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Ammonium and urea removal by *Spirulina platensis*

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Abstract Different concentrations either of ammonium chloride or urea were used in batch and fed-batch cultivations of *Spirulina platensis* to evaluate the possibility of substituting nitrate by cheaper reduced nitrogen sources in wastewaters biotreatment. The maximum nitrogen concentration able to sustain the batch growth of this microalga without inhibition was 1.7 mM in both cases. Ammonium chloride was limiting for the growth at lower concentrations, whereas inhibition took place at higher levels. This inhibition effect was less marked with urea, likely because the enzymatic hydrolysis of this compound by urease controlled the ammonia transfer into the cell. Fed-batch experiments carried out by pulse-feeding either ammonium or urea proved that the use of these compounds as nitrogen sources can sustain the long term-cultivation of *S. platensis*, provided that the conditions for their feeding are accurately optimized.

Keywords Urea · Ammonium · Removal · *Spirulina platensis* · Kinetics

Introduction

Microalgae mass culture appears to be a feasible way to remove from wastewaters inorganic nutrients, among which nitrogenous and phosphoric compounds, which are well-known primary agents of pollution and eutrophication. In the recent past, various systems and microbial species have been investigated to improve the effectiveness and economic feasibility of such a biotreatment. Moreover, the cultivation of photosynthetic microorganisms on adequately treated effluents can be an economically feasible process to produce high value-proteins for animal feeding and to obtain products of pharmaceutical interest [4].

Algal cultivation on wastewaters aims at producing biomass and, at the same time, removing organic and inorganic pollutants [17, 24, 29]. The use of microalgae can offer a valuable alternative to the conventional purification treatments and provides several advantages, among which: (a) it is not environmentally dangerous, because it rests on the principles of natural ecosystems; (b) biomass can be re-cycled reducing the causes of secondary pollution; and (c) algal growth on effluent removes heavy metals and xenobiotic substances and allows, under photosynthetic conditions, oxygen to be released, thus enhancing the auto-depuration potential of water bodies.

Different microalgae have been used with success for wastewater biotreatment [12–14, 16, 31, 32]. Although *Spirulina platensis* is the most widely studied photosynthetic microorganism, only a few attempts have been made up to now to utilize it for removal of nutrients from wastewaters. Particularly, its peculiar advantages, among which its preference for strongly alkaline environment that prevents external contamination [20], suggest its employment for environmental applications.

The conventional nitrogen source for this microalga is nitrate [22, 26, 33], although Stanca and Popovici observed that the use of reduced nitrogen improved biomass production [28]. The use of ammonium or urea is particularly attractive from the economic point of view, because these compounds are notoriously very cheap and are often present in wastewaters. In particular, the use of urea as a nitrogen source provides an energetic gain because of its possible hydrolysis to

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ammonia in the alkaline medium, thus being easily assimilated by *S. platensis*. On the contrary, feeding nitrate, the photosynthetic microorganism needs reducing it to nitrite (through the reaction catalyzed by nitrate reductase) and later to ammonia (through the reaction catalyzed by nitrite reductase) [15]. As this process requires energy, the alga prefers to use reduced nitrogen in the forms of ammonium or urea that however are toxic at high concentrations [1, 3]. Therefore, the nitrogen source amount to be supplemented to an algal culture medium has to be controlled; its excess can in fact lead to growth inhibition because of the toxic effect of ammonium, whilst too low levels hinder growth due to nitrogen limitation [25].

For removal of nutrients from wastewaters by *S. platensis*, it has been carried out research-work on using animal waste materials [7, 18, 21], wastewaters with high carbon/nitrogen ratio [23] and urban effluents [6]. In this sense, the cultivation of this microorganism can be considered a promising alternative for the removal of inorganic nitrogen and phosphorus from wastewater [10, 21]. However, these works were carried out on natural media, whose complexity, despite of the useful approach, can make the understanding of the process of reduced nitrogen uptake difficult.

Spirulina platensis was used in this work to compare urea and ammonium removal in a synthetic medium. These nitrogenous compounds were supplied to the culture medium according either to batch or fed-batch operation. Preliminary batch tests were carried out to evaluate the maximum levels of ammonium or urea able to sustain algal growth without inhibition. Because of its flexibility in supplying nutrients during cultivation, the fed-batch operation then allowed reducing the nitrogen source toxicity and promised to be suitable for long term operation.

Materials and methods

Microorganism and growth conditions

Spirulina platensis (UTEX 1926) was obtained from the University of Texas Culture Collection. Cells were maintained in the medium of Schlösser [26], having the following composition (per liter): 13.6 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4, 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4 \cdot 7\ H_2O$, 0.04 g $CaCl_2 \cdot 2\ H_2O$, pH 9.6. All nutrients were dissolved in distilled water containing 6.0 mL of solution A, 1.0 mL of solution B and 1.0 mL of a 1.5 μg L^{-1} B12 vitamin solution. The solution A composition was (per liter): 97.0 mg $FeCl_3 \cdot 6\ H_2O$, 41.0 mg $MnCl_2 \cdot 4\ H_2O$, 5.0 mg $ZnCl_2$, 2.0 mg $CoCl_2 \cdot 6\ H_2O$, 4.0 mg $Na_2MoO_4 \cdot 2\ H_2O$. The solution B composition was (per liter) 50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4 \cdot 5\ H_2O$, 44.0 mg $ZnSO_4 \cdot 7\ H_2O$, 20.0 mg $CoCl_2 \cdot 6\ H_2O$, 12.6 mg $MnCl_2 \cdot 4\ H_2O$, 12.6 mg $Na_2MoO_4 \cdot 2\ H_2O$.

