Algal Research, Volume 65, June 2022, Article number 102754
DOI:10.1016/j.algal.2022.102754

High rate algal systems for treating wastewater: a comparison

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Abstract

Algal systems can remove nitrogen (N) and phosphorus (P) from wastewater while producing valuable biomass. The microalga *Chlorella vulgaris* in three concentrated forms (suspended, entrapped in Ca-alginate gel beads and as a biofilm on supports and the macroalga *Oedogonium cardiacum* were compared for treating secondary effluent containing 15 mg/L of ammonium ($N\text{-}NH_4^+$), 6 mg/L of nitrate ($N\text{-}NO_3^-$), and 7 mg/L of total phosphorus (TP) with a hydraulic retention time of 12 h. Identical conditions and reaction vessels enabled a direct comparison of growth systems. The biofilm system was the most effective of the microalgal systems, decreasing concentrations to 1.9 mg/L TP and 0.5 mg/L $N\text{-}NO_3^-$ on average from day 3 to 24, and like the other microalgal systems, was not as effective for $N\text{-}NH_4^+$ removal (average of 9.0 mg/L). The macroalgal system decreased TP to 1.3 mg/L and $N\text{-}NH_4^+$ to ≤0.5 mg/L on average from day 16 to 30 and operated for longer than the other systems, but was not effective for $N\text{-}NO_3^-$ removal (average of 4.8 mg/L). Hence the minimum TN concentration of the effluent from the macroalgal system (7.1 mg/L) was lower than for the biofilm system (10.6 mg/L) from the feed of 24 mg/L. The biofilm system produced 56 mg/L/d and the macroalgae 102 mg/L/d of biomass. The production of the highest quality effluent for longer and of more biomass than the microalgal systems, combined with their larger cell size which facilitates reactor operation, demonstrates that macroalgae can compete with microalgae for wastewater remediation.

Keywords: Microalgae; Macroalgae; Biofilm; Immobilisation; Wastewater treatment; Biomass production

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1 Introduction

The use of algae to treat wastewater leads to low residual concentrations of nitrogen (N) and phosphorus (P) in the discharged effluent [1-3]. The nutrient-rich algal biomass grown in the wastewater can be harvested for resource recovery through the production of methane when the biomass is used as a feedstock for anaerobic digestion [4], fertiliser before or after digestion [5], feedstock materials for biopolymer production [6] or animal feed [7]. Thus the use of algae enables recovery of nutrients in wastewater which would otherwise be lost to the environment [8]. In addition, the use of algae offsets the use of chemicals and significant energy inputs that would otherwise be required to achieve similar low concentrations of nutrients in wastewater [9,10]. For example, removal of ammonium (NH_4^+) from wastewater with an algal system rather than an activated sludge process reduces aeration (the largest consumer of energy in a typical treatment plant [10]) and production of N_2O (a green-house gas by-product), thereby greatly reducing the overall environmental cost traditionally associated with ammonia removal.

The most commonly implemented algal system for treating wastewater is a microalgal suspension in an open pond due to its simplicity and low operating cost [11,12]. These systems have low concentrations of algae with slow algal growth rates and thus long treatment times (>4 days) [13,14]. Harvesting the dilute algae ($\leq$ 1 g/L) to prevent their contamination of the effluent and to recover the value-adding biomass can require the use of chemicals and substantial energy [15]. As nutrient removal is a consequence of biomass uptake and growth, it is strongly influenced by the algal biomass concentration maintained within the reactor [16]. Accordingly, intensification of the process can be accomplished through alternative designs that enable higher biomass levels. Of particular interest in this paper, entrapment of microalgae in calcium alginate beads enables biomass concentrations of 3.3 g/L and has been demonstrated to reduce required hydraulic residence times to several hours, while the beads rapidly settle simplifying the harvesting of the biomass [17,18]. Cultivation of algae attached to a surface as

a biofilm can also increase biomass concentrations, and thus facilitate simpler harvesting and potentially faster nutrient removal [19,20]. There is a deficiency of studies on the deliberate use of algal attachment on typical supports found in current conventional wastewater treatment, and the combination of such systems with artificial light. Macroalgal systems have been proposed to overcome the challenges of harvesting microalgae due to the greater macroalgal cell size, and have been tested in cultivation systems similar to open microalgal ponds [21,22]. However, there have been limited studies on wastewater treatment with macroalgae in a reactor designed to intensify nutrient removal rates and thus reduce treatment times by use of artificial light and retention of biomass.

Four systems of concentrated algae were of interest in this study: 1) suspended microalgae retained by a membrane [23-25], 2) passive immobilisation of microalgae by attachment to a surface as a biofilm [20,26,27], 3) active immobilisation by entrapment in Ca-alginate beads [18,28,29], and 4) suspended macroalgae [30] retained with a filter of larger pore size than needed for microalgae due to their larger cell size (14 by 30 μm) and filamentous nature [31,32]. Other systems such as granulation of microalgae could also result in easier biomass harvesting and greater nutrient removal rates [33], however were not included in this study. The different systems have different exposure patterns to the available light, offer different contact areas and mixing arrangements that affect mass transfer, nutrient uptake rates, energy value of the biomass and difficulty of harvesting. To date no direct comparison of these systems has been reported, restricting the understanding of the relative opportunity each system offers for wastewater treatment and which system may be the most effective for any given application.

This paper responds to this knowledge gap by presenting the first direct comparison of suspended, biofilm and entrapped microalgae, and suspended macroalgae, for wastewater remediation. This comparison is conducted under identical conditions for each growth system to demonstrate the impact of system selection on the transfer and utilisation of nutrients in

algae. This enables evaluation of the extent the fundamental behaviour of each system actually impacts the end goal of comparing their wastewater treatment performance. This provides evidence to help identify which system is the most favourable option for wastewater treatment and will help shape the focus of future research on algal technology and its application for wastewater treatment.

2 Materials and Methods

2.1 Algal cultivation

The microalga *Chlorella vulgaris* (211/11B) was obtained from the Culture Collection of Algae and Protozoa (CCAP, UK) and was cultivated in Jaworski medium under 100-150 µmol/m^2/s continuous light and aeration. For inoculation of experimental runs the algae were harvested by centrifugation (3000 x g) after 10 days of cultivation to be in exponential stage of growth, the algal pellet was collected and resuspended in deionised (DI) water. The macroalga *Oedogonium cardiacum* (511/1A) was also obtained from CCAP and cultivated in Jaworski medium with no mixing or aeration, under 50-100 µmol/m^2/s light with a 16:8 light:dark cycle. *C. vulgaris* was selected as it can treat wastewater as a monoculture in each microalgal growth system [25,34,35] and *O. cardiacum* due to its proven potential for municipal wastewater treatment [36]. The macroalgae were harvested by vacuum filtration (1.2 µm) and from the biomass cake formed the desired weight of inoculum was collected.

