

# The impact of wastewater characteristics, algal species selection and immobilisation on simultaneous nitrogen and phosphorus removal

Matthew Kube, Bruce Jefferson<sup>a</sup>, Linhua Fan and Felicity Roddick\*

Water: Effective Tools and Technology Centre, School of Engineering, RMIT University,

GPO Box 2476 Melbourne, 3001, Australia

<sup>a</sup>Cranfield University, Cranfield, MK43 0AL (United Kingdom)

\*Corresponding author:

Felicity Roddick

School of Engineering

RMIT University

GPO Box 2476

Melbourne, 3001, Australia

Tel: +613 9925 2080

Fax: +613 9639 0138

felicity.roddick@rmit.edu.au

## Highlights

- Review of factors that affect the ratio of nitrogen to phosphorus removal
- Interdependence of nitrogen and phosphorus on their simultaneous removal is discussed
- Analyses the impact of immobilisation and species selection
- Explores methods to improve remediation with minimal artificial lighting
- This knowledge assists the design of algal systems to be tailored to the wastewater

## **Abstract**

Nutrient removal from wastewater reduces the environmental impact of its discharge and provides opportunity for water reclamation. Algae can accomplish simultaneous nitrogen and phosphorus removal while also adding value to the wastewater treatment process through resource recovery. The application of algae to wastewater treatment has been limited by a low rate of nutrient removal and difficulty in recovering the algal biomass. Immobilising the algal cells can aid in overcoming both these issues and so improve the feasibility of algal wastewater treatment. Trends for nutrient removal by algal systems over different wastewater characteristics and physical conditions are reviewed. The impact that the selection of algal species and immobilisation has on simultaneous nutrient removal as well as the interdependence of nitrogen and phosphorus are established. Understanding these behaviours will allow the performance of algal wastewater treatment systems to be predicted, assist in their optimisation, and help to identify directions for future research.

**Keywords:** Algae, immobilisation, species, nitrogen, phosphorus.

## Contents

1	Introduction.....	5
1.1	Algae for wastewater treatment.....	5
1.2	Wastewater characteristics.....	7
1.3	Treatment systems.....	7
1.4	Species selection .....	8
2	Nutrients.....	10
2.1	Nitrogen.....	10
2.1.1	N removal mechanisms.....	10
2.1.2	N removal behaviour .....	10
2.2	Phosphorus.....	11
2.2.1	P removal mechanisms .....	11
2.2.2	P removal behaviour .....	12
2.3	Interdependence of N and P .....	13
2.3.1	Interaction of the wastewater and cellular N and P content .....	13
2.3.2	N impact on P.....	14
2.3.3	P impact on N.....	15
2.3.4	Impact of the magnitude of the wastewater N:P ratio .....	16
2.3.5	Impact of the total wastewater nutrient concentration .....	18
2.3.6	Species dependence .....	19
2.3.7	General trends for simultaneous N and P removal .....	20
2.4	Carbon .....	21
3	Environmental conditions .....	24
3.1	pH .....	24
3.2	Temperature .....	25
3.3	Light.....	26
4	Immobilisation.....	30
4.1	Methods of immobilisation.....	30
4.2	Impact of immobilisation .....	31
5	Conclusion .....	33
6	References.....	35

## **1 Introduction**

### **1.1 Algae for wastewater treatment**

Most sources of wastewater contain nitrogen (N) and phosphorus (P). If not removed, a build-up of these nutrients can cause eutrophication and toxicity in aquatic environments. Consequently there are limits on their discharge to prevent environmental damage, example allowable median concentrations in wastewater to inland water bodies are total nitrogen (TN) < 10 ppm and total phosphorus (TP) < 0.5 ppm [1]. Conventional biological, chemical and physical treatment methods that separate N and P from wastewater have several downsides not shared with the use of algae. Critically, they become disproportionately more expensive the lower the nutrient concentration, making removal to very low concentrations uneconomical. Separate unit operations or enhanced designs are needed to enable both N and P removal. The concentrated form of the nutrients cannot be readily reused and the potential formation of harmful by-products creates a further need for safe disposal of any waste sludge generated [2].

Rather than viewing the nutrients of N and P as a waste that must be disposed of, wastewater treatment with algae facilitates their recovery in a cost effective and environmentally friendly manner. For example, utilising algae as bioavailable fertiliser removes the need to synthesise fertiliser through energy intensive and resource depleting pathways. This is especially important for added food security as there is a current reliance of mining P from finite reserves. As well as treating wastewater by removing N and P, potentially aiding in providing alternative water supplies, algae consume carbon dioxide (CO<sub>2</sub>) and can be used to produce renewable energy, fuels and chemicals [3], thus reducing fossil fuel consumption and greenhouse gas emissions. Algae have the added benefit of removing additional

contaminants from wastewater such as heavy metals [4] and persistent organic pollutants [5]. Some of these micropollutants are not, or not sufficiently, removed by conventional treatment processes. The removal of micropollutants is important as they can build up in the ecosystem and have negative health and environmental impacts [6]. These environmental and economic benefits of using algae make it a promising tool to meet the growing demand for wastewater treatment, water and nutrient reclamation, and energy production in a sustainable manner.

Wastewater characteristics vary between locations and fluctuate with time for a single source, with the concentration of nutrients likely to vary independently of each other for a particular wastewater stream [7]. This is an obstacle in simultaneously removing N and P. The complex interaction of wastewater characteristics and environmental conditions creates discrepancies in reported removal behaviours. Careful analysis is required to take into account their compounding interaction and understand their influence on nutrient removal. This is reviewed, with a focus on the interaction between N and P, to help identify and direct strategies that will enable algal treatment of wastewater to be completed in an efficacious and cost effective manner.

Applying algal systems to wastewater treatment has been limited by a low degree of nutrient removal as well as problems with harvesting the algae [8]. Newly developed reactors and immobilisation systems show promise to overcome these issues [3]. However, more understanding is needed on the impact that selection of reactor type and algal species has on remediation performance. This review isolates compounding factors pertaining to wastewater characteristics and environmental conditions from the impact of species

selection and immobilisation. This approach can assist in improving treatment performance by allowing the design of the algal system to be tailored to the wastewater.

## **1.2 Wastewater characteristics**

Algae are an option to treat different wastewaters, including those from industrial, agricultural and municipal sources [9]; this review focuses on the treatment of municipal wastewater. The major nutrients required for algal growth include nitrogen, phosphorus and carbon, with their concentration and form being important [10]. In municipal wastewater N mostly occurs as ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), but it can also occur as nitrite ( $\text{NO}_2^-$ ) and organic nitrogen. The main form of P is orthophosphate ( $\text{PO}_4^{3-}$ ) along with organic phosphorus [6]. The concentration and form of C containing compounds also varies. Algae are autotrophic, and predominately grow photosynthetically on inorganic carbon (IC) [11]. Minor nutrients are also required for growth and include sulfur, calcium, potassium and several other metals. For municipal wastewater the minor nutrients are normally in sufficient concentrations to not substantially influence treatment performance [2].

## **1.3 Treatment systems**

Algal treatment systems can be categorised as suspended or immobilised. The former has cells growing freely in the medium. The low cost and simplicity of suspended systems are their main advantage, however they generally have low cell loadings leading to long hydraulic retention time (HRT) requirements [12]. Harvesting of suspended cells can be difficult causing inefficient resource recovery and risk of contaminating the discharged wastewater [8]. Due to the larger size of freshwater macroalgal cells, macroalgae are seen as an alternative to avoid the limitations in harvesting suspended microalgal systems [13].

Passive techniques of immobilisation, known as attached growth systems or biofilms, involve the algal cells accumulating on and attaching to a surface of a substrate through the excretion of extracellular polymeric substances [10; 14-16]. Active techniques of immobilisation involve physically separating the cells from the bulk liquid phase and include covalent coupling, adsorption, liquid-liquid emulsion, membrane separation, entrapment and encapsulation [4; 14; 17; 18]. In general, immobilisation allows for higher cell loading than suspended systems, giving a higher rate of nutrient removal. The resultant lower HRT and reduced reactor volume along with the ease of harvesting are the main advantages for wastewater treatment [19; 20]. These benefits need to be balanced against the additional costs of the immobilisation process and materials, and it should be noted that there have been few full scale applications of immobilised algae wastewater treatment systems.

#### **1.4 Species selection**

For the purpose of species selection many of the papers reviewed here are on pure monocultures to reduce interferences. In algal wastewater treatment systems the algae will exist as a consortium, and both bacteria and algae will participate in nutrient removal. Hence in real systems the influence of bacteria must also be considered [21; 22]. In addition, algae and bacteria form a synergistic relationship which can improve growth and nutrient removal, as the bacteria consume  $O_2$  and produce  $CO_2$ , while the algae consume  $CO_2$  and produce  $O_2$  [12].

Immobilisation can prevent contamination of the inoculated algae, which enables more control over the selection of species within the culture [14; 23]. This allows the most appropriate algal species to treat the wastewater to be selected and offers a pathway to decouple the role of algae from that of bacteria. Compared with algal monocultures, co-



cultures of selected algal and bacterial species, or of multiple algal species, can improve the rate and robustness of remediation [12]. Such co-cultures can be controlled by immobilisation, either by keeping each species in separate matrices or mixed within the same matrix [14; 24]. A carefully managed a co-culture of bacteria and algae may also be a way to improve nutrient removal during dark periods [25] or maintain the pH of the effluent [26].

Comparing monocultures, some algal species are equally capable of removing N and P and of adapting to different wastewaters [27-30]. In other cases the proportion of N to P removed differs significantly between algal species treating a single wastewater [31-33], indicating appropriate species selection can promote nutrient removal. There are species dependent responses to environmental conditions [34; 35] which mean in an open system seasonal variations can cause fluctuations in the species present and remediation rates [36]. One approach to selecting species that are suitable for the treatment conditions is to isolate them from the treatment plant or a local aquatic system [37; 38]. Starvation of algal cells before exposure to wastewater is a concept that may increase the rate of nutrient removal [39; 40]. Hernandez et al. [41] observed a higher P removal after starving alginate-immobilised *Chlorella sorokiniana*, but not with *C. vulgaris*, indicating caution needs to be applied as starvation leading to a positive effect is species-dependent.

## 2 Nutrients

### 2.1 Nitrogen

#### 2.1.1 N removal mechanisms

Nitrogen is essential to many of the functional components of algae, including structural proteins and enzymes, nucleic acids, chlorophyll and energy transport molecules [11; 42]. This leads to a high requirement for N, however most algal species do not exhibit non-structural N storage [42]. The algal cell can adapt to assimilate N as needed based on the form and concentration available [43]. In addition to direct assimilation,  $\text{NH}_4^+$  can be removed through volatilisation as ammonia ( $\text{NH}_3$ ) under basic conditions, where an elevated pH can be a consequence of algal growth.  $\text{NO}_3^-$  is more stable and hence not removed by volatilisation [11]. Based on finding no clear relationship between influent and effluent  $\text{NH}_4^+$  concentration from the compilation of a number of studies, Whitton et al. [19] suggested that the mechanism and rate of N removal depends on the treatment system and conditions.

