ENVIRONMENTAL BIOTECHNOLOGY

Kinetic exploration of nitrate-accumulating microalgae for nutrient recovery

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Abstract Within sustainable resource management, the recovery of nitrogen and phosphorus nutrients from waste streams is becoming increasingly important. Although the use of microalgae has been described extensively in environmental biotechnology, the potential of nitrate-accumulating microalgae for nutrient recovery has not been investigated yet. The ability of these marine microorganisms to concentrate environmental nitrate within their biomass is remarkable. The aim of this study was to investigate the application potential of nitrate-accumulating diatoms for nutrient recovery from marine wastewaters. The intracellular nitrate storage capacity was quantified for six marine diatom strains in synthetic wastewater. *Amphora coffeaeformis* and *Phaeodactylum tricornutum* stored the highest amount of nitrate with respectively 3.15 and 2.10 g N L^{-1} of cell volume, which accounted for 17.3 and 4.6 %, respectively, of the total nitrogen content. The growth and nitrate and phosphate uptake of both diatoms were further analyzed and based on these features *P. tricornutum* showed the highest potential for nutrient recovery. A mathematical model was developed which included intracellular nitrate storage and the kinetic parameters were derived for *P. tricornutum*. Furthermore, a simulation study was performed to compare the performance of a proposed microalgal nutrient recovery unit with a conventional denitrification system for marine wastewater treatment. Overall, this study demonstrates the potential application of *P. tricornutum* for saline wastewater treatment with concurrent nitrogen and phosphorus recycling.

Keywords Microalgae · Nitrogen recovery · Nutrient removal · Kinetics · Modeling · Wastewater

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Introduction

Within sustainable resource management, the recovery of nutrients such as nitrogen and phosphorus is becoming more and more critical (Verstraete and Vlaeminck 2011). In developing regions of Africa and Asia the insufficient access to local nutrient sources forms a severe constraint on human nutrition and economic development (Larsen et al. 2007). Efficient nutrient management, including the recycling of nitrogen and phosphorus from human, industrial and agricultural waste streams, is therefore seen as a necessary element of sustainable development.

In other regions, wastewater phosphorus and nitrogen compounds are considered as pollutants and nutrient management primarily focuses on nutrient removal within wastewater treatment plants to prevent discharge to sensitive surface waters (Larsen et al. 2007; Verstraete and Vlaeminck 2011). The recovery of nutrients is restricted to concentrated waste streams, such as urine, manure, or digestate, which hold reactive nitrogen and phosphorus compounds in concentrations of several grams of nitrogen per liter and up to a gram of phosphorus per liter (Mulder

2003). In this concentration range, physicochemical processes, such as struvite precipitation or ammonia stripping, are available which make nutrient recovery technically and economically feasible (Maurer et al. 2006).

In low-strength waste streams, such as domestic or aquaculture wastewater, nitrogen and phosphorus are present in concentrations of milligrams per liter. Biological or physicochemical nutrient removal processes are here the preferred choice of treatment. For phosphate, conventional removal involves precipitation using iron or aluminum salts. Phosphorus recovery is facilitated using enhanced biological phosphate removal (EBPR) systems using polyphosphate accumulating organisms (PAOs; Yuan et al. 2012). For nitrogen, no recovery options are implemented and biological nitrogen removal techniques based on nitrification/denitrification, nitritation/denitritation, or partial nitritation/anammox are the preferred choice of treatment (Vlaeminck et al. 2012). Despite of their excellent pollutant removal efficiencies, these treatment strategies result in the loss of valuable reactive nitrogen compounds, while the greenhouse and ozone-depleting gas N_2O is also co-emitted (Desloover et al. 2012). The nutrient recovery potential of low-strength wastewater is nonetheless substantial, as the volumes are sufficiently large to represent major nutrient fluxes (Mulder 2003; Yuan et al. 2012). The recycling of these nutrients, using sustainable biological nutrient recovery systems, could therefore reduce the dependency of inorganic fertilizers and generate revenues for wastewater treatment facilities (Verstraete et al. 2009).

The use of microalgae has been described extensively within environmental biotechnology. Microalgae efficiently utilize and remove the nitrogen and phosphorus present in wastewater and assimilate these in valuable algal biomass (Cai et al. 2013; Craggs et al. 1997). Also, the mitigation of flue gas can be included within the algal treatment process due to the ability to fix CO_2 (Van den Hende et al. 2012). Depending on the supplied wastewater, the nutrient-rich algal biomass can subsequently be valorized as a natural food source in aquaculture or as a feedstock for biorefineries (Hemaiswarya et al. 2011; Wijffels and Barbosa 2010).

The potential of nitrate-accumulating microalgae for nutrient recovery has not been investigated so far. These specific types of eukaryotic diatoms have been described in both the pelagic and benthic zones of marine ecosystems (Kamp et al. 2011). Nitrate-accumulating microalgae have the capacity to store nitrate in transitory cytoplasmic pools in concentrations up to several grams of nitrogen per liter of cell volume (Bode et al. 1997; Dortch et al. 1984; Kamp et al. 2011; Lomas and Glibert 2000; Needoba and Harrison 2004). The intracellular nitrate is then used as a source for assimilatory nitrate reduction, enabling the diatoms to overcome fluctuating nitrogen concentrations in the surrounding water and hence support growth when environmental nitrogen is depleted.