The inoculum for tests was prepared from a cell suspension maintained in the above Schlösser medium at 30°C under natural light conditions. Aliquots of this suspension were transferred to 0.5-L Erlenmeyer flasks containing 0.2 L of Schlösser medium, placed in a chamber thermostatted at 30°C and illuminated by three metal halide lamps, furnishing a photosynthetically active radiation of 55 μmol photons $m^{-2}\ s^{-1}$; cotton caps were used to minimize the risk of external contamination, although the very high alkalinity and salinity of the medium is considered to be a sufficient prevention even in outdoor ponds [8]. After about 3 days, the culture reached its exponential phase and the cells were harvested by centrifugation at 2,500 rpm. The pellet was subsequently washed with a 0.9% NaCl solution for three times to completely remove sodium nitrate. Finally, the cells were resuspended in the Schlösser medium without nitrate and used as inoculum.

Cultivation equipment and conditions

Batch and fed-batch cultures, inoculated by cell concentration of 0.4–0.6 g L^{-1} (dry weight), were carried out in triplicate in 3.0-L Erlenmeyer flasks, containing 1.0 L of Schlösser medium in which the traditional nitrogen source (nitrate) was substituted by ammonium or urea at variable levels, namely 1.1, 1.7, 2.2, and 2.8 mM. Culture mixing was ensured by a reciprocal shaker placed in a chamber thermostatted at 30°C and illuminated by three metal halide lamps, furnishing a photosynthetically active radiation of 55 μmol photons $m^{-2}\ s^{-1}$.

Fed-batch runs were carried out by pulse feeding the selected amounts either of ammonium or urea, selecting the time intervals so as to ensure the nearly complete consumption of nitrogen. Since the reactor volume did not appreciably vary during tests, most of the kinetic parameters were estimated, with reasonable approximation, as for the batch process [9], unless specified otherwise.

Analytical methods

Samples were withdrawn daily for determinations of cell and nitrogen concentrations. Cell concentration was determined by filtration of a sample (10 mL) through Millipore filters (0.45 μm pore diameter) after washing with 3.0 N acetic acid to eliminate salt precipitates; the filters were then dried in oven at 80°C for 2 h and weighed for determination of dry biomass concentration. Nitrate and ammonia concentrations were determined in the filtrate according to Standard Methods [2]. Urea was determined as ammonium after hydrolysis catalyzed by the enzyme urease by the Microquant urea test (Merck, Darmstadt, Germany).

Yields and kinetic parameter estimation

The experimental data of biomass and nitrogen compounds concentrations collected at variable time were treated for kinetic analysis based on the parameters and yields defined as follows.

The integral cell productivity of the batch runs, Q_X (mg L^{-1} day^{-1}) was calculated as:

$$Q_x = \frac{X_m - X_o}{t_m}, \quad (1)$$

where X_m (mg L^{-1}) is the maximum cell concentration obtained at the time t_m (days) needed to achieve X_m and X_o (mg L^{-1}) is the inoculum concentration.

The integral rate of nitrogen removal, Q_N (mg L^{-1} day^{-1}), was calculated as:

$$Q_N = \frac{N_o - N}{t_m}, \quad (2)$$

where N_o (mg L^{-1}) is the initial nitrogen concentration and N (mg L^{-1}) the nitrogen concentration remained in the medium to time t_m.

The molar nitrogen requirement to produce one C-mol of biomass, $Y_{N/C}$ (mol_N $Cmol_X^{-1}$), was defined as:

$$Y_{N/C} = \frac{Q_N}{Q_X} \quad (3)$$

being Q_N and Q_X expressed as mol_N L^{-1} day^{-1} and C-mol_X L^{-1} day^{-1}, respectively. The C-moles of biomass to use in this calculation were estimated from the experimental data of biomass concentration, X_m (mg L^{-1}), assuming for *S. platensis* the average biomass composition ($CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$) reported by Cornet et al. [11].

For the fed-batch pulse-feeding runs, the parameters Q_X and X_m were calculated as for the batch runs using the time required for the start-up achievement (t_f, days) instead of t_m. The average specific growth rate of these runs, μ_{av} (day^{-1}), was defined as:

$$\mu_{av} = \frac{1}{t_f} \ln \frac{X_m}{X_o} \quad (4)$$

while t_{av} (days) was the average time interval between one nitrogen addition and the subsequent one.

The statistical analysis of data was performed by comparison of standard deviations of X_m, Q_X, Q_N and $Y_{N/C}$ calculated with respect to the mean experimental values.

Results and discussion

Batch cultivations

The experimental protocol was aimed at verifying the ability of *S. platensis* to metabolize two different nitrogen sources: ammonium and urea. The difficulty of *S. platensis* to metabolize cheap reduced nitrogen was linked to the toxic effect of ammonia [1, 3]. Therefore, to optimize the growth of this cyanobacterium, it was necessary to preliminarily identify the maximum thresholds of reduced nitrogen concentration beyond which *S. platensis* growth was inhibited. For this purpose, tests were carried out at increasing starting equimolar nitrogen concentrations (N_o), namely 1.1, 1.7, 2.2, and 2.8 mM, either using ammonium chloride or urea as nitrogen sources.

Figures 1 and 2 show the nitrogen consumption and biomass growth observed using ammonium and urea as nitrogen sources, respectively, while Tables 1 and 2 summarize the kinetic results and removal yields of these experiments. A starting nitrogen concentration of 1.7 mM ensured the highest nitrogen removal and biomass growth, either feeding ammonium or urea. Under this condition, the cell productivity and nitrogen removal rate did in fact reach maximum values, whereas a fall in *S. platensis* growth was observed further increasing the nitrogen level. In both cases, $N_o < 1.7$ mM caused the growth to be limited by nitrogen shortage, while $N_o > 1.7$ mM led to inhibition due to excess nitrogen. These results agree with the inhibitory (1.7–2 mM) and toxic (10 mM) ammonium threshold levels reported in the literature for this microalga [1, 3, 8, 27].

