J Appl Phycol (2015) 27:1177–1184
DOI 10.1007/s10811-014-0416-1

Characterization of the growth, biochemical composition, and nutrient utilization of the cyanobacterium *Geitlerinema lemmermannii*

Flor Sánchez-Alejandro · M. del Pilar Sánchez-Saavedra

Received: 3 February 2014 / Revised and accepted: 15 September 2014 / Published online: 14 October 2014

Abstract *Geitlerinema lemmermannii* is a cosmopolitan cyanobacterium that is highly adapted to natural and artificial environments in which concentrations of phosphorus and nitrogen have been altered. *G. lemmermannii* was detected and isolated from the wastewater discharge of Mexican shrimp cultures. Its nutrition and potential incorporation into wastewater treatment were examined in a 168-h culture in an artificial wastewater medium at two concentrations each of nitrogen and phosphorus. *G. lemmermannii* showed a rapid phosphorus uptake (in the first 3 h of culture), ranging from 4.75 to 6.09 pg cell^{-1} h^{-1}. Nitrogen uptake peaked at 9 h of culture at 2.38 pg cell^{-1} h^{-1}. The total cellular lipid, carbohydrate, and protein content changed between treatments over culture time due to differences in uptake patterns but were similar at the end of the experiment. Additionally, the population and growth rate did not differ between culture media, indicating that the robust and rapid removal of nutrients from a synthetic medium occurred by this cyanobacterium.

Keywords Proximal composition · *Geitlerinema lemmermannii* · Growth · Nutrient uptake

Introduction

Organisms of the genus *Geitlerinema* exist in marine, terrestrial, and hypersaline environments; their presence is associated with low ammonia and orthophosphate concentrations (Major et al. 2005). *Geitlerinema* is also linked to ecosystems that have anthropogenic impacts (Vicente and Miracle 1992) and artificial environments, such as secondary wastewater plants that treat effluent from the paper industry, in which *Geilterinema*, *Phormidium*, and *Pseudoanabaena* predominate over heterotrophic bacteria and are the only photoautotrophic organisms (Kirkwood et al. 2001). The first report on the existence of *Geitlerinema* in Baja California, México, detected and isolated it from thermal ponds in 2001 (López-Cortés et al. 2001).

The species *Geitlerinema lemmermannii* (Woloszyńska) Anagnostidis was described as a freshwater planktonic cyanobacterium that is common to tropical swamps and marsh environments (Komárek and Anagnostidis 2005). In 2006, cells of this species were isolated from shrimp cultures that were grown in highly carbonated water in the Mexicali Valley, Baja California, México, where this species was the predominant species in winter at 26.3 °C, 8.8 mg oxygen L^{-1}, 0.08 mg ammonium L^{-1}, 0.001 mg nitrites L^{-1}, and undetectable nitrate levels (Fierro 2006).

There is little data on the biology and physiology of *G. lemmermannii*. Thus, we examined its growth characteristics, proximal composition, and capacity for phosphorus and nitrogen uptake.

Materials and methods

Geitlerinema lemmermannii, isolated from the tanks and effluent of shrimp farms in the Mexicali Valley, Baja California, Mexico (Fierro 2006), was identified using the taxonomic keys of Komárek and Anagnostidis (2005).

G. lemmermannii was maintained in triplicate, monospecific, nonaxenic batch cultures in 2.8-L Fernbach flasks that contained 2-L F Guillard medium or the 2 F variation with double the concentration of nutrients (Guillard and Ryther

F. Sánchez-Alejandro · M. del Pilar Sánchez-Saavedra (✉)
Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860 Ensenada, Baja California, México
e-mail: psanchez@cicese.mx

1962; Table 1). These media preparations had the same nitrogen and phosphate concentrations as the aquaculture wastewaters in Baja California (Aguilar-May and Sánchez-Saavedra 2009). The original nitrogen source of F Guillard medium was replaced by ammonium nitrate. The cultures were kept at 24±0.5 °C under continuous light at 100 μmol photons m^{-2} s^{-1} (Daylight fluorescent light F40D.EX, General Electric Company, USA). pH was not controlled and was pH 7±0.5 at the start of the cell culture and pH 9.3±0.2 at the end.

Growth assessment

For cultivation of *G. lemmermannii*, 81.3 g of glass pearls (2-mm diameter) was added in order to avoid the formation of films or flocks on the walls and bottoms of the flasks. The increase in cell density was measured using a hemocytomer. Clumps of cells were dislodged by ultrasonication for 2 min at 100 kHz with an L&R Solid State/Ultrasonic model T–9B (L&R Manufacturing Company, USA), as proposed by Voltolina (1985). The growth rate ($\mu = \frac{\log_2(N_2)-\log_1(N_1)}{t_2-t_1}$, defined by the number of cells N_2 at a second day t_2 after growing from initial values N_1 and t_1) and maximum of cell divisions (DP_{max} as the higher value of $DP=N_2-N_1$) were calculated according to Fogg and Thake (1987). The daily growth rate was added to that of the previous day to obtain the accumulated growth rate ($\Sigma\mu = \sum_{i\leq t}^{t} = \mu_1 + \mu_2 + \ldots + \mu_t$, according to Nieves et al. 1998).

