This article was published in Algal Research, 11, 350-358, 2015
http://dx.doi.org/10.1016/j.algal.2015.07.014

Towards sustainable microalgal biomass production by phycoremediation of a synthetic wastewater: a kinetic study

N.F.P. Silva[1,2], A.L. Gonçalves[1], F.C. Moreira[2], T.F.C.V. Silva[2], F.G. Martins[1], M.C.M. Alvim-Ferraz[1], R.A.R. Boaventura[2], V.J.P. Vilar[2], J.C.M. Pires[1*]

(1) LEPABE, (2) LSRE, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

* Corresponding author:
Telephone: +351 22 508 2262
Fax: +351 22 508 1449
E-mail address: jcpires@fe.up.pt (J.C.M. Pires)

Abstract

Microalgae are considered as one of the most promising sources of biomass for energy production. However, bioenergy production by microalgal culture is still not economically viable and it has high environmental impact (requirement of high amount of freshwater). These drawbacks can be surpassed by coupling microalgal biomass production with phycoremediation of wastewater. In this context, this study evaluates the kinetics of biomass production and nutrient removal by two microalgal species (*Chlorella vulgaris* and *Pseudokirchneriella subcapitata*) cultivated in different medium compositions.

The potential of microalgae for biomass production and their high efficiency on nutrients removal from medium, particularly nitrogen and phosphorus, was demonstrated. Maximum biomass productivity was observed for *C. vulgaris* (0.106±0.004 g L^{-1} d^{-1}), while *P. subcapitata* reached a maximum of 0.050±0.001 g L^{-1} d^{-1}. The value of N:P molar ratio that favoured microalgal growth was 8:1 for *C. vulgaris* and 16:1 for *P. subcapitata*. A complete removal (100%) of ammonium was measured and high removal efficiencies were observed for nitrate (above 95%) and phosphate (above 97%). Microalgae were also able to efficiently remove sulphates, presenting removal efficiencies from 54 to 100%. The removal kinetics for all the nutrients have been determined through application of pseudo-first-order kinetic model and modified Gompertz model. In conclusion, this work gives relevant data for culturing microalgae in wastewater, contributing to the bioprocess design of a sustainable and low-cost production of microalgal biomass.

Keywords: Biomass production; Microalgae; Nutrient uptake kinetics; Phycoremediation; Sustainable process; Wastewater treatment.

1. Introduction

Alternative sources of energy with lower carbon intensity and thus, more sustainable, should be studied. Biomass is a renewable energy resource that, with adequate management, can achieve high regeneration rates being considered sustainable (zero-emission energy source) [1-3]. In this context, microalgae appear as an important source of biomass. These photosynthetic microorganisms present higher growth rates and higher biomass productivities when compared to terrestrial crops [4-8]. Microalgae can be grown in non-arable land and require far less land than terrestrial crops, thus not competing with agriculture and not compromising food production and supply. Additionally, microalgae can grow in a wide variety of environmental conditions and also in low quality waters, reducing the requirements for freshwater [9, 10]. Due to their macromolecular composition, several commercial products can be achieved from microalgal biomass [11]: human food, animal feed, fine chemicals, biofuels and fertilizers. Microalgal cultures are already performed at large-scale, mainly for high-valued human nutritional products. However, bioenergy production is not economically viable yet; thus, several research efforts should be performed to reduce biomass production costs. Besides the search for the culture parameters corresponding to maximum growth rates, the process integration of biomass production with wastewater treatment (secondary or tertiary treatment) will provide a significant reduction on the requirement for freshwater and nutrients (whose price almost doubled in the last decade) [12, 13]. On the other hand, wastewater treatment using microalgae has several advantages over conventional treatments [14-16]: (i) nitrogen and phosphorus can be converted into biomass without the addition of organic carbon; (ii) the discharged effluent into water bodies is oxygenated; and (iii) high-valued products can be extracted from microalgal biomass. The main mechanisms for nutrient removal from microalgae include uptake into the cell and, in the case of ammonia, the stripping through elevated pH [17, 18]. However, tertiary treatment of wastewater with microalgae should guarantee that the discharge limits for urban wastewaters defined by the European Union (EU) Directives 91/271/EEC and 1998/15/EC are accomplished. Taking into account the definition of population equivalent (p.e.) presented in the EU legislation, the limits for effluent discharge are: (i) 2 $mg_P\ L^{-1}$ (for 10 to 100 thousand p.e.) or 1 $mg_P\ L^{-1}$ (for more than 100 thousand p.e.) for total phosphorus and a removal efficiency of this nutrient in the overall load of at least 80%; and (ii) 15 $mg_N\ L^{-1}$ (for 10 to 100 thousand p.e.) or 10 $mg_N\ L^{-1}$ (for more than 100 thousand PE) for total nitrogen

and a removal efficiency of this nutrient in the overall load of at least 70-80%. One or both parameters (values for concentrations or the percentage of reduction) may be applied depending on the local situation.

According to their source, wastewaters can present different compositions, some of them with compounds that inhibit microalgal growth. Several research studies were already performed with microalgal growth in wastewaters from different sources: (i) domestic wastewater [19-21]; (ii) anaerobic digestion wastewater [22-24]; (iii) livestock wastewater [25-27]; and (iv) agro-industrial wastewater [28, 29]. In almost all studies, microalgae were able to efficiently remove the monitored nutrients. Lundquist et al. [30] performed a techno-economic assessment of biofuel production by microalgae using wastewater as culture medium, selecting five case studies: two of them focused on wastewater treatment and the others on biofuel (biogas and biodiesel) production. Without integration with wastewater treatment, microalgal biofuels can exceed $400 per barrel, while this integration can lower the price to less than $30 per barrel. Thus, an important step to increase the competitiveness (promoting simultaneously the environmental sustainability) of microalgal biofuels over fossil fuels is the optimization of culture parameters using wastewater as culture medium.

Several phenomena should be studied to apply this technology at industrial scale. Kinetics of microalgal growth and nutrient removal are required to perform the bioprocess design. In addition, the influence of nitrogen to phosphorus (N:P) molar ratio on the growth of microalgae and the effect of fed nitrogen source (nitrate or ammonium) should be analysed. Therefore, this study aimed to evaluate the kinetics of biomass production and nutrient removal of microalgae grown under different experimental conditions. Specific objectives were: (i) to evaluate the effect of nitrogen to phosphorus (N:P) molar ratio and nitrogen source on the growth of two microalgae (*Chlorella vulgaris* and *Pseudokirchneriella subcapitata*); and (ii) to evaluate the kinetic parameters for biomass production and nutrient uptake from the culture medium.

2. Materials and methods

2.1. Microorganisms and culture medium

C. vulgaris and *P. subcapitata* were obtained from the Culture Collection of Algae and Protozoa (CCAP). The selection of these microorganisms was based on the following factors: (i) both microorganisms can be easily grown in laboratory cultures; (ii) different

studies have shown that microorganisms from the genus *Chlorella* have been effectively applied in nutrients removal from wastewaters from different sources [31-33]; and (iii) *P. subcapitata* is a green microalga commonly used as a chemical toxicity bioassay organism [34, 35] that has shown to be adapted to grow under different nitrogen and phosphorus concentrations [36]. Microalgae were inoculated in a modified standard medium [37] with the following composition (mg L^{-1}): 12 $MgCl_2 \cdot 6H_2O$; 18 $CaCl_2 \cdot 2H_2O$; 15 $MgSO_4 \cdot 7H_2O$; 20 KH_2PO_4; 0.08 $FeCl_3 \cdot 6H_2O$; 0.1 $Na_2EDTA \cdot 2H_2O$; 0.185 H_3BO_3; 0.415 $MnCl_2 \cdot 4H_2O$; 0.003 $ZnCl_2$; 0.0015 $CoCl_2 \cdot 6H_2O$; 10^{-5} $CuCl_2 \cdot 2H_2O$; 0.007 $Na_2MoO_4 \cdot 2H_2O$ and 1300 $NaHCO_3$. Different medium compositions regarding nitrogen (see Table 1) were applied to mimic the compositions of real effluents, which present a wide variability. NH_4Cl and $NaNO_3$ solutions were added at different molar ratios, to evaluate which nitrogen source (NH_4^+ and NO_3^-) results in an increased biomass productivity. Due to the variable composition of wastewaters, the use of a synthetic medium was considered more appropriate to reproduce the experiments at lab scale and to obtain mathematical models. N:P molar ratio is an important parameter in microalgal growth. Redfield ratio (16:1) was considered as middle value. Two additional ratios were selected, one higher (24:1) and one lower (8:1), to cover a wide range of values found in different wastewaters [38]: (i) poultry; (ii) swine; (iii) tannery and others. In addition, the selected concentrations of nitrogen and phosphorus are in the same order of magnitude of the values found in the same wastewaters [38].