The ability of these specific microorganisms to concentrate environmental nitrate within their biomass is remarkable and holds potential for biological nitrogen recovery in saline wastewaters, such as present in intensive recirculating aquaculture systems (van Rijn 2013). The aim of this study is to explore this potential of nitrate-storing diatoms for nutrient recovery from marine wastewater. A screening was performed to determine the nitrate storage capacity of six diatom species in synthetic wastewater. For the diatoms with the highest nitrate accumulation, the growth and nutrient uptake rates were further analyzed. Based on these features, the kinetic growth and nutrient uptake parameters were derived for the best performing diatom species using a mathematical model. Finally, a simulation study was performed to compare the performance of a proposed microalgal nutrient recovery unit with a conventional denitrification system for the treatment of aquaculture wastewater.

Material and methods

Strains and cultivation

The benthic diatom strains *Amphora coffeaeformis* (CCMP 127) and *Nitzschia punctata* (CCMP 561) and the pelagic strains *Skeletonema dohrnii* (CCMP 782), *Thalassiosira nordenskioeldii* (CCMP 995) and *Thalassiosira weissflogii* (CCMP 1336) were obtained from the Provasoli–Guillard National Center for Marine Algae and Microbiota (NCMA). The pelagic strain *Phaeodactylum tricornutum* (CCAP 1055/1) was obtained from the Flanders Institute for Biotechnology (VIB). In all experiments the diatoms were cultured axenically in sterile synthetic wastewater, modified from artificial seawater (ESAW) medium (Berges et al. 2001). Nitrate and phosphate concentrations were 100 mg NO_3^-–N L^{-1} and 44 mg PO_4^{3-}–P L^{-1}, respectively, i.e., a mass and molar N:P ratio of 2.3 and 5, respectively, to prevent phosphorus limitation in the medium. Cultures were aerated at 0.7 L_{air} L_{medium}^{-1} with 0.22 μm filter sterilized 2 % CO_2. The cultivation temperature was 20 °C, the pH was maintained at 8.0–8.2 and continuous illumination was provided from the top by means of cool white lamps (Osram, Dulux L) at a light intensity of 110 μmol photons m^{-2} s^{-1}. Bacterial contamination of the cultures was checked throughout the experiments using phase contrast microscopy.

Quantification of intracellular nitrate

Diatoms were cultured in 0.8 L batch tests in synthetic wastewater. Tests were performed in quadruplicate and growth was monitored by optical density (OD)

measurement at 450 and 670 nm. Intracellular nitrate storage was quantified under exponential and stationary growth phase. The cell disruption method was modified from Dortch (1982). 50 mL of algal suspension was filtered on a 0.45 μm Whatman glass fiber filter. Cells were washed three times with 50 mL of 3 % NaCl to remove extracellular nitrate. Filters were frozen at −80 °C for 15 min, after which cells were broken by adding three times 10 mL of hot (80 °C) distilled water to extract intracellular nitrate. Nitrate, nitrite, ammonium, and phosphate were determined after filtration as detailed below. Determination of the cell density was carried out using light microscopy with a cell counting chamber (KOVA® Glasstic, USA) at 100× magnification (Zeiss Axisoskop 2, Germany). The width and length of cells ($n=100$) was determined at 1,000× magnification using the image processing software tool ImageJ (Schneider et al. 2012). The cell volume was estimated by assuming a biconical cell shape for *P. tricornutum* and a cylindrical shape for the other five species (Kamp et al. 2011).

Kinetic experiments

Growth experiments were performed for *A. coffeaeformis* and *P. tricornutum* in 0.8 L batch tests. Cells in exponential growth were inoculated in synthetic wastewater at a cell density of 3×10^3 cells mL^{-1}. Tests were performed during 10 days and nitrate, nitrite, ammonium, and phosphate concentrations in the medium were measured daily. Growth was monitored daily by cell count (KOVA® Glasstic, USA), and cell concentrations were correlated to biomass concentrations by determining the dry weight (DW) content at different stages of the growth curve in parallel tests. Additional batch tests were performed for *P. tricornutum*, collected at the late stationary phase ($t=14$ days) after spiking with nitrate to reach the maximal cell density. Nutrient depleted cells at an initial cell density of 4×10^7 cells mL^{-1} were spiked with nitrate and phosphate to a final concentration of 100 mg N L^{-1} and 44 mg P L^{-1}. The cell density and nitrate, nitrite, ammonium, and phosphate in the medium were monitored during 3.5 days. All batch tests were performed in quadruplicate.

Analytical techniques

Nitrate, nitrite and phosphate were analyzed after sample filtration using anion chromatography (Metrohm 761 Compact IC, Switzerland). Ammonium (Nessler method), total Kjeldahl nitrogen, and total phosphorus (molybdene–vanadate method) were determined according to standard methods (Greenberg et al. 1992). The total carbon and nitrogen contents of the biomass were determined using an elemental analyzer (ANCA-GSL PDZ Europe, UK).