Proximal composition and nutrient removal

The cell density and proximal composition were measured at 0, 48, 120, and 168 h. Total ash-free dry weight and ash content were measured per Sorokin (1973). Proteins, carbohydrates, and lipids were measured in 30-mL samples that had been concentrated by filtration through 2.5-cm GF/C glass fiber filters with a nominal 1-μm pore size (Whatman, England). Proteins were extracted with 0.1 N NaOH at 100 °C for 15 min and quantified according to Lowry et al. (1951). Carbohydrates were extracted according to Whyte (1987) and quantified according to Dubois et al. (1956). Lipids were analyzed using the method of Bligh and Dyer (1959). Chlorophyll and carotenoids were measured according to Parsons et al. (1984).

To assess the nutrient levels in the culture medium, 50-mL samples were filtered through a 4.7-mm GF/C glass fiber filter to remove cells. Nutrient concentrations were spectrophotometrically measured at various times on a Hach DR/400 UV (Hach Company, USA)—nitrates were evaluated by cadmium reduction, nitrites by the diazotization method, ammonium by the silicate method, and phosphates by the ascorbic acid using the standard reagents and techniques of Hach (1997). As a control treatment, culture medium that lacked *G. lemmermannii* was used at similar nutrient concentrations as in the experimental treatments. The nutrient consumption was expressed as medium percentage concentrations, measured to each nutrient through time, as well as uptake rate in pg $cell^{-1}$ h^{-1}, which resulted from dividing the concentration of the nutrient consumed at a certain time, by the number of cells at the same period of time.

Table 1 Chemical composition of culture medium based on 1-L Guillard and Ryther (1962) medium

	F
$NaH_2PO_4 \cdot H_2O$	10.0 mg
Thiamine•HCl	0.2 mg
Biotin	1.0 μg
B_{12}	1.0 μg
$CuSO_4 \cdot 5H_2O$	0.0196 mg
$ZnSO_4 \cdot 7H_2O$	0.044 mg
$CoCl_2 \cdot 6H_2O$	0.020 mg
$MnCl_2 \cdot 4H_2O$	0.360 mg
$Na_2MoO_4 \cdot 2H_2O$	0.0126 mg
NH_4NO_3[a]	70.6 mg

[a] Modified, originally described as $NaNO_3$

Statistical analysis

Differences in growth, nutrient removal, chlorophyll *a* and carotenoid content, and proximal composition were evaluated by analysis of covariance (ANCOVA). The assumptions of the parametric statistics were verified, and Tukey's comparison test was used when significance was observed. When the data were nonparametric, a Kruskal-Wallis test was used. All statistical analyses were performed with Statistica®, version 6, and the significance level was $\alpha=0.05$. Student' *t* test and Kruskal-Wallis test were performed using MINITAB 15.

Results

Growth The growth of *G. lemmermannii* in either medium (F and 2 F) was not modified by the availability of nutrients. Its daily cellular density was similar between media ($t_{cal}=0.551$, $t_{crit}=2.101$; Fig. 1). The acclimation phase occurred during the first 9 h and was followed by an exponential phase at 96 h in both media (Fig. 1). The growth rate (μ) was similar between treatments ($p=0.610$) and its maximum value was that of the first day. Also, the accumulated growth rate (Σμ) ($t_{cal}=1.898$, $t_{crit}=2.024$) and daily production ($p=0.598$) were statistically similar (Table 2).

Proximate composition The total percentages of proteins ($p=0.323$), carbohydrates ($p=0.957$), and lipids ($p=0.414$) were similar between treatments, increasing over time in both media, with a higher protein and lipid