#### 2.1.2 N removal behaviour

As N is critical to many of the cellular components in algae it follows that the wastewater N concentration impacts N uptake. With alginate-entrapped *C. vulgaris*, when increasing the  $\text{NO}_3^-$  concentration Jeanfils et al. [44] found a proportional increase in the rate of  $\text{NO}_3^-$  removal. Increasing either  $\text{NO}_3^-$  or  $\text{NH}_4^+$  also led to an increased rate of N removal for *C. vulgaris* and *Pseudokirchneriella subcapitata* [45]. Removal of  $\text{NH}_4^+$  is especially important as it is the more toxic form of N in wastewater. Whitton [46] found that alginate immobilised *Scenedesmus obliquus* could adapt to 4.2 ppm  $\text{NH}_4\text{-N}$  and remove it to a residual of 0.06 ppm in 2 h bead contact time. *C. vulgaris* in attached growth exhibited greater N removal with increasing  $\text{NH}_4^+$  concentration [47], as did suspended *Chlamydomonas acidophila* to a lesser

extent [48], possibly due to inhibitory concentrations of  $\text{NH}_4^+$  being reached. An increase in N available in the wastewater results in more N uptake, with more of the vital components for cellular activity being produced, further contributing to a greater amount of N being transported and assimilated by the cells.

As algae can take up different forms of N, wastewater discharge requirements can be met when there are multiple sources of N present. It is well established that  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$  is preferentially consumed by algae. When both forms are present in the wastewater there is minimal consumption of  $\text{NO}_3^-$  until most of the  $\text{NH}_4^+$  has been removed [45]. Sanz-Luque et al. [43] explain that high  $\text{NH}_4^+$  concentrations will inhibit nitrate reductase (NR) activity, and hence lower  $\text{NO}_3^-$  uptake. This was demonstrated by a reduced NR cell content until  $\text{NH}_4^+$  was depleted [49]. Toxicity to algae can occur at high ammonium concentrations, negatively impacting remediation, with reduced growth occurring above 106 ppm  $\text{NH}_4\text{-N}$  for some Chlorophyceae [50]. This may be either from uncoupling of photophosphorylation or an increase in direct photodamage [50; 51]. The toxicity will depend on pH, as the un-ionised form of ammonia can be more rapidly transported across the cell membrane [52].

## **2.2 Phosphorus**

### **2.2.1 P removal mechanisms**

Phosphorus is utilised in algae for a number of components, including phospholipids, proteins and nucleic acids, and is critical to the energy cycle of algal cells [11; 42]. The uptake mechanisms as well as its use in algal cells depend on the concentration of P in the wastewater [39]. There is a common phenomenon of additional P uptake beyond what is required for cell growth, stored in the form of polyphosphate, known as luxury uptake [53]. In addition to direct assimilation a major mechanism of P removal from wastewater is  $\text{PO}_4^{3-}$  precipitation.

This is promoted when the pH is basic, which can occur because of algal growth [11]. A relationship between influent and effluent P concentration was found for a number of algal systems reviewed by Whitton et al. [19], who concluded that the P removal mechanism may not depend on the treatment system or conditions.

### **2.2.2 P removal behaviour**

With *S. obliquus* Martínez Sancho et al. [54] found that the rate of P removal rapidly increased with increasing P concentration until growth became limited. As well as through cell growth, P removal can depend on the amount of P accumulated per cell. With increasing P concentration in a *Scenedesmus*-dominated culture Powell et al. [53] observed an increased removal rate of P, with high concentrations triggering luxury uptake. A similar response was found to increasing P concentration by Zhu et al. [55] using *Chlorella* sp., with greater P removal a result of the algal cells containing a higher P content. With *S. quadricauda* Yao et al. [56] observed  $\text{PO}_4^{3-}$  first being absorbed onto the cell surface at a rapid rate and then slowly consumed by the cell. This surface-adsorbed P is in equilibrium with the surrounding wastewater, and could be released after a long algal retention time. This release should be prevented to maintain effluent quality and maximise the life of an algal culture.

The initial cellular P content can also impact P removal. Ruiz-Martínez et al. [57] used an algal culture where *Scenedesmus* sp. was the dominant species and found that the lower the initial cellular P, the faster the rate of P removal was for the first few hours. In practice, an algal culture will treat wastewater for several days. A high removal rate might not be sustained once the algae have reached their maximum quota of P per cell. Having a lower initial P content can not only increase the P uptake rate, but can also cause the algal cells to overshoot

and contain a higher P content than they would otherwise [56], suggesting that the improved P removal will be sustained for the life of the culture.

### **2.3 Interdependence of N and P**

It has been established that the wastewater and cellular N impact N removal, and similarly that the wastewater and cellular P impact P removal. The amount of N in wastewater and algal cells can also impact P removal, as the amount of P can impact N removal [58]. This interdependence is to be discussed, considering with the cellular N and P, the wastewater N:P ratio and the wastewater nutrient load.

#### **2.3.1 Interaction of the wastewater and cellular N and P content**

The cellular N:P of *C. vulgaris* can adapt to match the wastewater N:P [59]. Fulton [60] also reported that the average N:P ratio of the cells in a mixed algal culture was closely related to the N:P ratio of the feed. In this case the N (and P) content of the cells adjusted as a direct response to the N (and P) concentration of the wastewater respectively, with little interdependence on the other nutrient. Xin et al. [61] found that the proportion of N to P that *Scenedesmus* sp. removed gradually matched the effluent N:P ratio with increasing time, although this case may instead be a result of the nutrient concentration in the culture medium depleting over time. With increasing rate of supply of wastewater to *Scenedesmus* sp. Dickinson et al. [62] found an increase in the cellular N and P content, which promotes the use of short HRTs. The ability of algae to adjust their internal N and P content to the wastewater could form an important wastewater treatment strategy.

While the concentration of each nutrient can affect algal growth and hence uptake of other nutrients, the cellular content of algae will evidently adjust in response to the concentration and ratio of N and P in the wastewater. Changes in nutrient accumulation on a per cell basis

can have a large impact on remediation, with Beuckels et al. [7] finding on a mass basis the change in cellular N and P contributed equally to nutrient removal as did growth. This agrees with the finding of Whitton et al. [63] that a higher cellular N content of *S. obliquus* gave greater nutrient removal than *C. vulgaris* despite having lower growth. Changes in cellular content will also modify the metabolism of algae, further affecting the rate at which N and P are assimilated. This highlights the importance of understanding how cellular N and P contents change and affect N and P removal from wastewater.

### **2.3.2 N impact on P**

Beuckels et al. [7] explored this behaviour by varying the PO<sub>4</sub>-P concentration in five intervals from 2 to 10 ppm for five intervals of NO<sub>3</sub>-N concentration from 10 to 50 ppm, creating 25 trials. For both *S. obliquus* and *C. vulgaris* the percentage of P in the cells was dependent on the wastewater N concentration. This led to an increase in P removal with increasing N concentration. Furthermore, at high N, the concentration of P had a larger influence on cellular P content than at low N, showing that N limitation hinders uptake of P. This was explained by Beuckels et al. [7] through N being primarily contained in proteins which can also contain P and are essential for assimilation of further nutrients. N limitation can also decrease the thylakoid membrane, which governs photosynthesis [64]. This would result in less energy being available for P consumption. In addition, N limitation can cause phospholipid hydrolysis [65], which may explain why Fulton [60] found that when a mixed algal culture became aged P was released, with more released if the wastewater had lower N:P. This suggests that if the amount of N is low, the culture will have a reduced maximum uptake capacity of P.

A contrary behaviour was observed by Choi and Lee [59] using *C. vulgaris*. Increasing the N concentration led to a decrease in the cellular P content and P removal. This may be explained by the results of Kunikane et al. [58] using *S. dimorphus* where the wastewater N:P was modified by increasing  $\text{NO}_3^-$  while simultaneously decreasing  $\text{PO}_4^{3-}$  in a chemostat. At a low dilution rate, growth was limited, the response to increasing the wastewater N:P was more growth, which resulted in a lower cellular P content. When the dilution rate was high growth was not limited, an increase of N:P led to an increase in the cellular P content as, rather than for growth, the additional N was being utilised for P to be transported into the cells and metabolised. This shows that the culture conditions can change the impact that the wastewater N:P has on nutrient remediation.

### **2.3.3 P impact on N**

Kunikane et al. [58] observed that there was no accumulation of N in the algal cells when the effluent N:P changed. The decrease in P concentration may have limited N uptake. Otherwise a correlation of N with growth may have led to a stable cellular N content. In the study by Beuckels et al. [7] the percentage of N in the cells was relatively independent of P concentration for both *S. obliquus* and *C. vulgaris*. For Arbib et al. [66] lowering the N:P ratio with fixed N concentration using *S. obliquus* only slightly reduced the time taken to remove N, while the cellular N content was also barely affected. These studies suggest that P concentration has little impact on the rate of N removal; possibly as low P concentrations can promote—production of N containing transport proteins, allowing continued nutrient assimilation in low P environments [67]. This could be advantageous for controlling the rate of N removal if the influent P is low or unstable, and means factors other than P should first be considered to improve N removal.

#### 2.3.4 Impact of the magnitude of the wastewater N:P ratio

The interdependence of N and P becomes critical at extremely high or low N:P ratios as demonstrated by Wang et al. [68] using *Chlorella* sp. and *Micractinium* sp. Both species matched nutrient removal to the wastewater at a N:P mass ratio of 9 when treating primary effluent, but for sludge centrate with an N:P of 56 the increase in internal N of both species was not sufficient to match the higher N:P of the wastewater. In less extreme yet still a wide range of N:P, for example 2:1 to 50:1 [58], the concentration of one nutrient will still impact the rate of uptake of the other. It follows that the manner in which the wastewater N concentration impacts P removal, and vice-versa, depends on the magnitude of the wastewater N:P ratio. This N:P impact will be related to nutrient limitation or competition, hence it must also depend on the absolute concentration of the nutrients.

Nutrient removal was only limited at extreme N:P ratios for Liu and Vyverman [69], this is where the cells contain a maximum quota of one of the nutrients, and hence to increase the removal of this nutrient they require sufficient of the other nutrient to support further cell production. Once P was depleted, the algae could still consume N, while the opposite was not observed. N can be removed as long as there is sufficient P to meet a minimum cell quota for growth, as P is primarily only necessary for ribosomal RNA [70], and evidently algae are able to adjust the allocation of internal P resources to this component. On the other hand, N is needed to assist assimilation of P into the cell, which was suggested to be due to the importance of N in proteins used for the transport of P across the cell membrane [71]. As an essential component of all proteins [70], N is needed in order to have sufficient proteins to produce the P containing ribosomal RNA [72]. Effectively, low N can shift resource allocation to lipid production at the expense of components necessary for P assimilation and cell division



[61]. Xin et al. [61] observed that with a fixed  $\text{NO}_3\text{-N}$  of 10 ppm and varied  $\text{PO}_4\text{-P}$  from 0.1 ppm to 2 ppm, the rate of N removal by *Scenedesmus* sp. was not impacted until N:P exceeded 50. This is consistent with the idea that P has little effect on N uptake unless wastewater and cellular P are becoming depleted, which is when insufficient P is available to be allocated to ribosomal RNA.

Consistent with N being utilised to assist P assimilation, Zhang and Hong [73] reported that P removal by *Chlorella* sp. increased with increasing N concentration at low N:P ratios (2:1 to 8:1). However, at higher N:P ratios (8:1 to 20:1), when  $\text{NO}_3^-$  was increased with fixed  $\text{PO}_4^{3-}$ , P removal reduced. This is supported by the work of Arbib et al. [66] where increasing N:P from 5.9 to 15.8 with fixed P concentration increased the time taken for P removal despite P becoming more limiting. A high N content may change the metabolism of the cells and cause a reduction in P uptake. Klausmeyer et al. [74] stated that high N:P causes a shift to production of cellular components involved in resource acquisition, which was explained to be proteins and chloroplasts that are rich in N, but not P. The uptake of P may also reduce the removal of N under certain scenarios, with an increase from 0.3 ppm to 2 ppm  $\text{PO}_4\text{-P}$  with fixed  $\text{NH}_4\text{-N}$  of 5 ppm leading to a decrease in N removal for *C. vulgaris* and *S. obliquus* [63]. At higher concentrations of P the cells may have shifted resources to the creation of ribosomes rather than cellular components which have a greater N content. This shift may occur when nutrient concentrations are at an optimal proportion for cellular growth [74]. These results suggest competition between N and P that could be disadvantageous when trying to simultaneously remove both nutrients.