2.2 Wastewater treatment runs

2.2.1 Wastewater characteristics

The feed to the algal systems was sourced from the secondary treatment outlet of a municipal wastewater treatment plant. The wastewater used to feed the continuous system (Table 1) was supplemented with NH_4Cl, $NaNO_3$ and K_2HPO_4 to maintain initial concentrations of 15 mg/L of ammonium ($N\text{-}NH_4^+$), 6 mg/L of nitrate ($N\text{-}NO_3^-$) and 5 mg/L of phosphate ($P\text{-}PO_4^{3-}$). These

nutrients were added to explore the algal systems as alternatives to conventional wastewater treatment processes for N and P removal that emit more greenhouse gasses and that are more energy and chemical intensive.

Table 1 Secondary effluent quality after nutrient supplementation, concentration range provided throughout experimental runs.

Parameter	TDN	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	TP	$P\text{-}PO_4^{3-}$	pH	DIC	DOC	Turbidity
Unit	mg/L	mg/L	mg/L	mg/L	mg/L		mg/L	mg/L	NTU
Concentration	21.6-25.9	14.2-16.6	5.7-6.1	6.55-6.99	4.49-4.99	7.3-7.9	20-24	3-8	0.5-0.9

2.2.2 Algal Reactors

Duplicate runs for each algal system were completed in Algem^{TM} Labscale Photobioreactors (Algenuity, Stewartby, UK) which house 1 L Erlenmyer flasks with mixing provided by a gimbal system. The temperature was maintained at 20 °C and light was continuously provided to the base of the reactor at 180 µmol/m^2/s. The reactors contained 350 mL of wastewater which was continuously supplied to achieve a hydraulic retention time (HRT) of 12 h. This HRT was chosen as when using immobilised algae to treat secondary effluent a HRT of ≤ 12 h could achieve low N and P concentrations in the effluent [18]. Effluent samples were taken from the outlets of the reactors every 1 to 2 days. The runs were stopped at breakthrough as indicated by a loss in NO_3^-, NH_4^+ or PO_4^{3-} treatment performance.

For each system the biomass was physically retained in the reactor without recirculation back to the reactor from the effluent. The details of how the biomass were retained in the reactor are explained in the following sections as they were dependent on the growth system.

2.2.3 Suspended microalgae

The reactor was seeded with 1.5 x 10^6 cells/mL (11.7 mg/L dry weight) of *C. vulgaris*, which were retained in the reactor by a 1 µm cloth filter attached to the outlet tubing. The reactor was mixed at 120 rpm to ensure suspension of the algal cells. Culture samples (0.5 mL) were collected periodically to determine cell number, and on completion of the run the entire contents of the reactor were harvested by centrifugation.

2.2.4 Microalgal biofilm

An equal volume of biofilm supports (Biotube+, 12 x 8 mm, 1000 m^2/m^3, Warden Biomedia) and wastewater (350 mL) was used giving 205 supports and a total surface area of 0.35 m^2 available for algal growth.

Algal attachment for biofilm formation was completed by submerging the supports in 350 mL of Jaworski medium with a concentrated suspension of *C. vulgaris* for 1 week in the Algem™ Labscale Photobioreactors. After an initial dark period of 16 hours [37], illumination at 180 µmol/m^2/s with 16:8 hours light:dark cycle was used and gentle mixing (60 rpm) applied. After attachment the Jaworski medium was replaced with wastewater at 40 mL/min for 60 minutes (approximately 6 volume replacements) to remove the remaining suspended cells. The initial inoculum after attachment was 1.2 x 10^6 cells/mL (9.3 mg/L dry weight).

After attachment the flow rate to the reactor was reduced to achieve the HRT of 12 h. The mixing rate was kept at 60 rpm which facilitated transfer of nutrients to the cells without causing shear damage to the biofilm.

To determine the algal concentration two supports were removed from the reactor every second day. This rate of sampling was chosen to minimise net loss of reactive biomass over the treatment run. The supports were placed in DI water and agitated by a vortex mixer to remove the attached cells. After determining the number of algal cells on each support counting with a

haemocytometer, the algal concentration in the reactor was calculated based on the total number of supports in the reactor. At the end of the trial all remaining supports were collected and the algae harvested by vortex mixing for biomass dry weight and cell number analysis. The remaining effluent in the reactor was also collected for analysis of the algal biomass.

2.2.5 Entrapped microalgae

Immobilisation was conducted by dripping 2% Na-alginate (Sigma-Aldrich 71238) containing a known concentration of *C. vulgaris* into a gently mixed 2% $CaCl_2$ solution to form Ca-alginate beads approximately 3 mm in diameter. The beads were allowed to harden in the $CaCl_2$ solution in darkness overnight before rinsing by stirring in two batches of DI water. An equal volume of Na-alginate solution and wastewater was used (350 mL) to maximise the volume available for algal growth. This corresponded to approximately 35 beads/mL of wastewater. The reactor was mixed at 120 rpm to ensure suspension of the beads in the wastewater. The initial algal concentration was 1.2×10^6 cells/mL of wastewater (9.4 mg/L dry weight), which corresponded to 3.4×10^4 cells/bead.

Algae were sampled by collecting 15 beads from the reactor every 1 to 2 days and dissolving them in 2% Na-citrate. Cell concentration per bead was found by counting cell number with a haemocytometer and the cell concentration in the reactor calculated based on the total number of beads in the reactor. At the end of the trial all beads remaining in the reactor were collected, dissolved in 2% Na-citrate, and then centrifuged to collect the algal biomass.

2.2.6 Suspended macroalgae

To retain the macroalga *O. cardiacum* in the reactor an 85 µm nylon mesh was attached to the outlet tubing of the reactor. To enable appropriate comparison with the microalgal systems the initial concentration of *O. cardiacum* was proportionally adjusted based on preliminary determination that the growth rate was 16 times lower than *C. vulgaris* in Jaworski medium.

The reactor was mixed at 120 rpm. The initial concentration of macroalgae was 150 mg/L dry weight.