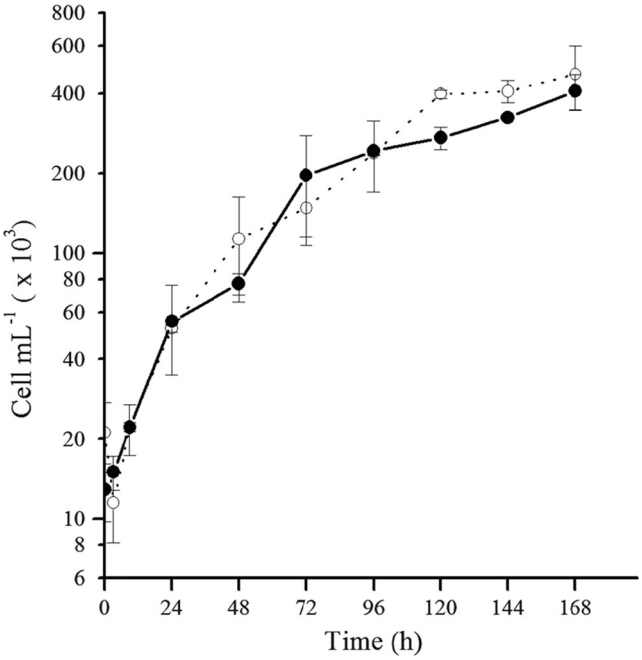

Fig. 1 Mean values of $\log_{10}$ numbers of *Geitlerinema lemmermannii* (cell number mL^{-1}). Cultures in F medium (*open circles*) and 2 F medium (*filled circles*). *Bars* indicate standard deviation (n=3)

percentage at 120 h in F medium (Fig. 2). The percentage of ash was similar between treatments (p=0.947) and decreased over time (Fig. 2). Chlorophyll *a* (p=0.159) and carotenoid (p=0.195) content increased over time and did not differ between treatments (Fig. 3).

Nutrient removal *G. lemmermannii* showed selective nitrogen consumption as species-specific ammonia consumer. The pH in this experiment was not controlled and ranged from pH 7.5 at the beginning to 9.5 at the end of both cultures.

The nitrate uptake rate was similar between treatments (p=0.738; Fig. 4b). Nitrite concentrations were similar between treatments (p=0.668) and increased with age in both media (F, p=0.001, and 2 F, p=0.002; Fig. 5). Ammonia uptake in F medium differed over time (p=0.001), decreasing steadily until 96 h (Fig. 4a). The total ammonia removal rate was 80.21 % in F medium and 61.81 % in 2 F (Table 4).

Phosphorous uptake differed between treatments (p=0.039) and over time (p=0.11; Fig. 4d). Phosphorous consumption by cells was favored in the first several hours in 2 F medium. The total orthophosphate removal was 90.99 % in F medium and 63.79 % in 2 F (Table 4).

Table 2 Mean values and standard deviation of cell density (cells mL^{-1}), growth rate during exponential phase (μ as divisions by day), accumulated growth rate (Σμ as divisions by day), and maximum daily production (DP_{max} as cells mL^{-1} day^{-1}) of *Geitlerinema lemmermannii* cells in F and 2 F media cultures

Growth parameters	F		2 F	
Initial cell density	18,750±5,000	b	12,917±3,146	a
Final cell density	310,208±75,830	a	407,917±60,484	b
μ	1.20±0.38	a	2.00±0.83	a
∑μ	4.08±0.48	b	5.06±0.19	a
DP_{max}	99,166±10,408	a	119,271±74,112	a

ANCOVA for initial and final cell density comparison and Mann-Whitney and Student's t tests for others; all evaluated at α=0.05; a<b are being compared between rows (n=3)

Discussion

To the best of our knowledge, this study represents the first report on *G. lemmermannii* growth in culture, and of its proximate composition and macronutrient consumption rates (Table 2). These values were lower than those for *Synechococcus elongatus* (Aguilar-May and Sánchez-Saavedra 2009) and similar to those for *Arthrospira maxima* (Cruz-Fraga 2003) which are two well-known cyanobacteria, when cultured under similar conditions. The growth rate calculated for *G. lemmermannii* was higher than the rate calculated for other microalgae that are commonly used in aquaculture (Table 3).

Chlorophyll *a* and carotenoid synthesis in *G. lemmermannii* is linked to their ability to perform oxygenic and nonoxygenic photosynthesis (Whitton and Potts 2000). Certain cyanobacteria synthesize pigments and proteins proportionally to the amount of nitrogen available (Loreto et al. 2003). Generally, the carotenoid content declines because of limited nitrogen availability (Rüker et al. 1995). In *G. lemmermannii*, chlorophyll *a* values were decreased at 168 h in F medium, but carotenoids continued to be synthesized until the experiment was terminated. In *S. elongatus*, the formation of chlorophyll-protein complexes increases as a consequence of a self-shading effect, regardless of the concentration of available nutrients in the media; this is a common response that occurs in high-density cyanobacteria cultures (Aguilar-May and Sánchez-Saavedra 2009). In this experiment, *G. lemmermannii* cells grew to form both dense conglomerates and free trichomes. Therefore, light limitation could occur in the cell conglomerates, producing a response similar to that observed for *S. elongatus*.