### 2.3.5 Impact of the total wastewater nutrient concentration

How N impacts P and vice-versa will not only depend on the N:P ratio of the wastewater but also on the nutrient load. This was demonstrated by Cabanelas et al. [75] with a strong correlation between wastewater N and P with N and P removal for various wastewaters using *C. vulgaris*, except when treating centrate effluents with higher nutrient concentrations. When excess nutrients are supplied, each species of algae may reach a certain N and P content in their cells. Klausmeyer et al. [74] explain that pooling of nutrients within algal cells beyond what is required for growth will be at the same N:P ratio of structural components. On the other hand, when the nutrient concentration is low algae are likely to exhibit a closer link to the wastewater N:P ratio.

With a biofilm Boelee et al. [76] found at low loads the algae adapted to the wastewater N and P concentration. With increasing  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  load there was an increase in cellular N and P, with decreasing internal N:P towards a ratio of 5.4:1. After cellular nutrient content peaked, cellular N remained constant and a gradual increase in P content occurred. This supports the concept that at high nutrient concentrations the cells will meet their desired N:P ratio, with slight pooling of P through luxury uptake decreasing cellular N:P beyond the structural N:P quota. Similar behaviour was observed for *C. vulgaris* when Liang et al. [26] increased the amount of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  with a fixed N:P of 5:1. This led to an increase in both N and P removal per cell until 3 ppm  $\text{PO}_4\text{-P}$  was reached, after which the rate of N removal decreased and the rate of P removal increased at a slower rate. This demonstrates an inability to simultaneously remediate both N and P at higher nutrient loads.

Also using *C. vulgaris*, with a fixed effluent N:P ratio of 2:1 Aslan and Kapdan [77] increased the concentration of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ . The removal of  $\text{NH}_4^+$  was proportional to the

concentration of  $\text{NH}_4^+$  in the culture media as at a low N:P there was sufficient P to support N removal. On the other hand,  $\text{PO}_4^{3-}$  removal increased until 71.6 ppm  $\text{PO}_4\text{-P}$  was reached then remained constant with increasing nutrient load. Biomass productivity still increased, indicating growth was not limited, suggesting N specifically limited P removal at higher nutrient concentrations. This indicates that having a stoichiometrically unfavourable N:P ratio will be a more significant factor at high nutrient loads, while at low loads algae can sufficiently adapt internal N and P content to be efficient at nutrient removal for a wider range of N:P ratios in wastewater.

### 2.3.6 Species dependence

How the wastewater N:P affects nutrient removal depends on the algal species. When decreasing N:P with a fixed  $\text{NO}_3^-$  concentration, Liu and Vyverman [69] found that N removal increased for *Klebsormidium* sp. and *Pseudanabaena* sp. but decreased for *Cladophora* sp. When increasing N:P with a fixed  $\text{PO}_4^{3-}$  concentration, only *Klebsormidium* sp. had a decrease in P removal. By changing N:P from 2.7:1 to 1:1.1 Whitton et al. [63] found that *Stigeoclonium* sp. adjusted with a decrease in internal N content while retaining a relatively fixed cellular P. More promisingly, *C. vulgaris* and *C. sorokiniana* adjusted with an increase in internal P content while retaining a comparatively fixed cellular N. Although each species in both studies adapted towards the wastewater N:P ratio, they did not all adapt in the same way. Species that are prone to increase nutrient uptake in response to changes in wastewater are better candidates for wastewater remediation.

Beuckels et al. [7] reported that *S. obliquus* had a lower N content while *C. vulgaris* had a lower P content. The concentration of P had a small influence on the rate of N removal for *C. vulgaris*, but none for *S. obliquus*. Analogously, the concentration of N had a greater effect on

P removal for *S. obliquus* than for *C. vulgaris*. This suggests that species with a lower N or P content may rely more on the external concentration of N and P. This could be beneficial, as the algae may have more flexibility in N and P removal, or not, as they may not be able to cope with varying wastewater quality. Whitton et al. [63] related the initial cellular N and P of *C. vulgaris* and *S. obliquus* to nutrient removal, with a higher cellular N:P of *S. obliquus* giving both better  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  removal. This provides another link between cellular content and nutrient remediation for the purpose of species selection. Of the species studied by Liu and Vyverman [69], when the media N:P was greater than 2 *Pseudanabaena* sp. removed N and P at the highest rate and also had the highest cellular N and P. At and below a N:P of 2 it could not adapt sufficiently to the wastewater and *Cladophora* sp., which had the lowest cellular N and P under most conditions, removed the most P. This highlights the importance of matching species with a suitable internal cellular content to the wastewater.

### **2.3.7 General trends for simultaneous N and P removal**

The foregoing discussion suggests several trends for the impact of N and P on their removal from wastewater. At low nutrient concentrations, the ability of algae to adapt their cellular N and P content to the wastewater enables simultaneous N and P removal. This can be limited at low wastewater N:P, as N is needed to promote P assimilation, creating reliance on luxury P uptake. At high wastewater N:P, N can be assimilated until the wastewater and cellular P levels are depleted, although some reduction in the rate of N removal may still occur. At higher nutrient concentrations, simultaneous removal of N and P can be limited by a competitive behaviour, with algae preferentially exerting resources to remove one nutrient over the other, as well as in a proportion to match their structural N:P requirement. This is summarised in Figure 1, as an indicative guide of expected behaviours. There are conflicting

results to these trends as a consequence of differences in conditions, species and reactor designs. These compounding factors also affect the nutrient concentrations and ratios for which each of these observations are relevant.

**Figure 1** Proposed impacts of the N and P ratio and concentration on their simultaneous removal.

## 2.4 Carbon

As carbon is usually the most abundant element in algae it is important to understand the mechanisms of C uptake, which are detailed by Markou et al. [78], and their impact on nutrient remediation. The C:N and C:P ratios in secondary effluent can be relatively low compared to an optimum fresh water algal cell content [29]. This indicates C could have a critical role in the rate of nutrient uptake. Indirect removal of nutrients by volatilisation and precipitation could also be affected by the wastewater C, with a rapid increase in pH largely from release of OH<sup>-</sup> during IC consumption observed in many experiments [19]. Furthermore, with organic carbon (OC) also present in wastewater, the pathways that algae take up C could influence nutrient remediation.

The C and N metabolism of algae are linked [79] and hence the uptake of N is expected to be influenced by the wastewater C:N ratio [80]. The wastewater P concentration impacts C assimilation [79] and many P containing cellular components have a related C content [81]. This suggests that the wastewater C content could impact both N and P removal. This is supported by an increase of the cellular N and P content with addition of CO<sub>2</sub> to *Scenedesmus*

sp. [82], possibly from IC enhancing light utilisation which leads to more energy being available for nutrient assimilation. An increase in IC concentration led to higher N and P removal by a mixed culture [80]. The response in biomass growth of each species in the consortium differed. While IC may directly impact N and P uptake, in an open system this can be compounded further by a change of species present.

IC may also affect the proportion of N to P removed. Increasing the wastewater N:P ratio caused an increase in alkalinity consumption by a mixed algal culture [60]. With a stronger link with N consumption than P consumption, IC may be more important for simultaneous nutrient removal from high N:P wastewater. Gonçalves et al. [83] also observed IC affecting the ratio of N:P removed, and further found the impact to depend on the algal species. For *Synechocystis salina* and *Microcystis aeruginosa* N removal depended on the CO<sub>2</sub> concentration, whereas for *C. vulgaris* and *P. subcapitata* it did not, while P removal by only *M. aeruginosa* was influenced by CO<sub>2</sub> concentration. Changes in the IC concentration caused a species-dependent impact on the cellular N and P content of several marine microalgae [84]. These studies suggest appropriate species selection can reduce the effect of unfavourable IC concentrations.

Improving algal growth, and subsequently nutrient removal, by supplying CO<sub>2</sub> is an option to overcome low C in wastewater that also provides the opportunity for CO<sub>2</sub> capture to reduce greenhouse gas emissions [85]. To take advantage of this a nearby source of flue gas would need to be available, such as through existing anaerobic energy recovery processes. Regardless, additional costs would be required to supply the gas [86]. Hence, unless the C source is available, and abatement is necessary, an algal system would ideally be designed to provide sufficient nutrient removal without the addition of external C.

To test *Chlorella* sp. in wastewater Wu et al. [87] used a light:dark cycle of 12:12 h and OC in the form of glucose. It was found that the rate of N removal increased with higher OC concentrations, with optimum remediation at C:N of 10. In another case, adjusting glucose concentration to vary OC had little impact on N and P removal by *C. vulgaris* [88], possibly due to use of a high light intensity. If assimilation of IC through photosynthesis is dominant OC would be expected to play less of a role [89]. In practice the impact of OC will be more complex than represented in these studies due to the variety of OC compounds that are present in wastewater.

### 3 Environmental conditions

The pH, temperature and light to which an algal culture is exposed can each affect nutrient removal. The influence of these parameters is interlinked, as evident with the temperature and pH changing the optimum light intensity for *S. obtusiusculus* [90]. Furthermore, the effect of each condition depends on the wastewater nutrients, one example being the optimum temperature to maximise biomass productivity of three *Chlorella* species depending on the N concentration [91]. This means that care must be taken when translating remediation performance to different conditions or wastewater sources.

#### 3.1 pH

The pH can affect the capability of algae to assimilate nutrients either by a change in the nature of the algal cell physiology or a change in the form of nutrients available, an example of the latter being the shift between dissolved carbon dioxide and carbonate based on pH. However, the predominant impact of pH on nutrient removal is through the indirect means of phosphate precipitation and  $\text{NH}_3$  volatilisation [19]. In some cases a large increase in pH can cause a release of nutrients [92] as well as cell rupture [93].

When using algae for wastewater treatment it is observed that the pH rapidly increases then remains at a relatively constant value. The results of Abdel Hameed [94] demonstrate this as the pH increased to around 9 in the initial stages of treatment with various configurations of immobilised *C. vulgaris*. When Zhang et al. [95] varied initial pH from 5 to 11 for a culture of *Chlorella* sp., removal of both N and P was not significantly affected. This may imply that the initial pH of wastewater has little influence on treatment performance, which was the case for Infante et al. [96] who found that algal growth increased alkalinity to a similar level regardless of the initial pH. These trends were observed for batch systems; in a continuous



reactor the rapid increase in pH may be less apparent. Whitton [46] observed that after an initial increase in pH the effluent from a continuous algal reactor adjusted towards the pH of the feed.

Often studies only investigate the impact of pH as a consequence of CO<sub>2</sub> sparging rate. Analysis of these results must take into account the IC concentration in combination with the IC, N and P form, which can each change depending on pH [97]. Interestingly, this scenario can lead to a balance of optimum pH for N removal that is dependent on the form of N present. If wastewater is rich in NH<sub>4</sub><sup>+</sup> a higher pH, through less CO<sub>2</sub> addition, may promote volatilisation as NH<sub>3</sub>. Conversely, if wastewater has a higher NO<sub>3</sub><sup>-</sup> concentration a lower pH, through more CO<sub>2</sub> addition, may encourage growth and hence improve N assimilation [80]. When not controlled by CO<sub>2</sub> addition pH had a slightly different impact on N removal. For immobilised *S. obliquus*, Whitton [46] found while a continuous culture had a high pH, the volatilisation of NH<sub>4</sub><sup>+</sup> as NH<sub>3</sub> allowed NO<sub>3</sub><sup>-</sup> to be preferentially assimilated. Once the pH decreased with time a transition to NH<sub>4</sub><sup>+</sup> assimilation occurred, reducing NO<sub>3</sub><sup>-</sup> consumption. It was also noted that the concentration of NO<sub>3</sub><sup>-</sup> compared with NH<sub>4</sub><sup>+</sup> in the influent impacted pH by the respective proportion of H<sup>+</sup> consumption and production during algal N assimilation. As the change in pH depends on which nutrients are present, there will be a wastewater-dependent pH impact on N and P removal.