A biomass wet weight (determined following removal of excess water by 1.2 μm vacuum filtration) to dry weight relationship (Eq. 1) was developed to facilitate measurement of the biomass for inoculation.

$$Dry\ weight = (0.13 \pm 0.05) \times wet\ weight + (0.03 \pm 0.03), R^2 = 0.8, n = 4 \quad \text{(Eq. 1)}$$

Samples of the biomass were not taken during the macroalgal run due to the destructive nature of sampling. The biomass in the reactor at the end of the trial was collected by vacuum filtration (1.2 μm).

2.3 Analytical methods

Effluent samples were analysed for pH, cell number, and total phosphorus (TP, which includes particulate and soluble P) with a Merck Spectroquant® cell test kit (1.14543). As there was a 1 μm filter on the outlet of the suspended microalgal system measurement of TP was not comparable to the other systems and so was not included in the analysis. Effluent samples were filtered (0.45 μm) for analysis of PO_4^{3-} and NO_3^- using a Thermo Scientific Dionex 1600 Ion Chromatography System; DIC, DOC and total dissolved nitrogen (TDN) using a Shimadzu TOC-V Analyzer with a TN unit (TNM-1); and total dissolved phosphorus (TDP) and NH_4^+ with Merck Spectroquant® cell test kits 1.14543 and 1.14558, respectively.

For analysis of the algae, whether collected from the reactor or the effluent, cell number was determined by counting with a haemocytometer with an optical microscope. For determining algal biomass productivity at the end of the treatment runs, collected algal biomass was centrifuged and the algal pellet freeze dried (ModulyoD Freeze Dryer) before measurement of dry weight.

2.4 Calculations and statistical analysis

The average of the duplicate runs are reported with the standard error (n = 2) presented with the results unless otherwise specified. Biomass productivity was calculated from the biomass increase over the duration of each run, from inoculation until breakthrough. Nutrient removal over the duration of each run was calculated as a percentage of biomass increase. This calculation included biomass lost in the effluent for each system and, for the biofilm, biomass that had sloughed off the supports and remained in the reactor. To calculate the dry weight of biomass in the reactors at a certain time the dry weight per cell at the end of the run was multiplied by the cell number throughout the run. The rate of nutrient transfer into the algal biomass could then be determined as the rate of nutrient removal normalised by the mass of algae in the reactor at that specific time.

3 Results and Discussion

3.1 Algal growth and biomass productivity

The macroalgal system (102±4 mg/L/d) and the entrapped microalgal system (106±2 mg/L/d) had similar biomass growth rates. This is reflective of the differences in the size of the initial seed population, confirming utilising a larger inoculum to compensate for the slower growth rate of *O. cardiacum* compared with *C. vulgaris* was appropriate. The growth rates for these systems were higher than the biofilm (56±3 mg/L/d) and suspended (36±5 mg/L/d) systems (Table 2). The biomass increase for the biofilm system included biomass that sloughed off the support carriers and remained settled in the reactor, which accounted for 32% (1.2±0.3 x 10^7 cells/mL) of total biomass. Considering just the biomass harvested from the carriers the growth rate was similar to that observed for the suspended system.

Each system was operated until nutrient removal stopped, which corresponds with the algal biomass accumulating to a maximum capacity in the reactor, and a greater proportion of cells breaking down or not being photosynthetically active. The duration of the treatment cycles of 6, 17, 27 and 30 days for the entrapped, suspended, biofilm and macroalgal systems, respectively, did not correlate with biomass growth rate. At the end of each treatment cycle cost is incurred by harvesting the biomass and replenishing the culture to begin a new treatment cycle, whereas value is returned from the recovery of the algal biomass. Hence, a system that has both a high rate of biomass production and long treatment duration is desirable. The rapid cell growth in the entrapped system was demonstrated by reaching a final cell concentration of $3.0\pm0.1 \times 10^7$ cells/mL by day 6, thus tending to favour cell production over the duration of the treatment run. This compared with a steady lower growth rate for the biofilm system which achieved $2.6\pm0.2 \times 10^7$ cells/mL attached to the supports by day 22, thus tending to favour longer cycle duration over cell production (Figure 1A). The macroalgal system achieved the dual benefit of less frequent culture replenishment (cost saving) and greater biomass recovery at the end of each cycle (increase in value return).

Despite growing well on the supports and accumulating in the reactor there was little control of cell loss from the biofilm which led to cell growth in suspension in the reactor and algal cells exiting in the effluent at 2.6 times the rate of biomass increase retained in the reactor (Figure 1B). This shows that the biofilm system acted as seed for additional algal growth and thus nutrient removal in the reactor, but did not prevent growth in suspension. Thus the biofilm *C. vulgaris* system would only be suitable for incorporation into wastewater treatment plants where downstream contamination of the effluent by algae was not an issue or, alternatively, in such cases an additional unit operation to harvest algal cells or recirculate them from the effluent would be required [38].

The loss of cells to the effluent represents a loss of treatment capacity due to a reduction in algal accumulation in the vessel and a loss in value through resource recovery at the end of the cycle. In contrast to the biofilm system, entrapment facilitated retention of nearly all microalgae (loss of 0.03 times the biomass retained in the reactor, before breakthrough), demonstrating it was an effective tool for preventing loss of value-adding biomass and reducing contamination of the effluent by algal cells (Figure 1B). The macroalgal system had no loss of biomass indicating the 85 μm filter was effective. There were also few, if any, microalgal cells in the effluent of the macroalgal system (Figure 1B), demonstrating that a dominant mass of macroalgae and a HRT of 12 h prevented accumulation of microalgal species and contamination of the culture, which can occur in open systems with longer treatment times [39]. The ease of biomass retention in the reactor for the macroalgal growth system relative to the other systems tested in this study significantly contributed to achieving the greatest biomass production and treatment duration.

Table 2 Overview of the performance of each system, including biomass productivity, operational duration and normalised nutrient removal.

	Unit	Suspended	Biofilm	Entrapped	Macroalgae
Initial cell number	cells/mL x 10^6	1.5 ± 0.1	1.2 ± 0.1	1.2 ± 0.4	N/A
Cell number at end of run	cells/mL x 10^7	1.2 ± 0.3	3.9 ± 0.6*	3.0 ± 0.1	N/A
Cycle duration	d	17	27	6	30
Mass per cell at end of run	x 10^{-11} g/cell	5.3 ± 0.6	6.2 ± 0.0	2.2 ± 0.1	N/A
Biomass productivity	mg/L/d	36 ± 5	56 ± 3*	106 ± 2	102 ± 4
Lowest concentration	mg TP/L	1.72 ± 0.06	1.32 ± 0.02	1.35 ± 0.06	0.63 ± 0.07
reached before breaktrough	mg TDN/L	9.79 ± 2.15	10.6 ± 0.4	8.92 ± 0.86	7.05 ± 1.19
Total nutrient removal based on biomass increase	% $\frac{\text{mg TP}}{\text{mg biomass increase}}$	1.8 ± 0.5	1.6 ± 0.1	1.9 ± 0.5	2.7 ± 0.4
	% $\frac{\text{mg TDN}}{\text{mg biomass increase}}$	9.0 ± 0.7	4.2 ± 0.3	7.9 ± 0.6	7.5 ± 0.1

*Including cells harvested from supports (68%) and settled in vessel (32%).

