | PO$_4$-P minimum residual concentration following initial start-up period and prior to breakthrough, peak pH and maximum removal contribution through precipitation (± Std error) |
|---------|-------------------------------------------------|-------------------------------------------------|---------|-----------------|-----------------|
| HRT (h) | Influent PO$_4$-P (mg·L$^{-1}$) | Min. effluent PO$_4$-P (mg·L$^{-1}$) | Peak pH | Max. precipitation contribution (%) |
| Site A  | 3 | 1.11 (0.02) | 0.09 (0.01) | 8.2 | 43.7 |
|         | 6 | 0.48 (0.00) | <0.01* | 9.1 | 50.7 |
|         | 12 | 0.85 (0.02) | 0.04 (0.04) | 9.0 | 80.5 |
|         | 20 | 0.54 (0.00) | <0.01* | 8.3 | 80.0 |
| Site B  | 3 | 5.39 (0.04) | 3.10 (0.03) | 7.3 | nd |
|         | 6 | 4.08 (0.00) | 0.88 (0.01) | 8.2 | 33.3 |
|         | 12 | 3.97 (0.01) | 0.49 (0.01) | 10.6 | 81.7 |
|         | 20 | 4.50 (0.00) | 0.20 (0.02) | 11.0 | 49.1 |
| Site C  | 3 | 1.07 (0.01) | 0.30 (0.01) | 8.6 | nd |
|         | 6 | 1.07 (0.04) | <0.01* | 9.7 | 80.3 |
|         | 12 | 1.07 (0.01) | <0.01* | 10.4 | 88.5 |
|         | 20 | 1.08 (0.01) | <0.01* | 11.1 | 44.0 |

nd = not determined, * = below limit of detection.
remediation profile for PO4-P (Figure 2) suggesting PO4-P to be the predominant form of phosphorus within the treated effluent. Microalgal remediation of PO4 is predominantly through a biological uptake pathway (Larsdotter 2006) and, as such, remediation during the initial stages of the cycles contributed to microalgal assimilation. However, as the operating cycle progressed, the residual TP concentration began to deviate with an increase in concentration observed on days 4, 7, 7 and 10 for 3, 6, 12 and 20 hours HRT runs, respectively (for site A sample). The concentration remained higher than the PO4-P residual and eventually returned to that of the feed when breakthrough (Figure 2(a) and 2(b)) was observed or the trial was stopped due to bead deterioration (Figure 2(c) and 2(d)). In addition, the effluent pH increased from an average of 7.7 to 8.2, 9.1, 9.0 and 8.3 for HRT of 3, 6, 12 and 20 h, respectively (Figure 2 and Table 2).

The change observed in TP:PO4-P during the cycle is congruent with a switch in the removal pathway from microalgal uptake in the initial stages to chemical precipitation during the remainder of the cycle. The precipitation pathway is driven by the increase in pH as a consequence of photosynthesis and its commensurate consumption of the inorganic carbon source, i.e., the bicarbonate ion (HCO3). This produces hydroxyl ions (OH−), creating a localised increase in pH (Nurdogan & Oswald 1995; Larsdotter et al. 2007; Kim et al. 2010) causing the onset of phosphate precipitation with calcium (Ca) and magnesium (Mg) cations found within the wastewater (Larsdotter et al. 2007). Precipitation then proceeds, even when the pH reduces to neutral, and continues to contribute to remediation (House 1999). Preliminary investigatory analysis of the precipitate revealed it to be predominately amorphous calcium phosphate.

An initial increase in the effluent pH subsequently followed by an increase in residual TP concentration was demonstrated in all wastewaters at all HRTs, with a maximum pH of 11.1 after 2 days observed for the 20 h HRT trial for the site C sample (Table 2). Across all the trials, precipitation accounted for a maximum of 33.3–88.5% for phosphorus removal (Table 2) equivalent to 0.001 to 0.06 mgP·mg biomass−1. Greater contribution through precipitation generally occurred at the longer HRTs; however, the percentage contribution by precipitation at 20 h HRT reduced to between 44 and 49% even though the pH peaked at 11 for samples from site B and C. Such observations of a reduction in pH following a peak, and a subsequent decrease in TP, are congruent with a switch in the relative competition for Ca between carbonate and phosphate. While the formation of calcium phosphate remains thermodynamically favourable, the relative precipitation kinetics significantly favours calcium carbonate formation reducing the amount of phosphate precipitate (Montastruc et al. 2003).