Although protein synthesis in *G. lemmermannii* increased in correlation with chlorophyll *a* content, the percentage of protein was stabilized at the stationary growth phase, as was the removal of ammonia and its incorporation into nitrogenous compounds. These trends might also be attributable to the high nitrite concentrations and limited CO_2 availability that were presented at the end of the experiment.

Cyanobacteria are commonly known to be high protein producers (Table 3) and, based on our present study, so is

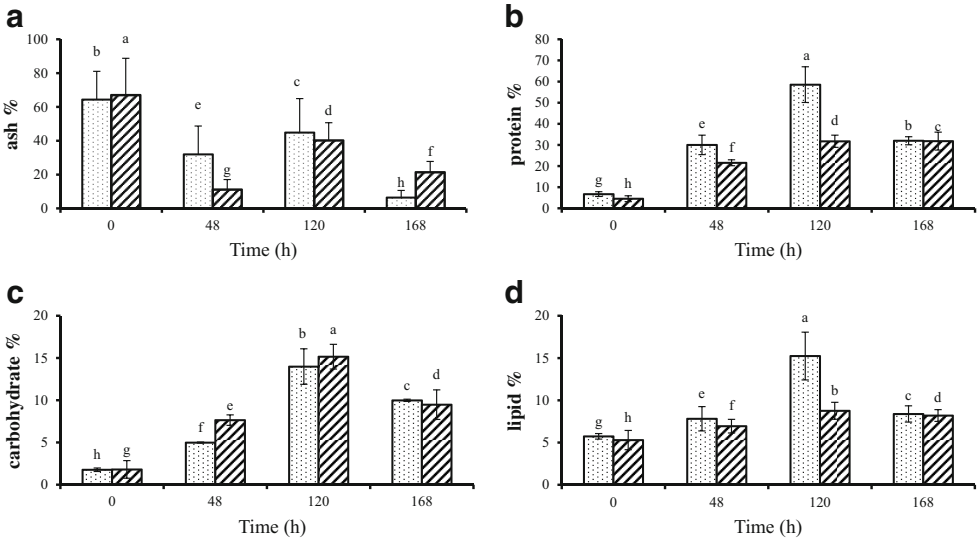

Fig. 2 Mean percentage of ash and biomolecules in *Geitlerinema lemmermannii* relative to organic dry weight obtained in F (*dotted bars*) and 2 F (*diagonal lined bars*) media cultures: a ash, b proteins, c carbohydrates, d lipids. *Lines* indicate standard deviation, and *a*>*b*>*c*>*d*>*e*>*f*>*g*>*h* (*n*=3)

G. lemmermannii. Certain species of *Chlorella* spp., which are maintained in batch cultures, show increased lipid content and reduced protein content over time (Pratt and Johnson 2006). However, for *G. lemmermannii*, the protein content remained threefold higher than the lipid content under both culture conditions (media F and 2 F), but the difference was smaller in the initial hours of culture. Limited carbon availability and low nitrogen assimilation at the stationary growth phase might have influenced biomolecules synthesis, which may explain the concurrent reduction in the assimilation of ammonia.

The high ash content present during the first days of *G. lemmermannii* culture could be attributed to the accumulation of minerals as the consequence of the high specific requirements of this group (Vonshak 1986). This observation could also be ascribed to salts present in the media that was used, which can accumulate in the intracellular space within the cyanobacteria (Zhu and Lee 1997). Ash content is one of the cell components that shows high variation, which is correlated with the age of the cyanobacteria cultures—i.e., 15 % at day 1 and 50 % at day 30 (Torres-Ariño and Mora-Heredia 2010). When cyanobacteria cells develop blooms, they produce low dry matter (0.2–14 %) and high ash content (31–71 %) (Roger et al. 1986; Roger 2005).

Inorganic nitrogen is a nutritional requirement of microalgae; nitrate is the most common dissolved form of nitrogen (Herrero et al. 1985). However, in certain conditions, microalgae prefer to use ammonia to conserve the energy of eight electrons from the photosynthetically reduced ferrodoxin, which is needed to reduce nitrate (Manzano et al. 1976; Miller and Castenholz 2001). It is also known that concentrations of ammonia greater than 0.5 to 1.0 μM can inhibit nitrate absorption (Darley 1987). Based on these findings, the nitrogen consumption pattern observed in *G. lemmermannii* cultures could have resulted from ammonia-dependent repression of gene expression including genes encoding functional enzymes, such as nitrate reductase (Nar) and nitrate permeases (Wang et al. 2000). Even the lowest concentration of ammonia detected in F medium, 22 μM (0.41 mg L^{-1}), can inhibit nitrate metabolism (Darley 1987). This finding is supported by a report that the *Geitlerinema* genus contains no nitrate consumers, as a consequence of nonfunctional reductases enzymes and no functional NtcA transcription factor that enhances the expression of *glnA*, a glutamine synthetase (GS) (Luque et al. 1994; Miller and Castenholz 2001). In freshwater ecosystem soil samples in which *Geitlerinema* sp. has been isolated, the