### **3.2 Temperature**

The temperature of wastewater depends on the local climate of the treatment plant as well as the sources of wastewater being delivered to the plant. The impact of temperature was demonstrated by Ruiz-Martínez et al. [98] using an algal mixture dominated by *Scenedesmus* sp. An optimum temperature for NH<sub>4</sub><sup>+</sup> removal of 31.3 °C achieved a removal rate of 17.5 mg

N/L/d. At 18 °C the removal was significantly lower, at 6.7 mg N/L/d. Much research has focused on the impact of temperature for biomass production, with small variations in temperature having a large influence [99; 100]. Algal growth will directly impact N and P removal, however, growth is not the only factor influenced by temperature. The metabolism of algal cells can also be affected [101] in terms of the allocation of resources within the cells [102] and the activity of enzymes, for example, nitrate reductase activity [103]. Consequently, temperature affects C, N and P assimilation [81].

As the effect of temperature on growth [104] and cellular nutrient content [101; 105] varies between algal species, selecting a species specifically for the expected wastewater temperature could enhance treatment performance. The stimulation of nutrient assimilation through increased intake of light energy can be a factor with low temperature replicating high light conditions for *C. vulgaris*, giving higher photosynthetic rates [106]. This was an explanation for higher N and P uptake at 15 °C than 25 °C observed also for *C. vulgaris* [92]. The link between temperature and photosynthesis could be exploited by using algae that exhibit this behaviour to treat lower temperature wastewater. For several algal species an increase in temperature led to a higher N:P removed [101; 105]. Improved growth at higher temperatures may increase N uptake due to the high requirement for N in functional components of algae while higher photosynthetic rates at low temperature may stimulate P uptake. With a different impact on N than P removal, temperature is an important consideration for simultaneous nutrient removal.

### **3.3 Light**

The method of providing light to an algal culture will significantly influence treatment. Harnessing sunlight, as the cheapest option for algal photosynthesis, can cause reduced

treatment rates if the intensity of light is too low or high [11]. The reactor design can be modified to improve the distribution of light intensity over the entire algal culture [11]. Alternatively, the use of artificial light allows more control over the light for the culture enhancing remediation. It has, however, been reported that for algal biomass production LEDs give 4-6% conversion of electrical to chemical energy [107]. This suggests that sufficient need for intensified nutrient removal is required to promote the use of artificial light, which enables the use of smaller reactors that are more effective for removing N and P. As a highly controllable part of an algal reactor there is significant potential to optimise the design of light provision to improve remediation rates and reduce costs. This has created recent interest in the use of different wavelengths, light-dark cycles, light intensities, and in flashing light at frequencies of the order of microseconds. The light actually received by algal cells will also be affected by the reactor design through factors such as depth, mixing, density and the absorption coefficient of the culture [108-110].

General ranges of light intensity for optimal growth are 200 to 400  $\mu\text{mol}/\text{m}^2/\text{s}$  with potential photo-inhibition at higher irradiance [111]. Each algal species can, however, have a different optimum light intensity [104], with Li et al. [112] finding maximum growth and N removal with *C. kessleri* at an intensity of 120  $\mu\text{mol}/\text{m}^2/\text{s}$  compared with *C. protothecoides* at 200  $\mu\text{mol}/\text{m}^2/\text{s}$ . The optimum light intensity also depends on factors such as temperature [103] and available  $\text{CO}_2$  [113], which can each influence photosynthetic activity, while using different intensities at different growth stages can be a strategy to reduce energy costs [88]. In addition, the optimum light intensity may not be the same for growth, N or P removal. An increase in N and P removal under increased illumination with no change in growth was found by Filippino et al. [92] using *C. vulgaris*, while for several marine algal species cellular C, N and

P content increased with increasing light intensity [81]. These studies suggest energy surplus to that required for general abiotic requirements can be used for additional N and P assimilation. When growth is being limited by another factor it may still be beneficial to increase light supply as this can increase the rate of nutrient removal. This may be more the case for P assimilation, as with several algal species Gonçalves et al. [114] found P uptake was promoted more by light than N uptake. This is possibly because P uptake can be related to energy storage in phospholipids [39]. To take advantage of this behaviour, a species that requires low light for growth but is tolerant to high light conditions could be selected.

Any improvements in nutrient removal and biomass productivity when increasing irradiance levels of artificial light must be balanced against the additional energy cost. This was the case for Whitton [46] using *S. obliquus* to treat wastewater under different intensities of light. Low intensity light at 50  $\mu\text{mol}/\text{m}^2/\text{s}$  had higher photosynthetic efficiency for N (146.8  $\mu\text{g N}/\text{mol photon}$ ) and P removal (38.6  $\mu\text{g P}/\text{mol photon}$ ) despite resulting in slower N and P removal. While one system may be able to operate using less light, another may be able to remove nutrients at greater rates with a more economically sized reactor despite less efficient use of light.

Appropriate selection of light wavelength can increase the rate of nutrient remediation while reducing the energy cost of providing light. Both Yan et al. [88] and Filippino et al. [92] observed that red light gave the highest growth and nutrient removal with *C. vulgaris*. On the contrary, for alginate-immobilised *S. obliquus* blue and white light led to similar  $\text{NH}_4^+$  remediation rates, and higher than for red light, while overall it was found that white light resulted in the greatest utilisation of light energy [46]. The latter result suggests that the use of a single wavelength of light will not provide the most efficient nutrient removal. Stöhr et

al. [115] found that blue light increased  $\text{NO}_3^-$  assimilation for *C. saccharophila* in the presence of background red light. This suggests that having some light in the blue region can improve N assimilation [116]. Kim et al. [117] built on this concept by optimising the rate of N and P removal based on selection of a red to blue light ratio. Using *Scenedesmus* sp. the ratio to maximise N removal was not the same as that for P removal. This supports the idea that different uptake mechanisms are stimulated by different wavelengths of light. It may be possible to utilise this result by optimising the lighting arrangement based on the wastewater N and P concentration.

Light intensity, light to dark period length, and ratio are interlinked, so should not be optimised independently [118]. To elaborate, the length of light period and light intensity should be enough to allow sufficient electron energy to be stored by the algal cell for continued function for the duration of the dark period. When considering duty cycles and flashing frequencies in the order of microseconds this leads to a balance based on light attenuation through electron pooling [119]. Furthermore, with a higher light intensity a more rapid decline in chlorophyll *a* content occurs, a shorter light period and longer dark period is required to allow for the cells to repair and sustain functionality [120]. Highlighting how the combination of several lighting parameters can be used to increase nutrient removal, Zhang et al. [121] investigated nutrient removal by *S. dimorphus* using LEDs with a fixed ratio of red to blue light and varied the light to dark ratios, light intensity and flashing frequency provided to the culture. Ratchford and Fallowfield [122] successfully tested photosynthetic  $\text{O}_2$  evolution of *C. vulgaris* and *Synechococcus* sp. with light and dark periods from 10 to 280 seconds. This has implications for mixing in an algal system; it may also demonstrate that a different order of light to dark length can be considered when optimising light provision.

## **4 Immobilisation**

### **4.1 Methods of immobilisation**

Of the methods of active immobilisation entrapment has a number of advantages and is one of the most commonly studied methods [11]. These advantages include a low cost and simplicity in manufacture that shows promise for large scale production as well meeting key criteria of intensifying the treatment process and allowing easy harvesting of the algal cells [18]. Using natural as opposed to synthetic polymers for entrapment reduces the requirement for hazardous materials in production and avoids additional contaminants being introduced to the wastewater [123]. If the entrapment matrix has a small pore size diffusion of nutrients can be restricted while the pressure exerted on the cells can reduce the number of viable cells and impact the cell morphology. This may occur more often with synthetic rather than natural polymers [123]. While materials such as chitosan, carrageenan and agar have been considered for entrapment, alginate has shown significant promise considering the simplicity of bead manufacture [18]. Entrapment requires cross-linking of the polymeric substance, generally with alginate this is completed by dripping the polymer solution into a polyvalent cation solution [4].

For systems of entrapped algae, Zhang et al. [40] and Castro-Ceseña et al. [124] found that lower cell densities led to higher nutrient removal, possibly due to more space being available for growth or because high cell loading can restrict nutrient movement [94]. As there is an inverse relationship between cell concentration and growth [125], there is an optimal balance of having sufficient initial cells for nutrient uptake while not having too many to slow down growth and hence nutrient assimilation [126]. Increasing the time that a culture of algae is used for treatment reduces the cost of replacing cells and the immobilisation material. For

entrapment, the culture life can be limited by damage to the beads. This can be through rupture from cell growth [40], making another benefit of having a lower initial cell density additional time that a culture can be used. Exposure to contaminants in the wastewater can also limit bead life, through ions that can denature the entrapment matrix or bacteria that lead to deterioration of the bead material [127]. Extending culture life is one of the main motivations behind enhancing entrapment techniques. Some methods employed to do this include selection of the type and concentration of both the polymer and cation used, or coating the beads in a resistant layer [128; 129].

#### **4.2 Impact of immobilisation**

A summary of results from several studies that compared the remediation performance between suspended and immobilised algae is presented in Table 1. As immobilisation can influence the metabolic activity and microenvironment of the cells [14] it will impact nutrient removal depending on the species, wastewater and environmental conditions. As a result, when suspended and entrapped systems were compared there was no common trend in biomass productivity or the ratio of N to P removed (Table 1). For example, Lau et al. [130] found little difference in the performance of suspended and chitosan entrapped *C. vulgaris*, while Fierro et al. [131] found a higher biomass productivity and lower proportion of N than P removed when *Scenedesmus* sp. was entrapped in chitosan. This suggests there is no direct method to predict the performance between suspended and immobilised systems if different conditions are being used.

Yan and Yu [132] found an increase in both N and P removal by immobilising *C. vulgaris* in alginate at all tested wastewater N concentrations. However, at low effluent N:P ratios entrapment caused a decrease in the ratio of N:P removed. At higher effluent N:P ratios

entrapment caused an increase in the ratio of N:P removed. At low ratios P is in excess, and entrapment aids in P removal, possibly by precipitation. At high ratios N is in excess, and entrapment aids in N removal. This may be explained by higher nitrate reductase activity for entrapped cells through either the gel absorbing  $\text{NO}_3^-$  or other changes to the microenvironment [49; 133]. This indicates that entrapment can aid the simultaneous removal of nutrients.

Lau et al. [130] demonstrated nutrient removal will be dependent on the entrapment material as immobilising *C. vulgaris* with carrageenan increased the N:P removed while when alginate was used the N:P removed decreased. This was the case for Jiménez-Pérez et al. [134], where the greater P removal by the immobilised cultures of both *S. intermedius* and *Nannochloris* sp. was mostly explained by precipitation with calcium used for hardening the alginate beads. On the other hand, Mallick and Rai [135] demonstrated a range of immobilisation materials for which the algal system adapted to the wastewater N:P ratio in a relatively consistent manner. That result may have been a consequence of the significantly lower wastewater N and P concentrations. In addition, the conditions used for an immobilisation material can impact nutrient removal, with higher concentrations of the alginate and calcium chloride solutions used for immobilising *S. elongatus* resulting in lower net N and P removal [124].

