Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment

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Abstract

Microalgae offer a promising technology to remove and re-use the nutrients N and P from wastewater. For effective removal of both N and P, it is important that microalgae can adjust the N and P concentration in their biomass to the N and P supply in the wastewater. The aim of this study was to evaluate to what extent microalgae can adjust the N and P concentrations in their biomass to the N and P supply in the wastewater, and to what extent supply of one nutrient influences the removal of the other nutrient. Using *Chlorella* and *Scenedesmus* as model organisms, we quantified growth and biomass composition in medium with different initial N and P concentrations in all possible combinations. Nutrient supply marginally affected biomass yield of both microalgae but had a strong influence on the composition of the biomass. The nutrient concentrations in the biomass ranged 5.0 – 10.1 % for N and 0.5 – 1.3 % for P in *Chlorella* and 2.9 – 8.4 % for N and 0.5 – 1.7 % for P in *Scenedesmus*. The concentrations of P in the biomass remained low and were relatively constant (0.6 – 0.8 % P) when the N concentration in the biomass was low. As a result, removal of P from the wastewater was influenced by the concentration of N in the wastewater. When the initial N concentration in the wastewater was above 40 mg L^{-1} the microalgae could remove up to 6 mg P L^{-1}, but this removal was only 2 mg P L^{-1} when the initial N concentration was below 20 mg L^{-1}. A lower N supply increased the carbohydrate concentration to about 40% and lipid concentration to about 30% for both species, compared to around 15% and 10% respectively at high N supply. Our results show that sufficiently high N concentrations are needed to ensure effective P removal from wastewater due to the positive effect of N on the accumulation of P.

Highlights

- N removal was independent of P supply, P removal improved with increased N supply
- Biomass N and P concentration was adjusted to the nutrient supply
- Biomass concentration ranged only a factor 2
- *Chlorella* has a higher N concentration and lower P concentration compared to *Scenedesmus*

Keywords: wastewater treatment, microalgae, nutrient removal, biomass composition

1. Introduction

Although the idea to use microalgae for wastewater treatment is not new and dates back almost half a century (Oswald et al., 1957), interest in this concept has revived in recent years. Microalgae-based wastewater treatment has several advantages over conventional wastewater treatment using activated sludge. First, microalgae-based wastewater treatment has a lower energy demand because oxygen is supplied through photosynthesis rather than energy-intensive electromechanical blowers (Green et al., 1995). Second, microalgae not only remove nitrogen (N) and phosphorus (P) from wastewater, but also recycle these nutrients in a biomass that can be converted into energy, raw chemicals or other products (Martins et al., 2010). These advantages are very attractive in times when there is a need to increase energy efficiency and to “close the loop” by recycling elements from waste streams. In this respect, the combination of microalgae wastewater treatment with the production of microalgal biofuels has attracted much attention in recent years (Craggs et al., 2013; Pittman et al., 2011).

In conventional wastewater treatment, N and P are removed from wastewater in two separate processes. Usually, N is converted to N_2 gas through coupled nitrification-denitrification while P is precipitated with metal salts. Microalgae, on the contrary, remove N and P from wastewater in a single process. Microalgae absorb N and P from wastewater and use these nutrients to produce biomass. Because microalgae require both N and P to produce new biomass, removal of one nutrient depends on the availability of the other: microalgae cannot remove N without the presence of P in the wastewater, or vice versa, because both nutrients are essential for their growth. The concentrations of N and P in wastewater are quite variable. In domestic wastewater, N concentrations vary between 15 and 90 mg L^{-1} and P concentrations between 4 and 20 mg L^{-1} (Abdelaziz et al., 2013; Cai et al., 2013; Christenson and Sims, 2011). Because the sources of N and P in wastewater are different, N and P concentrations often vary independently from each other. Human excreta are a source of both P and N while detergents, soaps and personal care products contain P but little N (Smil, 2000; Tjandraatmadja et al., 2010). Because N and P removal are coupled in microalgae, the fact that N and P concentrations in wastewater vary independent from each other poses a challenge when engineering nutrient removal from wastewater using microalgae.

Removal of N and P by microalgae depends on the concentrations of these nutrients in the microalgal biomass. The N and P requirements of microalgae have been an active research field in microalgal ecology and physiology for over half a century. In 1958, Redfield studied nutrient concentrations in marine microalgae and found a ratio of about 106:16:1 C:N:P (molar ratio) in the biomass, a ratio that is since then known as the Redfield ratio (Redfield, 1958). It soon became apparent, however, that this ratio is not fixed and that nutrient concentrations in microalgal biomass can be quite variable, particularly in freshwater species (Rhee, 1978). Microalgae have the ability to adjust the N and P concentration of their biomass depending on the supply of nutrients in the medium, resulting in a low nutrient concentration in the biomass when the supply of a nutrient is low and a higher concentration when the supply is high (e.g. Geider and La Roche, 2002; Rhee, 1978; Sterner and Elser, 2002). The microalgal P concentration can range from 0.03% to more than 3% of dry biomass and the microalgal N concentration can range from 3% up to 12% (Reynolds, 2006). Because N and P are used by microalgae to produce various biochemical compounds, changes in the concentrations of N and P in the biomass will influence the biochemical composition of the biomass (Klausmeier et al., 2004; Loladze and Elser, 2011). Nitrogen is predominantly used for synthesis of proteins while P is mainly incorporated into ribosomal RNA. When either N or P are limiting, the protein content of the cell is reduced and cell division slows down but C acquisition through photosynthesis continues. Therefore, cells tend to accumulate C-rich metabolites such as carbohydrates or lipids when the supply of N and P is low (González-

Fernández and Ballesteros, 2012; Smith et al., 2010). Induction of N or P limitation is commonly used in microalgal production for biofuels to increase the carbohydrate or lipid yield (Craggs et al., 2013).