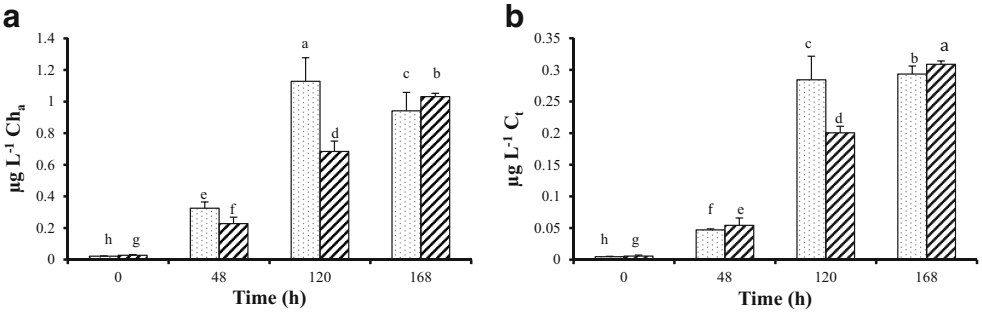

Fig. 3 Mean chlorophyll *a* (a Ch_a) and total carotenoid contnet (b Ct) in *Geitlerinema lemmermannii* cultures growth in F (*dotted bars*) and 2 F (*diagonal lined bars*) media. *Lines* indicate standard deviation (*n*=3)

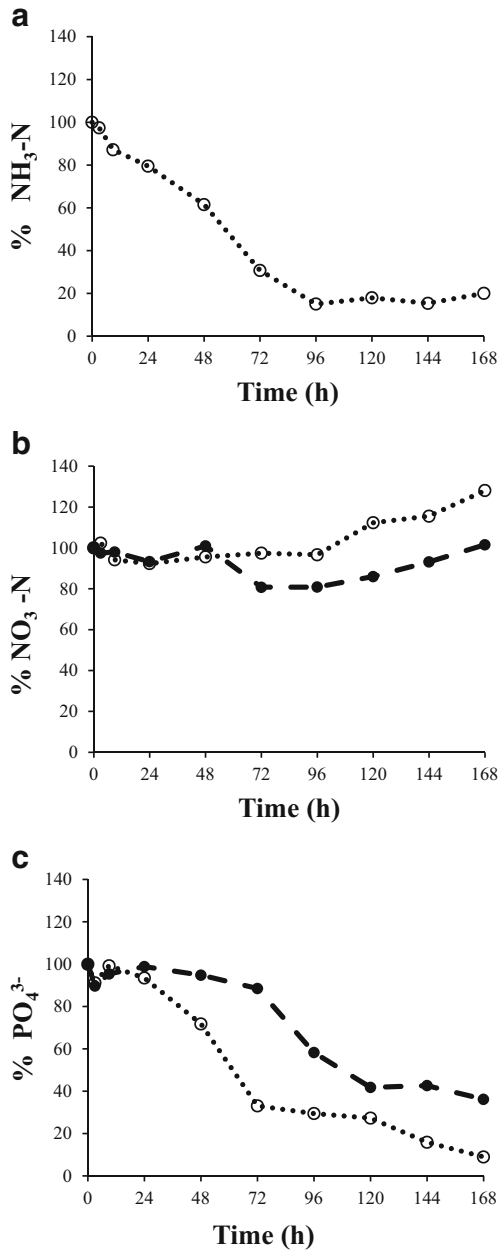

Fig. 4 Percentages of mean values of nutrient concentration in *Geitlerinema lemmermannii* cultures for ammonia (**a**), nitrate (**b**), and phosphate (**c**) (n=3). Cells were maintained in F (*open circles*) and 2 F (*filled circles*) media. 2 F data in **a** missing due to technical problems

nitrate and nitrite concentrations are high and vary more widely (250 to 600 μM) than ammonia concentrations (4 to 166 μM) (Major et al. 2005) (Fig. 5). Those observations suggest a more direct link of *Geitlerinema* spp. with ammonia than with nitrate.

Furthermore, *G. lemmermannii* produced an abundance of blue pigment, which was not measured but was evident visually. Blue pigment production has been implicated in an evolutionary inability to use nitrates in certain cyanobacteria (Miller and Castenholz 2001).