2.2. Experimental setup and culture conditions

Microalgae were inoculated in 1-L borosilicate glass flasks with an initial biomass concentration of approximately 20-30 mg L^{-1}. Cultures were performed at room temperature for 12 days using the above described medium. Agitation of the cultures was obtained by injection of atmospheric air at the base of the flasks, using air pumps Trixie TARP D-2463 (50-300 L) with an air flow of 90 L h^{-1}. Cultures were exposed to continuous light supply (provided by a set of four 18-W fluorescent lamps) with light intensity at the surface of the flasks between 2.5 and 3.0 klux. Light intensity was daily monitored using a light meter Isotech Lux-1335 – RS Components. The assays were performed in duplicates.

2.3. Analytical methods

The cultures were subjected to daily measurements of temperature, dissolved oxygen concentration (sensor Oxi 340i – WTW), pH (sensor pH 212 – Hanna Instruments) and optical density at 750 nm (OD_{750}). OD_{750} was measured using a spectrophotometer (Genesys 10S UV-Vis Scanning – Thermo Scientific). Biomass concentration was then calculated using the determined calibration curves for each microalga. The relationship between biomass dry weight ($g_{biomass}$ L^{-1}, x) and optical density (OD_{750}, y) was estimated using the following linear regressions: $y = (1.80 \pm 0.08)x + (0.04 \pm 0.07)$ (R^2=0.998; limits of quantification and detection were 0.15 and 0.04 g L^{-1}, respectively) for *C. vulgaris* and $y = (2.6 \pm 0.2)x + (0.1 \pm 0.1)$ (R^2=0.995; limits of quantification and detection were 0.16 and 0.05 g L^{-1}, respectively) for *P. subcapitata*.

To evaluate the temporal variation of the medium chemical composition, five samples were collected in different days. These samples were centrifuged for 15 minutes at 4000 rpm using a centrifuge by Hitachi Himac CT6E Koki Co., LMT and filtered through syringe filters of nylon membrane with a pore size of 0.45 μm (Acrodisc ®, Pall). The filtered solution was then analysed taking into account the following compounds: (i) sulphate, chloride, nitrate, phosphate and nitrite measured by ion chromatography using a Dionex ICS-2100 apparatus equipped with a IonPac® AS11-HC (4×250 mm) column at 30 ºC and an anion self-regenerating suppressor (ASRS® 300, 4 mm) under isocratic elution of 30 mM NaOH at a flow rate of 1.5 mL min^{-1}; (ii) sodium, potassium, ammonium, magnesium and calcium measured by ion chromatography using a Dionex DX-120 device equipped with a IonPac® CS12A (4×250 mm) column at room temperature and a cation self-regenerating (CSRS® Ultra II, 4 mm) suppressor under isocratic elution of 20 mM methanesulfonic acid at a flow rate of 1.0 mL min^{-1}; and (iii) dissolved organic carbon (DOC) concentration determined by combustion catalytic oxidation at 680 °C and non-dispersive infrared (NDIR) methods in a TOC-V_{CSN} analyser equipped with an ASI-V autosampler (Shimadzu). Total dissolved carbon (TDC) and dissolved inorganic carbon (DIC) were also measured and DOC was given by the difference between TDC and DIC (DOC=TDC-DIC).

2.4. Kinetic models and parameters

Biomass concentration (X, g L^{-1}) was used to determine specific growth rate (μ, d^{-1}) and biomass productivity (P_x, g L^{-1} d^{-1}) for both species in the different studied conditions. During the exponential growth phase, the specific growth rate was calculated according to Equation 1 [39, 40]:

$$\frac{dX}{dt} = \mu X \Leftrightarrow \mu = \frac{ln(X_1/X_0)}{t_1 - t_0} \quad (1)$$

where X_1 and X_0 are the biomass concentrations at time t_1 and t_0 (for this purpose, the end and beginning of exponential growth phase), respectively. Biomass productivity results from the difference in biomass concentration per unit time between two consecutive samples:

$$P_x = \frac{X_1 - X_0}{t_1 - t_0} \quad (2)$$

To compare this parameter among different cultures, maximum and average productivities ($P_{x\ max}$ and $P_{x\ av}$, respectively) were determined. Maximum productivity was calculated by rolling average of three consecutive values throughout the culture, considering the maximum value. The average productivity results from the ratio of overall produced biomass and elapsed time [41].

Regarding nutrient removal by microalgae, the removal efficiency (*RE*, %) was defined as:

$$RE\ (\%) = \frac{S_0 - S_f}{S_0} \times 100 \quad (3)$$

where S_0 and S_f are nutrient concentrations at the beginning and end of culture, respectively. In addition, removal rate (*RR*, mg L^{-1} d^{-1}) of the analysed nutrients was calculated as follows:

$$RR = \frac{S_0 - S_i}{t_i - t_0} \quad (4)$$

where S_i is the nutrient concentration at time t_i. In this work, the maximum and average values of this parameter for each culture were calculated.

A pseudo-first-order kinetic model was assumed to describe the temporal variation of nutrient concentrations in the cultures [40]. Accordingly, nutrient removal kinetics can be considered as:

$$S = S_0 \times e^{-kt} \quad (5)$$

Equation 5 can be linearized to determine the kinetic constant (*k*, d^{-1}). A plot of *ln(S)* as a function of *t* will yield a straight line with slope *–k*.

$$ln(S) = ln(S_0) - kt \quad (6)$$

The kinetic constant helps to identify the conditions where higher removal rates were obtained.

Based on the experimental data achieved in this work, it was observed that NO_3^- was not immediately assimilated by microalgae in some cultures. Therefore, the modified Gompertz model was applied to model the temporal variation of nutrient concentrations for those cultures [42, 43]. This model considered three distinct phases: (i) initial phase of adaptation (lag phase); (ii) exponential phase; and (iii) final stage of stagnation. It can be expressed as:

$$S = a \cdot exp[-exp(b - ct)] \quad (7)$$

where a is the upper asymptote, b ($b > 0$) sets the displacement along the x-axis and c ($c > 0$) sets the tangent at the inflection point. Taking into account that the nutrient removal follows a pseudo-first-order kinetic model, the following equation can be obtained [44]:

$$S(t) = S_0 + \left(S_f - S_0\right) * exp\{-exp[k * (\lambda - t) + 1]\} \quad (8)$$

where λ (d) is the lag time. The fitting of the modified Gompertz model to experimental data allows the estimation of the time delay taken by microalgae to assimilate NO_3^- in some cultures and the kinetic constant (k).

Biomass yield based on nutrient consumption (Y, $g_{biomass}\ g_{nutrient}^{-1}$) can be calculated by Equation 9. This parameter was calculated for NH_4^+, NO_3^-, PO_4^{3-} and SO_4^{2-}.

$$Y = \frac{X_f - X_0}{S_0 - S_f} \quad (9)$$

3. Results and discussion

Cultures of *C. vulgaris* and *P. subcapitata* were monitored taking into account the dual role of microalgae: biomass production and nutrient removal from the synthetic effluent. The achieved results are important in the design of bioreactors for the above referred applications.

3.1. Biomass production

The daily monitoring of biomass concentration in the different cultures allowed the characterization of their growth kinetics and analysis of the influence of nitrogen source and concentration in the medium (corresponding to different N:P molar ratio). Figure 1 shows the temporal variation of biomass concentration for *C. vulgaris* (Fig. 1a and 1c) and *P. subcapitata* (Figure 1b and 1d), for the tested N:P molar ratios and nitrogen sources (assays 1, 3, 4 and 6). In general, the cultures of *C. vulgaris* presented the same growth behaviour: (i) the lack of an adaptation phase was observed; (ii) the exponential phase started before completing the first day of culture; and (iii) microalgal growth stabilized after the seventh day. On the other hand, *P. subcapitata* presented a shorter exponential phase (96 h for *C. vulgaris* and 72 h for *P. subcapitata*). Table 2 presents the main kinetic parameters (X_{max}, μ, $P_{x\ max}$ and $P_{x\ av}$) determined for the different microalgal cultures. Concerning X_{max}, these values ranged between 0.19±0.04 and 0.71±0.02 g L^{-1}. *C. vulgaris* presented higher values (0.622±0.002 to 0.71±0.02 g L^{-1}), when compared to *P. subcapitata* (0.19±0.04 to 0.289±0.002 g L^{-1}). Maximum values were obtained for the N:P molar ratios 16:1 and 24:1. *C. vulgaris* presented specific growth rates between 0.55±0.03 and 0.85±0.05 d^{-1}, while *P. subcapitata* reached higher values (0.57±0.02 to 1.2±0.1 d^{-1}). These results are in agreement with several research studies that presented specific growth rates between 0.31 and 1.5 d^{-1} for *C. vulgaris* [41, 45, 46] and between 0.635 and 1.44 d^{-1} for *P. subcapitata* [46-48]. Concerning N:P molar ratio and the fed nitrogen source, *C. vulgaris* presented higher specific growth rates when both NH_4^+ and NO_3^- were present in the medium with N:P molar ratio of 8:1 (assay 2), while *P. subcapitata* presented higher specific growth rates when cultured in the medium containing only NH_4^+ with N:P molar ratio of 16:1 (assay 4). Hadj-Romdhane et al. [49] and Kapdan and Aslan [50] evaluated the influence of N:P molar ratio on *C. vulgaris* growth and both concluded that it should be near 8:1. These results show that the Redfield ratio [51] (N:P = 16:1) was not the optimal value for the growth of *C. vulgaris*, but it was the optimal one for *P. subcapitata* growth. A more recent study developed predictive models to determine the best N:P molar ratio [52]. This value can vary from 8.2 to 45.0, depending on the experimental conditions. The same research study considered that the Redfield ratio is an average of the values achieved for the different species. $P_{x\ av}$ values indicate the average temporal rate of biomass production in the cultures. However, in industrial context, microalgal cultures should be performed in continuous mode during the exponential growth phase, when high biomass productivities are achieved ($P_{x\ max}$). Thus, these values should be focused on the analysis of the optimal culture conditions. *C.*