Model development and parameter estimation

To describe the microalgal growth and nutrient uptake, a kinetic model was developed using the modeling and simulation platform WEST® (Vanhooren et al. 2003). The model was constructed based on the experimental observations and the algal kinetics model developed by Decostere et al. (2013). The latter describes inorganic carbon uptake, oxygen production, and algal growth and decay and was extended in this study with nitrate and phosphate uptake and intracellular nitrate storage. Growth on nitrate and phosphate were expressed by a Monod equation (Aslan and Kapdan 2006; Baldia et al. 1991). Growth on intracellular stored nitrate when the ambient nitrate becomes limiting was included and defined by the same kinetics as direct nitrate assimilation. This growth on intracellular stored nitrate will only occur when the concentration of intracellular stored nitrate becomes small compared to the ambient nitrate concentration, as indicated by the term $\left(\frac{X_{STN}}{S_{NO_3^-}+X_{STN}}\right)$. As other nitrogen species, such as ammonium and nitrite, were not detected, only kinetics for nitrate were considered.

Growth limitation due to the self-shading effect was described by an empirical function, based on the observed growth rate which was determined by cell count (Eq. 1). The microalgal biomass concentration is represented by X_{ALG} (g DWL^{-1}). The biomass inhibition constant K_{i_X} (g DWL^{-1}) and the inhibition exponent n_X were set to 1 and 10, respectively, in order to describe the observed reduction in growth rate at higher biomass concentrations.

$$f(X) = \frac{K_{i\_x}}{K_{i\_x} + (X_{ALG})^{n_x}} \qquad (1)$$

The nitrate storage kinetics were expressed as a function of the algal biomass concentration, the ambient nitrate concentration $S_{NO_3^-}$ (mg NO$_3^-$–N L^{-1}) and the nitrate storage constant k_{STO} (L g^{-1} DW d^{-1}; Eq. 2). A nitrate accumulation limitation factor was also included and was based on the P-uptake model by Henze et al. (2000). This limitation factor is a function of the maximum nitrate uptake capacity K_{cap} (mg NO$_3^-$–N g^{-1} DW), the microalgal biomass concentration, and the effective concentration of nitrate stored in the algal pools X_{STN} (mg NO$_3^-$–N L^{-1}; Table 1):

$$\rho_{STO}^{NO_3^-} = k_{STO}\ S_{NO_3^-}\ X_{ALG}\ \frac{K_{cap}}{K_{cap}+\dfrac{X_{STN}}{X_{ALG}}} \qquad (2)$$

Table 1 Gujer matrix for the kinetic growth model

| Process	X_{alg} (g DW.L^{-1})	$S_{HCO_3^-}$ (mg HCO$_3^-$.L^{-1})	S_{CO_2} (mg CO$_2$.L^{-1})	$S_{CO_3^{2-}}$ (mg CO$_3^{2-}$.L^{-1})	S_{O_2} (mg O$_2$.L^{-1})	$S_{NO_3^-}$ (mg NO$_3^-$-N L^{-1})	$S_{PO_4^{3-}}$ (mg PO$_4$-P L^{-1})	X_{STN} (mg NO$_3$-N L^{-1})	Process rate
1. Growth on HCO$_3^-$ and nutrients	1	$-1/Y_1$			Y_3	$-1/Y_4$	$-1/Y_5$		$\mu_{max}\left(\dfrac{S_{HCO_3^-}}{K_{CO_2}+S_{HCO_3^-}}\right)\left(\dfrac{K_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\dfrac{S_N}{K_{s,N}+S_N}\right)\left(\dfrac{S_P}{K_{s,P}+S_P}\right)\left(\dfrac{Ki\_x}{Ki\_x+(X_{ALG})^{n_x}}\right)X_{ALG}$
2. Growth on CO$_2$ and nutrients	1		$-1/Y_2$		Y_3	$-1/Y_4$	$-1/Y_5$		$\mu_{max}\left(\dfrac{S_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\dfrac{S_N}{K_{s,N}+S_N}\right)\left(\dfrac{S_P}{K_{s,P}+S_P}\right)\left(\dfrac{Ki\_x}{Ki\_x+(X_{ALG})^{n_x}}\right)X_{ALG}$
3. Microalgal growth on stored NO$_3$–N, PO$_4$ and HCO$_3^-$	1	$-1/Y_2$			Y_3		$-1/Y_5$	$-1/Y_4$	$\mu_{max}\left(\dfrac{S_{HCO_3^-}}{K_{CO_2}+S_{HCO_3^-}}\right)\left(\dfrac{K_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\dfrac{X_{STN}}{Ks,N+X_{STN}}\right)\left(\dfrac{Ki\_x}{Ki\_x+(X_{ALG})^{n_x}}\right)$ $\left(\dfrac{S_P}{K_{s,P}+S_P}\right)\left(\dfrac{X_{STN}}{S_N+X_{STN}}\right)X_{ALG}$
4. Microalgal growth on stored NO$_3$–N, PO$_4$ and CO$_2$	1		$-1/Y_1$		Y_3		$-1/Y_5$	$-1/Y_4$	$\mu_{max}\left(\dfrac{S_{CO_2}}{K_{CO_2}+S_{CO_2}}\right)\left(\dfrac{X_{STN}}{Ks,N+X_{STN}}\right)\left(\dfrac{Ki\_x}{Ki\_x+(X_{ALG})^{n_x}}\right)\left(\dfrac{S_P}{K_{s,P}+S_P}\right)$ $\left(\dfrac{X_{STN}}{S_N+X_{STN}}\right)X_{ALG}$
5. Storage of NO$_3$ – N						-1		1	$k_{STO}\,S_N\left(\dfrac{K_{cap}}{K_{cap}+\frac{X_{STN}}{X_{ALG}}}\right)X_{ALG}$