Chevalier and de la Noüe [136] compared the nutrient removal of *S. obliquus* by suspended and carrageenan-entrapped cells under non-buffered pH 9 and buffered pH 7.7 conditions. It was suggested that  $\text{NH}_4^+$  is attracted to the anionic carrageenan matrix, leading to higher N removal for the entrapped cells when buffered. In addition, the form of IC is mostly  $\text{HCO}_3^-$  at pH 9, consequently it may be repelled by the matrix, causing slower growth and hence influencing  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  removal. As the form of nutrients can influence uptake in



conjunction with the immobilisation material used, the impact of immobilisation will be dependent on the wastewater being treated. With both *C. vulgaris* and *S. obliquus* Ruiz-Marin et al. [137] found that immobilisation in alginate increased the rate of N removal, especially when in real effluent, possibly as immobilisation protects the algae [3], allowing greater growth and N assimilation. Net P removal increased when entrapped for synthetic medium but decreased for real effluent, possibly as in real wastewater P precipitation chemistry is more complex. Regardless, in all cases investigated by Ruiz-Marin et al. [137] entrapment led to a proportional N and P removal closer to that of the effluent N:P, supporting the idea that entrapment can assist in simultaneous nutrient removal.

**Table 1** Comparison of the proportion of N to P removed between suspended and entrapped algal systems.

## 5 Conclusion

The remediation performance of each algal system will be uniquely impacted by wastewater characteristics and environmental conditions. When the objective is to remove both N and P the lowest removal rate will define reactor costs. Hence, it is important to design the algal system for simultaneous removal of these nutrients.

The impact of inorganic and organic C on simultaneous N and P removal has not yet been fully explored. N and P removal is complex as it depends on the amount and ratio of both nutrients in both the wastewater and the algal cells, with algae exhibiting an ability to match cellular content to the wastewater N:P. Identification of this behaviour allows the design of algal systems to be tailored to the wastewater, enabling the treatment process to be intensified, increasing the rate of remediation. This can be harnessed with immobilisation of algal species

selected for their ability to mimic the N:P of the wastewater being treated, allowing for the greatest rate of both N and P removal to occur simultaneously. While further improvement is possible via this method for species selection, the ability of algae to adapt to a range of wastewater nutrient concentrations under a variety of conditions demonstrates their potential to add value to the wastewater treatment process.

When artificial lighting is being provided, optimising light utilisation is of particular interest due to the significant cost savings that can be achieved and the flexibility in design options. Continuing to investigate concurrent optimisation of parameters such as wavelength, intensity and cycling of light will help with this. Areas to focus on include how optimal settings change depending on the type of reactor and species being used, and how to customise lighting to target the wastewater being treated. Methods to deliver the same illumination for minimal capital and operating costs should also be explored.

The practicality of scaling up each algal system cannot be neglected, which includes methods of cell harvesting and resource recovery as well as considering for immobilised systems the frequency and cost of regenerating the culture and any associated materials. Understanding of the cost benefit balance between increasing biomass production and value versus maximising nutrient removal would help to direct optimisation strategies. More work is needed on how to harness some of the additional benefits during nutrient remediation of organic carbon, heavy metal and persistent organic pollutant removal. This would make using algae a more attractive prospect for wastewater treatment.

## 6 References

- [1] EPA Victoria (1995), *Managing sewage discharges to inland waters*, Publication 473, Victoria.
- [2] Christenson, L. and Sims, R. (2011), 'Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts', *Biotechnology Advances*, vol. 29, no. 6, pp. 686-702.
- [3] Zeng, X., Guo, X., Su, G., Danquah, M. K., Zhang, S., Lu, Y., Sun, Y. and Lin, L. (2015), 'Bioprocess considerations for microalgal-based wastewater treatment and biomass production', *Renewable and Sustainable Energy Reviews*, vol. 42, pp. 1385-1392.
- [4] Mallick, N. (2002), 'Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review', *BioMetals*, vol. 15, no. 4, pp. 377-390.
- [5] Wu, Y. H., Hu, H. Y., Yu, Y., Zhang, T. Y., Zhu, S. F., Zhuang, L. L., Zhang, X. and Lu, Y. (2014), 'Microalgal species for sustainable biomass/lipid production using wastewater as resource: A review', *Renewable and Sustainable Energy Reviews*, vol. 33, pp. 675-688.
- [6] Metcalf and Eddy (2003), *Water engineering: treatment and reuse*, 4th edn, revised by G. Tchobanoglous, F. Burtun and H. Stensel, McGraw-Hill, New York.
- [7] Beuckels, A., Smolders, E. and Muylaert, K. (2015), 'Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment', *Water Research*, vol. 77, pp. 98-106.
- [8] Prajapati, S. K., Kaushik, P., Malik, A. and Vijay, V. K. (2013), 'Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges', *Biotechnology Advances*, vol. 31, no. 8, pp. 1408-1425.

- [9] Chiu, S. Y., Kao, C. Y., Chen, T. Y., Chang, Y. B., Kuo, C. M. and Lin, C. S. (2015), 'Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource', *Bioresource Technology*, vol. 184, pp. 179-189.
- [10] Kesaano, M. and Sims, R. C. (2014), 'Algal biofilm based technology for wastewater treatment', *Algal Research*, vol. 5, no. 1, pp. 231-240.
- [11] Cai, T., Park, S. Y. and Li, Y. (2013), 'Nutrient recovery from wastewater streams by microalgae: Status and prospects', *Renewable and Sustainable Energy Reviews*, vol. 19, pp. 360-369.
- [12] Pires, J. C. M., Alvim-Ferraz, M. C. M., Martins, F. G. and Simões, M. (2013), 'Wastewater treatment to enhance the economic viability of microalgae culture', *Environmental Science and Pollution Research*, vol. 20, no. 8, pp. 5096-5105.
- [13] Neveux, N., Magnusson, M., Mata, L., Whelan, A., de Nys, R. and Paul, N. A. (2016), 'The treatment of municipal wastewater by the macroalga *Oedogonium* sp. and its potential for the production of biocrude', *Algal Research*, vol. 13, pp. 284-292.
- [14] Cohen, Y. (2001), 'Biofiltration - The treatment of fluids by microorganisms immobilized into the filter bedding material: a review', *Bioresource Technology*, vol. 77, no. 3, pp. 257-274.
- [15] Schnurr, P. J. and Allen, D. G. (2015), 'Factors affecting algae biofilm growth and lipid production: A review', *Renewable and Sustainable Energy Reviews*, vol. 52, pp. 418-429.
- [16] Hoh, D., Watson, S. and Kan, E. (2016), 'Algal biofilm reactors for integrated wastewater treatment and biofuel production: A review', *Chemical Engineering Journal*, vol. 287, pp. 466-473.

- [17] Moreno-Garrido, I. (2008), 'Microalgae immobilization: Current techniques and uses', *Bioresource Technology*, vol. 99, no. 10, pp. 3949-3964.
- [18] de-Bashan, L. E. and Bashan, Y. (2010), 'Immobilized microalgae for removing pollutants: Review of practical aspects', *Bioresource Technology*, vol. 101, no. 6, pp. 1611-1627.
- [19] Whitton, R., Ometto, F., Pidou, M., Jarvis, P., Villa, R. and Jefferson, B. (2015), 'Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment', *Environmental Technology Reviews*, vol. 4, no. 1, pp. 133-148.
- [20] Hoffmann, J. P. (1998), 'Wastewater treatment with suspended and nonsuspended algae', *Journal of Phycology*, vol. 34, no. 5, pp. 757-763.
- [21] Muñoz, R. and Guieysse, B. (2006), 'Algal–bacterial processes for the treatment of hazardous contaminants: A review', *Water Research*, vol. 40, no. 15, pp. 2799-2815.
- [22] Gonçalves, A. L., Pires, J. C. M. and Simões, M. (2017), 'A review on the use of microalgal consortia for wastewater treatment', *Algal Research*, vol. 24, pp. 403-415.
- [23] Covarrubias, S. A., De-Bashan, L. E., Moreno, M. and Bashan, Y. (2012), 'Alginate beads provide a beneficial physical barrier against native microorganisms in wastewater treated with immobilized bacteria and microalgae', *Applied Microbiology and Biotechnology*, vol. 93, no. 6, pp. 2669-2680.
- [24] de-Bashan, L. E., Hernandez, J. P., Morey, T. and Bashan, Y. (2004), 'Microalgae growth-promoting bacteria as "helpers" for microalgae: A novel approach for removing ammonium and phosphorus from municipal wastewater', *Water Research*, vol. 38, no. 2, pp. 466-474.

- [25] Lee, C. S., Lee, S. A., Ko, S. R., Oh, H. M. and Ahn, C. Y. (2015), 'Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater', *Water Research*, vol. 68, pp. 680-691.
- [26] Liang, Z., Liu, Y., Ge, F., Liu, N. and Wong, M. (2015), 'A pH-dependent enhancement effect of co-cultured *Bacillus licheniformis* on nutrient removal by *Chlorella vulgaris*', *Ecological Engineering*, vol. 75, pp. 258-263.
- [27] Abou-Shanab, R. A. I., Kim, S. H., Ji, M. K., Lee, S. H., Roh, H. S. and Jeon, B. H. (2013), 'Municipal wastewater utilization for biomass and biodiesel production by *Scenedesmus obliquus* HM103382 and *Micractinium reisseri* JN169781', *Journal of Renewable and Sustainable Energy*, vol. 5, no. 5.
- [28] Tam, N. F. Y. and Wong, Y. S. (1989), 'Wastewater nutrient removal by *Chlorella pyrenoidosa* and *Scenedesmus* sp', *Environmental Pollution*, vol. 58, no. 1, pp. 19-34.
- [29] Ji, M. K., Abou-Shanab, R. A. I., Kim, S. H., Salama, E. S., Lee, S. H., Kabra, A. N., Lee, Y. S., Hong, S. and Jeon, B. H. (2013), 'Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO<sub>2</sub> for nutrient removal and biomass production', *Ecological Engineering*, vol. 58, pp. 142-148.
- [30] Park, K. C., Whitney, C., McNichol, J. C., Dickinson, K. E., MacQuarrie, S., Skrupski, B. P., Zou, J., Wilson, K. E., O'Leary, S. J. B. and McGinn, P. J. (2012), 'Mixotrophic and photoautotrophic cultivation of 14 microalgae isolates from Saskatchewan, Canada: Potential applications for wastewater remediation for biofuel production', *Journal of Applied Phycology*, vol. 24, no. 3, pp. 339-348.

- [31] Mennaa, F. Z., Arbib, Z. and Perales, J. A. (2015), 'Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability', *Water Research*, vol. 83, pp. 42-51.
- [32] Rasoul-Amini, S., Montazeri-Najafabady, N., Shaker, S., Safari, A., Kazemi, A., Mousavi, P., Mobasher, M. A. and Ghasemi, Y. (2014), 'Removal of nitrogen and phosphorus from wastewater using microalgae free cells in batch culture system', *Biocatalysis and Agricultural Biotechnology*, vol. 3, no. 2, pp. 126-131.
- [33] Shaker, S., Nemati, A., Montazeri-Najafabady, N., Mobasher, M. A., Morowvat, M. H. and Ghasemi, Y. (2015), 'Treating urban wastewater: Nutrient removal by using immobilized green algae in batch cultures', *International Journal of Phytoremediation*, vol. 17, no. 12, pp. 1177-1182.
- [34] Singh, S. P. and Singh, P. (2015), 'Effect of temperature and light on the growth of algae species: A review', *Renewable and Sustainable Energy Reviews*, vol. 50, pp. 431-444.
- [35] Teoh, M. L., Phang, S. M. and Chu, W. L. (2013), 'Response of Antarctic, temperate, and tropical microalgae to temperature stress', *Journal of Applied Phycology*, vol. 25, no. 1, pp. 285-297.
- [36] Mehrabadi, A., Farid, M. M. and Craggs, R. (2016), 'Variation of biomass energy yield in wastewater treatment high rate algal ponds', *Algal Research*, vol. 15, pp. 143-151.
- [37] de-Bashan, L. E., Trejo, A., Huss, V. A. R., Hernandez, J. P. and Bashan, Y. (2008), 'Chlorella sorokiniana UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater', *Bioresour. Technol.*, vol. 99, no. 11, pp. 4980-4989.