By comparing batch tests carried out with ammonium or urea, it is evident that these two nitrogen sources exerted different effects on biomass growth as well as on their own removals. Using ammonium chloride up to 1.7 mM, both cell productivity and nitrogen removal rate increased, thus pointing out that nitrogen was immediately metabolized by biomass as the likely result of glutamine synthetase activity [5]. Both cell productivity and ammonium removal rate decreased in the presence of urea, thus suggesting some limitation of the activity of urease, the enzyme responsible for urea hydrolysis to ammonia. Opposite effects were observed using ammonium chloride or urea at higher levels ($N_o > 1.7$ mM). With the former nutrient, ammonia availability in the medium likely exceeded biomass requirements; therefore, it became inhibitory for microbial growth and its excess was likely lost by off-gassing. On the contrary, urea was hydrolyzed at rates consistent with nitrogen microbial needs because of the enzymatic control of ammonia transfer into the cell and thus exhibited a less significant inhibitory effect.

The values of the nitrogen requirement to produce one C-mol of biomass ($Y_{N/C}$) appear to be consistent with these observations. Using ammonium as nitrogen source at $N_o > 1.7$ mM (Table 1), $Y_{N/C}$ increased up to 0.20–0.21 mol_N C-mol_X^{-1}. These values are appreciably higher than the one calculated in this study (0.17 mol_N C-mol_X^{-1}) using the *S. platensis* composition reported by Cornet et al. [11]. On the other hand, at $N_o = 1.7$ mM, this parameter reached a value ($Y_{N/C} = 0.16$ mol_N C-mol_X^{-1}) almost coincident to the calculated one, while at $N_o = 1.1$ mM it was even lower

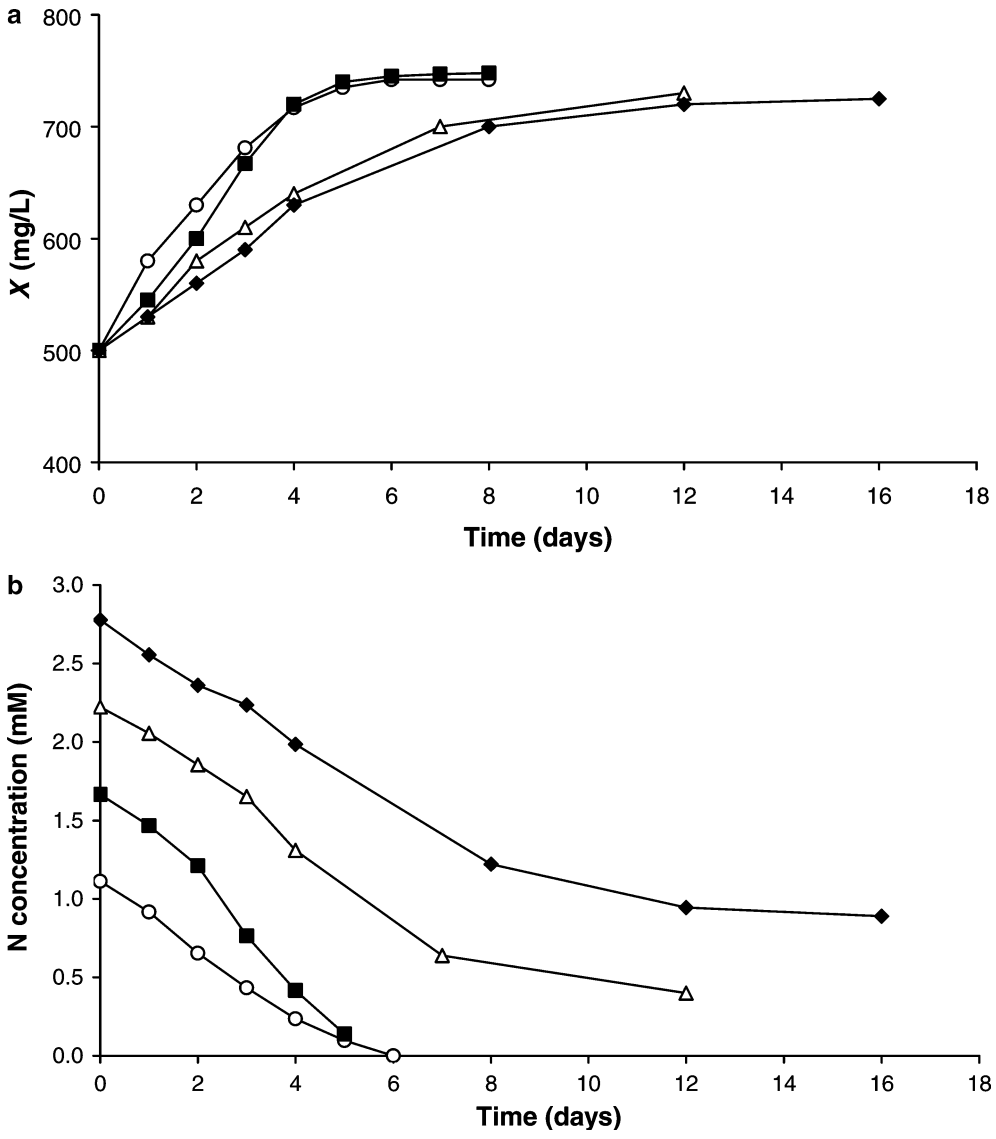

Fig. 1 Biomass (**a**) and nitrogen (**b**) concentrations profiles during batch cultivations of *S. platensis* on the Schlösser medium where nitrate had been substituted by NH_4Cl as nitrogen source at different starting nitrogen concentrations (mM): (*open circle*) 1.1; (*filled square*) 1.7; (*open triangle*) 2.2; (*filled diamond*) 2.8