The *dark grey* shaded parts refer to the processes from the model developed by Decostere et al. (2013). The *light grey* shaded parts refer to the processes that were derived from literature. The remaining *white* parts refer to new model developments related to this paper

The half-saturation coefficient for nitrate and the nitrate storage rate were fitted to the experimental data by using the Simplex algorithm (Nelder and Mead 1965), while the maximum specific growth rate μ_{max} (1.05 d^{-1}) and the half-saturation coefficient for phosphate K_{PO4} (8.3×10^{-3} mg P L^{-1}) were obtained from literature (Fawley 1984; Tyrrell 1999). An overview of the modeled processes and the reaction rates developed in this research is given in the Gujer matrix of the model presented in Table 1. Microalgae decay, gas–liquid transfer of O_2 and CO_2 and inorganic carbon equilibrium were also incorporated in the model, but are not given in Table 1 as these processes were taken over from Decostere et al. (2013).

The goodness-of-fit between experimental and simulated values was quantified by calculating the Theil's inequality coefficient (TIC), in which y_i represents simulated data and $y_{m,i}$ represents measured data points (Eq. 3). A TIC value lower than 0.3 hereby indicates a good agreement between model predictions and measured data (Audenaert et al. 2010; Zhou 1993):

$$TIC = \frac{\sqrt{\sum_i (y_i - y_{m,i})^2}}{\sqrt{\sum_i y_i^2} + \sqrt{\sum_i y_{m,i}^2}} \qquad (3)$$

Furthermore, a local sensitivity analysis was performed. The relative sensitivity function (RSF) was adopted to evaluate the sensitivity of the model output (concentration of nitrate, phosphate and algal biomass) to a change of the model parameters. The RSF was calculated out of the sensitivity function (SF) by the finite forward difference method with a relative perturbation factor of 0.1 % (De Pauw and Vanrolleghem 2006). Model parameters are defined as not (RSF<0.25), moderately (0.25<RSF<1), very (1<RSF<2) or extremely (RSF>2) influential (Jiang et al. 2005).
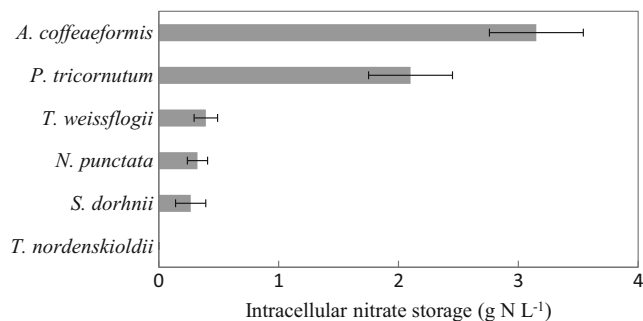
Fig. 1 Intracellular nitrate storage capacity of the six diatoms during exponential growth

Results

Intracellular nitrate storage capacity of the diatom species

A stepwise screening was performed to analyze which diatom species has the highest potential for nutrient recovery. First, the intracellular nitrate storage capacity was determined for six marine diatom species during both exponential and stationary growth and was normalized to their cell volume. A large variation in nitrate storage was observed between the different species (Fig. 1). *A. coffeaeformis* and *P. tricornutum* stored the highest amount of nitrate with respectively 3.15 g NO_3^-–N L^{-1} of cell volume and 2.10 g NO_3^-–N L^{-1} of cell volume. The capacity to accumulate nitrate was not correlated to the cell size or the habitat type (benthic or pelagic) of the studied diatoms. Furthermore, no significant difference ($p<0.05$) in the intracellular nitrate concentration between the exponential growth phase and the stationary growth phase was observed. Although phosphate was never limiting in the medium, intracellular phosphate storage was not detected for any of the diatoms. Moreover, nitrite and ammonium were not observed to be stored intracellularly.