The flexibility of the N and P concentration of microalgal biomass allows microalgae to adjust their intracellular N and P concentration to the supply of N and P in the wastewater. This flexibility is essential to ensure simultaneous removal of N and P from wastewater. Although there have been a large number of studies on N and P removal from wastewater by microalgae, relatively few studies have specifically studied the flexibility of the N and P concentration in microalgal biomass in response to the supply of N or P in the wastewater. Several studies have shown that a lower N or P supply in wastewater results in a lower N or P concentration of the microalgal biomass and this is often associated with an accumulation of carbohydrates and/or lipids (Akerström et al., 2014; Arbib et al., 2013; Aslan and Kapdan, 2006; Dickinson et al., 2013; Samorì et al., 2013; Xin et al., 2010). Other studies have investigated to what extent microalgae can accumulate excess nutrients in their biomass, known as luxury uptake, which is interesting for removal of nutrients from high-strength wastewaters. Some species are capable of accumulating P as polyphosphate granules when the P supply is high and can accumulate up to 3% P in the biomass (Eixler et al., 2006; Powell et al., 2009). Other species of microalgae, such as some diatom species, can accumulate nitrate in their central vacuole (Coppens et al., 2014). In most studies, the concentration of only one nutrient is varied while the other is maintained constant (Arbib et al., 2013; Samorì et al., 2013; Xin et al., 2010; Zhang and Hong, 2014) or the concentration of both nutrients is varied simultaneously without changing their ratio (Akerström et al., 2014; Aslan and Kapdan, 2006). No studies have investigated to what extent the supply of one nutrient influences the uptake of the other nutrient.

The aim of this study was to evaluate to what extent microalgae can adjust the concentration of N and P in their biomass to the supply of these nutrients in the wastewater. Because nutrient availability has a profound influence on the biochemical composition of microalgae, we expect that availability of one nutrient may have an influence the uptake of the other nutrient. Therefore, we used a central composite design in which the supply of N and P to microalgae was varied independently. We tested this for the two microalgae species that are most frequently observed in microalgae-based wastewater treatment systems: *Chlorella* and *Scenedesmus* (Craggs et al., 2013; Pittman et al., 2011). We also evaluated to what extent changes in wastewater N and P supply influenced the biochemical composition of the biomass because this has important implications for the valorisation of the biomass.

2. Material and methods

2.1. *Microalgae cultivation*

Chlorella vulgaris (SAG 211-11 B) and *Scenedesmus obliquus* (CCAP 276/3A) stock cultures were maintained in 2 L glass photobioreactors aerated with 0.2 µm filtered air and mixed by stirring. The medium was based on Wright's Cryptophyte (WC) medium and contained 8.7 mg L^{-1} K_2HPO_4, 85.0 mg L^{-1} $NaNO_3$, 36.8 mg L^{-1} $CaCl_2.2H_2O$, 37.0 mg L^{-1} $MgSO_4.7H_2O$, 12.6 mg L^{-1} $NaHCO_3$, trace metals and vitamins (Guillard and Lorenzen, 1972). The pH of the culture was buffered at 8 by addition of Tris buffer. The cultures were irradiated from one side with daylight fluorescent tubes, giving a light intensity of 60 µE m^{-2} s^{-1} at the surface of the reactors.

2.2. Experimental setup

To evaluate how the concentration of N influences the removal of P and vice versa, *Chlorella* and *Scenedesmus* were cultured in medium with varying concentrations of N and P. The range of N and P concentrations used here represents the range of concentrations observed in weak to medium strength domestic wastewaters (Abdelaziz et al., 2013). The medium had the same composition as the WC medium (see above) but with modified $NaNO_3$ and K_2HPO_4 concentrations. A total of 25 treatments were prepared with different concentrations of N (10, 20, 30, 40 and 50 mg N L^{-1}, as $NaNO_3$) and P (2, 4, 6, 8 and 10 mg P L^{-1}, as K_2HPO_4) in all possible combinations. The design of the experiment was based on a central composite design: each combination of N and P concentration was tested once while the centre point (30 mg N L^{-1} and 6 mg P L^{-1}) was replicated three times to provide information about the within-treatment variability. Tris buffer (4.1 mM) was added to the medium to buffer the pH at 8 and to prevent precipitation of calcium phosphates at elevated pH levels. Preliminary experiments had shown that both species were not capable of using Tris as a N source. The treatments were prepared in 1 L round bottom flasks that were bubbled with 0.2 µm filtered and humidified air to mix the culture and to supply CO_2. For each treatment, 1000 mL of medium was inoculated with 50 mL stock culture to obtain an initial optical density (OD) of 0.05 at 750 nm. The contribution of N and P to the medium through the addition of this inoculum was negligible compared to the N and P concentration already present in the treatments (< 3% N and < 2% P). The flasks were continuously irradiated from one side with daylight fluorescent tubes, giving a light intensity of 120 µE m^{-2} s^{-1} at the surface. The temperature of the culture room was kept constant at 20 ±1 °C. All glassware and media were autoclaved before use and the inoculum was added under a sterile hood to ensure cultures were unialgal. The experiment was terminated after 8 days, when all treatments had reached the onset of the stationary phase. All analyses were performed on samples collected on day 8.