- [38] Ruiz-Martinez, A., Martin Garcia, N., Romero, I., Seco, A. and Ferrer, J. (2012), 'Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent', *Bioresource Technology*, vol. 126, pp. 247-253.
- [39] Solovchenko, A., Verschoor, A. M., Jablonowski, N. D. and Nedbal, L. (2016), 'Phosphorus from wastewater to crops: An alternative path involving microalgae', *Biotechnology Advances*, vol. 34, no. 5, pp. 550-564.
- [40] Zhang, E., Wang, B., Wang, Q., Zhang, S. and Zhao, B. (2008), 'Ammonia-nitrogen and orthophosphate removal by immobilized *Scenedesmus* sp. isolated from municipal wastewater for potential use in tertiary treatment', *Bioresource Technology*, vol. 99, no. 9, pp. 3787-3793.
- [41] Hernandez, J. P., de-Bashan, L. E. and Bashan, Y. (2006), 'Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*', *Enzyme and Microbial Technology*, vol. 38, no. 1-2, pp. 190-198.
- [42] Procházková, G., Brányiková, I., Zachleder, V. and Brányik, T. (2014), 'Effect of nutrient supply status on biomass composition of eukaryotic green microalgae', *Journal of Applied Phycology*, vol. 26, no. 3, pp. 1359-1377.
- [43] Sanz-Luque, E., Chamizo-Ampudia, A., Llamas, A., Galvan, A. and Fernandez, E. (2015), 'Understanding nitrate assimilation and its regulation in microalgae', *Frontiers in Plant Science*, vol. 6, no. 10.
- [44] Jeanfils, J., Canisius, M. F. and Burlion, N. (1993), 'Effect of high nitrate concentrations on growth and nitrate uptake by free-living and immobilized *Chlorella vulgaris* cells', *Journal of Applied Phycology*, vol. 5, no. 3, pp. 369-374.



- [45] Silva, N. F. P., Gonçalves, A. L., Moreira, F. C., Silva, T. F. C. V., Martins, F. G., Alvim-Ferraz, M. C. M., Boaventura, R. A. R., Vilar, V. J. P. and Pires, J. C. M. (2015), 'Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: A kinetic study', *Algal Research*, vol. 11, pp. 350-358.
- [46] Whitton, R. (2016), *Algae reactors for wastewater treatment*, EngD thesis, Cranfield University, Cranfield.
- [47] Kapdan, I. K. and Aslan, S. (2008), 'Application of the Stover-Kincannon kinetic model to nitrogen removal by *Chlorella vulgaris* in a continuously operated immobilized photobioreactor system', *Journal of Chemical Technology and Biotechnology*, vol. 83, no. 7, pp. 998-1005.
- [48] Escudero, A., Blanco, F., Lacalle, A. and Pinto, M. (2014), 'Ammonium removal from anaerobically treated effluent by *Chlamydomonas acidophila*', *Bioresource Technology*, vol. 153, pp. 62-68.
- [49] Yan, G. A. and Yu, J. Y. (1997), 'Comparison of the effects of nitrogen concentration on nutrient removal, growth and physiological characteristics of immobilized and free *Chlorella vulgaris*: II. Chlorophyll content, fluorescence spectra and nitrate reductase activity', *Toxicological and Environmental Chemistry*, vol. 58, no. 1-4, pp. 75-84.
- [50] Collos, Y. and Harrison, P. J. (2014), 'Acclimation and toxicity of high ammonium concentrations to unicellular algae', *Marine Pollution Bulletin*, vol. 80, no. 1-2, pp. 8-23.
- [51] Collier, J. L., Lovindeer, R., Xi, Y., Radway, J. C. and Armstrong, R. A. (2012), 'Differences in growth and physiology of marine *Synechococcus* (cyanobacteria) on nitrate versus ammonium are not determined solely by nitrogen source redox state', *Journal of Phycology*, vol. 48, no. 1, pp. 106-116.

- [52] Källqvist, T. and Svenson, A. (2003), 'Assessment of ammonia toxicity in tests with the microalga, *Nephroselmis pyriformis*, Chlorophyta', *Water Research*, vol. 37, no. 3, pp. 477-484.
- [53] Powell, N., Shilton, A., Chisti, Y. and Pratt, S. (2009), 'Towards a luxury uptake process via microalgae - Defining the polyphosphate dynamics', *Water Research*, vol. 43, no. 17, pp. 4207-4213.
- [54] Martínez Sancho, M. E., Jiménez Castillo, J. M. J. and El Yousfi, F. (1997), 'Influence of phosphorus concentration on the growth kinetics and stoichiometry of the microalga *Scenedesmus obliquus*', *Process Biochemistry*, vol. 32, no. 8, pp. 657-664.
- [55] Zhu, S., Wang, Y., Xu, J., Shang, C., Wang, Z., Xu, J. and Yuan, Z. (2015), 'Luxury uptake of phosphorus changes the accumulation of starch and lipid in *Chlorella* sp. under nitrogen depletion', *Bioresource Technology*, vol. 198, pp. 165-171.
- [56] Yao, B., Xi, B., Hu, C., Huo, S., Su, J. and Liu, H. (2011), 'A model and experimental study of phosphate uptake kinetics in algae: Considering surface adsorption and P-stress', *Journal of Environmental Sciences*, vol. 23, no. 2, pp. 189-198.
- [57] Ruiz-Martínez, A., Serralta, J., Romero, I., Seco, A. and Ferrer, J. (2015), 'Effect of intracellular P content on phosphate removal in *Scenedesmus* sp. Experimental study and kinetic expression', *Bioresource Technology*, vol. 175, pp. 325-332.
- [58] Kunikane, S., Kaneko, M. and Maehara, R. (1984), 'Growth and nutrient uptake of green alga, *Scenedesmus dimorphus*, under a wide range of nitrogen/phosphorus ratio-I. Experimental study', *Water Research*, vol. 18, no. 10, pp. 1299-1311.
- [59] Choi, H. J. and Lee, S. M. (2015), 'Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater', *Bioprocess and Biosystems Engineering*, vol. 38, no. 4, pp. 761-766.

- [60] Fulton, L. (2009), Nutrient removal by algae grown in CO<sub>2</sub>-enriched wastewater over a range of nitrogen-to-phosphorus ratios, Masters thesis, California Polytechnic State University, San Luis Obispo.
- [61] Xin, L., Hong-ying, H., Ke, G. and Ying-xue, S. (2010), 'Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp.', *Bioresource Technology*, vol. 101, no. 14, pp. 5494-5500.
- [62] Dickinson, K. E., Whitney, C. G. and McGinn, P. J. (2013), 'Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga *Scenedesmus* sp. AMDD', *Algal Research*, vol. 2, no. 2, pp. 127-134.
- [63] Whitton, R., Le Mével, A., Pidou, M., Ometto, F., Villa, R. and Jefferson, B. (2016), 'Influence of microalgal N and P composition on wastewater nutrient remediation', *Water Research*, vol. 91, pp. 371-378.
- [64] Wettern, M. (1980), 'Lipid variation of the green alga *Fritschiella tuberosa* during growth in axenic batch culture', *Phytochemistry*, vol. 19, no. 4, pp. 513-517.
- [65] Muro-Pastor, M. I., Reyes, J. C. and Florencio, F. J. (1996), 'The NADP<sup>+</sup>-isocitrate dehydrogenase gene (*icd*) is nitrogen regulated in cyanobacteria', *Journal of Bacteriology*, vol. 178, no. 14, pp. 4070-4076.
- [66] Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J. and Perales, J. A. (2013), 'Photobiotreatment: Influence of Nitrogen and Phosphorus ratio in wastewater on growth kinetics of *Scenedesmus obliquus*', *International Journal of Phytoremediation*, vol. 15, no. 8, pp. 774-788.
- [67] Rausch, C. and Bucher, M. (2002), 'Molecular mechanisms of phosphate transport in plants', *Planta*, vol. 216, no. 1, pp. 23-37.

- [68] Wang, M., Kuo-Dahab, W. C., Dolan, S. and Park, C. (2014), 'Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, *Chlorella* sp. and *Micractinium* sp., in wastewater treatment', *Bioresource Technology*, vol. 154, pp. 131-137.
- [69] Liu, J. and Vyverman, W. (2015), 'Differences in nutrient uptake capacity of the benthic filamentous algae *Cladophora* sp., *Klebsormidium* sp. and *Pseudanabaena* sp. under varying N/P conditions', *Bioresource Technology*, vol. 179, pp. 234-242.
- [70] Sterner, R. W. and Elser, J. J. (2017), *Ecological Stoichiometry - The Biology of Elements from Molecules to the Biosphere*, Princeton University Press, Princeton.
- [71] Perini, V. and Bracken, M. E. S. (2014), 'Nitrogen availability limits phosphorus uptake in an intertidal macroalga', *Oecologia*, vol. 175, no. 2, pp. 667-676.
- [72] Loladze, I. and Elser, J. J. (2011), 'The origins of the Redfield nitrogen-to-phosphorus ratio are in a homeostatic protein-to-rRNA ratio', *Ecology Letters*, vol. 14, no. 3, pp. 244-250.
- [73] Zhang, Q. and Hong, Y. (2014), 'Effects of stationary phase elongation and initial nitrogen and phosphorus concentrations on the growth and lipid-producing potential of *Chlorella* sp. HQ', *Journal of Applied Phycology*, vol. 26, no. 1, pp. 141-149.
- [74] Klausmeyer, C. A., Litchman, E., Daufreshna, T. and Levin, S. A. (2004), 'Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton', *Nature*, vol. 429, no. 6988, pp. 171-174.
- [75] Cabanelas, I. T. D., Ruiz, J., Arbib, Z., Chinalia, F. A., Garrido-Pérez, C., Rogalla, F., Nascimento, I. A. and Perales, J. A. (2013), 'Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal', *Bioresource Technology*, vol. 131, pp. 429-436.