Based on their nitrate storage capacity *A. coffeaeformis* and *P. tricornutum* were selected and their potential for nutrient recovery was further examined by analyzing growth and nutrient uptake of the two species. Inocula from both diatoms in their exponential growth phase were incubated and the evolution of the cell density and nitrate and phosphate concentrations was analyzed. Although both microalgae showed a similar growth curve, the nitrate uptake rate was higher for *P. tricornutum* (Fig. 2). Moreover, the total nitrogen content in exponential growth of *P. tricornutum* (8.1 %) was found to be higher compared to *A. coffeaeformis* (3.0 %) (Table 2). The intracellular nitrate pool of *P. tricornutum* only accounted for 4.6 % of the total nitrogen content, while it accounts for 17.3 % of total nitrogen for *A. coffeaeformis*. The initial decrease in phosphate concentration in both experiments is the result of precipitation rather than assimilation, which was visually noticeable and also shown by a small discrepancy in the phosphorus mass balance of 2.6 mg P L^{-1} (*P. tricornutum*) and 6.6 mg P L^{-1} (*A. coffeaeformis*; Fig. 2 and Table 2).

To enable validation of the kinetic model, additional batch experiments were performed for *P. tricornutum* in which nitrogen-depleted cells in the late stationary phase at maximal cell density (1.55 g DW L^{-1}) were spiked with nitrate and phosphate to a final concentration of 100 mg N L^{-1} and 44 mg P L^{-1} (Fig. 3, right). Nitrate was removed completely from the medium after 3 days, resulting in an increase of the total nitrogen content of the biomass by 34.2 % (Table 2). Also 36 % of the phosphate was removed from the medium, which increased the phosphorus content in the biomass by
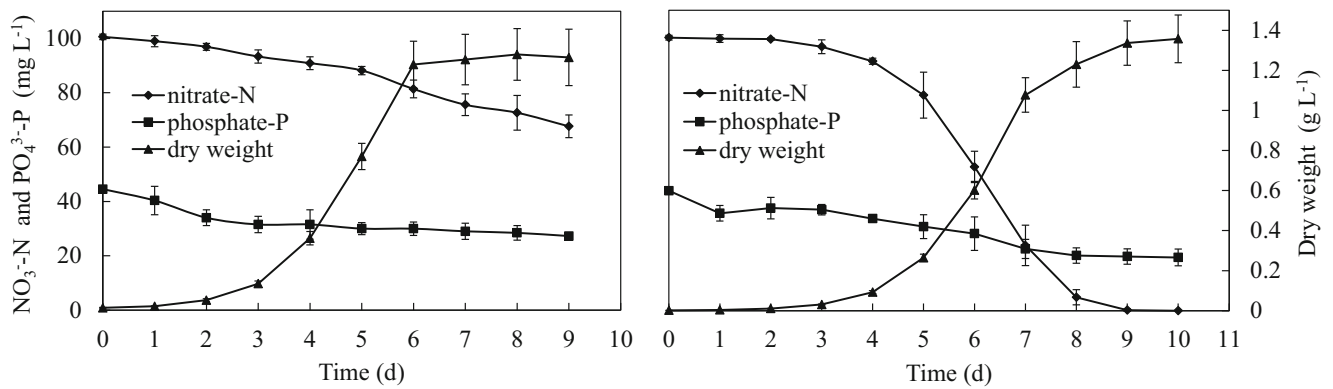
Fig. 2 Evolution of the cell dry weight and nitrate and phosphate concentration in the medium for *Amphora coffeaeformis* (*left*) and *Phaeodactylum tricornutum* (*right*)

6.4 %. However, a difference of 12 mg P L^{-1} between the phosphate removed from the medium and the increase in phosphorus in the biomass indicated that also physicochemical precipitation occurred.

Kinetic parameter estimation for P. tricornutum

A mathematical model was developed for *P. tricornutum* to determine the kinetic parameters using the results of the exponential growth experiment. Model validation was conducted using the stationary growth test. The half-saturation coefficient for nitrate K_{NO3} was estimated at 0.02 mg N L^{-1}, and the nitrate storage rate k_{STO} at 29 L g^{-1} DW d^{-1} with a negligible error (Table 3). Due to identifiability issues, the values for μ_{max} (1.05 d^{-1}) and the half-saturation coefficient for phosphate (8.3×10^{-3} mg P L^{-1}) were chosen from literature and not estimated. Using these assigned values, the kinetic model was able to describe the algal metabolism accurately for the exponential growth experiment (Fig. 3), as confirmed by the low TIC values for nitrate (TIC=0.02), phosphate (TIC= 0.06), and algal biomass (TIC=0.04).

The model validation using the stationary growth experiment showed a larger deviation, as the simulated environmental nitrate concentration at the end of the experiment reached 13 mg N L^{-1}, instead of the experimental 0 mg N L^{-1}. This can be explained by an increase of the dry weight concentration in the system and the increase of the organic nitrogen content, which was unaccounted for in the model.