2.3. *Measurements and biomass analysis*

The algal biomass was monitored daily by measuring the absorbance at 750 nm (Griffiths et al., 2011). At the end of the experiment, the dry weight biomass concentration in the medium was determined gravimetrically by filtering a known volume of culture on pre-weighed GF/F filters. The N and P remaining in the medium at the end of the experiment were measured as nitrate and phosphate in the supernatant (centrifugation 10 min, 4500 rpm) using a microflow segmented flow analysis system (QuAAtro Seal Analytical, Bran+Luebbe, Germany) following the application notes of the manufacturer. At the end of the experiment, the microalgal biomass was harvested by centrifugation (15 min, 9500 g) for analysis of the nutrient, lipid and carbohydrate concentration of the biomass. The pellet was freeze-dried and stored at -80°C until further analysis. The freeze-dried biomass was disrupted by tip sonication and the carbohydrates were extracted in 1M sulphuric acid. The extracted carbohydrates were measured using the Dubois method (DuBois et al., 1956). The fatty acid methyl ester (FAME) concentration was analysed using a direct transesterification method (adapted from Cho et al., 2013) (Trace GC Ultra, Thermo Scientific, Interscience, Louvain-la-Neuve, Belgium). The FAME concentration is a good measure for the potential biodiesel yield of the biomass (Laurens et al., 2012). For analysis of the N and P concentration of the biomass, the biomass was digested using an alkaline persulphate digestion (Koroleff, 1983). The N and P concentration in the digestate was measured as nitrate and phosphate as described above.

2.4. *Statistical analysis*

The results were analysed in R using response surface modelling (RSM) based on a central composite design (Lenth, 2009). The RSM design consisted of a full factorial design for the two variables (N and P), with five levels for each variable and three replications for the centre points. The calculated second order model included the first order terms, the two-way interaction term and the quadratic terms of the variables N and P. The obtained quadratic polynomials for the different response variables were visualised in contour plots.

3. Results

3.1. Nutrient uptake by the microalgae

The initial concentrations of N and P in the medium were measured. These concentrations corresponded well (< 6% deviation) to the theoretical concentrations, of 10 to 50 mg L^{-1} N and 2 to 10 mg L^{-1} P, used in the response surface model.

Mass balances were calculated to verify whether removal of N and P from the medium was matched by accumulation of N and P in the microalgal biomass (total biomass concentrations times N or P concentration of the biomass). On average, the mass balance was 85% ± 10% for N and 100% ± 22% for P in the *Chlorella* experiment and 92% ± 5% for N and 98% ± 8% for P in the *Scenedesmus* experiment.

The N and P concentration of the microalgal biomass varied considerably with the supplied N and P concentrations (Figure 1). In *Chlorella*, the N concentration varied between 5.0 and 10.1 % while the P concentration varied between 0.5 and 1.3 %. *Scenedesmus* contained more P (0.5 to 1.7 %) and less N (2.9 to 8.4 %) than *Chlorella* when compared pairwise for each nutrient combination (paired t-test, p= 0.02 for P and p <0.01 for N) (Figure 2). For both species, the biomass N concentration increased quadratically with the supply of N (Table 1). The N concentration of the biomass was unaffected by the supply of P. As for N, the biomass P increased with increasing P supply in the medium, and this increase was linear in *Scenedesmus* and quadratic in *Chlorella*. The P concentration of the biomass was also influenced by the N supply in the medium. In both species, the P concentration of the biomass was higher when the N supply was high. When the P concentration of the biomass is plotted versus the biomass N concentration, it is clear that the P concentration varies over a wide range when biomass N concentration is high, whereas biomass P concentration is confined to a narrow range when biomass N concentration is low (Figure 2).

The nutrient concentrations remaining in the medium after 8 days of cultivation were measured (Figure 3). The residual N concentrations ranged from < 1% to 49% of the initial values while the residual P concentrations ranged from <1% to 80%. The residual N and P concentrations increased quadratically with the N and P supply, indicating that high residual N and P concentrations were limited to the treatments with the highest N or P supply (Table 1). For both species, N and P were removed below the current EU emission standards (10 mg L^{-1} for N and 2 mg L^{-1} for P, 91/271/CEE and 98/15/EC Urban Wastewater Treatment Directive, (European Commission Directive, 1998) when the N supply was 40 mg L^{-1} or less or when the P supply was 4 mg L^{-1} or less. For both species, P removal was more effective when the N supply was high. At high N supply, P was removed to below the emission standard of 2 mg L^{-1} up to a P supply of 6 mg L^{-1} for *Chlorella* and up to a P supply of even 8 mg L^{-1} by *Scenedesmus*. The removal of N by *Scenedesmus* was not influenced by the P supply in the medium. The N removal by *Chlorella* was slightly reduced when the P supply was low, but this effect was relatively small. The removal of N was only influenced by the P supply at the highest N supply level, on average the N removal was 22% lower at the lowest P supply compared to the highest P supply level.