($Y_{N/C} = 0.12$ mol_N C-mol_X^{-1}). These results suggest that at low ammonium concentration ($N_o < 1.7$ mM), nitrogen available in the medium was insufficient to sustain regular biomass synthesis, hence becoming limiting for the growth; as a consequence, biomass was characterized by low protein and nitrogen content (data not shown). Such a mechanism of cell adaptation to the nitrogen content of the medium varying its composition is consistent with the results of Tam and Wong with *Chlorella vulgaris* [30]. This microorganism did in fact show a decrease in its protein content when cultivated for 2 days after complete ammonium depletion, thus suggesting that additional growth was only possible at the expense of its internal nitrogen reserve.

As suggested by Boussiba [5], ammonia is introduced into the cell by means of a diffusion process driven by the pH gradient between the medium (pH about 9–10) and the cellular cytoplasm (pH about 8.5). This system could support the inward movement of the uncharged ammonia and lead to its intracellular accumulation up to toxic level. Such an undesired effect can be partially avoided when using urea as nitrogen source; in fact, as shown in Table 2, a low urea level ($N_o < 1.7$ mM) was responsible for nitrogen depletion in the medium and low nitrogen content of biomass ($Y_{N/C} = 0.12$ mol_N C-mol_X^{-1}), whilst at $N_o \geq 1.7$ mM the alga was able to consume more effectively the nitrogen source, thereby reducing its loss by off-gassing and keeping the nitrogen content of biomass at normal level ($Y_{N/C} = 0.16$ mol_N C-mol_X^{-1}). This better behavior of urea with respect to ammonium, demonstrated by the higher biomass volumetric productivity at $N_o \geq 2.2$ mM, was likely due to the urease activity in this alga, which hydrolyzed urea to ammonium according to its metabolic requirements. Therefore, feeding urea as a nitrogen source in fed-batch or continuous processes was expected to furnish advantages with respect to ammonium chloride.

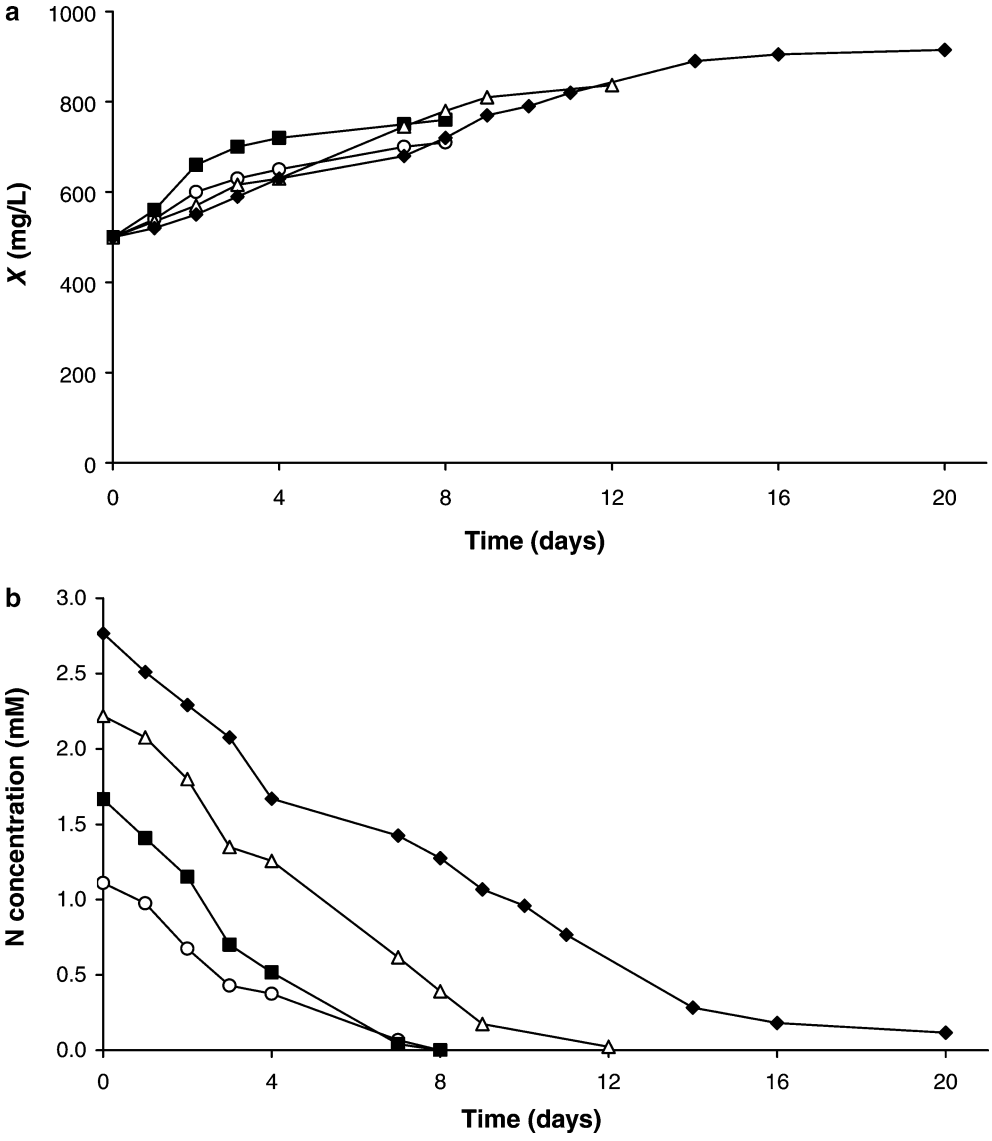

Fig. 2 Biomass (**a**) and nitrogen (**b**) concentrations profiles during batch cultivations of *S. platensis* on the Schlösser medium where nitrate had been substituted by urea as nitrogen source at different starting nitrogen concentrations (mM): (*open circle*) 1.1; (*filled square*) 1.7; (*open triangle*) 2.2; (*filled daimond*) 2.8