In addition, a local sensitivity analysis of the kinetic model was executed in order to get an insight in the influence of the parameters on the model simulations (Fig. 4). The exponential growth experiment was used for this local sensitivity analysis and the same experimental conditions and (calibrated) parameter values were applied. From this local sensitivity analysis, it became clear that mainly the relative sensitivity functions related to μ_{max} were influential (RSF>1, Fig. 4a). This stresses the need for a meticulous selection of this chosen parameter to be able to obtain an accurate prediction of the algal metabolism. Results showed that the nitrate concentration was only influenced by the nitrate storage rate at the end of the experiments when nitrate was mostly depleted (Fig. 4b). Furthermore, the sensitivity function for the

Table 2 Biomass composition of *Amphora coffeaeformis* and *Phaeodactylum tricornutum* in exponential growth and stationary growth

	Exponential growth experiment		Stationary growth experiment	
	A. coffeaeformis (*t*=2 days)	*P. tricornutum* (*t*=2 days)	*P. tricornutum* (*t*=0 days)	*P. tricornutum* (*t*=3 days)
C (%)	18.98±1.55	44.44±0.78	47.07±1.29	62.13±2.08
N (%)	3.03±0.27	8.09±0.92	7.25±0.10	11.01±1.49
Organic N (%)	2.51±0.61	7.72±0.72	7.20±0.36	10.64±1.54
NO$_3^-$–N (%)	0.52±0.07	0.37±0.07	0.05±0.02	0.36±0.02
NH$_4^+$–N (%)	ND	ND	ND	0.012±0.003
P (%)	0.80±0.15	1.65±0.21	1.60±0.28	1.71±0.33
NO$_3^-$–N/TN (%)	17.29	4.56	0.70	3.22
C:N:P (mol)	61:8:1	70:11:1	86:10:1	94:14:1
C:N (mol)	7.6	6.4	8.6	6.7
C:N (g)	6.3	5.5	6.5	5.6

ND not detected
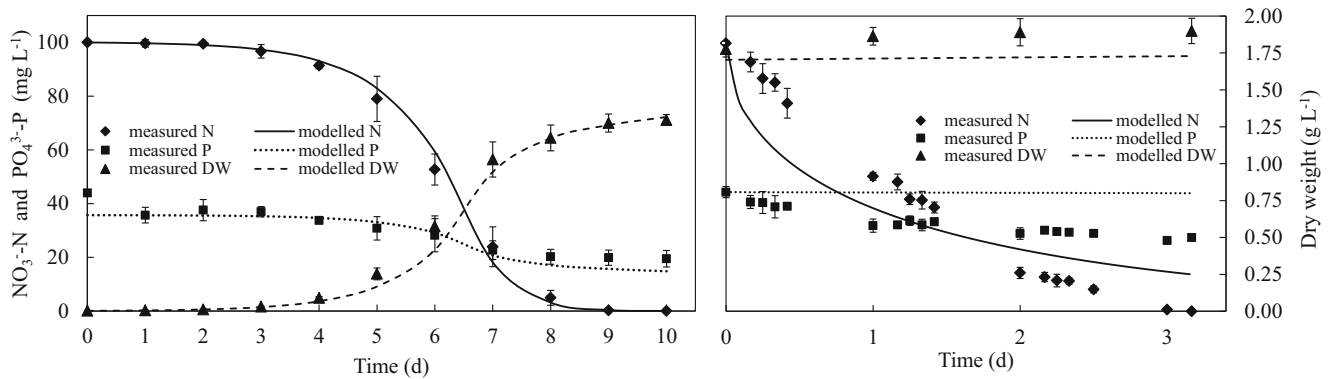
Fig. 3 Model simulation and experimental data of *Phaeodactylum tricornutum* for the exponential growth (*left*) and stationary growth experiment (*right*)

half-saturation coefficients for nitrate and phosphate almost equaled to zero during the experiment, which is explained by the high initial nitrate and phosphate concentrations in the synthetic wastewater.

Model-based comparison of the nutrient recovery system with a conventional denitrification system

The potential for nutrient recovery of *P. tricornutum* was further assessed by simulating a microalgal nutrient recovery system using the calibrated kinetic model. The performance of the proposed nutrient recovery unit, which requires marine conditions, was compared to a conventional submerged moving bed biofilm reactor for seawater denitrification (Labelle et al. 2005). The algal biomass concentration in the system

was controlled at 1 g L^{-1}, while both influent characteristics and reactor volume were derived from Labelle et al. (2005).

The conventional denitrification system achieved a nitrate removal efficiency of 88 % at a hydraulic retention time (HRT) of 1 h (Table 4). The simulated microalgal nutrient recovery system resulted in a nitrate removal efficiency of 92 % at a HRT of 36 h. Also a 55 % phosphate removal efficiency was obtained. The increase of the HRT lowered the possible volumetric loading rate of the reactor from 1.3 to 0.04 g N L^{-1} d^{-1} compared to Labelle et al. (2005) (Fig. 5). A maximal algal biomass production of 109 g DW d^{-1} was obtained at an HRT of 24 h, which results in a nitrate and phosphate removal efficiency of 67 and 37 %, respectively (Fig. 5).