3.2. Microalgal biomass concentration and biomass composition

The final dry weight microalgal biomass yield was about 10% higher for *Scenedesmus* than for *Chlorella* when compared pairwise for each nutrient combination (paired t-test, p= 0.03). The final microalgal biomass yield varied between 245 and 475 mg L^{-1} for *Chlorella* and between 304 and 564 mg L^{-1} for *Scenedesmus*. For *Chlorella*, the final biomass yield decreased in a quadratic way with the N supply in the medium and was reduced by about 40 % at the lowest N supply level (Figure 4, Table 1). The final biomass yield was also significantly reduced at lower P supply in the medium, but only by about 20 %. The final biomass yield of *Scenedesmus* was unaffected by the medium composition. This is due to the relatively large measurement error on the dry weight biomass measurement for *Scenedesmus* (standard error is 56 mg L^{-1}, i.e. more than 10% of the mean, Table 1). In contrast to *Chlorella* cells, *Scenedesmus* formed small flocs. This complicated subsampling of the cultures and resulted in higher variability between replicate biomass measurements.

The supplied nutrient concentrations in the medium had a strong influence on the biochemical composition of the biomass. The carbohydrate concentration ranged from 18 to 40 % for *Chlorella* and from 13 to 44 % for *Scenedesmus*, indicating that both species accumulated starch as a result of nutrient starvation (González-Fernández and Ballesteros, 2012; Markou et al., 2012) (Figure 4). For both species, the carbohydrate concentration of the biomass decreased linearly with increasing N supply in the medium (Table 1). In *Chlorella*, the carbohydrate concentration also decreased (about 25%) with increasing P supply (> 2 mg P L^{-1}) in the medium, but the response was weaker when compared to that of N. For both species, the FAME concentration responded in a quadratic way to the N supply in the medium (Table 1), being highest when N supply was lowest. The increase in FAME was mainly due to an increase in C16:0 (palmitic acid) and C18:1 (oleic acid).

4. Discussion

The aim of this study was to evaluate to what extent microalgae can adjust the N and P concentration in their biomass to the supply of these nutrients in the wastewater and to test whether the supply of one nutrient influences the uptake of the other nutrient. In both *Chlorella* and *Scenedesmus*, the N and P concentration in the biomass responded to the supply of these nutrients, being high when the supply in the medium is high and low when the supply in the medium is low. This is in agreement with previous research (Akerström et al., 2014; Arbib et al., 2013; Dickinson et al., 2013; Halfhide et al., 2014). Interestingly, in both species, the uptake of P not only depended on the supply of P but also on the supply of N. At high N supply, the concentration of P in the biomass was a function of the supply of P. At low N supply, the concentration of P in the biomass was low, irrespective of the supply of P in the medium. Conversely, the N concentration in the biomass was independent of the P supply and was only influenced by the N supply in the medium.

This observation can be explained by the roles of N and P in the cellular metabolism. In microalgae, N is mainly integrated in proteins and a low N supply will thus limit the synthesis of proteins (Geider and La Roche, 2002; Loladze and Elser, 2011). A reduction in protein synthesis will result in a reduction in the number of ribosomes as well as the amount of ribosomal RNA. Because most P in the cell is stored in the ribosomal RNA, a reduction in ribosomes will result in a lower cellular P concentration (Sterner and Elser, 2002). If this is indeed the physiological explanation for the influence of N on P uptake, this phenomenon may not only apply to the model species used in this study, but also to other species of microalgae. Indeed, in experiments with other species of microalgae, it has been noted that a reduction in the N supply not only resulted in a reduction of the N concentration but also of the P concentration in the biomass e.g. in *Phaeodactylum* and *Tetraselmis* (Goiris et al., 2015).

This study confirmed that the N and P concentration in microalgal biomass can deviate strongly from the concentrations predicted by the Redfield ratio. For *Chlorella*, the N:P ratio in the biomass varied between 15 - 42 and for *Scenedesmus* between 7 - 32. Models for nutrient uptake by microalgae based on a fixed Redfield stoichiometry are therefore not reliable for estimating the capacity of microalgae to remove N and P removal from wastewater. More flexible models that take into account a variable N and P concentration in the biomass should be applied, such as the Droop model (Droop, 1973). In these models, the N concentration in the biomass is only dependent on the N supply in the medium. According to our results, these models may be refined in a way that the N concentration in the biomass not only depends on the N supply in the medium, but also on the P supply.

