Title:

Biotechnological exploration of nitrate-accumulating microalgae for nutrient recovery from saline wastewaters

Introduction

Within sustainable resource management, the recovery of nitrogen and phosphorus nutrients from human and agricultural waste streams is becoming more and more important. Biological treatment systems that allow nutrient recovery can provide sustainable alternatives for the conventional nutrient removal treatments of diluted wastewaters. The use of microalgae has been described extensively within environmental biotechnology, as they efficiently utilize and remove the nitrogen and phosphorus present in wastewater and assimilate these in valuable algal biomass (Cai et al. 2013). However, the potential of nitrate-accumulating microalgae for nutrient recovery so far has not been investigated yet. The ability of these specific micro-organisms to concentrate environmental nitrate within their biomass is nonetheless remarkable and holds potential for biological nitrogen recovery in wastewaters where physicochemical techniques are lacking.

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 the microalgal nutrient recovery unit with a conventional denitrification system for the treatment of aquaculture wastewater.

Material and Methods

Six diatoms strains, *A. coffeaeformis*, *N. punctata*, *S. dohrnii*, *T. nordenskioeldii*, *T. weissflogii* and *P. tricornutum* were cultured in axenic batch cultures in artificial marine seawater containing 100 mg/L NO_3^--N and 44 mg/L PO_4^{3-}-P. Intracellular nitrate storage was quantified in the exponential and stationary growth phase by filtering cells, freezing them for 15minutes at -80 °C and extracting intracellular nitrate with hot (80°C) distilled water.

Exponential growth experiments were performed for *A. coffeaeformis* and *P. tricornutum* in the same medium. The cell density, nitrate, nitrite, ammonium and phosphate were monitored daily. Additional batch tests were performed for *P. tricornutum*, collected at the late stationary phase at maximal cell density to further assess the nutrient uptake.

To describe the microalgal growth and nutrient uptake, a kinetic model was developed using the modelling and simulation platform WEST®. The algal kinetics model describes inorganic carbon uptake, oxygen production, algal growth and decay, nitrate and phosphate uptake and intracellular nitrate storage.

Results and discussion

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. *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.

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. Although both microalgae showed a similar growth curve, the nitrate uptake rate was higher for *P. tricornutum.* Moreover, the total nitrogen content in exponential growth of *P. tricornutum* (8.1%) was found to be higher compared to *A. coffeaeformis* (3.0%). The intracellular nitrate pool of *P. tricornutum* accounted for 4.6% of the total nitrogen content, while it accounts for 17.3% of total nitrogen for *A. coffeaeformis*.

A mathematical model was developed for *P. tricornutum* to determine the kinetic parameters using the results of the exponential growth experiment (Table 1). Model validation was conducted using the stationary growth test (Figure 1). A local sensitivity analysis showed that the kinetic model was able to describe the algal metabolism accurately for the exponential growth experiment, as confirmed by the low TIC (Theil's inequality coefficient)-values for nitrate (TIC = 0.02), phosphate (TIC = 0.06) and algal biomass (TIC = 0.04).

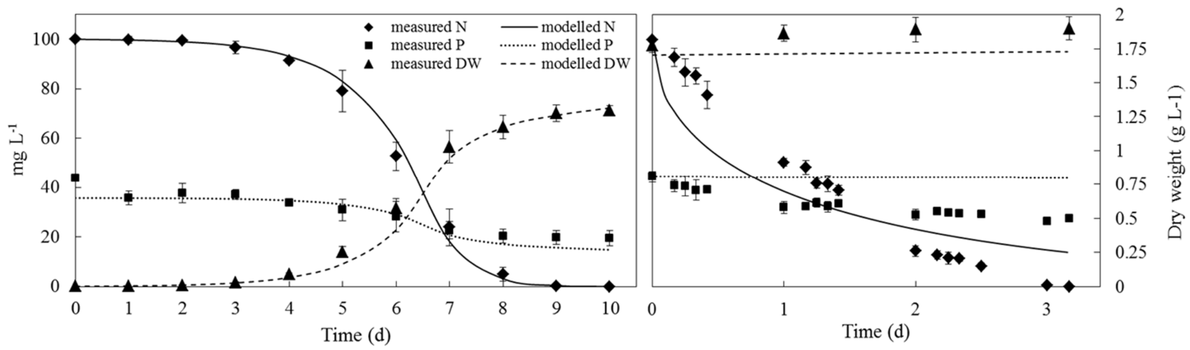

Figure 1. Model simulation and experimental data of *P. tricornutum* for the exponential growth (left) and stationary growth experiment (right)