Table 3 Growth rate (μ as division by day) and percentage (%) mean values of proximal composition in *Geitlerinema lemmermannii* cultures compared with other microalgae

	Geitlerinema lemmermannii (this work)	*Synechococcus* sp. (Campa-Ávila 2002)	*Arthrospira maxima* (Mexia-Bernal 2011)	*Chaetoceros* sp. (Sánchez-Saavedra and Voltolina 1994)	*Nannochloropsis* sp. (Campa-Ávila 2002)	*Chlorella vulgaris* (Sánchez-Saavedra and Velasco-Arriaga in prep.)
Phylum	Cyanophyceae Cyanobacteria	Cyanophyceae Cyanobacteria	Cyanophyceae Cyanobacteria	Coscinodiscophyceae Diatom	Eustigmatophyceae Chlorophyta	Trebouxiophyceae Chlorophyta
Culture media	F	F/2	S	F	F/2	F/2
Proteins	58.56	44.40	30–47	31.93	27.66	42.00
Carbohydrates	13.99	16.68	10–35	9.40	11.15	37.00
Lipids	15.24	6.22	10–16	22.23	11.94	17.00
μ	1.20	1.82	0.92	0.73	1.02	0.58

F medium described by Guillard and Ryther (1962), F/2 half of the nutrients added to the F medium, S Spirulina medium described by Vonshak (1986)

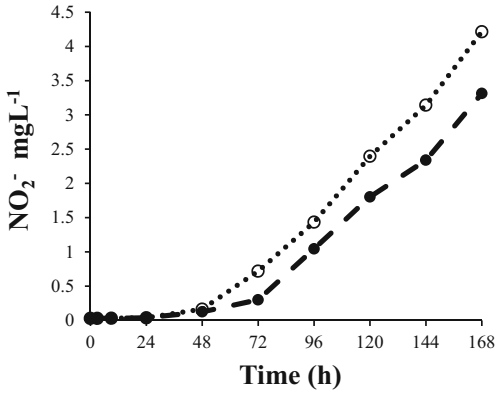

Fig. 5 Nitrite production in *Geitlerinema lemmermannii* cultures in F (*open circles*) and 2 F (*filled circles*) media (n=3)

Nitrite excretion has been attributed to limited CO_2 availability that is induced by high intracellular ammonia content and the inhibition of nitrite reductase (Nir) when ammonia or nitrates are available in the medium (Reyes et al. 1993; Suzuki et al. 1995). The decrease in medium dissolved ammonia and the nitrite excretion observed for *G. lemmermannii* cultures indicate a biological effect of moving nitrogen from the extracellular to intracellular environment.

Regarding the phosphate depletion observed in this study, there are some reports for *Geitlerinema* in natural environments that suggest some members of the genus to be biological drivers of phosphate removal: They inhabit environments with an important dynamic of phosphates and become predominant species when these ions had decreased at the lowest levels, 0.04 and 1 μM (Major et al. 2005). This is similar to what occurs in a mesohaline hypereutrophic environment, in which appears when phosphorus starts to be depleted and becomes dominant when nutrient concentrations decline. In contrast, when *G. amphibium* appeared but was replaced by another genus, such as *Planktothrix* and *Pseudoanabaena*, the phosphorus content did not decline (Chomérat et al. 2007). One of the most limiting factors for photosynthetic growth is low phosphate availability. Therefore, when phosphate is available, cells tend to store it intracellularly in the cytoplasm, to use it directly for energy, or to form cellular structures.

The nutrient removal measured for *G. lemmermannii* was similar to that described for *S. elongatus*, for which phosphorus removal is more efficient than nitrogen removal (Aguilar-May and Sánchez-Saavedra 2009). In this study, the total phosphorus uptake by *G. lemmermannii* was nearly equivalent in both media (F=1.79 mg L^{-1} and 2 F= 1.80 mg L^{-1}), although 2 F media had a higher concentration of phosphorus. Nevertheless, a higher rate of uptake was observed in 2 F media, which indicates that a rapid environmental response occurs during the first 3 h. Additionally, lower phosphorus concentrations and equivalent rates of phosphorus uptake were observed at the end of the experiment, indicating species-specific consumption of phosphorus (Table 4). As mentioned previously, this pattern suggests a rapid intracellular storage response triggered by the presence of phosphorus rather than cellular assimilation.

It is well known that two of the most water quality hazards are the N and P, which are introduced into water bodies worldwide directly or by poorly treated discharges. Releases of N and P are predicted to increase by about 60 % by 2050 (MEA 2005). For the first time, our study shows that the cyanobacterium *G. lemmermannii*, cultured in medium with high nitrogen and phosphorus content, drives a biological process that results in the removal of at least 60 % of N and P. This effect was achieved without any sophisticated engineered method, using a single-batch cycle of 168 h. The use of microalgae in water treatment is not new and is still in a research level in most cases. *G. lemmermannii*, similar to many microalgae, represents a candidate for secondary or tertiary treatment processes aimed at the removal of N and P (Metcalf et al. 2004), in addition to oxygenation and CO_2 fixation. Notably, because of biomass quality production measured in this study for *G. lemmermannii*, there are additional secondary metabolites of biotechnological interest.