The amount of N and P that can be removed from wastewater by microalgae depends on the amount of microalgal biomass that is produced as well as on the N and P concentration in that biomass. In our experiments, variability in biomass concentration and variability in nutrient concentrations in the biomass contributed about equally to the nutrient removal from the medium. The final biomass concentration in the treatments varied by about a factor of two between the different treatments, while the N and P concentration in the biomass varied by a factor of two to three. Because the N and P concentration in the biomass influences the growth rate, there is a link between the N and P concentration in the biomass and the biomass productivity. When the N and P supply is high, microalgae accumulate more N and P in their biomass and less C-rich metabolites, and cell division rates and thus biomass productivity are high. When the N and P supply is reduced, microalgae accumulate more C-rich metabolites and less N and P, and cell division rate and biomass productivity are reduced. The accumulation of C-rich metabolites under low nutrient supply was evident from the accumulation of carbohydrates and lipids in the biomass, particularly in treatments with a low N supply. The carbohydrate concentration of the biomass decreased significantly with the N concentration in both *Chlorella* (Pearson correlation -0.681, $p < 0.001$, $n = 27$) and *Scenedesmus* (Pearson correlation -0.936, $p < 0.001$, $n = 27$). Photosynthetic products are redirected from protein synthesis to carbohydrate synthesis when nutrients become limiting (González-Fernández and Ballesteros, 2012). At the lowest N supply treatments (10 mg L^{-1}), lipids were accumulated: the FAME concentration of the biomass increased from about 12 % to 32 – 36 % in *Chlorella* and from about 10 % to 25 – 31 % in *Scenedesmus*. P limitation had no influence on the FAME concentration of both species but had some influence on the carbohydrate concentration in *Chlorella*. It is known that microalgae first accumulate carbohydrates and start accumulating lipids only when nutrient stress is more advanced (Procházková et al., 2014; Zhu et al., 2014). The biomass productivity was also significantly affected by the nutrient supply in Chlorella. In *Scenedesmus* no significant effect of nutrient supply on biomass productivity was detected, but this can ascribed to the high variability in biomass analyses for this species, which were due to aggregate formation.

The dependence of the biomass P concentration on the N supply in the medium had implications for the removal of P from the medium. The removal of P was limited when the N supply was low. P was removed below the EU emission level standard of 2 mg L^{-1} only when the P supply was 4 mg L^{-1} or less. When the N supply was high, however, P could also be removed below 2 mg L^{-1} in treatments with a P supply of 6 or even 8 mg L^{-1}. On the contrary, N removal was independent of the P supply. N was removed to below 10 mg L^{-1} (the EU emission level for N) in all treatments where the N supply was 40 mg L^{-1} and even in some of the treatments with a supply of 50 mg N L^{-1}. Because the model species used here are often dominant in HRAP systems used for microalgae-based wastewater treatment, *Chlorella* and *Scenedesmus*, it is likely that the same phenomenon may apply to large-scale systems. Further research is needed to investigate to what extent this link between N and P accumulation in the biomass is influenced by C assimilation, which is in turn influenced by irradiance and CO_2 supply. An increase in C assimilation will most likely increase the C:N and C:P ratio in the biomass (Sterner and Elser, 2002), but it is not known whether this will influence the balance between N and P in the cell.

There were differences between the two model species that were used with respect to the N and P concentration in the biomass. *Chlorella* was capable of accumulating more N than *Scenedesmus* (5 to 10 % as opposed to 3 to 8%). *Scenedesmus*, on the contrary, accumulated more P than *Chlorella* (0.5 to 1.7 % as opposed to 0.5 to 1.3 %). This makes *Chlorella* a more attractive candidate for treatment of wastewaters that are rich in N, while *Scenedesmus* may be more suitable for waters rich in P. The flexibility in the N and P concentration of the biomass that was observed for these species in this study fell well within the range of concentrations that have been reported in previous studies (Akerström et al., 2014; Arbib et al., 2013; Dickinson et al., 2013). Differences between this and other studies may be due to differences in the N and P supply, as well as differences in time of exposure to nutrient depletion. When nutrient supply is lower, this may result in lower biomass nutrient concentrations than observed in our study. When microalgae are exposed to nutrient stress for a prolonged time, either in batch or in continuous cultures, the nutrient concentration in the biomass may also be lower than observed in this study e.g. (Dickinson et al., 2013).

Conclusion

Chlorella and *Scenedesmus* are capable of adjusting the N and P concentration in their biomass to the N and P supply in the wastewater, which is important to achieve simultaneous removal of both N and P in wastewaters with a variable N and P loading. The degree to which both species adjusted the P concentration in their biomass, however, depended on the N concentration in the biomass. Microalgae can accumulate more P when N concentrations are high but P uptake is reduced when N concentrations are low. This implies that a sufficiently high N concentration in the wastewater is a prerequisite for effective P removal. *Scenedesmus* can accumulate more P in its biomass compared to *Chlorella,* while *Chlorella* can accumulate more N than *Scenedesmus*. Because microalgae adjust the N and P concentration in the biomass, the biomass yield varies relatively little with differences in wastewater nutrient concentration. The biochemical composition of the biomass, on the contrary, is strongly influenced by the wastewater nutrient concentration, with a two- to four-fold increase in lipids or carbohydrates when the wastewater N concentration is low.