vulgaris achieved values between 0.077±0.001 and 0.106±0.004 g L^{-1} d^{-1} that were significantly higher than *P. subcapitata* (0.033±0.001 to 0.050±0.001 g L^{-1} d^{-1}). Despite having lower specific growth rates, *C. vulgaris* achieved higher biomass concentrations and higher productivities (due to the longer duration of their exponential growth phase – 96 h) than *P. subcapitata*, thus showing higher potential for biomass production.

Besides the monitoring of biomass concentration in the different cultures, three other culture parameters were daily monitored: (i) pH; (ii) temperature; and (iii) dissolved oxygen concentration. Table 3 shows the average values and standard deviations of these culture variables for all assays. Temporal variation profiles were very similar for all cultures. The initial value of pH was 8.2±0.2, which increased in the first day of culture to 9.6±0.2 for *C. vulgaris* and 9.4±0.2 for *P. subcapitata*, then presenting a slight decrease tendency until the end of the cultures. The observed increase occurred at the beginning of the exponential growth phase. In autotrophic growth, microalgae uptake dissolved CO_2, which leads to a pH rise and a new chemical equilibrium in the medium is then established. In the remaining days of culture, no significant pH change was observed; thus, CO_2 uptake rate by microalgae was equal to gas-to-liquid mass transfer rate of this compound. On the other hand, temperature did not present significant variation (not controlled variable), being equal to 25±1 ºC. Regarding dissolved oxygen concentration, an increase was expected due to the photosynthetic activity of microalgae. Thus, this variable should have similar behaviour than the one observed for culture pH. Cultures of *C. vulgaris* showed higher values, presenting an increase in the first day of culture from 6.7±0.4 mg L^{-1} to 7.9±0.3 mg L^{-1} and showing a slight decrease until the end of culture with final value of 7.2±0.1 mg L^{-1}. High dissolved oxygen concentrations may have a negative effect on the growth of microalgae. However, air bubbling promotes the removal of photosynthetic produced oxygen from the cultures, avoiding the negative impact of excessive concentrations of dissolved oxygen. In the case of *P. subcapitata*, no significant variations in this variable were observed for all cultures, due to their low biomass concentration.

3.2. Nutrient uptake

The value of N:P molar ratio is considered as one of the most important parameters for nutrient removal in biological treatment systems. Limitation in one of these important nutrients may reduce the removal of other nutrients [38]. In this study, the influence of N:P molar ratio and nitrogen source on nutrient uptake by microalgae was analysed.

Besides the monitoring of biomass concentration, pH, temperature and dissolved oxygen concentration, culture samples were collected in five time periods to evaluate the chemical composition of the medium, taking into account the following nutrients: (i) carbon (DIC and DOC); (ii) nitrogen (NH_4^+ and NO_3^-); (iii) phosphorus; and (iv) sulphur.

3.2.1. Carbon

Microalgae can use organic or inorganic forms of carbon. In this study, culture medium only contained soluble carbonates (HCO_3^-) that were assimilated by microalgae, as well as the atmospheric carbon dioxide that was injected to the cultures to promote their mixing. However, organic and inorganic carbon were analysed in all cultures, showing a similar behaviour for all studied conditions. Figure 2 shows, as example, the temporal variation of DIC (Figure 2a and 2b) and DOC (Figure 2c and 2d) concentrations for the assays 2 (*C. vulgaris*, Figure 2a and 2c) and 5 (*P. subcapitata*, Figure 2b and 2d) for all tested N:P molar ratios. These temporal profiles are representative for all cultures. DIC concentration decreased in the first days about 20-40 mg L^{-1} (approximately 10%). This decrease occurred in the beginning of exponential growth phase, in which a pH increase was also observed. On the other hand, DOC concentration increased to 15±2 mg L^{-1} for *C. vulgaris* and 31±2 mg L^{-1} for *P. subcapitata* during the same time period. The presence of organic forms of carbon can be justified by compounds produced and excreted by microalgae [53-55]. Hulatt and Thomas [55] determined the amount of dissolved organic matter exuded by microalgae, achieving the values of 6.4% and 17.3% of the total organic carbon in the cultures of *C. vulgaris* and *Dunaliella tertiolecta*, respectively. In this study, this percentage was about 2.3% for *C. vulgaris* and 12% for *P. subcapitata*.

3.2.2. Nitrogen

Nitrogen is an essential nutrient for all organisms. Microalgae require this nutrient to produce important biological substances, such as proteins, chlorophylls, energy transfer molecules (ADP and ATP) and genetic materials (RNA and DNA). In this study, microalgal cultures were prepared with different concentrations of N-NH_4^+ and N-NO_3^-, aiming the analysis of the effect of N:P molar ratio on nutrient removal kinetics. In addition, cultures were also performed using both nitrogen sources (assays 2 and 5), to evaluate which one (N-NH_4^+ or N-NO_3^-) improves biomass productivities. In these assays, the culture medium had the same molar concentration of N-NH_4^+ and N-NO_3^-. Table 4 shows the removal kinetic parameters and efficiencies of N-NH_4^+ and N-NO_3^- for

all assays. Regarding $N\text{-}NH_4^+$, microalgal cultures presented removal efficiencies of 100% (values achieved in less than 48 h of culture). The highest removal rate was 13.92 $mg_N\ L^{-1}\ h^{-1}$, achieved by *P. subcapitata* in the first day of culture with the highest $N\text{-}NH_4^+$ concentration. The kinetic constant (*k*) varied between 0.5±0.1 and 3.86±0.05 d^{-1}, being the highest value achieved for N:P molar ratio of 8:1 for both microalgae. Lower concentrations of this nutrient in the culture medium may induce the increase of removal kinetics by microalgae. This effect took more relevance for *C. vulgaris*, in which significant differences in removal kinetic constants were achieved for different N:P molar ratios in assay 1 (corresponding to the highest concentration of ammonia). In the case of *P. subcapitata*, the increase of removal kinetics with the decrease of N:P molar ratio was only significant in assay 5 (corresponding to the lowest concentration of ammonia). Thus, the results showed that *C. vulgaris* requires higher nitrogen concentrations in culture medium than *P. subcapitata*. Different values can be found in literature for kinetic constant of $N\text{-}NH_4^+$ uptake by microalgae: (i) 0.05-0.16 d^{-1} (*Chlorella* sp. and *Micractinium* sp.) [40]; and (ii) 2.5 d^{-1} (*C. vulgaris*) [56]. Concerning the yield of biomass based on ammonium consumption, the highest values were also obtained for all cultures with N:P molar ratio of 8:1.