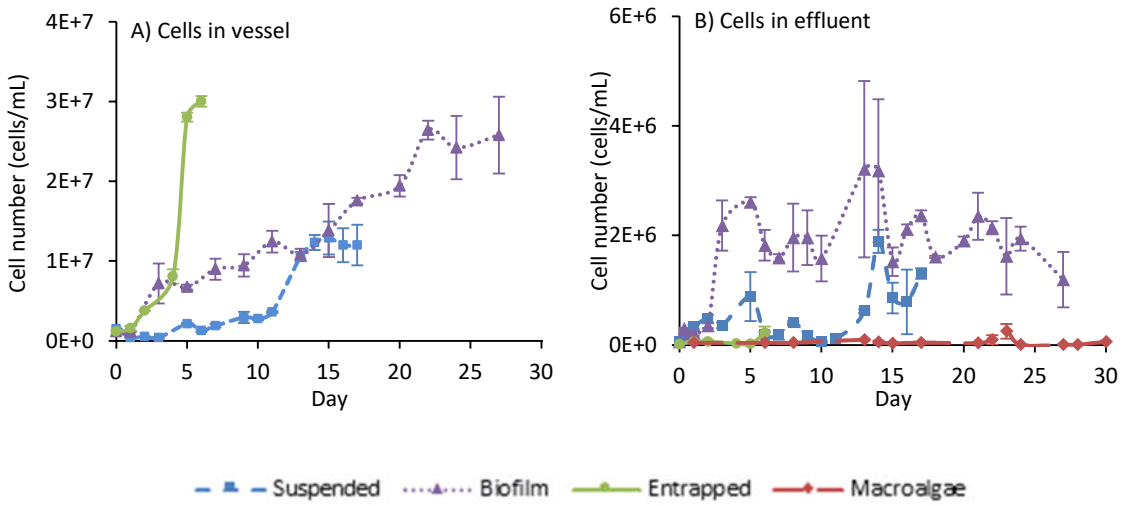


Figure 1 Microalgal cell concentration A) growing in the reactor for the three microalgal systems, and B) in the treated effluent for all four algal systems (no *O. cardiacum* cells were observed in the effluent, the cell number stated is for microalgal cells).

3.2 Removal of phosphorus

TP, TDP and P-PO_4^{3-} removal were closely correlated to each other over the duration of the run (Figure 2), suggesting that the main mechanism of P removal was by algal uptake rather than precipitation. This pH was > 8 for each system (Figure 4), which can promote precipitation of phosphate with Ca^{2+} [40]. This implies of the P removed from the wastewater, any that was not taken up by the algae was precipitated and retained in the reactor. P precipitate may have been retained by the membrane for the suspended system, captured on the alginate matrix for the entrapped system, attached to the surface of the biofilm and macroalga, or potentially settled in the reactors.

From a feed concentration of 7.0±0.5 mg TP/L (6.8±0.6 mg TDP/L) the suspended microalgal system treated the wastewater to 2.8±1.1 mg TDP/L (on average, days 9-14), the entrapped system to 1.4±0.1 mg TP/L (days 3-4), the biofilm system to 1.9±0.4 mg TP/L (days 3-24) and the macroalgal system to 1.3±0.6 mg TP/L (days 15-30). For the suspended system, the long start-up time for P removal and short treatment duration was reflected in the rate of algal accumulation in the reactor, with a long lag phase (days 0-9) and early onset of stationary phase (days 14-17). For the entrapped system, a substantial increase in DOC from a feed of 5.2±0.8 mg/L to 63.5± 36.3 mg/L in the effluent on day 6 (Figure 4C) and a visually observed decrease in bead size and an apparent weakening of the beads (not quantified) shows that the decline in P removal coincided with bead deterioration. The resultant release of algal cells from the beads (Figure 1B), and additional potential for P to release from algal cells [41], would have contributed to the effluent P concentration returning to that of the influent. For the biofilm system, the sustained P removal coincided with consistent algal growth from days 1 to 22 (Figure 1A), after which a decline in algal growth led to TP removal stopping by day 27. This demonstrates that of the microalgal systems attachment to supports enabled the most effective removal of P from wastewater with 7 mg TP/L in a reactor operating with a 12 h HRT. The

longer start-up time for the macroalgal system may indicate there were lower rates of nutrient transfer into the macroalgal biomass due to a lower surface area in contact with the wastewater (*O. cardiacum* is approximately 14 by 30 µm in cell size [31] compared with *C. vulgaris* which is spherical with a diameter of 2-6 µm [32]), and so more biomass was needed to achieve the same level of nutrient removal as the microalgal systems. However, achieving the lowest P concentration (0.67±0.19 mg TP/L) indicates by utilisation of the 85 µm filter to retain the macroalgal biomass, concentrations sufficient to compensate for the slower nutrient transfer were achieved and sustained after this start-up period, enabling effective P removal at a HRT of 12 h.

There was little variation in the amount of P removed based on biomass increase between the microalgal systems (1.6-1.9% mg P/mg biomass produced). The similarity of P uptake per unit of biomass in the three microalgal systems indicates that the differences in P removal from the wastewater were related to algal growth. This is shown by the lowest effluent P concentrations for the greatest duration occurring for the biofilm system as it best facilitated cell accumulation within the reactor. The amount of P in the biomass for the microalgal systems was higher than the 1.3% P content of suspended *C. vulgaris* when grown in synthetic media with 10 mg/L of $P\text{-}PO_4^{3-}$ in batch culture [42], possibly due to the continuous supply of wastewater in the present study, meaning more nutrients were being fed to the algae. The macroalgal system removed 2.7% mg P/mg biomass produced, signifying that uptake per unit of biomass and likely utilisation of P within *O. cardiacum* was higher than for *C. vulgaris*. The superior P removal performance for the macroalgal system can thus be attributed to both the higher P content in the algal biomass and the greater sustained rate of biomass production compared with the microalgal systems.