Parameter	Description	Assigned value	Unit
μ_{max}	Max. specific growth rate (1)	1.05	d^{-1}
Y_4	Growth yield on N (2)	14.4	g DW g^{-1} N
Y_5	Growth yield on P (2)	66	g DW g^{-1} P
$K_{NO_3^-}$	Half saturation coefficient for NO_3^- (3)	2.0 10^{-2}	mg N L^{-1}
$K_{PO_4^{3-}}$	Half saturation coefficient for PO_4^{3-} (1)	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

Table 1 Estimated kinetic parameter values for *P. tricornutum*

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 (53 mg NO_3—N L^{-1} and 20 mg PO_4^{3-}—P L^{-1}) and reactor volume (110L) 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. The simulation study showed that *P. tricornutum* can obtain a 92% nitrate removal efficiency by increasing the HRT of the system from 1 h to 36 h (figure 1), thereby reducing the volumetric loading rate of the reactor to 0.04 g N L^{-1} d^{-1}. Additionally, a 55% phosphate removal efficiency was obtained with the algal recovery unit. 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.

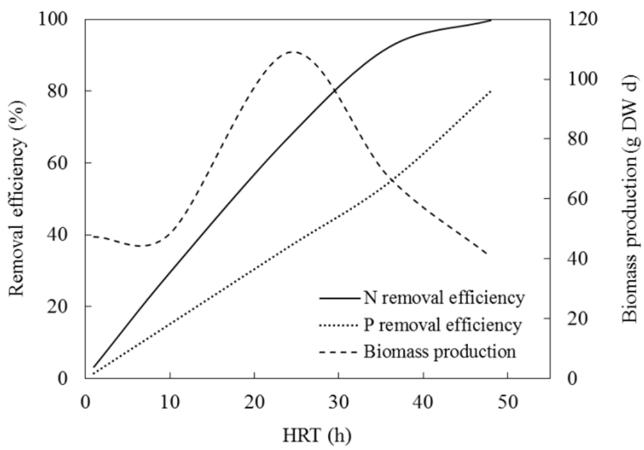

Figure2 Simulation of the nitrate removal, phosphate removal and algal biomass production in function of the hydraulic retention time in the reactor

Due to the large reactor volume required for the algal recovery unit, this system is only feasible at treatment sites where the surface area is not constrained. Here the algal system is competitive with conventional periphyton beds, applied in intensive aquaculture (Crab et al. 2007). Additionally the high protein content observed of *P. tricornutum* (39.7 to 59.3% of the dry weight) is similar to that of *Chlorella* (60%), demonstrating the potential of *P. tricornutum* for applications as single cell protein and its valorization as feed in aquaculture.

Conclusion

This case study demonstrates the potential use of the nitrate-accumulating diatom *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 set-up using real wastewater and a natural light regime is indispensable to illuminate the potential of these nitrate-accumulating microalgae for nutrient recovery.

Cai T, Park SY, Li YB (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew Sust Energ Rev 19:360-369 doi:10.1016/j.rser.2012.11.030

Crab R, Avnimelech Y, Defoirdt T, Bossier P, Verstraete W (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270(1-4):1-14 doi:10.1016/j.aquaculture.2007.05.006

Labelle MA, Juteau P, Jolicoeur M, Villemur R, Parent S, Comeau Y (2005) Seawater denitrification in a closed mesocosm by a submerged moving bed biofilm reactor. Water Res 39(14):3409-3417 doi:10.1016/j.watres.2005.06.001