ELSEVIER

FEMS Microbiology Ecology 54 (2005) 197–203

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

Cultivation factors and population size control the uptake of nitrogen by the microalgae *Chlorella vulgaris* when interacting with the microalgae growth-promoting bacterium *Azospirillum brasilense*

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Received 18 January 2005; received in revised form 28 March 2005; accepted 29 March 2005

First published online 6 May 2005

Abstract

Growth of and the capacity to take up nitrogen in the freshwater microalgae *Chlorella vulgaris* were studied while varying the concentrations of ammonium and nitrate, the pH and the source of carbon in a synthetic wastewater growth medium when co-immobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Analyses of 29 independent experiments showed that co-immobilization of the microalgae with *A. brasilense* could result in two independent phenomena directly affected by cultivation factors, such as nitrogen species, pH and presence of a carbon source. First, growth of the microalgal population increased without an increase in the capacity of the single cells to take up nitrogen, or second, the capacity of cells to take up nitrogen increased without an increase of the total microalgal population. These phenomena were dependent on the population density of the microalgae, which was in turn affected by cultivation factors. This supports the conclusion that the size of the microalgal population controls the uptake of nitrogen in *C. vulgaris* cells – the higher the population (regardless the experimental parameters), the less nitrogen each cell takes up.

Keywords: Microalgae; Plant growth-promoting bacteria; *Azospirillum brasilense*; *Chlorella vulgaris*; Nitrogen uptake

1. Introduction

Microalgae are used in wastewater bio-treatments [1], as food for humans and animals [2], as feed in aquacultures [3,4], for the production of pigments [5] and in agriculture [6]. Naturally grown microalgae are always associated with bacteria [7,8]. The positive effects of bacteria on microalgae were reported decades ago [9,10]. More recently, the plant growth-promoting bacterium (PGPB) *Flavobacterium* sp. was found to promote the growth of a marine microalgae (the diatom *Chaetoceros gracilis*), which is used as feed in pearl oyster hatcheries [11,12]. Inoculation of freshwater aquaculture ponds with the PGPB *Azospirillum* sp. and *Azotobacter* sp. significantly increased the phytoplankton population, and consequently, fish yields [13]. When the two microorganisms (bacteria and microalgae) are growing together, there are mutually beneficial effects between them that can be explained in several ways. Microalgae are known to produce and release enough exogenous oxygen to

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0168-6496/$22.00
doi:10.1016/j.femsec.2005.03.014

fulfill most aerobic bacterial requirements, and they can also release large amounts of organic compounds that can be assimilated by bacteria [8]. In return, bacteria can stimulate algal growth by the release of vitamins and plant hormones [7,8,14], or they can be a source of CO_2, especially during periods of limited carbon. Some associations are not necessarily beneficial for microalgae; the natural associative bacterium *Phyllobacterium myrsinacearum* accelerates senescence of the microalgae *Chlorella vulgaris* when grown together [15].

Immobilized microalgae in alginate or carrageenan beads can remove N, P and heavy metals from wastewater [16–19]. In recent studies, we showed that co-immobilization in alginate beads of the freshwater microalgae *C. vulgaris* or *C. sorokiniana* with the microalgae growth-promoting bacterium (MGPB) *Azospirillum brasilense* strain Cd, commonly used as an inoculant in agriculture [20], significantly increases growth parameters over microalgae immobilized alone [14,15,21]. Furthermore, we have demonstrated that microalgae are able to take up higher amounts of nitrogen (ammonium and nitrate) and phosphorus when cultured together with the bacteria than when cultured alone under laboratory conditions in synthetic wastewater or municipal wastewater [22,23].

Our working hypothesis was that the bioreactor cultivation factors (type of nitrogen, pH and carbon source) affect the microalgal population, in addition to the effects caused by the interaction with the MGPB. Consequently, the developing population of microalgae possibly controls the uptake of nitrogen from the wastewater at culture or at individual cell levels. Therefore, this study examined cultivation factors and population densities governing the uptake of nitrogen by *C. vulgaris*, when co-immobilized in alginate beads with the MGPB *A. brasilense* Cd.

2. Materials and methods

2.1. Microorganisms and axenic growth conditions

The unicellular microalgae *C. vulgaris* Beijerinck (UTEX 2714, University of Texas, Austin, TX) was used. Before immobilization in alginate beads, the microalgae were cultured in sterile mineral medium (C30) for 5 days, following methods described previously [24]. *A. brasilense* Cd (DMS 1843, Braunschweig, Germany) was used in co-immobilization experiments. The bacterium was grown in nutrient broth (Sigma) at 30 ± 2 °C for 18 h in a rotary shaker at 120 rpm.