The alginate matrix did not limit the rate of transfer of nutrient into the algal cells with P being transferred into each unit of biomass at the same rate for the entrapped (0.22±0.02 mg P/d/mg

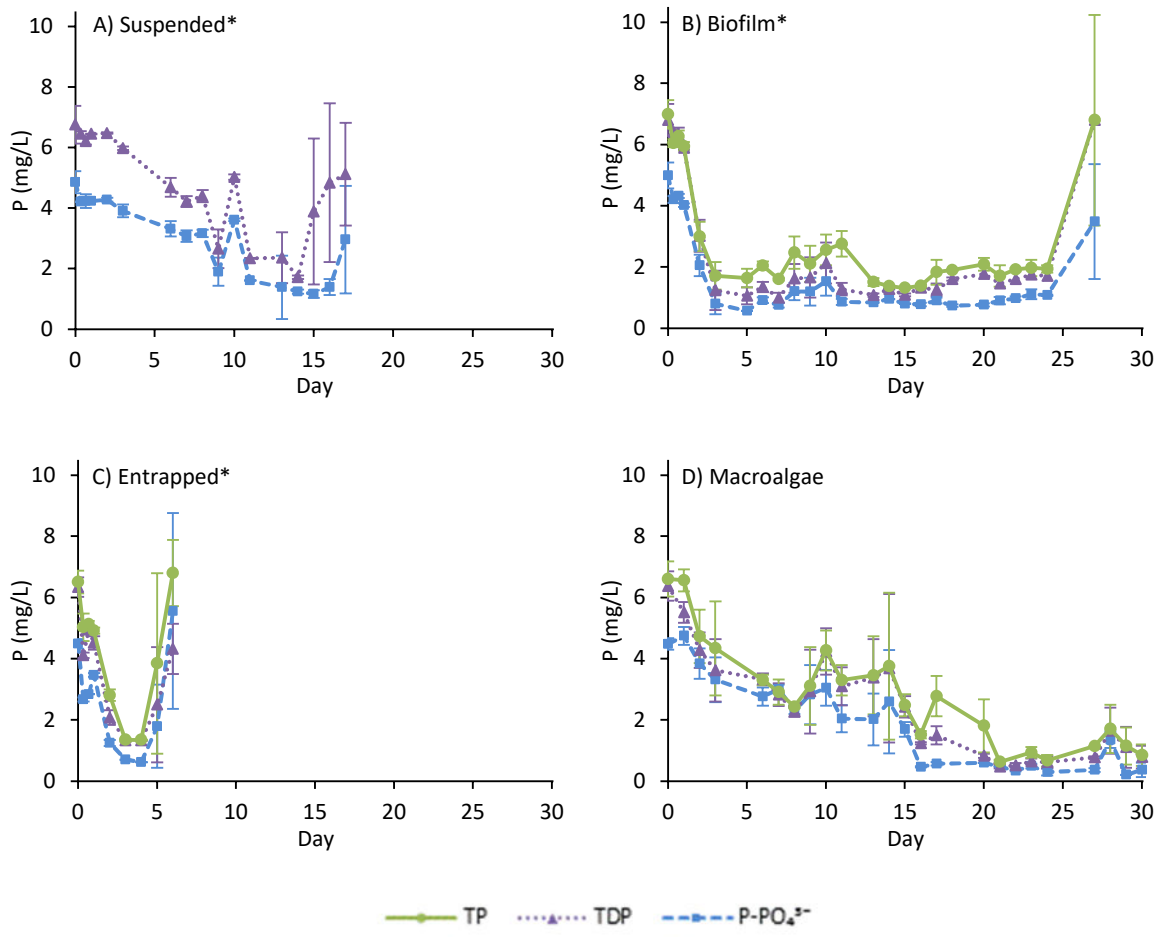
biomass) and suspended (0.21±0.07 mg P/d/mg biomass) systems at day 3. With the same rate of transfer, entrapment facilitating rapid cell production can explain why lower concentrations of P were reached sooner than for the suspended system (Figure 2). There was slower P mass transfer into each unit of biomass for the biofilm systems (0.07±0.03 mg P/d/mg biomass at day 3) than the other microalgal systems. The reduced rate of nutrient transfer resulted from low light transmissivity through the depth of the biofilm restricting algal activity to close to the biofilm surface [43] and the smaller surface area of the algal culture in contact with the wastewater. Despite this constraint, the supports enabled rapid and continuous cell accumulation in the reactor, which led to P removal for the biofilm system being sustained at the highest rate for the longest period for the microalgal systems.

Treatment of synthetic secondary effluent with a membrane (0.1 μm) photobioreactor inoculated with *C. vulgaris* over long term continuous operation reduced 6.1 mg/L of P to 0.61 mg/L (SRT 10 d, HRT 12 h) and 0.98 mg/L (SRT 5 d, HRT 12 h) [24]. The greater P removal reported by Xu et al. [24] than for the suspended system in this study can be explained by their use of synthetic effluent creating a better growth environment for the algae, and their use of a smaller pore size filter which better retained the algae within the reactor.

At a HRT of 12 h, entrapped *Scenedesmus obliquus* treated effluent with 0.7 mg/L P-PO_4^{3-}, 3.2 mg/L N-NH_4^+ and 20.3 mg/L N-NO_3^- for 19 days, reaching a minimum of 0.04 mg/L P-PO_4^{3-}, <0.001 mg/L N-NH_4^+ and 1 mg/L N-NO_3^- before bead deterioration [18]. The longer run duration than the entrapped system in the current study was due to the use of an algal species with a lower growth rate which would lead to less physical pressure on the alginate matrix of the beads, and the lower P concentration in the wastewater feed which would reduce disruption of the Ca-alginate matrix by removal of the bridging agent Ca^{2+}.

A mixed consortium biofilm growing in a flow cell (a flat surface supporting algal growth over which wastewater ran) with a HRT of 12 h treated synthetic effluent with 10 mg/L of $N\text{-}NO_3^-$ and 1.1 mg/L of $P\text{-}PO_4^{3-}$ to below 2.2 mg/L of $N\text{-}NO_3^-$ and 0.15 mg/L of $P\text{-}PO_4^{3-}$ for 6 days [44]. Utilisation of 3-dimensional supports for the biofilm system in the current study meant there was a lower nutrient load per biofilm area of 0.051 g $N/m^2/d$ and 0.014 g $P/m^2/d$ compared with 1.01 g $N/m^2/d$ and 0.094 g $P/m^2/d$ for Boelee et al. [44], which can explain the greater nutrient removal performance over the longer duration. The algal biomass that sloughed off the supports and remained in the reactor would also have contributed to nutrient removal, and so the reactor may have been operating as a combined suspended-biofilm system, leading to improved performance compared with the flow cell biofilm system.