In conclusion, a rapid phosphorus uptake was observed by *G. lemmermannii* that was isolated from an anthropogenic environment. In response to the culture conditions, it changed its proximal composition over time but caused no difference by the end of the culture period. However, the population and growth rate did not change, indicating a high nutrient storage capacity for this cyanobacterium. *G. lemmermannii*, at 120 h of culture, shows a high proximal composition (58.56 % proteins, 13.99 % carbohydrates, and 15.24 % lipids) when grown in F medium. Based on the removal of N and P

Table 4 Phosphorus and nitrogen consumption of *Geitlerinema lemmermannii* in F and 2 F media

	PO_4^{3-} total consumption		PO_4^{3-} uptake rate (pg cell^{-1} h^{-1})		NH_3-N total consumption		NH_3-N uptake rate (pg cell^{-1} h^{-1})	
	(mg L^{-1})	Medium %	lower	higher	(mg L^{-1})	Medium %	Lower	Higher
F	1.79	90.99	0.02 (168 h)	4.75 (3 h)	2.19	80.21	0.01 (144 h)	2.38 (9 h)
2 F	1.80	63.79	0.02 (168 h)	6.09 (3 h)	2.38	61.81	0.02 (168 h)	[a]

Lower and higher concentrations of phosphorus were found at the start (3 h) and end of the cultures (168 h)

[a] The nitrogen uptake rate in 2 F could not be measured due to technical problems

observed in this study, *G. lemmermannii* represents a good candidate for secondary or tertiary water treatment processes.

Acknowledgments This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACyT 130074). The first author acknowledges a CONACYT special scholarship. Socorro Jiménez contributed to the taxonomic identification of *G. lemmermannii*. N. Flores-Acevedo and J. Dávila-Ortiz gave technical assistance. Thanks to the editorial bureau Blue Pencil Science for English editing of this manuscript.

References

Aguilar-May B, Sánchez-Saavedra MP (2009) Growth and removal of nitrogen and phosphorus by free-living and chitosan-immobilized cells of the marine cyanobacterium *Synechococcus elongatus*. J Appl Phycol 21:353–360

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Phys 37:911–917

Campa-Ávila MA (2002) Evaluación del efecto en el valor nutricio del rotifero *Brachionus plicatilis* alimentado con una microalga y una cianobacteria. Aquaculture Department M.Sc. Thesis. Centro de Investigación Científica y de Educación Superior de Ensenada, México, p 97

Chomérat N, Garnier R, Bertrand C, Casaubon A (2007) Seasonal succession of cyanoprokaryotes in a hypereutrophic oligo-mesohaline lagoon from the South of France. Estuar Coast Shelf Sci 72:591–602

Cruz-Fraga CE (2003) Uso de la cianobacteria *Arthrospira maxima* para remover macronutrientes de efluentes producidos por cultivos. Aquaculture Department M.Sc. Thesis. Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada, Baja California, p 83

Darley MW (1987) Biología de las Algas. Enfoque fisiológico. México D.F. Editorial Limusa S.A. de C.V. pp 236

Dubois M, Gilies KA, Halmiton JD, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Fierro S (2006) Informe de actividades de aislamiento y caracterización de cepas aisladas de granjas camaronicolas de Mexicali. Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). Ensenada, Baja California, p 103

Fogg GE, Thake BJ (1987) Algal Cultures and Phytoplankton Ecology. University of Wisconsin Press, London, p 448

Guillard RRL, Ryther JH (1962) Studies on marine planktonic diatoms I. *Cyclotella nana* Huestedt and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hach (1997) Water Analysis Handbook. HACH Company Loveland, USA, p 1309

Herrero A, Flores E, Guerrero M (1985) Regulation of nitrate reductase celular levels in the cyanobacteria *Anabaena variabilis* and *Synechocystis* sp. FEMS Microbiol Lett 26:21–25

Kirkwood AE, Nalewajko C, Fulthorpe RR (2001) The occurrence of cyanobacteria in pulp and paper waste-treatments systems. Can J Microbiol 47:761–766

Komárek I, Anagnostidis K (2005) Cyanoprokaryota. 2nd Part *Oscillatoriales*. Elsevier Spektrum Akademischer Verlag, Italia, p 757

López-Cortés A, García-Pichel F, Nübel U, Vázquez-Juárez R (2001) Cyanobacterial diversity in extreme environments in Baja California, Mexico: a polyphasic study. Int Microbiol 4:222–236