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Tables and figures

	N	P	N^2	P^2	N*P	Standard error
Chlorella						
Residual N (mg L^{-1})	**< 0.001**	**0.011**	**< 0.001**	0.370	**0.044**	1.50
Residual P (mg L^{-1})	**< 0.001**	**< 0.001**	**0.024**	**0.001**	**0.018**	0.46
Biomass N (%)	**< 0.001**	0.186	**< 0.001**	0.602	0.407	0.23
Biomass P (%)	**0.008**	**0.031**	0.351	**0.007**	0.290	0.08
Dry weight (mg L^{-1})	**< 0.001**	**0.022**	**0.001**	0.580	0.271	10.14
Carbohydrate (%)	**< 0.001**	**0.018**	0.845	0.132	0.986	1.35
Fame (%)	**< 0.001**	0.929	**< 0.001**	0.773	0.452	0.96
Scenedesmus						
Residual N (mg L^{-1})	**< 0.001**	0.216	**< 0.001**	0.917	0.120	0.06
Residual P (mg L^{-1})	**0.001**	**< 0.001**	0.613	0.481	**0.001**	0.41
Biomass N (%)	**< 0.001**	0.318	**< 0.001**	0.417	0.467	0.68
Biomass P (%)	**< 0.001**	**< 0.001**	0.157	0.612	**< 0.001**	0.03
Dry weight (mg L^{-1})	0.970	0.982	0.287	0.654	0.236	56.05
Carbohydrate (%)	**< 0.001**	0.254	0.428	0.695	0.322	4.49
Fame (%)	**< 0.001**	0.142	**< 0.001**	0.792	0.515	0.30

Table 1 Significance (p-value) of the regression parameters N, P, N^2, P^2 and N*P in the response surface models and standard error of the 3 centre point replications (30 mg N L^{-1} and 6 mg P L^{-1}). The standard error was calculated as the standard deviation, of the response variable for the replicated centre points, divided by the square of 3 (number of centre points).

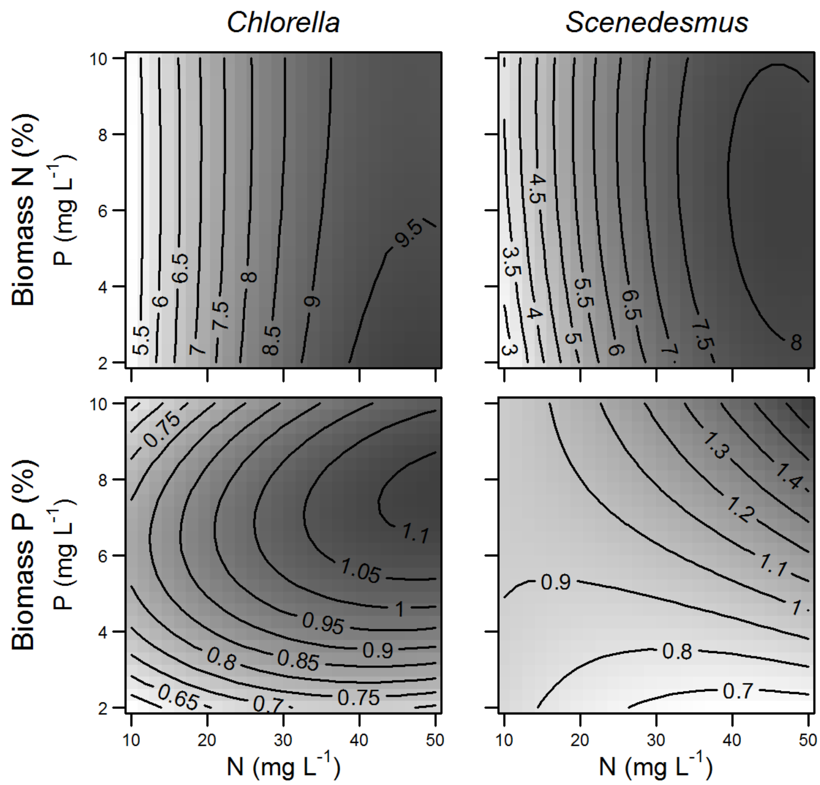

Figure 1. Biomass N and P concentrations (% of dry weight), at day 8 of the batch culture, as a function of the supplied N and P concentrations.