In an open system at 80 m^3 scale treating TN of 4.0 mg/L and TP of 0.8 mg/L, *O. cardiacum* grew at 7.8 mg/L/d and removed nutrients on average to 2.6 mg/L TN and 0.3 mg/L TP with a HRT of 1 day [45]. The lower nutrient removal and growth than in the current study was because the macroalgae were not retained with a filter in the reactor nor provided with continuous light.



*Disintegration of cells toward end of the runs resulted in increased organic matter in the effluent which increased the variability of P measurement.

Figure 2 Effluent TP, TDP and P-PO_4^{3-} of the A) suspended, B) biofilm and C) entrapped microalgal systems and the D) macroalgal system.

3.3 Removal of nitrogen

No microalgal system consistently removed TDN to <10 mg/L (Figure 3A-C) indicating that for the majority of the operational period a HRT of 12 h was insufficient for TDN removal. Removal of N-NO_3^- was rapid, with the concentration in the effluent reaching and remaining below 1 mg/L of N-NO_3^- by day 3 for the suspended, and day 2 for the biofilm and entrapped systems. N-NH_4^+ removal followed the same trend for each microalgal system, with the

greatest removal only achieved after bead deterioration for the entrapped system, and P removal had ceased for the biofilm and suspended systems. Hence the poor TDN removal was related to poor $N\text{-}NH_4^+$ removal.

The microalgal systems were hence more efficient for removal of $N\text{-}NO_3^-$ than $N\text{-}NH_4^+$. Generally *C. vulgaris* preferentially takes up NH_4^+ rather than NO_3^- when both were in a synthetic growth medium [46]. However, the strain of an algal species and the culture conditions can affect which of NO_3^- or NH_4^+ is preferentially assimilated [47]. This is due to the relative abundance of different genes that impact NO_3^- transport, assimilation and regulation in the algal cell [48]. With the strain of *C. vulgaris* used in the current study treatment of wastewater with a high NO_3^- rather than NH_4^+ concentration thus leads to more favourable performance.

The pH (Figure 4) varied for each system and was not correlated to NH_4^+ removal, this suggests volatilisation of NH_4^+ as NH_3 was not a significant mechanism of removal in this study. In addition, in the latter stages of the treatment cycle for the biofilm and suspended systems TDN did not decrease in the effluent as much as $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. For these systems there was a longer stationary phase (Figure 1) where cell growth had stopped. During this phase as there was lower photosynthetic activity it is suggested that bacteria from the wastewater became a more dominant mechanism of NH_4^+ removal and generated NO_2^- (not quantified) or deteriorating cells and bacteria led to higher organic N in the effluent.

The macroalgal system was not effective for removing $N\text{-}NO_3^-$, with an average concentration in the effluent of 4.2 mg/L and a minimum of 2.2 mg/L. Furthermore, increase in biomass over time did not increase $N\text{-}NO_3^-$ removal, $N\text{-}NO_3^-$ removal did not increase even when $N\text{-}NH_4^+$ was depleted (to < 0.25 mg/L), and the $N\text{-}NO_3^-$ concentration increased to equal or greater than that of the feed from day 27 (Figure 3D). This provides evidence of nitrifying bacterial activity,

which involves conversion of NH_4^+ to NO_3^-. This would mean there was a symbiotic relationship in which the macroalgae produced O_2, which was then consumed by the bacteria, and the bacteria produced CO_2 which can enhance the photosynthetic activity of the macroalgae [49]. This would have improved biomass production and nutrient depletion rates, and arose from bacteria attaching to the surface of the macroalgae, providing the opportunity for their accumulation within the reactor.

TDN removal for the macroalgal system was at its most efficient from days 14-23, achieving 8.5±1.5 mg/L in the effluent. Over this period the effluent had low N-NH_4^+ of < 1.1 mg/L, low DIC of < 5.4 mg/L (Figure 4D) and less N-NO_3^- than in the feed, suggesting autotrophic algal growth was the dominant mechanism of nutrient removal. This indicates *O. cardiacum* preferentially assimilated NH_4^+ than NO_3^-, which was in contrast to the *C. vulgaris* systems. The DIC increased to >15.4 mg/L for days 24-30, N-NH_4^+ removal was still efficient to <0.5 mg/L and N-NO_3^- concentration increased. This suggests an increase in bacterial activity proportional to photosynthetic activity in the latter stages of the run. This led to a decline in TDN removal, with 12.2±0.9 mg/L in the effluent from days 24-30. While bacterial-algal symbiosis can be advantageous, this indicates the correct balance and system maintenance is needed to ensure efficient N removal. However, the macroalgal system reached a minimum concentration of <0.25 mg N-NH_4^+/L in the effluent at day 14 and remained below 1.1 mg N-NH_4^+/L until the end of the run, indicating it could operate at a HRT of 12 h to effectively remove NH_4^+ from wastewater containing 15 mg/L of N-NH_4^+ during periods of both photosynthetic and bacterial dominance.

The microalgal systems assimilated 4.2-9.0% mg N/mg biomass produced over each run (Table 2). This was consistent with the 5.0-10.1% N content of *C. vulgaris* when grown in synthetic medium with 10-50 mg/L of N-NO_3^- [42]. The biofilm system took up much less N (4.0% mg N/mg biomass produced) than the other microalgal systems, suggesting a change in resource

allocation due to the different growth system. Suspended algae led to the greatest assimilation of N (9.0% mg N/mg biomass produced), indicating that being suspended and not contained in a matrix (biofilm or entrapped) promoted uptake and allocation of N within the algae. The difference in N allocation within the biomass between the microalgal systems demonstrates N removal was less dependent on biomass production than was found for P removal. The *O. cardiacum* biomass assimilated 7.5% mg N/mg biomass produced, within the same range of the *C. vulgaris* microalgal systems.