Table 4 also shows the removal efficiency and kinetics of $N\text{-}NO_3^-$. Both microalgae were able to efficiently uptake this nutrient, presenting removal efficiencies above 95%. All microalgal cultures fulfilled the limit defined by EU legislation for nitrogen concentration in discharged effluents (10 $mg_N\ L^{-1}$). The removal rates increased with the increase of initial NO_3^- concentration. The maximum values occurred in cultures with N:P molar ratio of 16:1 for *C. vulgaris* and 24:1 for *P. subcapitata*. Analysing the temporal variation of $N\text{-}NO_3^-$ concentration in assays 2 and 5 (cultures also having $N\text{-}NH_4^+$ in medium composition), this value did not change significantly in the beginning of cultivation time (see Figure 3). For these cultures, the modified Gompertz model was applied to describe the evolution of $N\text{-}NO_3^-$ concentration in the microalgal cultures. The observed delay of $N\text{-}NO_3^-$ uptake showed that these species prefer $N\text{-}NH_4^+$ rather than $N\text{-}NO_3^-$. These results were expected since $N\text{-}NH_4^+$ is directly assimilated by microalgae, whereas $N\text{-}NO_3^-$ requires the previous reduction of $N\text{-}NO_3^-$ into $N\text{-}NH_4^+$ [38, 57]. In addition, this delay increases with the increase of the initial $N\text{-}NH_4^+$ concentration, taking more relevance in cultures of *C. vulgaris* (maximum delay of 3.26±0.05 d) comparing to *P. subcapitata* (maximum delay of 1.5±0.6 d). For assays 3 and 6 (only $N\text{-}NO_3^-$ as nitrogen source), the

nutrient uptake follows a pseudo-first-order kinetic equation. Higher kinetic constants were obtained for lower N-NO_3^- concentrations (N:P molar ratio of 8:1). This behaviour was observed for both species. Despite the high removal efficiencies, kinetic constants of N-NO_3^- removal were lower than the ones achieved by Ruiz et al. [56] (1.4-1.7 d^{-1}). Moreover, these values were also lower than the N-NH_4^+ uptake rates obtained in this work. This phenomenon is justified by the mechanism adopted by microalgae to assimilate different nitrogen sources. Biomass yields based on nitrogen consumption for *C. vulgaris* and *P. subcapitata* decreased with the increase of N:P molar ratio. *C. vulgaris* presented higher biomass yields than *P. subcapitata* for both nitrogen sources: (i) 13.5-37.9 $g_{biomass}$ g_N^{-1} (assay 1 – *C. vulgaris*, N-NH_4^+) and 4.8-12.5 $g_{biomass}$ g_N^{-1} (assay 4 – *P. subcapitata*, N-NH_4^+); and (ii) 13.2-35.5 $g_{biomass}$ g_N^{-1} (assay 3 – *C. vulgaris*, N-NO_3^-) and **11.4-22.0** $g_{biomass}$ g_N^{-1} (assay 6 – *P. subcapitata*, N-NO_3^-). Biomass yields achieved with N-NH_4^+ **in assay 4 were very low. For example, the value 4.8 $g_{biomass}$ g_N^{-1} corresponds to a percentage of nitrogen in biomass of about 20%. This value is usually 6.8-12.4% [58]. These results indicate that ammonia stripping might have occurred. This phenomenon has high probability of occurrence with pH values higher than 8 (which was verified for all cultures).**

3.2.3. Phosphorus

Phosphorus is one of the key elements for microalgal growth, as it is used in the energy metabolism, playing an important role on cell growth [38]. Microalgae preferably uptake the inorganic forms $H_2PO_4^-$ and HPO_4^{2-}. In addition, microalgae have a second mechanism for phosphorus removal, called luxury uptake. Luxury uptake is the storage of phosphorus within the biomass in the form of polyphosphates [59, 60]. Table 5 shows the phosphorus (P-PO_4^{3-}) removal efficiencies and kinetic parameters. Microalgal cultures presented high removal efficiencies, achieving phosphorus concentrations below the limit defined by EU legislation (1 mg_P L^{-1}). Maximum removal rates were in the range of 0.48-2.61 mg_P L^{-1} d^{-1}. Temporal variation of its concentration was similar in all cultures, following the tendency described by pseudo-first-order kinetic equation. Applying this kinetic model, the maximum phosphorus uptake rates were obtained in the assays 2 and 5 (corresponding to cultures of *C. vulgaris* and *P. subcapitata*, respectively), when both nitrogen sources were present in the culture medium. The achieved kinetic constants were in the same order of magnitude than the ones presented by Wang et al. [40] (0.17-0.32 d^{-1}), but significantly lower than those presented by Ruiz et al. [56] (2.0-

3.2 d^{-1}). Higher values obtained in the last study are justified by the feed of CO_2 at higher concentrations (5%) when compared with this study, which promoted microalgal growth and, consequently, nutrient removal from the culture medium using atmospheric CO_2 concentrations. Biomass yields based on phosphorus consumption did not vary significantly in cultures of *C. vulgaris* (130.2-150.2 $g_{biomass}$ g_P^{-1}). Cultures of *P. subcapitata* presented higher biomass yields (between 37.0 and 59.8 $g_{biomass}$ g_P^{-1}) for higher N:P molar ratios. These values showed that the mass percentages of phosphorus in *C. vulgaris* are lower (0.67-0.77%) than those in *P. subcapitata* (1.7-2.7%). These results suggest that *P. subcapitata* may remove phosphorus by luxury uptake, as they contain a percentage of phosphorus greater than 1% [59]. This removal mechanism may take more importance in the media with lower N:P molar ratio, in which higher phosphorus mass concentrations were achieved.

3.2.4. Sulphur

The consumption of sulphur was significantly lower than other studied nutrients. Table 6 shows the sulphur ($S\text{-}SO_4^{2-}$) removal efficiencies and kinetic parameters. *C. vulgaris* presented higher removal efficiencies (75-100%) when compared with *P. subcapitata* (54-92%). The removal rates did not significantly vary in the different cultures, presenting a maximum of 0.821 mg_S L^{-1} d^{-1}. The analysis of temporal variation of $S\text{-}SO_4^{2-}$ concentration in the medium was also performed by fitting the pseudo-first-order kinetic equation to the experimental results. The kinetic constants were in the range of 0.139±0.005 to 0.42±0.03 d^{-1}. Biomass yields based on sulphur consumption were between 338.0 and 397.1 $g_{biomass}$ g_S^{-1} for *C. vulgaris* and between 93.3 and 207.9 $g_{biomass}$ g_S^{-1} for *P. subcapitata*.

4. Conclusions

This study showed the potential of *C. vulgaris* and *P. subcapitata* for biomass production and simultaneous nutrient removal from a synthetic effluent. Regarding biomass production, *C. vulgaris* led to higher biomass concentrations and higher productivities than *P. subcapitata*, showing higher potential for biomass production. The value of N:P molar ratio that favoured microalgal growth was 8:1 for *C. vulgaris* and 16:1 for *P. subcapitata*. Taking into account these results and typical compositions of different wastewaters, it can be concluded that *C. vulgaris* can be grown in wastewaters from the dairy and swine industries and in anaerobic digestion effluents from dairy manure,

whereas *P. subcapitata* can be grown in poultry wastewaters. The nutrient uptake by microalgae from the culture medium was also analysed, focusing inorganic carbon, nitrogen (ammonium and nitrate), phosphorus and sulphur. Inorganic carbon presented only a slightly decrease (about 10%) in the first day of the cultures. Both microalgae efficiently removed nitrogen and phosphorus from the medium (almost all cultures presented removal efficiencies above 95%). Higher uptake rates were determined for ammonium, which complete removal from culture medium was observed at the second day of culture. The cultures fed with both nitrogen sources (ammonium and nitrate) showed that ammonium was preferably assimilated by *C. vulgaris*. The removal efficiencies of sulphates were significantly lower, presenting values between 54 and 100%. Thus, both microalgae showed high potential for nutrient removal from wastewater, mainly nitrogen and phosphorus, accomplishing the limits defined by EU legislation. Thus, microalgal culture using wastewater as culture medium lowers the cost of biomass production, improving the economic competitiveness of microalgae-based products.

Acknowledgements

This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors – COMPETE, ON.2 - O Novo Norte - North Portugal Regional Operational Programme and National Funds through FCT - Foundation for Science and Technology under the projects: Pest-C/EQB/UI0511, Pest-C/EQB/LA0020/2013, NORTE-07-0124-FEDER-000026 and NORTE-07-0162-FEDER-000050. Ana L. Gonçalves, Francisca C. Moreira and Tânia F. C. V. Silva acknowledge their Ph.D. fellowships (with the references SFRH/BD/88799/2012, SFRH/BD/80361/2011 and SFRH/BD/73510/2010, respectively) supported by FCT, POPH-QREN and European Social Fund (FSE). Vítor .J.P. Vilar acknowledges the FCT Investigator 2013 Programme (IF/01501/2013). José C.M. Pires acknowledges QREN and FCT for his fellowship FEUP-ON2-26-FM-Engª Sistemas of the project SAESCTN-PIIC&DT\1\2011.

451 **References**

452 [1] D.P. Serrano, J. Dufour, D. Iribarren, On the feasibility of producing hydrogen with
453 net carbon fixation by the decomposition of vegetable and microalgal oils, Energ Environ
454 Sci, 5 (2012) 6126-6135.

455 [2] R. Luque, L. Herrero-Davila, J.M. Campelo, J.H. Clark, J.M. Hidalgo, D. Luna, J.M.
456 Marinas, A.A. Romero, Biofuels: a technological perspective, Energ Environ Sci, 1
457 (2008) 542-564.

458 [3] T.W.R. Powell, T.M. Lenton, Future carbon dioxide removal via biomass energy
459 constrained by agricultural efficiency and dietary trends, Energ Environ Sci, 5 (2012)
460 8116-8133.

461 [4] M. Mikkelsen, M. Jorgensen, F.C. Krebs, The teraton challenge. A review of fixation
462 and transformation of carbon dioxide, Energ Environ Sci, 3 (2010) 43-81.

463 [5] R.H. Wijffels, M.J. Barbosa, An Outlook on Microalgal Biofuels, Science, 329 (2010)
464 796-799.