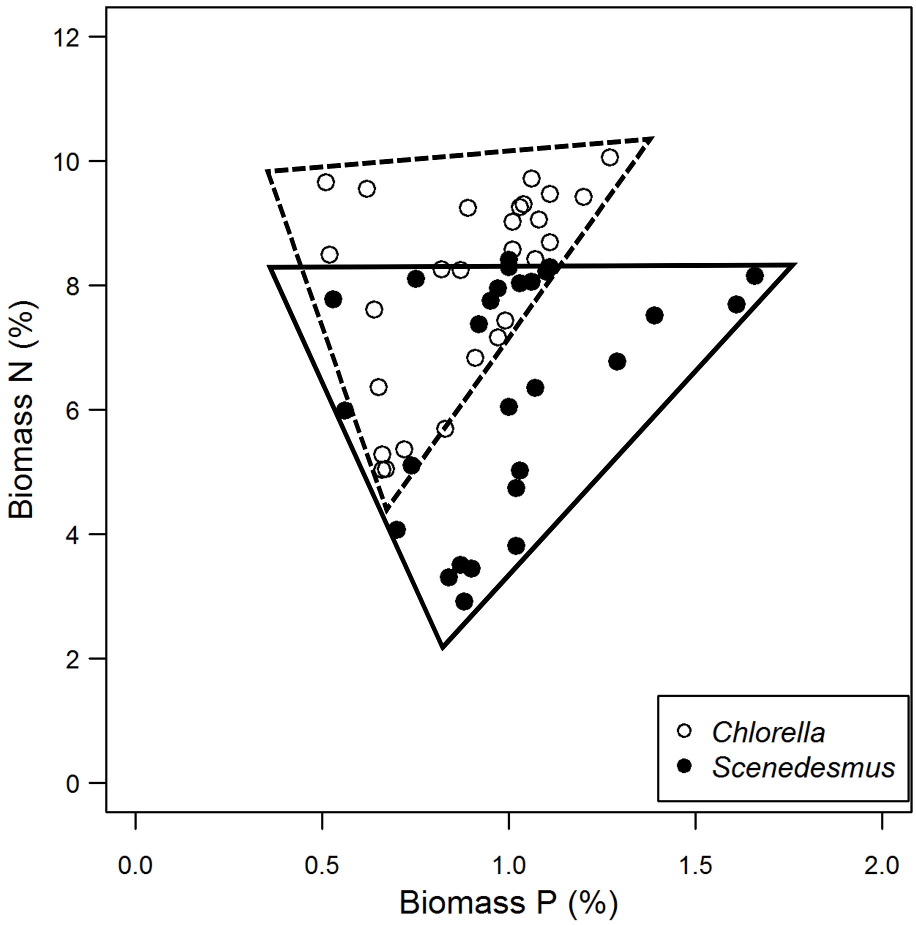

Figure 2: The biomass N concentrations (% of dry weight) as a function of biomass P concentrations (% of dry weight).

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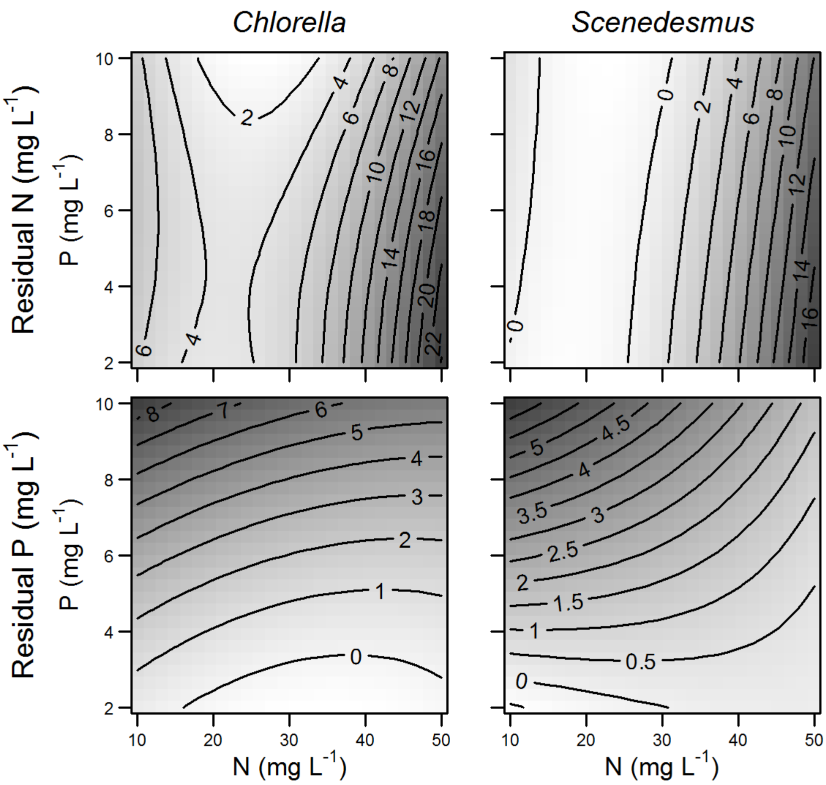

Figure 3. Residual medium N and P concentrations (mg L^{-1})
at day 8 of the batch culture, as a function of the supplied N and P concentrations.