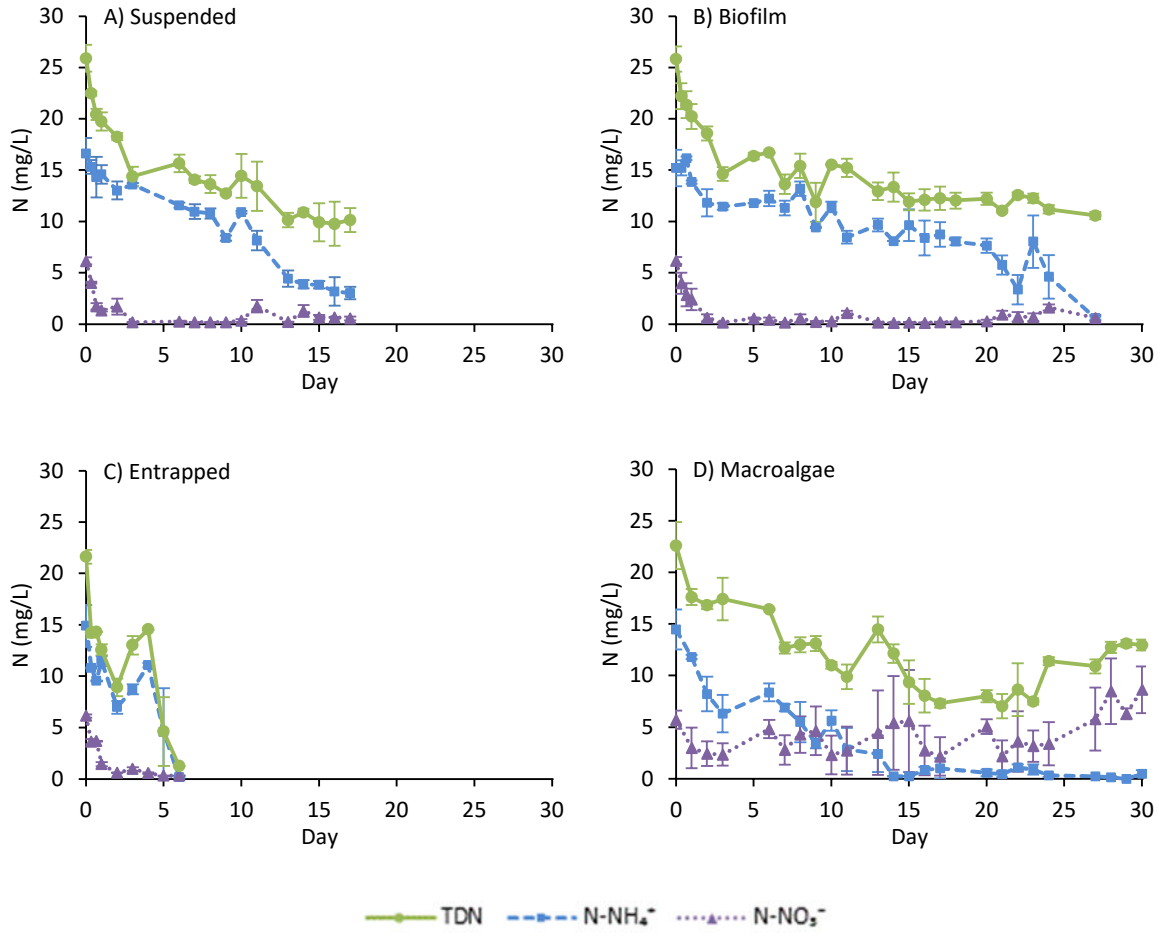


Figure 3 Effluent TDN, N-NH_4^+ and N-NO_3^- of the A) suspended, B) biofilm and C) entrapped microalgal systems and the D) macroalgal system.

3.4 Wastewater carbon and pH

The DIC decreased and DOC of the wastewater increased for each system (Figure 4) for both *C. vulgaris* and *O. cardiacum* indicating both species were growing autotrophically. The effluent DIC decreased from 34.7±5.3 mg/L to a minimum of 0.2-0.5 mg/L for the suspended, biofilm and macroalgal systems, suggesting that the DIC concentration of the wastewater feed was limiting for at least part of the run. The entrapped system was an exception, with the minimum DIC of 4.0±2.1 mg/L in the effluent being less limited and contributing to production of biomass at a greater rate than the other microalgal systems. The removal of DIC from wastewater by algal uptake results in an increase in pH during treatment [50]. The suspended system resulted in the lowest level of alkalisation of the effluent (maximum pH of 8.5), the other systems reached a pH of 9.3-9.7 due to greater rates of algal growth (Table 2) and so the effluent alkalinity would need to be reduced before discharge to prevent harm to the receiving water body and for water reuse.

The DOC increased from 5.9±0.7 mg/L to a maximum of 11.7±3.3, 13.8±3.2 and 12.4±0.5 mg/L for the suspended (Figure 4A), biofilm (Figure 4B) and macroalgal systems (Figure 4D), respectively. This indicates that under these growth conditions of low DOC concentration in the wastewater and continuous provision of light the algal systems will cause a net DOC increase in the effluent. If a disinfection process were to follow the algal treatment harmful by-products can form from the released algal organic matter (AOM), for example chlorination of AOM from *C. vulgaris* yielded trihalomethanes of 21-27 µg/mg-DOC and haloacetic acids of 24-30 µg/mg-DOC [51]. However, this negative impact depends on the nature of the DOC [52], with the yield of harmful by-products related to cellular composition [53] and thus cultivation conditions [54]. The DOC of the effluent from the entrapped system was 14.4±4.3 mg/L on day 5 which increased to 63.5±36.3 mg/L on day 6 (Figure 4C). No other system showed a similar spike in DOC at the end of the run, suggesting it was mostly due to the

degradation of the Ca-alginate beads rather than the release of organics from the algal cells. The beads may have degraded from a combination the binding of the alginate matrix being weakened by Ca^{2+} reacting with anions in the wastewater and cell growth applying pressure to the matrix [55]. The entrapped system would therefore need to be stopped and harvested before this increase in DOC.