Fed-batch cultivations

On the basis of the above considerations, fed-batch experiments were carried out by pulse-feeding ammonium or urea, to avoid the toxic effect of ammonia notoriously occurring when the total amount of the nitrogen source required for the growth is one-step added at the start of a batch process. Two parallel series of fed-batch tests were carried out at the same starting concentrations of nitrogen as for batch runs, whose results in terms of biomass growth and nitrogen concentration profiles are illustrated in Fig. 3 and 4, respectively. The complete removal of 1.1 mM ammonia requested 3 days, while 4 days were necessary to remove

Table 1 Results of batch cultivations of *S. platensis* using ammonium chloride as a nitrogen source at variable starting concentration (N_o)

N_o (mM)	1.1	1.7	2.2	2.8
Q_X (mg L^{-1} day^{-1})[a]	40 ± 2	41 ± 2	19 ± 1	14 ± 1
X_m (mg L^{-1})[b]	742 ± 35	745 ± 31	730 ± 29	725 ± 28
t_m (days)[c]	6	6	12	16
Q_N(mg L^{-1} day^{-1})[d]	0.19 ± 0.01	0.26 ± 0.02	0.15 ± 0.01	0.12 ± 0.01
$Y_{N/C}$(mol_N C-mol_X^{-1})[e]	0.12 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	0.21 ± 0.03

[a] Integral cell productivity calculated at $t = t_m$
[b] Maximum cell concentration[c] Time of achievement of maximum cell concentration
[d] Integral rate of nitrogen removal calculated at $t = t_m$
[e] Nitrogen requirement to produce one C-mol of biomass
Values on the right are standard deviations with respect to the mean value

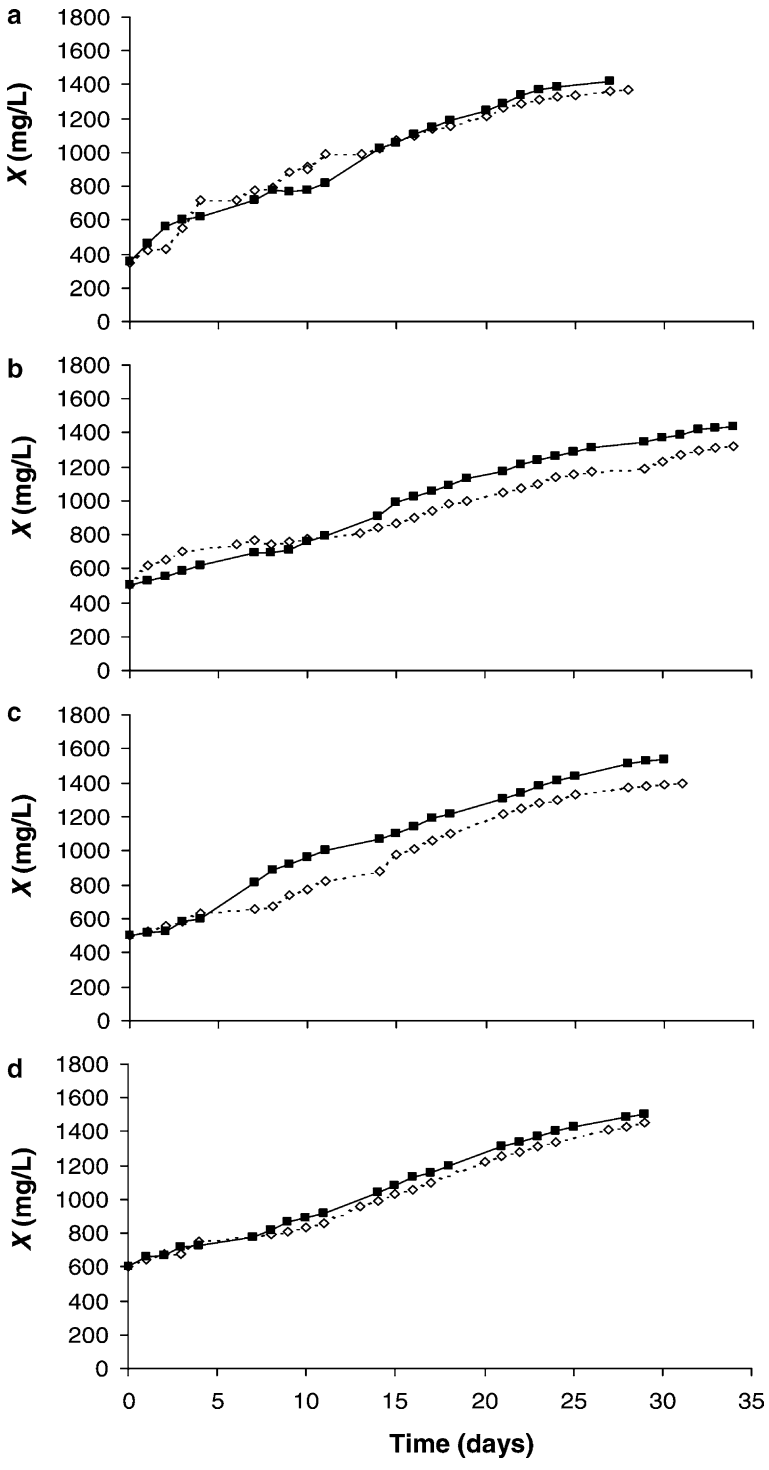

Fig. 3 Biomass concentration versus time during fed-batch cultivations of *S. platensis* performed by pulse feeding (*open diamond*) ammonium chloride or (*filled square*) urea as nitrogen sources at different starting nitrogen concentrations (mM): **a** 1.1; **b** 1.7; **c** 2.2; **d** 2.8