- [76] Boelee, N. C., Temmink, H., Janssen, M., Buisman, C. J. and Wijffels, R. H. (2011), 'Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms', *Water Research*, vol. 45, no. 18, pp. 5925-5933.
- [77] Aslan, S. and Kapdan, I. K. (2006), 'Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae', *Ecological Engineering*, vol. 28, no. 1, pp. 64-70.
- [78] Markou, G., Vandamme, D. and Muylaert, K. (2014), 'Microalgal and cyanobacterial cultivation: The supply of nutrients', *Water Research*, vol. 65, pp. 186-202.
- [79] Hu, H. and Zhou, Q. (2010), 'Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in the *Nannochloropsis* sp', *World Journal of Microbiology and Biotechnology*, vol. 26, no. 5, pp. 957-961.
- [80] Sutherland, D. L., Howard-Williams, C., Turnbull, M. H., Broady, P. A. and Craggs, R. J. (2015), 'The effects of CO<sub>2</sub> addition along a pH gradient on wastewater microalgal photo-physiology, biomass production and nutrient removal', *Water Research*, vol. 70, pp. 9-26.
- [81] Cade-Menun, B. J. and Paytan, A. (2010), 'Nutrient temperature and light stress alter phosphorus and carbon forms in culture-grown algae', *Marine Chemistry*, vol. 121, no. 1-4, pp. 27-36.
- [82] Posadas, E., Morales, M. D. M., Gomez, C., Ación, F. G. and Muñoz, R. (2015), 'Influence of pH and CO<sub>2</sub> source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways', *Chemical Engineering Journal*, vol. 265, pp. 239-248.
- [83] Gonçalves, A. L., Rodrigues, C. M., Pires, J. C. M. and Simões, M. (2016), 'The effect of increasing CO<sub>2</sub> concentrations on its capture, biomass production and wastewater

- bioremediation by microalgae and cyanobacteria', *Algal Research*, vol. 14, pp. 127-136.
- [84] Burkhardt, S., Zondervan, I. and Riebesell, U. (1999), 'Effect of CO<sub>2</sub> concentration on C:N:P ratio in marine phytoplankton: A species comparison', *Limnology and Oceanography*, vol. 44, no. 3 I, pp. 683-690.
- [85] Razzak, S. A., Hossain, M. M., Lucky, R. A., Bassi, A. S. and De Lasa, H. (2013), 'Integrated CO<sub>2</sub> capture, wastewater treatment and biofuel production by microalgae culturing - A review', *Renewable and Sustainable Energy Reviews*, vol. 27, pp. 622-653.
- [86] Judd, S., van den Broeke, L. J. P., Shurair, M., Kuti, Y. and Znad, H. (2015), 'Algal remediation of CO<sub>2</sub> and nutrient discharges: A review', *Water Research*, vol. 87, pp. 356-366.
- [87] Wu, H. H., Gau, S. H., Li, M. G., Chen, Y. J. and Sun, C. J. (2015), 'Effects of *Chlorella* sp. on nutrient treatment in cultures with different carbon to nitrogen ratios', *Water Science and Technology*, vol. 71, no. 11, pp. 1597-1603.
- [88] Yan, C., Zhang, L., Luo, X. and Zheng, Z. (2013), 'Effects of various LED light wavelengths and intensities on the performance of purifying synthetic domestic sewage by microalgae at different influent C/N ratios', *Ecological Engineering*, vol. 51, pp. 24-32.
- [89] Ogbonna, J. C., Yoshizawa, H. and Tanaka, H. (2000), 'Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms', *Journal of Applied Phycology*, vol. 12, no. 3-5, pp. 277-284.
- [90] Cabello, J., Toledo-Cervantes, A., Sánchez, L., Revah, S. and Morales, M. (2015), 'Effect of the temperature, pH and irradiance on the photosynthetic activity by *Scenedesmus obtusiusculus* under nitrogen replete and deplete conditions', *Bioresource Technology*, vol. 181, pp. 128-135.

- [91] Ördög, V., Stirk, W. A., Bálint, P., Aremu, A. O., Okem, A., Lovász, C., Molnár, Z. and van Staden, J. (2016), 'Effect of temperature and nitrogen concentration on lipid productivity and fatty acid composition in three *Chlorella* strains', *Algal Research*, vol. 16, pp. 141-149.
- [92] Filippino, K. C., Mulholland, M. R. and Bott, C. B. (2015), 'Phycoremediation strategies for rapid tertiary nutrient removal in a waste stream', *Algal Research*, vol. 11, pp. 125-133.
- [93] Martínez, M. E., Sánchez, S., Jiménez, J. M., El Yousfi, F. and Muñoz, L. (2000), 'Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*', *Bioresource Technology*, vol. 73, no. 3, pp. 263-272.
- [94] Abdel Hameed, M. S. (2007), 'Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal', *African Journal of Biotechnology*, vol. 6, no. 10, pp. 1185-1191.
- [95] Zhang, Q., Wang, T. and Hong, Y. (2014), 'Investigation of initial pH effects on growth of an oleaginous microalgae *Chlorella* sp. HQ for lipid production and nutrient uptake', *Water Science and Technology*, vol. 70, no. 4, pp. 712-719.
- [96] Infante, C., León, I., Florez, J., Zárate, A., Barrios, F. and Zapata, C. (2013), 'Removal of ammonium and phosphate ions from wastewater samples by immobilized *Chlorella* sp', *International Journal of Environmental Studies*, vol. 70, no. 1, pp. 1-7.
- [97] Shene, C., Chisti, Y., Bustamante, M. and Rubilar, M. (2016), 'Effect of CO<sub>2</sub> in the aeration gas on cultivation of the microalga *Nannochloropsis oculata*: Experimental study and mathematical modeling of CO<sub>2</sub> assimilation', *Algal Research*, vol. 13, pp. 16-29.

- [98] Ruiz-Martínez, A., Serralta, J., Seco, A. and Ferrer, J. (2015), 'Effect of temperature on ammonium removal in *Scenedesmus* sp', *Bioresource Technology*, vol. 191, pp. 346-349.
- [99] Renaud, S. M., Thinh, L. V., Lambrinidis, G. and Parry, D. L. (2002), 'Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures', *Aquaculture*, vol. 211, no. 1-4, pp. 195-214.
- [100] Teoh, M. L., Chu, W. L. and Phang, S. M. (2010), 'Effect of temperature change on physiology and biochemistry of algae: A review', *Malaysian Journal of Science*, vol. 29, no. 2, pp. 82-97.
- [101] Gonçalves, A. L., Pires, J. C. M. and Simões, M. (2016), 'The effects of light and temperature on microalgal growth and nutrient removal: An experimental and mathematical approach', *RSC Advances*, vol. 6, no. 27, pp. 22896-22907.
- [102] Raven, J. A. and Geider, R. J. (1988), 'Temperature and algal growth', *New Phytologist*, vol. 110, no. 4, pp. 441-461.
- [103] Vona, V., di Martino Rigano, V., Lobosco, O., Carfagna, S., Esposito, S. and Rigano, C. (2004), 'Temperature responses of growth, photosynthesis, respiration and NADH: Nitrate reductase in cryophilic and mesophilic algae', *New Phytologist*, vol. 163, no. 2, pp. 325-331.
- [104] Talbot, P., Thébault, J. M., Dauta, A. and de la Noüe, J. (1991), 'A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment', *Water Research*, vol. 25, no. 4, pp. 465-472.



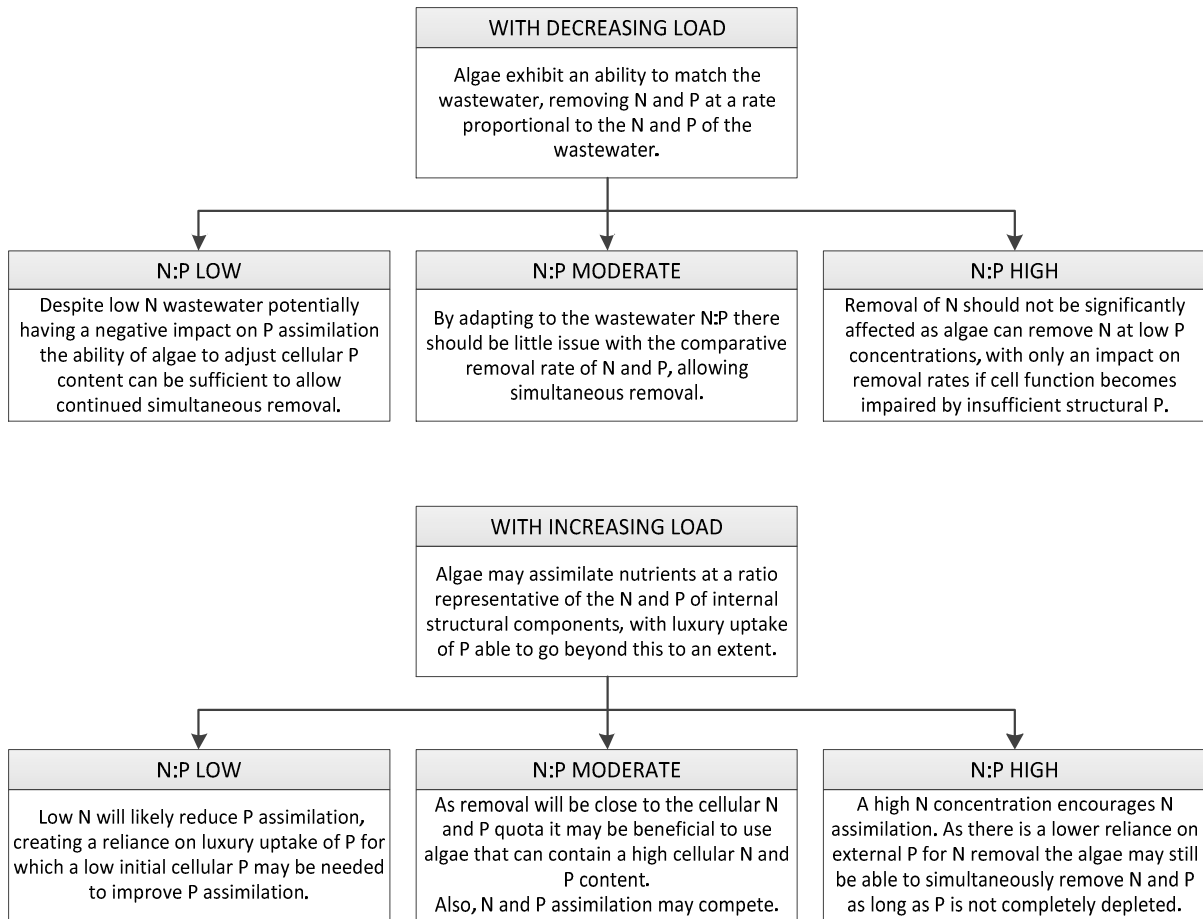
- [105] de la Noüe, J. and Bassères, A. (1989), 'Biotreatment of anaerobically digested swine manure with microalgae', *Biological Wastes*, vol. 29, no. 1, pp. 17-31.
- [106] Maxwell, D. P., Falk, S., Trick, C. G. and Huner, N. P. A. (1994), 'Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*', *Plant Physiology*, vol. 105, no. 2, pp. 535-543.
- [107] Blanken, W., Cuaresma, M., Wijffels, R. H. and Janssen, M. (2013), 'Cultivation of microalgae on artificial light comes at a cost', *Algal Research*, vol. 2, no. 4, pp. 333-340.
- [108] Béchet, Q., Shilton, A. and Guieysse, B. (2013), 'Modeling the effects of light and temperature on algae growth: State of the art and critical assessment for productivity prediction during outdoor cultivation', *Biotechnology Advances*, vol. 31, no. 8, pp. 1648-1663.
- [109] Huesemann, M. H., Van Wagenen, J., Miller, T., Chavis, A., Hobbs, S. and Crowe, B. (2013), 'A screening model to predict microalgae biomass growth in photobioreactors and raceway ponds', *Biotechnology and Bioengineering*, vol. 110, no. 6, pp. 1583-1594.
- [110] Sutherland, D. L., Montemezzani, V., Howard-Williams, C., Turnbull, M. H., Broady, P. A. and Craggs, R. J. (2015), 'Modifying the high rate algal pond light environment and its effects on light absorption and photosynthesis', *Water Research*, vol. 70, pp. 86-96.
- [111] Gordon, J. M. and Polle, J. E. W. (2007), 'Ultrahigh bioproductivity from algae', *Applied Microbiology and Biotechnology*, vol. 76, no. 5, pp. 969-975.
- [112] Li, Y., Zhou, W., Hu, B., Min, M., Chen, P. and Ruan, R. R. (2012), 'Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains *Chlorella kessleri* and *Chlorella protothecoide* cultivated in highly concentrated