Table 3 Assigned and estimated kinetic parameter values for *Phaeodactylum tricornutum*

Parameter	Description	Assigned value	Unit
μ_{max}	Max. specific growth rate[1]	1.05	d^{-1}
Y_4	Growth yield[2] on N	14.4	g DW g^{-1} N
Y_5	Growth yield[2] on P	66	g DW g^{-1} P
$K_{NO_3^-}$	Half-saturation coefficient[3] for NO_3^-	2.0×10^{-2}	mg N L^{-1}
$K_{PO_4^{3-}}$	Half-saturation coefficient[1] for PO_4^{3-}	8.3×10^{-3}	mg P L^{-1}
K_{i_x}	Biomass inhibition constant[4]	1	g DW L^{-1}
n_x	Biomass inhibition exponent[4]	10	–
K_{cap}	Nitrate uptake capacity[2]	4	mg N g^{-1} DW
k_{STO}	Storage constant[3]	29	L g^{-1} DW d^{-1}

[1] Assigned value from literature (Fawley 1984; Tyrrell 1999)

[2] Calculated from experimental biomass composition

[3] Parameter estimation

[4] Empirically determined based on biomass concentration

Discussion

A screening was performed to determine the nitrate storage capacity of six marine diatoms in synthetic wastewater. The applied nutrient concentrations are representative for the wastewater in marine recirculating aquaculture systems (RAS) (Hamlin et al. 2008; Menasveta et al. 2001; Suzuki et al. 2003). The capacity to accumulate nitrate varied strongly between the diatom species, but was in line with values for microalgal nitrate accumulation in literature (De La Rocha et al. 2010; Dortch et al. 1984; Kamp et al. 2011; Lomas and Glibert 2000; Needoba and Harrison 2004). However, previous research was performed under ecologically relevant conditions, with nitrate concentrations varying from 1 mg N L^{-1} (Lomas and Glibert 2000) to 13 mg N L^{-1} (Kamp et al. 2011), while the applied nitrate concentration of 100 mg N L^{-1} in this study is representative for aquaculture wastewaters (van Rijn 2013). This did not result in a larger intracellular nitrate pool (Fig. 1). No nitrate accumulation was observed for *T. nordenskioeldii*. As also a low growth rate was observed for this diatom, the lack of nitrate storage resulted from the suboptimal cultivation temperature for this diatom, which
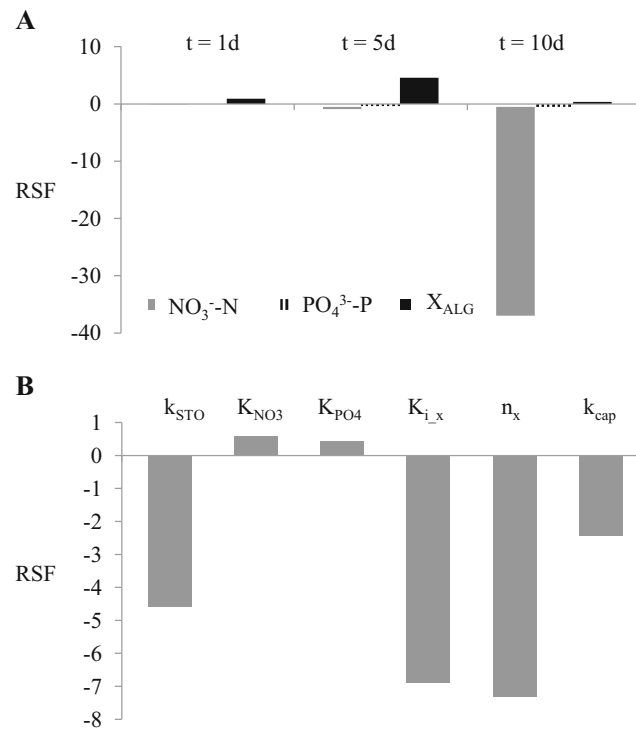
Fig. 4 Sensitivity function of μ_{max} toward the nitrate, phosphate, and biomass concentrations (**a**) and values of the relative sensitivity functions at $t = 10$ days (**b**) during the exponential growth experiment

blooms in cold water habitats (2–15 °C; Hoppenrath et al. 2007). *A. coffeaeformis* and *P. tricornutum* stored the highest amount of nitrate. The evolution of the biomass composition (Table 2) also indicates that nitrate is stored as long as environmental nitrate is not depleted. However, the difference in biomass composition between the two species showed not only that the capacity to store nitrate can vary between species but also that its contribution towards the total nitrogen content is flexible. A range of 0.1 to 4 % of inorganic N of the total N is typically described in literature (Dortch et al. 1984), yet inorganic nitrogen can account for up to 20 % of the total cellular nitrogen (Bode et al. 1997). This is confirmed in this

study by the high ratio of intracellular nitrate to total nitrogen of 17.3 % for *A. coffeaeformis* (Table 2).