equimolar amount of urea; likewise, $N_o = 1.7$, 2.2 and 2.8 mM required 4, 6 and 7 days for ammonia and 6, 9 and 15 days for urea removals, respectively (Tables 3 and 4). This result is consistent with the fact that ammonium was directly metabolized by the microalga, while urea, before its assimilation, had to be hydrolyzed to ammonia by urease, and the activity of this enzyme likely became limiting for the growth at the highest urea level. As reported by Muro-Pastor and Florencio [19], the photosynthetic organisms, in the absence of ammonia, are in fact obliged to display different strategies to assimilate nitrogen.

The kinetic results of these fed-batch tests demonstrate that the fed-batch pulse-feeding operation minimized the impact of the nitrogen source on the system. In fact, only little variations were observed in both the specific and volumetric growth rates either varying N_o or the type of nitrogen source (for ammonium: $0.033 \leq \mu$

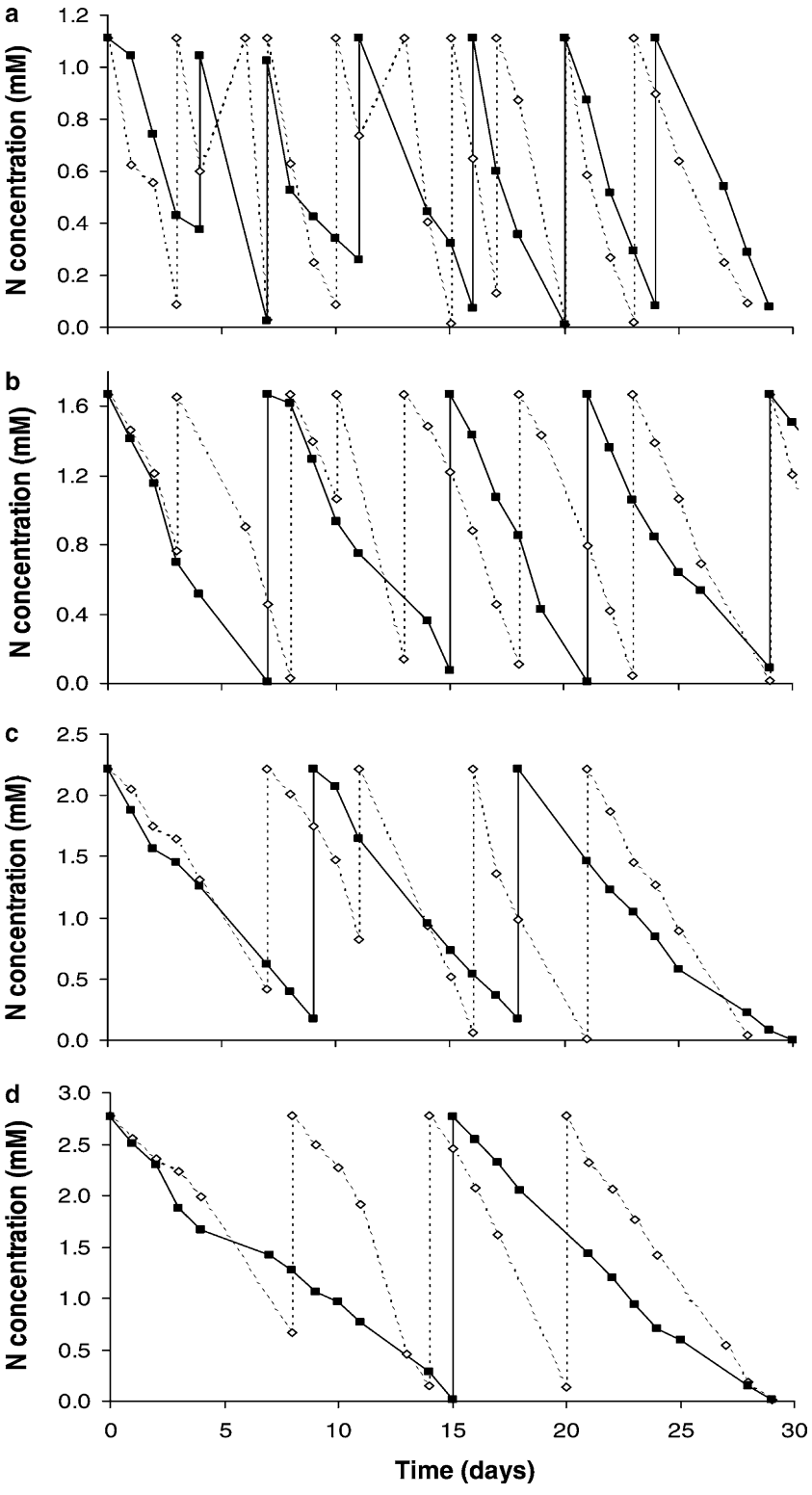

Fig. 4 Nitrogen concentration versus time during fed-batch cultivations of *S. platensis* performed by pulse feeding (*open daimond*) ammonium chloride or (*filled square*) urea as nitrogen sources at different starting nitrogen concentrations (mM): **a** 1.1; **b** 1.7; **c** 2.2; **d** 2.8