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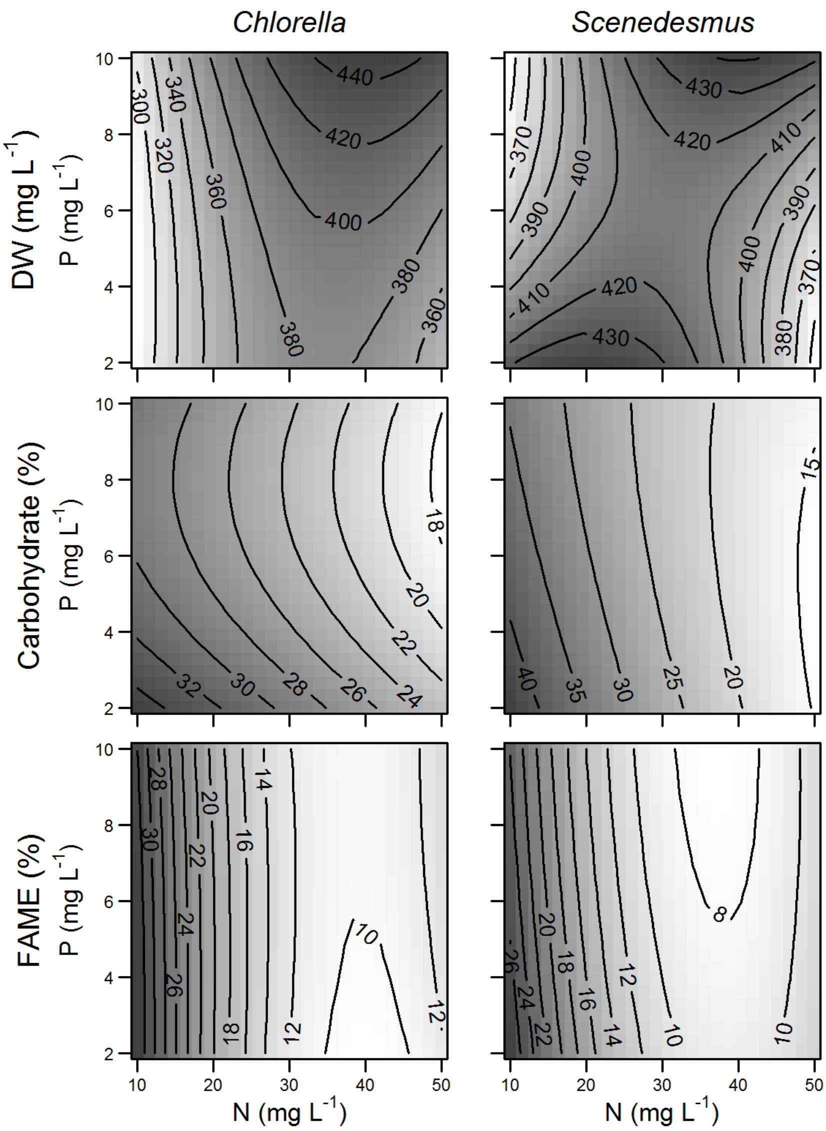

403 Figure 4. Density measured as dry weight (mg/L) and the biomass carbohydrate and FAME concentrations (% of dry weight), at day 8 of the batch culture, as a function of the supplied N and P concentrations.

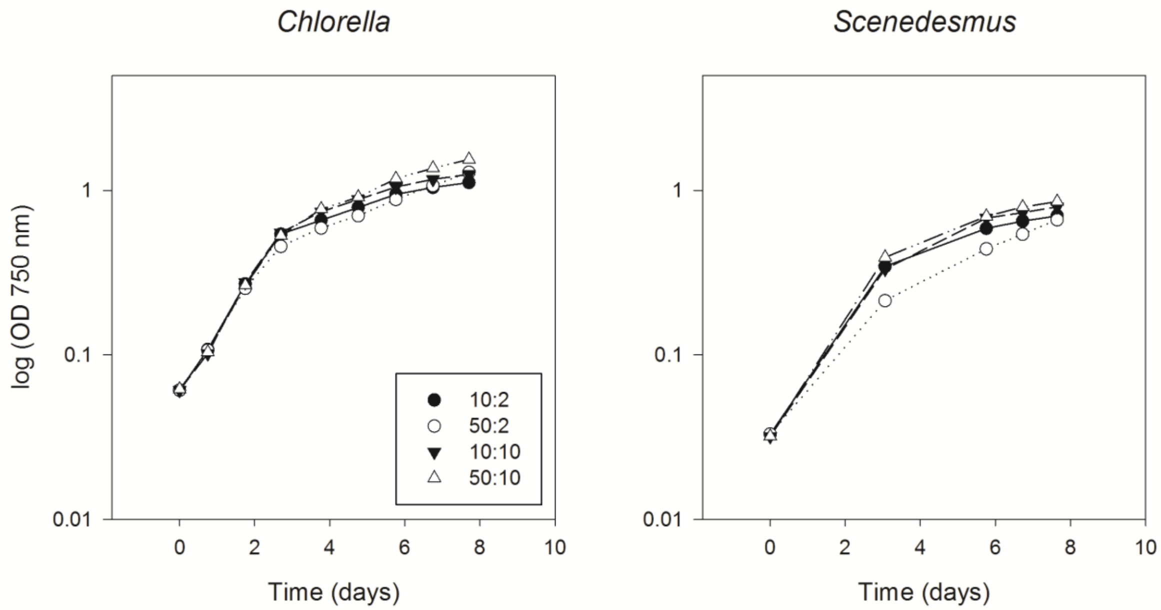

Figure S1. The growth curves represent the logarithmic tranformation of the optical density, measured at 750 nm, for Chlorella and Scenedesmus at a function of time. The treatments with 10 and 50 mg/L N in combination with 2 and 10 mg/L P are shown. The labels show the N:P concentrations eg. label 10:2 is the treatment with 10 mg/L N and 2 mg/L P. After 6 to 8 days the difference between the treatments had stabilised and the algal biomass was harvested at day 8.

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