- municipal wastewater', *Biotechnology and Bioengineering*, vol. 109, no. 9, pp. 2222-2229.
- [113] Chang, H. X., Huang, Y., Fu, Q., Liao, Q. and Zhu, X. (2016), 'Kinetic characteristics and modeling of microalgae *Chlorella vulgaris* growth and CO<sub>2</sub> biofixation considering the coupled effects of light intensity and dissolved inorganic carbon', *Bioresource Technology*, vol. 206, pp. 231-238.
- [114] Gonçalves, A. L., Simões, M. and Pires, J. C. M. (2014), 'The effect of light supply on microalgal growth, CO<sub>2</sub> uptake and nutrient removal from wastewater', *Energy Conversion and Management*, vol. 85, pp. 530-536.
- [115] Stöhr, C., Glogau, U., Mätschke, M. and Tischner, R. (1995), 'Evidence for the involvement of plasma-membrane-bound nitrate reductase in signal transduction during blue-light stimulation of nitrate uptake in *Chlorella saccharophila*', *Planta*, vol. 197, no. 4, pp. 613-618.
- [116] Aparicio, P. J., Roldán, J. M. and Calero, F. (1976), 'Blue light photoreactivation of nitrate reductase from green algae and higher plants', *Biochemical and Biophysical Research Communications*, vol. 70, no. 4, pp. 1071-1077.
- [117] Kim, T. H., Lee, Y., Han, S. H. and Hwang, S. J. (2013), 'The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment', *Bioresource Technology*, vol. 130, pp. 75-80.
- [118] Yan, C. and Zheng, Z. (2013), 'Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella* sp', *Bioresource Technology*, vol. 139, pp. 292-299.

- [119] Vejrazka, C., Streefland, M., Wijffels, R. H. and Janssen, M. (2015), 'The role of an electron pool in algal photosynthesis during sub-second light-dark cycling', *Algal Research*, vol. 12, pp. 43-51.
- [120] Shriwastav, A. and Bose, P. (2015), 'Algal growth in photo-bioreactors: Impact of illumination strategy and nutrient availability', *Ecological Engineering*, vol. 77, pp. 202-215.
- [121] Zhang, S., Kim, T. H., Han, T. H. and Hwang, S. J. (2015), 'Influence of light conditions of a mixture of red and blue light sources on nitrogen and phosphorus removal in advanced wastewater treatment using *Scenedesmus dimorphus*', *Biotechnology and Bioprocess Engineering*, vol. 20, no. 4, pp. 760-765.
- [122] Ratchford, I. A. J. and Fallowfield, H. J. (2003), 'The effect of light:dark cycles of medium frequency on photosynthesis by *Chlorella vulgaris* and the implications for waste stabilisation pond design and performance', *Water Science and Technology*, vol. 48, pp. 69-74.
- [123] Leenen, E. J. T. M., Dos Santos, V. A. P., Grolle, K. C. F., Tramper, J. and Wijffels, R. H. (1996), 'Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment', *Water Research*, vol. 30, no. 12, pp. 2985-2996.
- [124] Castro-Ceseña, A. B., del Pilar Sánchez-Saavedra, M. and Ruíz-Güereca, D. A. (2015), 'Optimization of entrapment efficiency and evaluation of nutrient removal (N and P) of *Synechococcus elongatus* in novel core-shell capsules', *Journal of Applied Phycology*, pp. 1-9.

- [125] Aravantinou, A. F., Theodorakopoulos, M. A. and Manariotis, I. D. (2013), 'Selection of microalgae for wastewater treatment and potential lipids production', *Bioresource Technology*, vol. 147, pp. 130-134.
- [126] Choi, H. J. and Lee, S. M. (2012), 'Effects of microalgae on the removal of nutrients from wastewater: Various concentrations of *Chlorella vulgaris*', *Environmental Engineering Research*, vol. 17, no. S1, pp. 3-8.
- [127] Cruz, I., Bashan, Y., Hernández-Carmona, G. and de-Bashan, L. E. (2013), 'Biological deterioration of alginate beads containing immobilized microalgae and bacteria during tertiary wastewater treatment', *Applied Microbiology and Biotechnology*, vol. 97, no. 22, pp. 9847-9858.
- [128] Moreira, S. M., Moreira-Santos, M., Guilhermino, L. and Ribeiro, R. (2006), 'Immobilization of the marine microalga *Phaeodactylum tricornutum* in alginate for in situ experiments: Bead stability and suitability', *Enzyme and Microbial Technology*, vol. 38, no. 1-2, pp. 135-141.
- [129] Vílchez, C., Garbayo, I., Markvicheva, E., Galván, F. and León, R. (2001), 'Studies on the suitability of alginate-entrapped *Chlamydomonas reinhardtii* cells for sustaining nitrate consumption processes', *Bioresource Technology*, vol. 78, no. 1, pp. 55-61.
- [130] Lau, P. S., Tam, N. F. Y. and Wong, Y. S. (1997), 'Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris*', *Environmental Technology*, vol. 18, no. 9, pp. 945-951.
- [131] Fierro, S., del Pilar Sánchez-Saavedra, M. and Copalcúa, C. (2008), 'Nitrate and phosphate removal by chitosan immobilized *Scenedesmus*', *Bioresource Technology*, vol. 99, no. 5, pp. 1274-1279.

- [132] Yan, G. A. and Yu, J. Y. (1997), 'Comparison of the effects of nitrogen concentration on nutrient removal, growth and physiological characteristics of immobilized and free *Chlorella vulgaris*: I. Growth and nutrient removal', *Toxicological and Environmental Chemistry*, vol. 58, no. 1-4, pp. 63-74.
- [133] Petrovič, A. and Simonič, M. (2015), 'The effect of carbon source on nitrate and ammonium removal from drinking water by immobilised *Chlorella sorokiniana*', *International Journal of Environmental Science and Technology*, vol. 12, no. 10, pp. 3175-3188.
- [134] Jiménez-Pérez, M. V., Sánchez-Castillo, P., Romera, O., Fernández-Moreno, D. and Pérez-Martínez, C. (2004), 'Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure', *Enzyme and Microbial Technology*, vol. 34, no. 5, pp. 392-398.
- [135] Mallick, N. and Rai, L. C. (1994), 'Removal of inorganic ions from wastewaters by immobilized microalgae', *World Journal of Microbiology & Biotechnology*, vol. 10, no. 4, pp. 439-443.
- [136] Chevalier, P. and de la Noüe, J. (1985), 'Wastewater nutrient removal with microalgae immobilized in carrageenan', *Enzyme and Microbial Technology*, vol. 7, no. 12, pp. 621-624.
- [137] Ruiz-Marin, A., Mendoza-Espinosa, L. G. and Stephenson, T. (2010), 'Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater', *Bioresource Technology*, vol. 101, no. 1, pp. 58-64.
- [138] Lau, P. S., Tam, N. F. Y. and Wong, Y. S. (1998), 'Effect of carrageenan immobilization on the physiological activities of *Chlorella vulgaris*', *Bioresource Technology*, vol. 63, no. 2, pp. 115-121.



**Figure 2** Proposed impacts of the N and P ratio and concentration on their simultaneous removal.



**Table 1** Comparison of the proportion of N to P removed between suspended and entrapped algal systems.

Design wastewater batch duration initial inoculum	Conditions temperature light intensity light:dark period	Species	Initial Concentration (ppm)			Immobilisation	Removal N/P	Productivity (inoculum units per day)	Reference		
			TN (NH <sub>4</sub> -N/NO <sub>3</sub> -N)	P	N:P						
Synthetic 30 days ~0.035 (µg chl <i>a</i> )/mL	20 °C 100 µmol/m <sup>2</sup> /s 24:0 h	<i>Synechococcus elongatus</i>	13.0 (4.8/8.2)	3.2	4.1	Suspended	0.5	0.01	[124]		
						Alginate Entrapped (2/1)*	1.6	0.4			
						Alginate Entrapped (2/2)*	1.4	0.4			
						Alginate Entrapped (4/2)*	2.1	0.18			
Synthetic 106 h 10 <sup>6</sup> cells/mL	32 °C 43 24:0 h	<i>Scenedesmus</i> sp.	44 (0/44)	6	7.3	Suspended	8.6	2x10 <sup>5</sup>	[131]		
						Chitosan Entrapped	5.4	5x10 <sup>5</sup>			
Synthetic 10 days 29.6 (µg chl <i>a</i> )/mL	20 °C 120 µmol/m <sup>2</sup> /s 14:10 h	<i>S. intermedius</i>	38.8 (0/38.8)	49.9	0.78	Suspended	4.0	40	[134]		
		<i>Nannochloris</i> sp.				Alginate Entrapped	0.5	10			
						Suspended	5.5	24			
						Alginate Entrapped	0.6	11			
Primary settled 1st day 10 <sup>6</sup> cells/mL	24 °C 90 µmol/m <sup>2</sup> /s 16:8 h	<i>C. vulgaris</i>	37.5-47.5 (37.5-47.5, ~0)	3.1-4.3	11.5	Suspended	9.7	3x10 <sup>5</sup>	[130]		
						Alginate Entrapped	5.0	3x10 <sup>5</sup>			
						Carrageenan Entrapped	13.4	3x10 <sup>5</sup>			
Synthetic 4 days 5x10 <sup>6</sup> cells/mL	25 °C 100 µmol/m <sup>2</sup> /s 16:8 h	<i>C. vulgaris</i>	11.5 (0/11.5)	5.6	2.1	Suspended	4.2	4.5x10 <sup>6</sup>	[138]		
						Chitosan Entrapped	4.2	4.5x10 <sup>6</sup>			
Synthetic 48 h ~2x10 <sup>6</sup> cells/mL  Secondary effluent 48 h ~2x10 <sup>6</sup> cells/mL	25 °C 135 µmol/m <sup>2</sup> /s 24:0 h	<i>C. vulgaris</i>	30.1 (30.1/0)	3.4	8.9	Suspended	22.3	2.2x10 <sup>6</sup>	[137]		
		<i>S. obliquus</i>				Alginate Entrapped	14.1	6.6x10 <sup>6</sup>			
						Suspended	5.0	3.9x10 <sup>6</sup>			
						Alginate Entrapped	11.1	1.8x10 <sup>6</sup>			
	<i>C. vulgaris</i>	Suspended	6.6	1.7x10 <sup>6</sup>							
		Alginate Entrapped	12.5	5.4x10 <sup>6</sup>							
		Suspended	3.4	1.9x10 <sup>6</sup>							
		Alginate Entrapped	15.1	2.9x10 <sup>6</sup>							
Synthetic 6 days 6x10 <sup>6</sup> cells/mL	22 °C 3 klx 24:0 h	<i>C. vulgaris</i>	10 (3.5/2)	10	1	Suspended	1.0	1.4x10 <sup>6</sup>	[132]		
			80 (28/16)			10	8	Alginate Entrapped		0.7	1.3x10 <sup>6</sup>
								Suspended		4.2	8.5x10 <sup>5</sup>
								Alginate Entrapped		8.0	9.2x10 <sup>5</sup>
Wastewater 24 h ~1.5 (µg chl <i>a</i> )/mL	26 °C 72 µmol/m <sup>2</sup> /s 14:10 h	<i>C. vulgaris</i>	0.96 (0.33/0.47)	1.0	0.95	Suspended	1.3	2.7	[135]		
						Chitosan Entrapped	1.3	3.2			
						Carrageenan Entrapped	1.5	2.8			
						Agar Entrapped	0.8	1.3			

\*Concentration mass percentage of alginate and calcium chloride, respectively