≤ 0.039 day^{-1} ; $29 \pm 2 \leq Q_X \leq 36 \pm 2$ mg L^{-1} day^{-1}; for urea: $0.036 \leq \mu \leq 0.042$ day^{-1}; $31 \pm 3 \leq Q_X \leq 39 \pm 4$ mg L^{-1} day^{-1}). Nevertheless, some significant differences can be evidenced between the two systems. Notwithstanding at the highest nitrogen level ($N_o = 2.8$ mM) a longer time was needed to uptake urea ($t_{av} = 15$ days) rather than ammonium ($t_{av} = 7$ days), the former system allowed reaching a higher biomass productivity ($Q_X = 31 \pm 3$ mg L^{-1} day^{-1}), which suggests that ammonia loss by off-gassing could have affected biomass growth more than the urease limitation of nitrogen source availability.

Tests performed using ammonium chloride or urea in mixture with nitrate (results not shown) demonstrated

Table 2 Results of batch cultivations of *S. platensis* using urea as a nitrogen source at variable starting concentration (N_o)

N_o (mM)	1.1	1.7	2.2	2.8
Q_X (mg L^{-1} day^{-1})[a]	26 ± 1	32 ± 3	29 ± 2	21 ± 1
X_m (mg L^{-1})[b]	711 ± 44	759 ± 40	847 ± 39	915 ± 53
t_m (days)[c]	8	8	12	20
Q_N (mg L^{-1} day^{-1})[d]	0.14 ± 0.01	0.21 ± 0.02	0.18 ± 0.01	0.13 ± 0.01
$Y_{N/C}$(mol_N $Cmol_X^{-1}$)[e]	0.12 ± 0.01	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01

[a] Integral cell productivity calculated at $t = t_m$
[b] Maximum cell concentration
[c] Time of achievement of maximum cell concentration
[d] Integral rate of nitrogen removal calculated at $t = t_m$
[e] Nitrogen requirement to produce one C-mol of biomass
Values on the right are standard deviations with respect to the mean value

Table 3 Results of fed-batch cultivations of *S. platensis* using ammonium chloride as nitrogen source at variable starting concentration (N_o)

N_o (mM)	1.1	1.7	2.2	2.8
Q_X (mg L^{-1} day^{-1})[a]	36 ± 2	30 ± 2	29 ± 2	29 ± 1
X_m (mg L^{-1})[b]	1518 ± 70	1403 ± 64	1396 ± 75	1352 ± 54
t_f (days)[c]	31	30	31	26
t_{av} (days)[d]	3	4	6	7
μ_{av}(day^{-1})[e]	0.039	0.035	0.033	0.034

[a] Cell productivity calculated at the end of start-up
[b] Maximum cell concentration
[c] Time needed to complete the start-up
[d] Average time interval between one nitrogen addition and the subsequent one
[e] Average specific growth rate
Values on the right are standard deviations with respect to the mean value

Table 4 Results of fed-batch cultivations of *S. platensis* using urea as nitrogen source at variable starting concentration (N_o)

N_o (mM)	1.1	1.7	2.2	2.8
Q_X (mg L^{-1} day^{-1})[a]	39 ± 4	34 ± 3	35 ± 4	31 ± 3
X_m (mg L^{-1})[b]	1570 ± 124	1475 ± 114	1540 ± 124	1405 ± 96
t_f (days)[c]	30	29	30	26
t_{av} (days)[d]	4	6	9	15
μ_{av}(day^{-1})[e]	0.042	0.037	0.037	0.036

[a] Cell productivity calculated at the end of start-up
[b] Maximum cell concentration
[c] Time needed to complete the start-up
[d] Average time interval between one nitrogen addition and the subsequent one
[e] Average specific growth rate
Values on the right are standard deviations with respect to the mean value

that, in both cases, nitrate started to be uptaken only after complete depletion of the reduced nitrogen source, thus confirming the preference of this microalga for the reduced forms of nitrogen [5]. No temporary lack of nitrogen in the medium took place in these experiments during fed-batch pulse feeding operation, thanks to the simultaneous feeding of nitrate present in the Schlösser medium.

Conclusions

Batch and fed-batch cultivations of *Spirulina platensis* were performed using ammonium chloride and urea as cheap nitrogen sources alternative to nitrate. $N_o = 1.7$ mM was shown to be the maximum threshold of reduced nitrogen concentration beyond which *S. platensis* growth was inhibited.

Fed-batch experiments, carried out feeding these nitrogen sources by pulse-feeding operation, showed that, at equimolar levels of nitrogen in the feed, ammonium was uptaken more quickly than urea, but it is likely that most of the former nitrogen source was lost by off-gassing under the alkaline conditions of these cultivations. These results are consistent with the fact that ammonium is directly metabolized, while urea, before its assimilation, has to be hydrolyzed to ammonia,

which allowed preventing the inhibitory accumulation of ammonia in the medium.

The use of urea for *S. platensis* cultivation can be particularly attractive from the economic point of view, because this nutrient, which is usually present in wastewaters or cattle-breeding wastes, can be profitably employed instead of nitrate as cheap and alternative nitrogen source.