Equivalent findings have been reported in suspended systems with, for instance, precipitation accounting for 16–63% P removal for a suspended culture of Monoraphidium species at a HRT of 5 days growing within sterile filtered domestic wastewater (Larsdotter et al. 2007). This extends previous discussions concerning the significant role of indirect remediation (Mesplé et al. 1996; Garcia et al. 2000) to include systems based on immobilised microalgae. The increased contribution in the immobilised systems compared to suspended systems is attributed to the greater algal biomass concentrations involved; with suspended systems up to an order of magnitude less concentrated than immobilised systems.

**Ammonium remediation**

Remediation of NH4-N exhibited a similar pattern of remediation to PO4-P with a period of initial decrease to a plateau which was consistent for all site samples and HRTs. To illustrate, the site A sample achieved greater than 70% removal following an initial start-up period of 1, 3 and 5 days for 6, 12 and 20 h reactor HRT, respectively, with corresponding averaged residual concentrations of 0.06, 0.25 and 0.35 mg·L−1 (Figure 3). This pattern of performance was not seen for the 3 h reactor HRT trial, presumably due to the high NH4-N loading of approximately 20.2 mg·d−1 in comparison to <10.1 mg·d−1 for HRTs of 6–20 h. As such, the 3 h HRT resulted in only a 14.3% NH4-N reduction.
after a period of 4 days followed by an increase in the residual concentration which returned to that of the feed by day 7 (Figure 3). The enhanced uptake and improved residual concentrations for NH₄-N in comparison to PO₄-P during these trials are consistent with the greater nitrogen content of algal biomass, at around ten times that of phosphorus. The increased nitrogen composition leads to an enhanced assimilation of nitrogen in comparison to phosphorus (Nurdogan & Oswald 1995). This was further demonstrated through increased biomass productivity ranging between 0.04 g·L⁻¹·d⁻¹ and 0.2 g·L⁻¹·d⁻¹. The higher rates of productivity were observed for the shorter HRTs (i.e., 3 hours) for sites with a higher NH₄-N concentration (i.e., sites A and C) in comparison to a longer HRT (i.e., 20 hours) for sites characterised by a lower NH₄-N concentration (i.e., site B).

Unlike PO₄-P, the minimum residual effluent NH₄-N concentration, following the start-up period and prior to breakthrough, did not show improvement with increasing HRT (Table 3). A residual concentration of ≤0.001 mg·L⁻¹ NH₄-N was observed for the majority of wastewaters despite varying influent NH₄-N concentration (excluding site A sample and 3 h HRT). Treatment was comparable to >90% TN remediation efficiency observed by Filippino et al. (2015) for immobilised C. vulgaris at HRTs of 6.5 and 20 h, and consistent with the system remaining non-limiting up to loading rates of at least 20.8 g·m⁻³·d⁻¹.