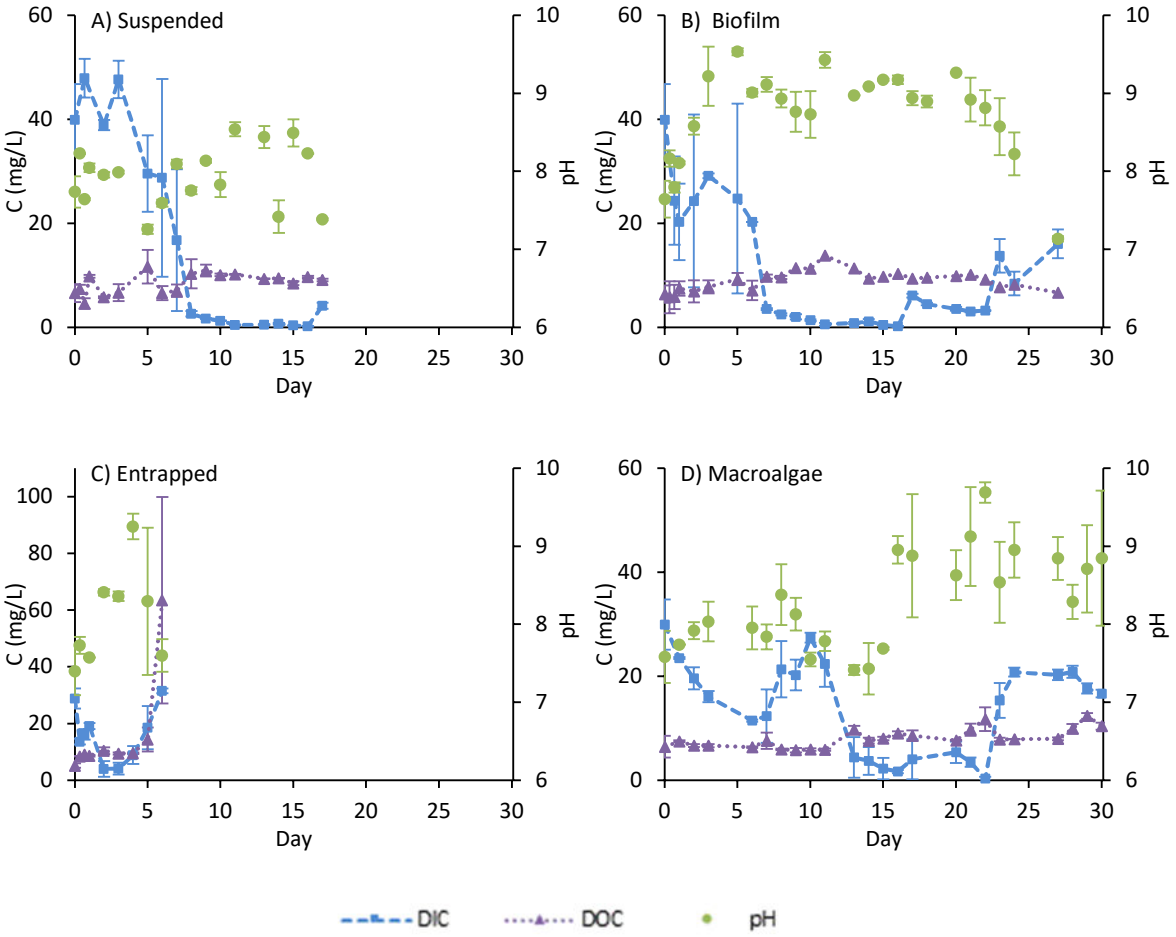


Figure 4 Effluent DIC, DOC and pH of the A) suspended, B) biofilm and C) entrapped microalgal systems and the D) macroalgal system.

3.5 System comparison

Treating wastewater with each growth system under identical reactor conditions enabled the relative importance of fundamental differences in their nutrient removal and biomass production performance to be identified.

The supports for the biofilm system were not being moved around the reactor which meant light distribution was not even for all of the culture. In addition, reduced light penetration and nutrient transfer through the thickness of the biofilm led to that system having lower rates of P and N transfer into the microalgae per unit of biomass than the other microalgal systems. By comparison, for the entrapped system the alginate beads were well mixed throughout the reactor and so the entire culture was exposed to the same level of light. Mixing for the biofilm system at higher rates to suspend the supports is not practical as this would cause shear damage to the biofilm. The entrapped system had the equal highest N and P transfer rates per unit biomass as the suspended system, demonstrating that the alginate matrix caused no restriction in cellular nutrient uptake. The macroalgae had a lower area of contact with the wastewater per unit biomass than the microalgal systems due to their larger cells. This led to the longer start up time for nutrient removal, with the macroalgal biomass also containing the lowest N and P per unit of biomass, indicating more biomass was needed to achieve the same level of nutrient removal.

Despite the restrictions of nutrient transfer rates for the biofilm and macroalgal systems, accumulation of biomass in the reactor was proven to be more important to the treatment performance. Considering full scale application, both these systems are also simpler to operate compared with a microporous filter required to retain microalgae in a suspended system (meaning greater pressure differential is needed and filter backwash and cleaning requirements) and the use of alginate beads for the entrapped system (which adds cost at initiation of each treatment cycle).

The macroalgal system also showed further advantage over the biofilm system with no biomass being lost from the reactor, meaning less contamination of the treated wastewater and less loss of value-adding biomass. Furthermore, the growth of the macroalgae was not restricted to a support surface, unlike the biofilm system which contributed to its having a shorter treatment

cycle before growth stagnated and thus the need for more frequent harvesting than the macroalgal system. The ability to control the macroalgal biomass concentration in the reactor more effectively may mean that the symbiosis between the algae and bacteria can be controlled to further improve nutrient removal efficiency. Further work to adjust operational parameters such as the macroalgal biomass concentration, mixing rates and light provision would help enhance this symbiosis to enable maximum nutrient removal under the lowest artificial light requirements and/or shortest treatment times.

4 Conclusions

Four algal systems utilising artificial light with algal biomass retained within a reactor (three of the microalga *C. vulgaris* and one of the macroalga *O. cardiacum)* were demonstrated to treat wastewater with 15 mg/L $N\text{-}NH_4^+$, 6 mg/L $N\text{-}NO_3^-$ and 7 mg/L TP at a HRT of 12 h. Treatment performance was related more to the ability of the system to accumulate cells in the reactor than to the effect of system selection on nutrient transfer into the biomass. The biofilm system performed better than the suspended and entrapped microalgal systems in terms of treatment duration (27 days), achieving the lowest TP (1.9 mg/L, average in the effluent for days 3-24), $N\text{-}NO_3^-$(0.5 mg/L) and to a lesser extent $N\text{-}NH_4^+$ (9.0 mg/L) and TDN (10.6 mg/L). The macroalgal system achieved the multiple benefits of equal highest biomass production (102±4 mg/L/d), longest operating duration (30 days) and, although not being effective for NO_3^- removal, achieved the lowest TP (1.3 mg/L, on average days 16-30), $N\text{-}NH_4^+$ ($\leq$0.5 mg/L), and TDN (<7.1 mg/L) concentrations of all the algal systems. This demonstrates that treatment performance can be significantly enhanced by the combined utilisation of biomass retention and artificial light, and suggests that macroalgal systems are very promising for this purpose.

Acknowledgement

This study was funded by Water Research Australia (grant 4531-17), South East Water and RMIT University.

Conflict of interest

The authors report no commercial or proprietary interest in any product or concept discussed in this article.

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Kube M, Fan L, Roddick F, et al., (2022) High rate algal systems for treating wastewater: a comparison. Algal Research, Volume 65, June 2022, Article number 102754

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