465 [6] Y. Zhou, L. Schideman, G. Yu, Y.H. Zhang, A synergistic combination of algal
466 wastewater treatment and hydrothermal biofuel production maximized by nutrient and
467 carbon recycling, Energ Environ Sci, 6 (2013) 3765-3779.

468 [7] J.C.M. Pires, M.C.M. Alvim-Ferraz, F.G. Martins, M. Simoes, Carbon dioxide capture
469 from flue gases using microalgae: Engineering aspects and biorefinery concept, Renew
470 Sust Energ Rev, 16 (2012) 3043-3053.

471 [8] Y. Chisti, Biodiesel from microalgae, Biotechnol Adv, 25 (2007) 294-306.

472 [9] J. Lu, C. Sheahan, P.C. Fu, Metabolic engineering of algae for fourth generation
473 biofuels production, Energ Environ Sci, 4 (2011) 2451-2466.

474 [10] L. Lardon, A. Helias, B. Sialve, J.P. Steyer, O. Bernard, Life-Cycle Assessment of
475 Biodiesel Production from Microalgae, Environ Sci Technol, 43 (2009) 6475-6481.

476 [11] E.M. Grima, E.H. Belarbi, F.G.A. Fernandez, A.R. Medina, Y. Chisti, Recovery of
477 microalgal biomass and metabolites: process options and economics, Biotechnol Adv, 20
478 (2003) 491-515.

[12] V. Vasudevan, R.W. Stratton, M.N. Pearlson, G.R. Jersey, A.G. Beyene, J.C. Weissman, M. Rubino, J.I. Hileman, Environmental Performance of Algal Biofuel Technology Options, Environ Sci Technol, 46 (2012) 2451-2459.

[13] M.Y. Menetrez, An Overview of Algae Biofuel Production and Potential Environmental Impact, Environ Sci Technol, 46 (2012) 7073-7085.

[14] J.K. Pittman, A.P. Dean, O. Osundeko, The potential of sustainable algal biofuel production using wastewater resources, Bioresource Technol, 102 (2011) 17-25.

[15] J. Pires, M. Alvim-Ferraz, F. Martins, M. Simoes, Wastewater treatment to enhance the economic viability of microalgae culture, Environ Sci Pollut R, 20 (2013) 5096-5105.

[16] I. Rawat, R.R. Kumar, T. Mutanda, F. Bux, Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production, Appl Energ, 88 (2011) 3411-3424.

[17] J.P. Hoffmann, Wastewater treatment with suspended and nonsuspended algae, J Phycol, 34 (1998) 757-763.

[18] N.N. Bich, M.I. Yaziz, N.A.K. Bakti, Combination of Chlorella vulgaris and Eichhornia crassipes for wastewater nitrogen removal, Water Res, 33 (1999) 2357-2362.

[19] M.E. Martinez, S. Sanchez, J.M. Jimenez, F. El Yousfi, L. Munoz, Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus, Bioresource Technol, 73 (2000) 263-272.

[20] A. Ruiz-Marin, L.G. Mendoza-Espinosa, T. Stephenson, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater, Bioresource Technol, 101 (2010) 58-64.

[21] T.Y. Zhang, Y.H. Wu, H.Y. Hu, Domestic wastewater treatment and biofuel production by using microalga Scenedesmus sp ZTY1, Water Sci Technol, 69 (2014) 2492-2496.

[22] A.M. Akerstrom, L.M. Mortensen, B. Rusten, H.R. Gislerod, Biomass production and nutrient removal by Chlorella sp as affected by sludge liquor concentration, J Environ Manage, 144 (2014) 118-124.

[23] E. Ficara, A. Uslenghi, D. Basilico, V. Mezzanotte, Growth of microalgal biomass on supernatant from biosolid dewatering, Water Sci Technol, 69 (2014) 896-902.

509 [24] E. Uggetti, B. Sialve, E. Latrille, J.P. Steyer, Anaerobic digestate as substrate for
510 microalgae culture: The role of ammonium concentration on the microalgae productivity,
511 Bioresource Technol, 152 (2014) 437-443.

512 [25] L.E. Gonzalez, R.O. Canizares, S. Baena, Efficiency of ammonia and phosphorus
513 removal from a Colombian agroindustrial wastewater by the microalgae Chlorella
514 vulgaris and Scenedesmus dimorphus, Bioresource Technol, 60 (1997) 259-262.

515 [26] M.V. Jimenez-Perez, P. Sanchez-Castillo, O. Romera, D. Fernandez-Moreno, C.
516 Perez-Martinez, Growth and nutrient removal in free and immobilized planktonic green
517 algae isolated from pig manure, Enzyme Microb Tech, 34 (2004) 392-398.

518 [27] I. de Godos, V.A. Vargas, S. Blanco, M.C.G. Gonzalez, R. Soto, P.A. Garcia-Encina,
519 E. Becares, R. Munoz, A comparative evaluation of microalgae for the degradation of
520 piggery wastewater under photosynthetic oxygenation, Bioresource Technol, 101 (2010)
521 5150-5158.

522 [28] S. Chinnasamy, A. Bhatnagar, R.W. Hunt, K.C. Das, Microalgae cultivation in a
523 wastewater dominated by carpet mill effluents for biofuel applications, Bioresource
524 Technol, 101 (2010) 3097-3105.

525 [29] H.Y. Su, Y.L. Zhang, C.M. Zhang, X.F. Zhou, J.P. Li, Cultivation of Chlorella
526 pyrenoidosa in soybean processing wastewater, Bioresource Technol, 102 (2011) 9884-
527 9890.

528 [30] T.J. Lundquist, I.C. Woertz, N. Quinn, J.R. Benemann, A realistic technology and
529 engineering assessment of algae biofuel production, Energy Biosciences Institute, 2010.

530 [31] J. Yang, X. Li, H.Y. Hu, X. Zhang, Y. Yu, Y.S. Chen, Growth and lipid accumulation
531 properties of a freshwater microalga, Chlorella ellipsoidea YJ1, in domestic secondary
532 effluents, Appl Energ, 88 (2011) 3295-3299.

533 [32] A.M. Silva-Benavides, G. Torzillo, Nitrogen and phosphorus removal through
534 laboratory batch cultures of microalga Chlorella vulgaris and cyanobacterium
535 Planktothrix isothrix grown as monoalgal and as co-cultures, J Appl Phycol, 24 (2012)
536 267-276.

537 [33] L.D. Zhu, Z.M. Wang, Q. Shu, J. Takala, E. Hiltunen, P.Z. Feng, Z.H. Yuan, Nutrient
538 removal and biodiesel production by integration of freshwater algae cultivation with
539 piggery wastewater treatment, Water Res, 47 (2013) 4294-4302.

[34] M. DellaGreca, M.R. Iesce, F. Cermola, M. Rubino, M. Isidori, Phototransformation of carboxin in water. Toxicity of the pesticide and its sulfoxide to aquatic organisms, J Agr Food Chem, 52 (2004) 6228-6232.

[35] C.J. McLarnon-Riches, C.E. Rolph, D.L.A. Greenway, P.K. Robinson, Effects of environmental factors and metals on Selenastrum capricornutum lipids, Phytochemistry, 49 (1998) 1241-1247.

[36] P. Fergola, M. Cerasuolo, A. Pollio, G. Pinto, M. DellaGreca, Allelopathy and competition between Chorella vulgaris and Pseudokirchneriella subcapitata: Experiments and mathematical model, Ecol Model, 208 (2007) 205-214.

[37] OECD, Freshwater alga and cyanobacteria, growth inhibition test. Test Guideline 201, Organisation for Economic Co-operation and Development, 2011.

[38] T. Cai, S.Y. Park, Y.B. Li, Nutrient recovery from wastewater streams by microalgae: Status and prospects, Renew Sust Energ Rev, 19 (2013) 360-369.

[39] A.L. Goncalves, M. Simoes, J.C.M. Pires, The effect of light supply on microalgal growth, CO2 uptake and nutrient removal from wastewater, Energ Convers Manage, 85 (2014) 530-536.

[40] M. Wang, W.C. Kuo-Dahab, S. Dolan, C. Park, Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, Chlorella sp and Micractinium sp., in wastewater treatment, Bioresource Technol, 154 (2014) 131-137.

[41] M.J. Griffiths, R.P. van Hille, S.T.L. Harrison, The effect of nitrogen limitation on lipid productivity and cell composition in Chlorella vulgaris, Appl Microbiol Biot, 98 (2014) 2345-2356.

[42] M.S. Mohamed, J.S. Tan, S. Kadkhodaei, R. Mohamad, M.N. Mokhtar, A.B. Ariff, Kinetics and modeling of microalga Tetraselmis sp FTC 209 growth with respect to its adaptation toward different trophic conditions, Biochem Eng J, 88 (2014) 30-41.