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References

1. Abeliovich A, Azov Y (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol 31:801–806
2. APHA, AWWA, WEF (1992) Standard methods for the examination of water and wastewater, 18th edn. APHA, Washington
3. Belkin S, Boussiba S (1991) High internal pH conveys ammonia resistance in *S. platensis*. Biores Technol 38:167–169
4. Borowitzka MA (1988) Vitamins and fine chemicals from microalgae. In: Borowitzka MA, Borowitzka LJ (eds) Microalgal biotechnology. Cambridge University Press, Cambridge, pp 153–196
5. Boussiba S (1989) Ammonia uptake in the alkalophilic cyanobacterium *Spirulina platensis*. Plant Cell Physiol 30:303–308
6. Bustos Aragon A, Fiestas Ros de Ursinos JA, Borja Padilla R (1992) Algal cultures with effluents from biological treatment of urban wastewater by anaerobiosis. Res Conserv Recycl 6:303–312
7. Cañizares-Villanueva RO, Domínguez AR, Cruz MS, Ríos-Leal E (1995) Chemical-composition of cyanobacteria grown in diluted, aerated swine waste-water. Biores Technol 51:111–116
8. Carvalho JCM, Francisco FR, Almeida KA, Sato S, Converti A (2004) Cultivation of *Arthrospira (Spirulina) platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially increasing feeding rate. J Phycol 40:589–597
9. Carvalho JCM, Sato S (2001) Fermentação descontínua alimentada. In: Schmidell W, Lima UA, Aquarone E, Borzani W (eds) Biotecnologia industrial, vol. 2. Edgar Blücher, São Paulo, pp 205–218
10. Chuntapa B, Powtongsook S, Menasveta P (2003) Water quality control using *Spirulina platensis* in shrimp culture tanks. Aquaculture 220:355–366
11. Cornet JF, Dussap CG, Cluzel P, Dubertret G (1992) A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: II. Identification of kinetic parameters under light and mineral limitations. Biotechnol Bioeng 40:826–834
12. Craggs RJ, Smith VJ, McAuley PJ (1995) Wastewater nutrient removal by marine microalgae cultured under ambient conditions in mini-ponds. Water Sci Technol 31:151–160
13. de-Bashan LE, Moreno M, Hernandez J-P, Bashan Y (2002) Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Water Res 36:2941–2948
14. Garbisu C, Gil JM, Bazin MJ, Hall DO, Serra JL (1991) Removal of nitrate from water by foam-immobilized *Phormidium laminosum* in batch and continuous-flow bioreactors. J Appl Phycol 3:221–234
15. Hatori A, Myers J (1966) Reduction of nitrate and nitrite by subcellular preparations of *Anabaena cylindrica*. I. Reduction of nitrite to ammonia. J Plant Physiol 41:1031
16. Jiménez-Pérez MV, Sánchez-Castillo P, Romera O, Fernández-Moreno D, Pérez-Martínez C (2004) Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. Enzyme Microb Technol 34:392–398
17. Martínez ME, Sánchez S, Jiménez JM, El Yousfi F, Muñoz L (1999) Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Biores Technol 73:263–272
18. Mitchell SA, Richmond A (1988) Optimization of a growth medium for *Spirulina* based on cattle waste. Biol Wastes 25:41–50
19. Muro-Pastor MI, Florencio FJ (2003) Regulation of ammonium assimilation in cyanobacteria. Plant Physiol Biochem 41:595–603
20. Olguín EJ, Galicia S, Camacho R, Mercado G, Pérez T (1997) Production of *Spirulina* sp. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. Appl Microbiol Biotechnol 48:242– 247
21. Olguín EJ, Galicia S, Mercado G, Pérez T (2003) Annual productivity of *Spirulina (Arthrospira)* and nutrient removal in a pig wastewater recycling process under tropical conditions. J Appl Phycol 15:249–257
22. Paoletti C, Pushparaj B, Tomaselli Feroci L (1975) Ricerche sulla nutrizione minerale di *Spirulina platensis*. In: Italian Society of Microbiology (ed) Atti del XVII congresso della Società Italiana de Microbiologia. Padua, Italy, pp 833–839
23. Phang SM, Miah MS, Yeoh BG, Hashim MA (2000) *Spirulina* cultivation in digested sago starch factory wastewater. J Appl Phycol 12:395–400
24. Proulx D, Lessard P, De La Noüe J (1994) Traitment tertiaire d'un effluent domestique secondaire par culture intensive de la cyanobactérie *Phormidium bohneri*. Environ Technol 15:449–458
25. Sassano CEN, Carvalho JCM, Gioielli LA, Sato S, Torre P, Converti A (2004) Kinetics and bioenergetics of *Spirulina platensis* cultivation by fed-batch addition of urea as nitrogen source. Appl Biochem Biotechnol 112:143–150
26. Schlösser UG (1982) Sammlung von Algenkulturen. Ber Deutsch Bot Ges 95:181–276
27. Soletto D, Binaghi L, Lodi A, Carvalho JCM, Converti A (2005) Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. Aqualculture 243:217–224
28. Stanca D, Popovici E (1996) Urea as nitrogen source in modified Zarrouk medium. Rev Roum Biol 41:25–31
29. Talbot P, Thébault JM, Dauta A, De La Noüe J (1991) A comparative study and mathematical modelling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. Wat Res 24:465–472
30. Tam NFY, Wong YS (1996) Effect of ammonia concentrations on growth of *Chlorella vulgaris* and nitrogen removal from media. Biores Technol 57:45–50
31. Vílchez C, Garbayo I, Markvicheva E, Galván F, Leon R (2001) Studies on the suitability of alginate-entrapped *Chlamydomonas reinhardtii* cells for sustaining nitrate consumption processes. Biores Technol 78:55–61
32. Voltolina D, Gómez-Villa H, Correa G (2005) Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. Biores Technol 96:359–362
33. Zarrouk C (1966) Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima (Setch. Et Gardner) Geitler*. PhD Thesis, University of Paris, Paris