Based on the growth and biomass composition, *P. tricornutum* proved to be the most suitable for nutrient recycling. This diatom is studied extensively in biotechnology, as it functions as a model organism in genomic and transcriptomic research (Fabris et al. 2012). However, most research concerning the cultivation of *P. tricornutum* focuses on nitrate- and/or phosphate-depleted conditions. In such conditions, *P. tricornutum* is known to accumulate lipids to concentrations of up to 19 % of the dry weight, making it a valuable resource for biofuel production (Chen et al. 2011; Valenzuela
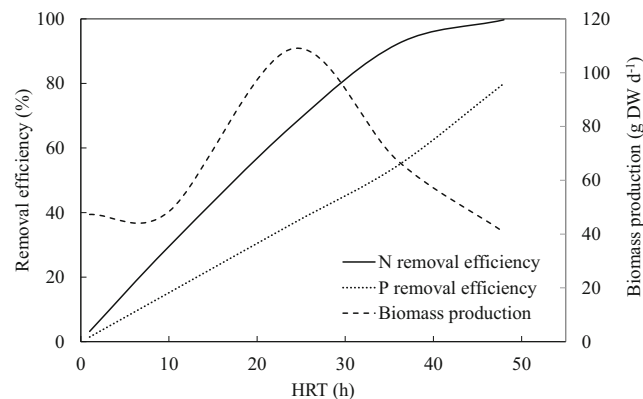
Fig. 5 Simulation of the nitrate removal, phosphate removal and algal biomass production in function of the hydraulic retention time in the reactor

Table 4 Comparison of the simulated microalgal nutrient recovery unit with a conventional seawater denitrification system

Parameter	Labelle et al. (2005)	This study
Influent NO_3^- (mg N L^{-1})		53
Influent PO_4^{3-} (mg P L^{-1})		20
Reactor volume (L)		110
N loading rate (g N L^{-1} d^{-1})	1.3	0.04
HRT (h)	1	36
N removal efficiency (%)	88	92
P removal efficiency (%)	NA	55

ND not analyzed

et al. 2013). In contrast, this study demonstrated the application potential of *P. tricornutum* in conditions with elevated nitrate and phosphate concentrations. In these conditions, *P. tricornutum* reached a total nitrogen content of 8.1 to 11.0 % of the dry weight (Table 2). According to the specific total nitrogen-to-protein conversion factors for *P. tricornutum*, this corresponds to a protein content of 39.7 to 59.3 % of the dry weight, which is higher than the measured 35 % protein content in exponential growth described by Lourenco et al. (2004). The protein content is similar to that of *Chlorella* (60 %) and *Spirulina* (70 %), demonstrating the potential of *P. tricornutum* for applications as single cell protein (Cai et al. 2013; Habib et al. 2008; Lourenco et al. 2004)

To determine the kinetic parameters of *P. tricornutum*, a mathematical model was built based on Decostere et al. (2013). Most microalgal kinetic models describe the photosynthetic activity as a function of a single factor such as light intensity, inorganic carbon or temperature (Ogbonna et al. 1995). Only a few models take into account the combination of different environmental conditions (Bernard and Remond 2012; Bougaran et al. 2010; Filali et al. 2011). The model applied in this work includes the inorganic carbon equilibrium and uptake of inorganic carbon, nitrate, and phosphate. For the first time, intracellular nitrate accumulation was included to describe the algal metabolism. The parameters related to growth on nitrate and nitrate storage were calibrated using the experimental data, and the low TIC values showed that a good model agreement was obtained. The derived half-saturation coefficient for nitrate (0.02 mg N L^{-1}) is in the range of the values for diatoms obtained by Collos et al. (2005) (0.028–0.14 mg N L^{-1}) and is lower than the values of denitrification systems (0.2–0.5 mg N L^{-1}; Henze et al. 1986). This shows that the algal system is superior for mitigation at low nitrogen concentrations.

To further assess the potential of *P. tricornutum* for nutrient recovery and wastewater treatment, a microalgal nutrient recovery unit was simulated for the treatment of aquaculture wastewater and its performance was compared to a conventional denitrification system (Labelle et al. 2005). The simulation study showed that *P. tricornutum* can obtain the same nitrate removal efficiency as the denitrification reactor by increasing the HRT of the system from 1 to 36 h, thereby reducing the loading rate of the system. The microalgal system on the other hand also results in simultaneous phosphate recovery. This valuable nutrient stays untreated in the denitrification reactor, which necessitates further treatment with iron or aluminum salts to remove it from the effluent before discharge. Also the dosage of an additional carbon source under the form of methanol is not required in the microalgal system. From a wastewater treatment perspective the algal unit is however unable to compete with the denitrification system due to the large reactor volume required. This restricts the system in the proposed configuration to treatment sites where the surface area is not constrained. Here, the algal system is competitive with conventional periphyton beds, applied in intensive aquaculture, which achieve 23 % protein recovery with a hydraulic retention time of several days (Crab et al. 2007). The algal biomass can subsequently be valorized as feed in aquaculture or can be commercially applied as a slow-release fertilizer (Mulbry et al. 2005).

From a sustainable resource management perspective, the transition from a nutrient removal to a nutrient recycling approach is inevitable. This case study demonstrates the potential use *P. tricornutum* for marine wastewater treatment and the production of renewable resources under the form of microalgal biomass. Future research is, nonetheless, required to further assess the feasibility of *P. tricornutum* for nutrient recovery. A continuous reactor setup using real wastewater and a natural light regime is indispensable to illuminate the potential of these nitrate-accumulating microalgae for nutrient recovery.

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