[43] M.I. Queiroz, M.O. Hornes, A.G. da Silva-Manetti, E. Jacob-Lopes, Single-cell oil production by cyanobacterium Aphanothece microscopica Nageli cultivated heterotrophically in fish processing wastewater, Appl Energ, 88 (2011) 3438-3443.

[44] M.H. Zwietering, I. Jongenburger, F.M. Rombouts, K. Vantriet, Modeling of the Bacterial-Growth Curve, Appl Environ Microb, 56 (1990) 1875-1881.

570 [45] M.F. Blair, B. Kokabian, V.G. Gude, Light and growth medium effect on *Chlorella*
571 *vulgaris* biomass production, Journal of Environmental Chemical Engineering, 2 (2014).

572 [46] J.C.M. Pires, A.L. Gonçalves, F.G. Martins, M.C.M. Alvim-Ferraz, M. Simões,
573 Effect of light supply on CO_2 capture from atmosphere by *Chlorella vulgaris* and
574 *Pseudokirchneriella subcapitata*, Mitigation and Adaptation Strategies for Global
575 Change, 19 (2014) 1109-1117.

576 [47] M. Moreira-Santos, A.M.V.M. Soares, R. Ribeiro, An in situ bioassay for freshwater
577 environments with the microalga Pseudokirchneriella subcapitata, Ecotox Environ Safe,
578 59 (2004) 164-173.

579 [48] B.G. Terigar, C.S. Theegala, Investigating the interdependence between cell density,
580 biomass productivity, and lipid productivity to maximize biofuel feedstock production
581 from outdoor microalgal cultures, Renew Energ, 64 (2014) 238-243.

582 [49] F. Hadj-Romdhane, P. Jaouen, J. Pruvost, D. Grizeau, G. Van Vooren, P. Bourseau,
583 Development and validation of a minimal growth medium for recycling Chlorella
584 vulgaris culture, Bioresource Technol, 123 (2012) 366-374.

585 [50] K. Kapdan, S. Aslan, Application of the Stover-Kincannon kinetic model to nitrogen
586 removal by Chlorella vulgaris in a continuously operated immobilized photobioreactor
587 system, J Chem Technol Biot, 83 (2008) 998-1005.

588 [51] R.J. Geider, J. La Roche, Redfield revisited: variability of C : N : P in marine
589 microalgae and its biochemical basis, Eur J Phycol, 37 (2002) 1-17.

590 [52] C.A. Klausmeier, E. Litchman, T. Daufresne, S.A. Levin, Optimal nitrogen-to-
591 phosphorus stoichiometry of phytoplankton, Nature, 429 (2004) 171-174.

592 [53] A.K. Lee, D.M. Lewis, P.J. Ashman, Microbial flocculation, a potentially low-cost
593 harvesting technique for marine microalgae for the production of biodiesel, J Appl
594 Phycol, 21 (2009) 559-567.

595 [54] B.J. Bellinger, A.S. Abdullahi, M.R. Gretz, G.J.C. Underwood, Biofilm polymers:
596 relationship between carbohydrate biopolymers from estuarine mudflats and unialgal
597 cultures of benthic diatoms, Aquat Microb Ecol, 38 (2005) 169-180.

598 [55] C.J. Hulatt, D.N. Thomas, Dissolved organic matter (DOM) in microalgal
599 photobioreactors: A potential loss in solar energy conversion?, Bioresource Technol, 101
600 (2010) 8690-8697.

601 [56] J. Ruiz, Z. Arbib, P.D. Alvarez-Diaz, C. Garrido-Perez, J. Barragan, J.A. Perales,
602 Photobiotreatment model (PhBT): a kinetic model for microalgae biomass growth and
603 nutrient removal in wastewater, Environ Technol, 34 (2013) 979-991.

604 [57] O. Perez-Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic
605 cultures of microalgae: Metabolism and potential products, Water Res, 45 (2011) 11-36.

606 [58] C.V.G. Lopez, M.D.C. Garcia, F.G.A. Fernandez, C.S. Bustos, Y. Chisti, J.M.F.
607 Sevilla, Protein measurements of microalgal and cyanobacterial biomass, Bioresource
608 Technol, 101 (2010) 7587-7591.

609 [59] N. Powell, A.N. Shilton, S. Pratt, Y. Chisti, Factors influencing luxury uptake of
610 phosphorus by microalgae in waste stabilization ponds, Environ Sci Technol, 42 (2008)
611 5958-5962.

612 [60] N. Powell, A. Shilton, Y. Chisti, S. Pratt, Towards a luxury uptake process via
613 microalgae - Defining the polyphosphate dynamics, Water Res, 43 (2009) 4207-4213.

614

615 **Table 1**. Concentrations of NH_4Cl and $NaNO_3$ for the different assays.

Assay	Microalgae	Nitrogen source	Mass concentration (mg L^{-1})			NH_4^+:NO_3^- molar ratio
			C1	C2	C3	
1	*C. vulgaris*	NH_4Cl	63	126	189	2:0
2		$NH_4Cl/NaNO_3$	31.5/50	63/100	94.5/150	1:1
3		$NaNO_3$	100	200	300	0:2
4	*P. subcapitata*	NH_4Cl	63	126	189	2:0
5		$NH_4Cl/NaNO_3$	31.5/50	63/100	94.5/150	1:1
6		$NaNO_3$	100	200	300	0:2

616 Mass concentrations C1, C2 and C3 corresponded to N:P molar ratio of 8:1, 16:1 and 24:1, respectively.

617

618 **Table 2**. Microalgal growth parameters.

Assay	N:P molar ratio	X_{max} (g L^{-1})	μ (d^{-1})	P_X (g L^{-1} d^{-1})	
				$P_{x\,max}$	$P_{x\,av}$
1	8:1	0.658±0.002	0.68±0.01	0.106±0.003	0.058±0.001
	16:1	0.71±0.02	0.60±0.02	0.105±0.003	0.062±0.002
	24:1	0.70±0.02	0.55±0.03	0.096±0.002	0.062±0.002
2	8:1	0.64±0.05	0.85±0.05	0.093±0.001	0.057±0.006
	16:1	0.68±0.04	0.83±0.03	0.106±0.004	0.060±0.001
	24:1	0.66±0.01	0.76±0.01	0.100±0.001	0.058±0.001
3	8:1	0.622±0.002	0.66±0.03	0.082±0.002	0.058±0.001
	16:1	0.636±0.007	0.67±0.04	0.079±0.003	0.060±0.001
	24:1	0.64±0.01	0.61±0.03	0.077±0.001	0.060±0.001
4	8:1	0.219±0.003	0.98±0.08	0.046±0.002	0.019±0.001
	16:1	0.255±0.009	1.2±0.1	0.049±0.003	0.022±0.001
	24:1	0.245±0.009	0.74±0.04	0.033±0.001	0.022±0.001
5	8:1	0.25±0.01	0.57±0.02	0.046±0.002	0.020±0.001
	16:1	0.288±0.002	0.77±0.05	0.049±0.001	0.025±0.001
	24:1	0.284±0.007	0.57±0.01	0.041±0.004	0.024±0.001
6	8:1	0.19±0.04	0.68±0.04	0.041±0.002	0.015±0.003
	16:1	0.28±0.01	0.77±0.05	0.050±0.001	0.024±0.001
	24:1	0.289±0.002	0.77±0.06	0.047±0.001	0.025±0.001

619 X_{max} – maximum biomass concentration; μ – specific growth rate; $P_{x\,max}$ – maximum value of biomass productivity; $P_{x\,av}$ – average value of biomass productivity.

620 **Table 3**. Microalgal culture parameters.

Assay	N:P molar ratio	pH	T (ºC)	DO (mg L^{-1})
1	8:1	9.7±0.2	23.6±0.6	7.9±0.5
	16:1	9.5±0.2	23.6±0.5	7.9±0.3
	24:1	9.4±0.2	23.6±0.6	7.7±0.4
2	8:1	9.7±0.1	25.3±0.5	7.3±0.2
	16:1	9.5±0.1	25.3±0.5	7.3±0.2
	24:1	9.7±0.2	25.7±0.5	7.5±0.3
3	8:1	9.6±0.1	25.8±0.9	5.0±0.4
	16:1	9.7±0.1	26.0±0.8	5.0±0.4
	24:1	9.6±0.1	25.8±0.6	5.0±0.3
4	8:1	9.3±0.1	24.7±0.5	4.7±0.2
	16:1	9.3±0.1	24.7±0.5	4.7±0.2
	24:1	9.3±0.1	24.7±0.5	4.7±0.1
5	8:1	9.7±0.2	25±2	4.5±0.3
	16:1	9.6±0.2	25±2	4.4±0.1
	24:1	9.5±0.1	25±2	4.4±0.1
6	8:1	9.3±0.1	25.4±0.9	4.3±0.3
	16:1	9.2±0.1	25.1±0.8	4.4±0.3
	24:1	9.4±0.1	25.4±0.7	4.5±0.4

621 Values are presented as the mean ± standard deviation; DO – dissolved oxygen.

622

623 **Table 4**. Nitrogen ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) uptake by microalgae: kinetics and efficiency.