Loreto C, Rosales N, Bermúdez J, Morales E (2003) Producción de pigmentos y proteínas de la cianobacteria *Anabaena* PCC 7120 en relación a la concentración de nitrógeno e irradiancia. Gayna Bot 60: 83–90

Lowry OH, Rosebrought NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

Luque I, Flores E, Hierro E (1994) Molecular mechanism for the operation of nitrogen control in cyanobacteria. EMBO J 13: 2862–2869

Manzano C, Candau P, Gómez-Moreno C, Relimpio AM, Losada M (1976) Ferrodoxin-dependent photosintetic reduction of nitrate and nitrite by particles of *Anacystis nidulans*. Mol Cell Biochem 10: 161–169

Major KM, Kirkwood AE, Major CS, McCreadie JW, Henley WJ (2005) In situ studies of algal biomass in relation to physicochemical characteristics of the Salt Plains National Wildlife Refuge, Oklahoma, USA. Saline Systems 1(11):10

Metcalf L, Eddy HP, Tchobanoglous G (2004) Wastewater engineering: treatment and reuse, Fourth edition. McGraw-Hill, New York, p 1819

Mexia-Bernal KH (2011) Efecto de la salinidad e irradiancia en el crecimiento, fotosíntesis y composición de *Spirulina maxima* para su utilización en la acuicultura. Bachelor Thesis, Universidad de Occidente, Unidad Guasave, Sinaloa, México, p 96

MEA (Millennium Ecosystem Assessment) (2005) Ecosystems and human well-being: synthesis. Island Press, Washington, DC, p 80

Miller SR, Castenholz RW (2001) Ecological physiology of *Synechococcus* sp. strain SH-94-5, a naturally occurring cyanobacterium deficient in nitrate assimilation. Appl Environ Microbiol 67: 3002–3009

Nieves M, Voltolina D, Barrera A (1998) A new parameter for comparison of microalgae growth. Riv Ital Acquacolt 33: 177–184

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford, p 173

Pratt R, Johnson E (2006) Production of protein and lipid by *Chlorella vulgaris* and *Chlorella pyrenoidosa*. J Pharm Sci 52:979–984

Reyes JC, Chávez S, Muro-Pastor MI, Candau P, Florencio FJ (1993) Effect of glucose utilization on nitrite excretion by the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. Appl Environ Microbiol 59:3161–3163

Roger PA, Tirol A, Santiago-Ardales S, Watanabe I (1986) Chemical composition of cultures and natural samples of N_2-fixing blue-green algae from rice fields. Biol Fert Soils 2:131–146

Roger PA (2005) N2-fixing cyanobacteria as biofertilizers in rice fields. In: Richmond a (ed) Handbook of Microalgal Culture. Biotechnology and Applied Phycology. Blackwell Publishing, Oxford, pp 392–402

Rüker J, Kohl J, Kaiser K (1995) Responses of carotenoids and to variations of growth-limiting factors in three filamentous blue-green algae. Algol Stud 77:51–65

Sánchez-Saavedra MP, Voltolina D (1994) The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. Comp Biochem Physiol 107B:30–44

Sorokin C (1973) Dry weight, packed cell volumen and optical density. In: Stein JR (ed) Handbook of phycological methods. Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, UK, pp 321–344

Suzuki I, Horie N, Sugiyama T, Omata T (1995) Identification and characterization of two nitrogen-regulated genes of the cyanobacterium *Synechococcus* sp. strain PCC7942 required

for maximum efficiency of nitrogen assimilation. J Bacteriol 177:290–296

Torres-Ariño A, Mora-Heredia E (2010) Isolation and characterization of potentially toxic or harmful cyanobacteria from Oaxaca and Chiapas, Mexico. J Environ Sci Heal A 45:128–136

Vicente E, Miracle MR (1992) The coastal lagoon Albufera de Valencia: an ecosystem under stress. Limnetica 8:87–100

Vonshak A (1986) Laboratory techniques for the cultivation of microalgae. In: Richmond DA (ed) CRC handbook of microalgal mass culture. Boca Raton, Florida, pp 117–145

Voltolina D (1985) Biomass evaluation in cultures of benthic diatoms: an experimental review of methodology. Coast Marine Science Laboratory Internal Manuscript Series 85:4 Royal Roads Military College, Victoria, B.C. Cánada, p 86

Wang Q, Li H, Post AF (2000) Nitrate assimilation genes of the marine diazotrophic, filamentous cyanobacteria *Trichodesmium* sp. strain WH9601. J Bacteriol 182:1764–1767

Whitton BA, Potts M (2000) The ecology of cyanobacteria: their diversity in time and space. Dordrecht, Kluwer, p 669

Whyte JNC (1987) Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 6:231–241

Zhu CJ, Lee YK (1997) Determination of biomass dry weight of marine microalgae. J Appl Phycol 9:189–194