**Nitrate remediation**

The remediation of nitrate (NO₃-N) was observed in parallel to the uptake of NH₄-N offering the potential of the immobilised process to provide TN remediation (Figure 3). Average residual NO₃-N concentrations for the complete cycle for the site A sample of 14.1, 9.8, 2.4 and 3.0 mg·L⁻¹ was observed for 3, 6, 12 and 20 h HRT, respectively. The residual concentration was maintained throughout the trial and showed a deterioration in remediation performance following the addition of fresh feed (>20 mg·L⁻¹ NO₃-N) (Figure 3(b)). The increased TN load contributed to bead deterioration after 19 days of treatment.
The impact of the relative removal of both nitrogen species was observed in relation to the evolution of pH (Figure 4). The sample from site A, with an influent concentration of 5.6 mg·L\(^{-1}\) NH\(_4\)-N and 18.8 mg·L\(^{-1}\) NO\(_3\)-N peaked at a maximum pH of 9.1 before reducing to an average of 7.9 for the remainder of the run for the 6 h HRT (Figure 4(a)). Whereas the sample from site B, with a lower NH\(_4\)-N influent concentration of 0.5 mg·L\(^{-1}\) and a higher NO\(_3\)-N concentration of 27.5 mg·L\(^{-1}\), peaked at a maximum pH of 10.6 before averaging 9.2 for the remainder of the trial for the 12 h HRT (Figure 4(b)). The pH of the effluent mirrored that of the NO\(_3\)-N residual concentration for site B (Figure 4(b)), with a reduction from a pH of 10.0 to 7.2 after 19 days following an increase in NO\(_3\)-N residual from 2.4 to 20.1 mg·L\(^{-1}\). The ratio of NH\(_4\)-N to NO\(_3\)-N influences pH as assimilation of NO\(_3\) results in a net H\(^+\) uptake by co-transportation through the ATPase extrusion pump within microalgal cells whereas assimilation of NH\(_4\) creates a net release of H\(^+\) (Ullrich 1983). Furthermore, while not directly measured, the remediation opportunity related to the ammonia speciation pKa estimates volatilisation of free ammonia contributing between 1 and 98% at the peak pH values of 7.3–11.1 (Table 2). The presumed elimination of NH\(_3\) through volatilisation at the higher pH values would further facilitate alkalisation due to the microalgae assimilating NO\(_3\) in the absence of

<table>
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<tr>
<th>HRT (h)</th>
<th>Influent NH(_4)-N (mg·L(^{-1}))</th>
<th>Min. effluent NH(_4)-N (mg·L(^{-1}))</th>
<th>Influent NO(_3)-N (mg·L(^{-1}))</th>
<th>Min. effluent NO(_3)-N (mg·L(^{-1}))</th>
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<td></td>
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<td>3</td>
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<td>3</td>
<td>0.20 (0.02)</td>
<td>&lt;0.001*</td>
<td>40.90 (0.70)</td>
<td>19.3 (0.10)</td>
</tr>
<tr>
<td>6</td>
<td>0.51 (0.00)</td>
<td>&lt;0.001*</td>
<td>21.00 (0.00)</td>
<td>1.00 (0.00)</td>
</tr>
<tr>
<td>12</td>
<td>0.52 (0.02)</td>
<td>&lt;0.001*</td>
<td>27.50 (0.30)</td>
<td>1.55 (0.05)</td>
</tr>
<tr>
<td>20</td>
<td>0.33 (0.01)</td>
<td>&lt;0.001*</td>
<td>33.65 (0.35)</td>
<td>0.40 (0.10)</td>
</tr>
<tr>
<td>Site C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.61 (0.09)</td>
<td>&lt;0.001*</td>
<td>1.90 (0.10)</td>
<td>nd</td>
</tr>
<tr>
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<td>&lt;0.001*</td>
<td>1.35 (0.05)</td>
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<tr>
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<td>2.75 (0.00)</td>
<td>&lt;0.001*</td>
<td>nd</td>
<td>nd</td>
</tr>
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<td>&lt;0.001*</td>
<td>3.30 (0.10)</td>
<td>0.4 (0.00)</td>
</tr>
</tbody>
</table>

nd = not determined, * = below limit of detection.
NH₄⁺ through co-transportation with H⁺, resulting in a further increase in pH of the localised medium (Whitton et al. 2015).