Nutrient	Assay	N:P Molar Ratio	RE (%)	RR		Kinetic Model			Y ($g_{biomass}\ g_N^{-1}$)
				Maximum ($mg_N\ L^{-1}\ h^{-1}$)	Average ($mg_N\ L^{-1}\ d^{-1}$)	k (d^{-1})	λ (d)	R^2	
$N\text{-}NH_4^+$	1	8:1	100	0.83	1.53	3.86±0.05 [a]	-	1.000	37.9
		16:1	100	1.51	3.05	2.55±0.07 [a]	-	1.000	20.4
		24:1	100	2.18	4.58	2.2±0.2 [a]	-	0.997	13.5
	2	8:1	100	2.74	0.75	*	*	*	75.2
		16:1	100	5.12	1.51	*	*	*	40.1
		24:1	100	7.41	2.26	*	*	*	25.5
	4	8:1	100	4.89	1.50	1.2±0.1 [a]	-	0.987	12.5
		16:1	100	9.70	2.99	1.2±0.2 [a]	-	0.978	7.4
		24:1	100	13.92	4.49	0.5±0.1 [a]	-	0.968	4.8
	5	8:1	100	2.15	0.75	3.75±0.02 [a]	-	1.000	26.7
		16:1	100	4.21	1.50	1.63±0.08 [a]	-	0.998	16.5
		24:1	100	5.90	2.25	1.1±0.2 [a]	-	0.963	10.6
$N\text{-}NO_3^-$	2	8:1	100	0.095	0.82	1.1±0.1 [b]	0.86±0.04	0.999	71.0
		16:1	98	0.337	1.67	3.1±0.5 [b]	2.85±0.04	0.998	38.5
		24:1	99	0.156	2.60	1.2±0.1 [b]	3.26±0.05	0.996	24.4
	3	8:1	100	0.250	1.49	0.63±0.06 [a]		0.988	35.5
		16:1	100	0.311	3.01	0.27±0.03 [a]		0.979	18.2
		24:1	92	0.238	4.22	0.19±0.02 [a]		0.981	13.2
	5	8:1	99	0.198	0.95	1.8±0.8 [b]	1.0±0.2	0.956	25.5
		16:1	100	0.100	1.49	0.34±0.07 [b]	0.3±0.6	0.984	15.6
		24:1	69	0.257	1.67	0.4±0.1 [b]	1.5±0.6	0.959	14.4
	6	8:1	92	0.361	1.15	1.0±0.2 [a]		0.976	**22.0**
		16:1	96	0.398	2.51	0.55±0.06 [a]		0.987	**16.6**
		24:1	97	0.448	4.00	0.35±0.02 [a]		0.994	**11.4**

624 RE – Removal Efficiency; RR – Removal Rate; Y – Yield of biomass based on nutrient consumption.
625 a – pseudo-first-order kinetic model; b – modified Gompertz model; * – not enough data to determine model parameters.
626

627 **Table 5**. Phosphorus ($P\text{-}PO_4^{3-}$) uptake by microalgae: kinetics and efficiency.

Assay	N:P molar ratio	RE (%)	RR		Pseudo-First-Order Kinetic Model		Y ($g_{biomass}\ g_P^{-1}$)
			Maximum ($mg_P\ L^{-1}\ h^{-1}$)	Average ($mg_P\ L^{-1}\ d^{-1}$)	k (d^{-1})	R^2	
1	8:1	99	0.073	0.42	0.54±0.04	0.996	139.1
	16:1	98	0.048	0.41	0.44±0.06	0.984	150.2
	24:1	100	0.051	0.42	0.32±0.06	0.974	147.1
2	8:1	97	0.073	0.39	0.55±0.05	0.989	139.6
	16:1	98	0.090	0.38	0.68±0.04	0.995	147.8
	24:1	97	0.070	0.38	0.61±0.06	0.987	141.9
3	8:1	99	0.084	0.45	0.55±0.09	0.996	130.2
	16:1	100	0.079	0.45	0.37±0.08	0.960	131.6
	24:1	100	0.088	0.46	0.48±0.03	0.997	131.9
4	8:1	99	0.031	0.39	0.21±0.05	0.965	45.5
	16:1	100	0.041	0.42	0.27±0.07	0.938	53.9
	24:1	99	0.020	0.24	0.25±0.07	0.982	52.8
5	8:1	100	0.094	0.36	0.91±0.04	0.999	48.3
	16:1	100	0.109	0.38	0.778±0.004	1.000	59.7
	24:1	100	0.098	0.39	0.58±0.01	1.000	57.4
6	8:1	100	0.082	0.26	0.47±0.08	0.992	37.0
	16:1	100	0.081	0.27	0.50±0.07	0.995	57.5
	24:1	100	0.078	0.27	0.52±0.06	0.997	59.8

628 RE – Removal Efficiency; RR – Removal Rate; Y – Yield of biomass based on nutrient consumption.

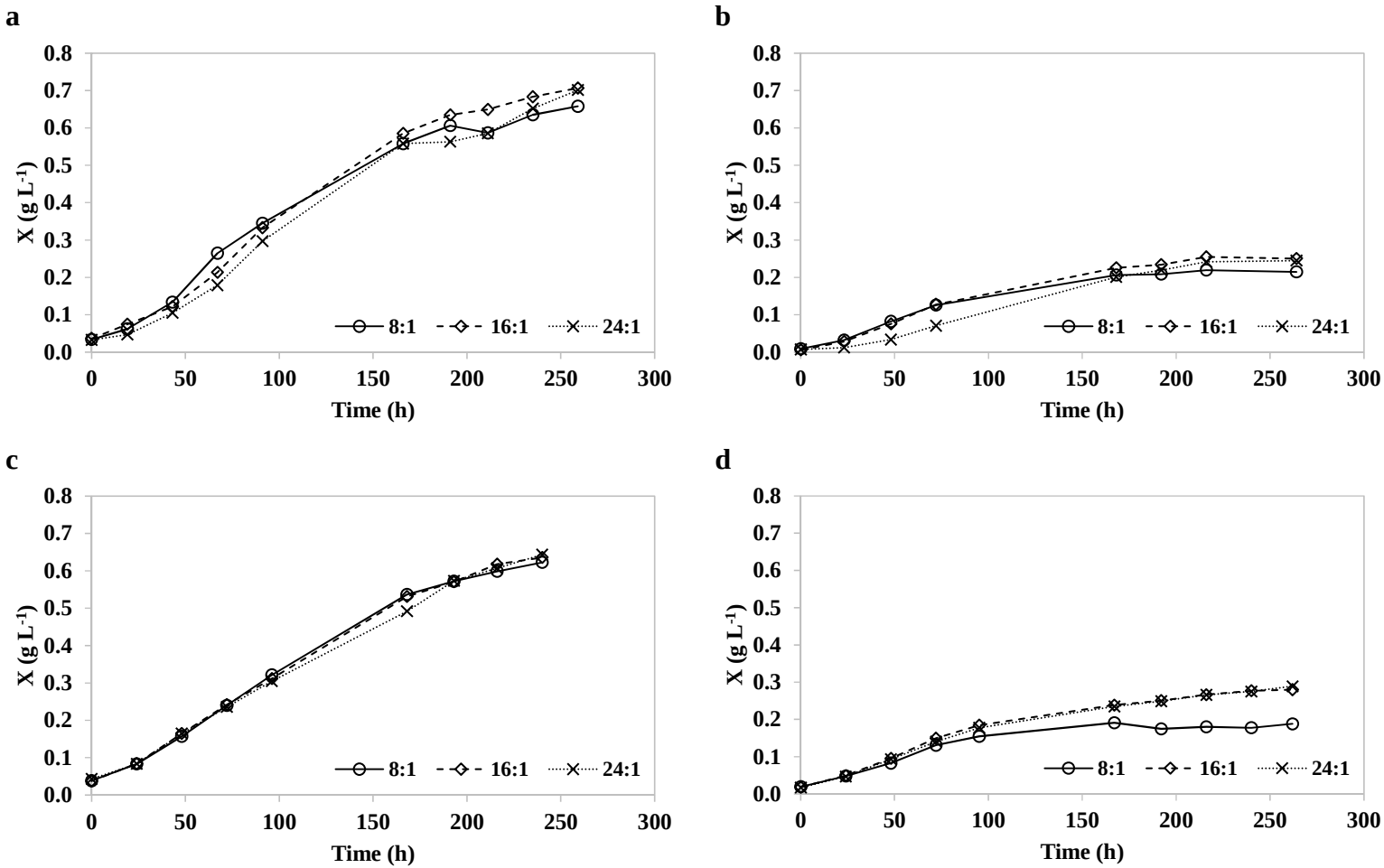
629

630 **Table 6**. Sulphur ($S\text{-}SO_4^{2-}$) uptake by microalgae: kinetics and efficiency.

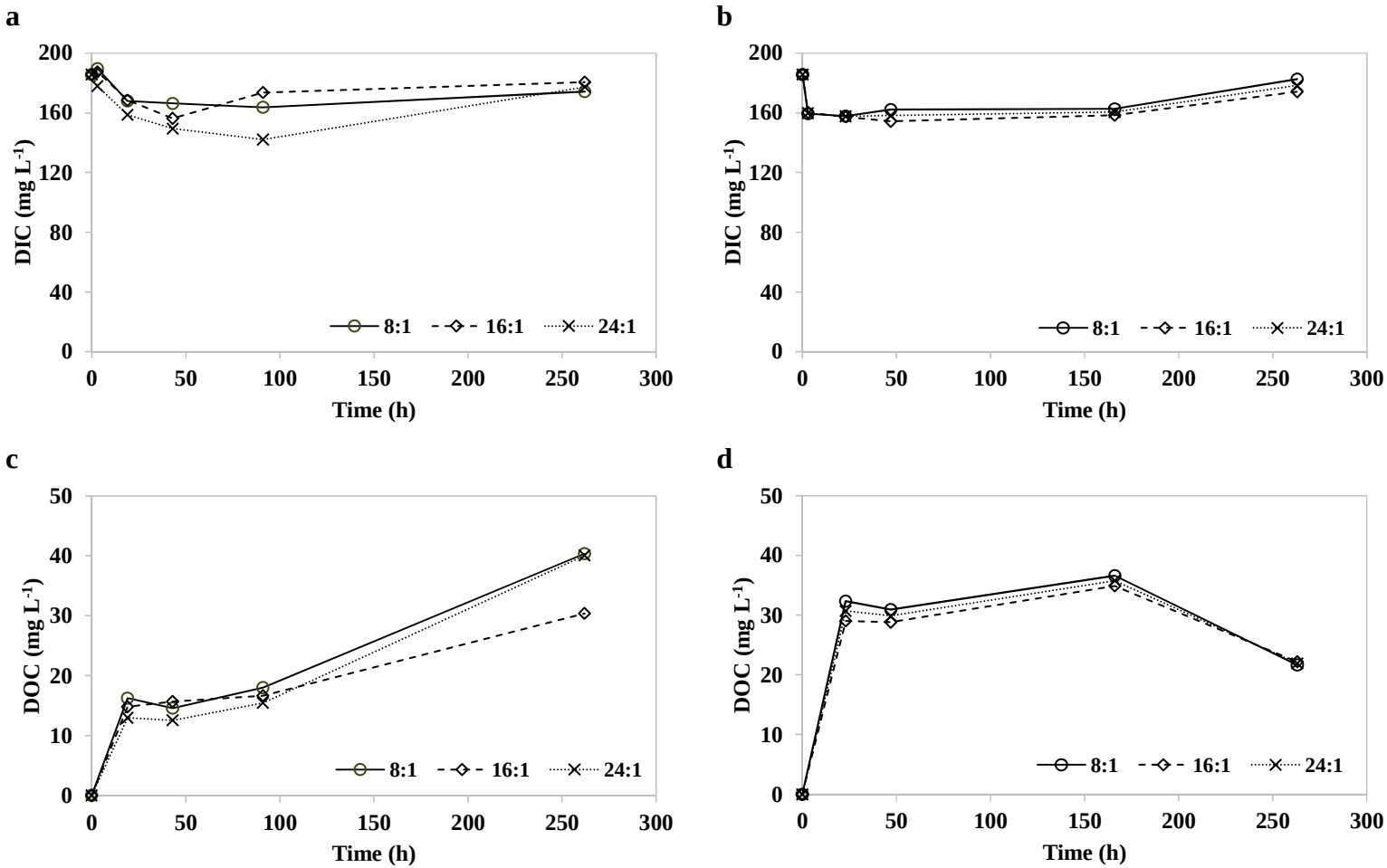
Assay	N:P molar ratio	RE (%)	RR		Pseudo-First-Order Kinetic Model		Y ($g_{biomass}\ g_S^{-1}$)
			Maximum ($mg_S\ L^{-1}\ h^{-1}$)	Average ($mg_S\ L^{-1}\ d^{-1}$)	k (d^{-1})	R^2	
1	8:1	81	0.033	0.440	0.31±0.05	0.945	393.8
	16:1	94	0.029	0.508	0.36±0.02	0.994	366.0
	24:1	100	0.034	0.542	0.42±0.04	0.994	342.7
2	8:1	89	0.011	0.314	0.17±0.02	0.991	356.0
	16:1	100	0.021	0.363	0.42±0.03	0.994	338.0
	24:1	85	0.015	0.275	0.38±0.04	0.983	380.5
3	8:1	75	0.016	0.394	0.21±0.02	0.966	397.1
	16:1	85	0.028	0.543	0.25±0.04	0.962	359.1
	24:1	87	0.014	0.422	0.21±0.02	0.976	352.6
4	8:1	54	0.013	0.231	0.23±0.04	0.934	194.8
	16:1	63	0.012	0.279	0.207±0.003	1.000	199.8
	24:1	78	0.007	0.342	0.139±0.005	0.998	156.0
5	8:1	56	0.034	0.344	0.33±0.06	0.917	200.1
	16:1	81	0.014	0.377	0.24±0.02	0.987	172.2
	24:1	64	0.019	0.333	0.225±0.008	0.998	207.9
6	8:1	92	0.026	0.444	0.30±0.04	0.975	93.3
	16:1	65	0.025	0.334	0.31±0.03	0.982	207.7
	24:1	88	0.028	0.463	0.32±0.04	0.973	159.2

631 RE – Removal Efficiency; RR – Removal Rate; Y – Yield of biomass based on nutrient consumption.

632



633 **Figure 1**. Temporal variation of biomass concentration (*X*) for *Chlorella vulgaris* (*a* and *c*) and *Pseudokirchneriella subcapitata* (*b* and *d*) cultivated
634 with ammonium (*a* and *b*) and nitrate (*c* and *d*).



635 **Figure 2**. Temporal variation of dissolved inorganic (*a* and *b*) and organic (*c* and *d*) carbon concentrations in the assays 2 (*a* and *c*) and 5 (*b* and
636 *d*).

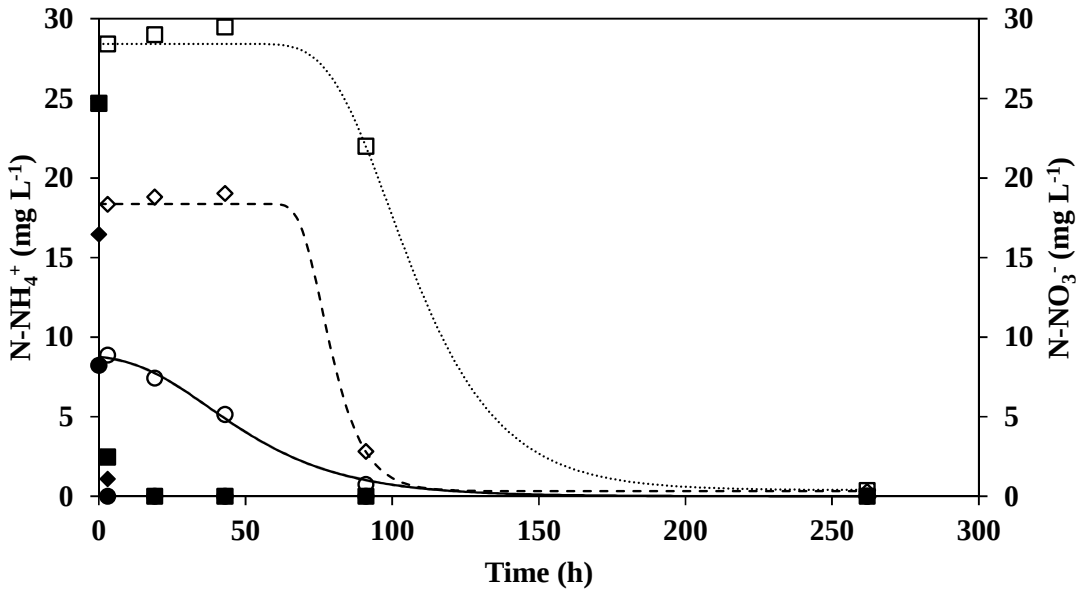


Figure 3. Temporal variation of ammonium ($N\text{-}NH_4^+$ – filled symbols) and nitrate ($N\text{-}NO_3^-$ – open symbols) concentrations in the assay 2: (i) circles – N:P molar ratio of 8:1; (ii) diamonds – N:P molar ratio of 16:1; and (iii) squares – N:P molar ratio of 24:1. Modified Gompertz model (lines) was determined with $N\text{-}NO_3^-$ concentration data for the tested N:P molar ratios.