Bead remediation characteristics

The nutrient removal rate ranged from 0.03 to 74.6 mgP·h⁻¹·10⁶ beads⁻¹ (equivalent to 0.0003 to 0.75 μgP·h⁻¹·10⁶ cells⁻¹) (Figure 5(a)) and 0.03 to 142.7 mgNH₄-N·h⁻¹·10⁶ beads⁻¹ (equivalent to 0.0003 to 1.43 μgNH₄-N·h⁻¹·10⁶ cells⁻¹) (Figure 5(b)) with an average of 0.16 μgP·h⁻¹·10⁶ cells⁻¹ and 0.43 μgNH₄-N·h⁻¹·10⁶ cells⁻¹ (when considering only those trials which were halted through performance breakthrough). Such data demonstrate enhanced cell uptake rates when compared to previous studies using microalgae in the treatment of urban wastewater. For example, for a feed concentration of 32.5 mg·L⁻¹ NH₄-N and 2.5 mg·L⁻¹ PO₄-P operating in 50 h batch trials, uptake rates for suspended S. obliquus of 0.05 μgP·h⁻¹·10⁶ cells⁻¹ and 0.18 μgNH₄-N·h⁻¹·10⁶ cells⁻¹ were observed (Ruiz-Marín et al. 2010).

The bead PO₄-P uptake rate increases until a loading of approximately 20 g·m⁻³·d⁻¹, after which the rate levels out (Figure 5(a)) with results suggesting a maximum uptake rate of approximately 75 mgP·h⁻¹·10⁶ beads⁻¹. A similar relationship was observed for NH₄-N (Figure 5(b)); however, a rapid decrease was observed at 33.6 g·m⁻³·d⁻¹ concurrent with performance breakthrough within 8 days for the site A sample at 3 h HRT. Accordingly, the data suggest a maximum effective loading rate between 20.8 g·m⁻³·d⁻¹ and 33.6 g·m⁻³·d⁻¹, corresponding to a maximum uptake rate of approximately 116 mgNH₄-N·h⁻¹·10⁶ beads⁻¹.

The effective cycle time was defined by either performance breakthrough or loss of bead integrity through deterioration. In cases of lower nutrient loading, the cycle time was defined by performance breakthrough. This occurred at all HRTs for site C samples and at HRTs of 3 and 6 h for site A samples and 12 and 20 h for site B samples with a reduction in cycle run time from 24 days to 3 days as the beads treated 0.1 to 0.8 μgP·bead⁻¹·d⁻¹ (Figure 6(a)). In such cases, an increase in residual effluent concentration was observed over a period of 2–5 days, providing a signal that the operating cycle had ended, representing a convenient method for cycle time control. No such relationship between specific growth rate or the final bead biomass concentration and the cycle run time was evident.

Site A samples at 12 and 20 h HRT and site B samples at 3 and 6 h HRT demonstrated bead deterioration and continued to remediate the influent wastewater due to the release of the suspended cells into the reactor. Consistent with expectations, bead integrity was breached due to the increase in cell numbers to approximately 10⁶ cells·bead⁻¹ and reduction in bead stability through chemical and biological interactions with components within the effluent (Cruz et al. 2013). The beads which did not deteriorate retained form and function for a maximum period of 24 days at a 20 h
HRT (Figure 6(b)). This is a significantly longer survival period than experienced by others. For instance, substantial deterioration after 96 hours was observed by Cruz et al. (2013).

Implementation of the technology would require that the cycle is terminated and the beads harvested prior to bead deterioration. The harvested beads can then be either applied to land as a fertiliser or converted into methane through anaerobic digestion (San Juan et al. 2017). Additional consideration needs to be given to the reactor design to prevent washout of the calcium phosphate precipitate, as the precipitated phosphate will continue to contribute to the total phosphate residual and further presents a useful resource for recovery.

CONCLUSIONS

- Microalgal immobilisation process enables effective tertiary nutrient removal at contact times significantly lower than in alternative algal reactor systems. Effective removal to <0.5 mg·L\(^{-1}\) phosphate, aligned to the WFD, was observed at reactor HRTs of ≤12 h, in addition to residual ammonia concentrations of <0.001 mg·L\(^{-1}\).
- Indirect removal through a pH-induced precipitation process is a significant phosphate remediation pathway.
- The maximum rate of bead performance can be defined through the phosphate loading rate with effective treatment up to a maximum loading rate of 0.8 μgP·bead\(^{-1}\)·d\(^{-1}\).
- The effective cycle time of operation is linked to the total phosphate treated with cycle times up to 24 days when operating under low loadings.

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