2.2. Immobilization of microorganisms in alginate beads

Microorganisms were immobilized following methods described previously [23]. Briefly, 20 ml of axenically grown cultures of *C. vulgaris*, containing 6.0×10^6 cells ml^{-1}, were harvested by centrifugation at 2000*g* and washed twice with sterile saline solution (0.85% NaCl). Afterwards, the cells were mixed with 80 ml of sterile, 6000-cP (1 cP = 0.001 Pa s) 2% alginate solution (a solution made of alginate mixed at 14,000 and 3500 cP) and stirred for 15 min. The solution dripped from a sterile syringe into a 2% $CaCl_2$ solution with slow stirring. The beads that were formed were left for 1 h at 22 ± 2 °C for curing and then washed in sterile saline solution. *A. brasilense* cultures (approximately 10^9 cfu ml^{-1}) were immobilized similarly. Because immobilization normally reduces the number of organisms in the beads, a second incubation step was necessary. This was carried out overnight in diluted nutrient broth (1:10). Where co-cultures of *A. brasilense* and the microalgae were used, the same concentration of each microorganism as used in pure cultures was mixed prior to incorporation with alginate and bead formation, but the volume of each microbial culture was reduced to 10 ml before adding the alginate.

2.3. Growth medium

Synthetic wastewater growth medium (SGM) was prepared with the following components (in milligrams per liter): NaCl (7), $CaCl_2$ (4), $MgSO_4 \cdot 7H_2O$ (2), K_2HPO_4 (21.7), KH_2PO_4 (8.5), Na_2HPO_4 (33.4) and NH_4Cl (3). According to the experiment, the concentration of NH_4Cl was increased up to 25.2 mg l^{-1} to obtain a final maximum concentration of 13 mg l^{-1} NH_4^+. Nutrient broth, used by both microorganisms (2.7 mg l^{-1}), or arabinose (1 g l^{-1}) used by *A. brasilense*, but not by *Chlorella* spp. [25], were added when indicated. In some assays, the source of nitrogen was changed from ammonium to nitrate (KNO_3, 24 mg l^{-1}), or the pH was adjusted to 8.0 with a 0.2 M phosphate buffer solution.

2.4. Culture conditions and experiments

Microorganisms solitary immobilized or co-immobilized were grown under semi-continuous conditions during which changing the growth solution, but not the immobilized microorganism (every 48 h). The experiments were: (i) Growth of microorganisms in SGM at different concentrations of ammonium (3 and 13 mg l^{-1} NH_4^+), at pH 7.0; (ii) Growth of microorganisms in SGM at an initial 13 mg l^{-1} concentration of ammonium at pH 8.0; (iii) Growth of microorganisms in SGM supplemented with a source of carbon at an initial 13 mg l^{-1} concentration of ammonium; and (iv) Growth of microorganisms in SGM with nitrate as a source of nitrogen. All experiments lasted 8 days. The cultures were incubated in 250-ml unbaffled Erlenmeyer flasks (100 ml medium with 4 g beads) at 28 ± 2 °C, 120 rpm and constant light at 30 μmol m^{-2} s^{-1} photon flux den-

sity. Every 48 h, 5 beads were sampled for microbial counts by dissolving the beads in 5 ml 4% sodium bicarbonate (Sigma) for 30 min. Cell counting and measuring the individual cell size of the microalgae were both done with help of an image analyzer (Image Pro-Plus 4.1, Media Cybernetics, Silver Spring, MD). Because the chloroplasts of microalgae have natural fluorescence, the image analyzer was equipped with an Olympus BX41 epifluorescence microscope, allowing automatic counting of microalgae and measurement of individual cells by the software. Dry weight of the microalgae was measured after the beads were dissolved and the cultures were washed twice with 0.85% saline solution. The samples were filtered in pre-weighed nitrocellulose membranes of 0.45 μm pore size (Millipore HAWP 04700, Bedford, MA) and the filter was dried at 60 °C for 72 h. At the same time, 25-ml aliquots of SGM for ion analysis (ammonium and nitrate) were taken. Ammonium and nitrate ion contents were measured using standard water analysis techniques [26] and a Hach DR/2000 spectrophotometer (Hach Co., Loveland, CO). Ammonium was analyzed by the salicylate method and nitrate by the cadmium reduction method. Kits developed by the Hach Company were used for the specific detection of ions. The pH was measured by a pH-electrode (Horiba-twin pH, Irvine, CA). Total nitrogen content of cells was measured by automatic micro-Kjeldahl after digestion (Digestion System 12.1009, and Kjeltec Auto 1030 Analyzer, Tecator, Höganäs, Sweden).

2.5. Experimental design and statistical analysis

Semi-continuous cultures were prepared in triplicate, where a single flask served as one replicate. Each experiment was repeated two or three times. Bacterial counts were done in five replicates, and counts of microalgae and ion analyses were done in triplicate. Controls were prepared similarly, but without microorganisms in the beads. Results of triplicates of the experiment at different pH, combined and analyzed together by ANOVA, were followed by Tukey's post-hoc analysis at $P \leqslant 0.05$ and Student's t-test at $P \leqslant 0.05$. Results for weight and size of the microalgae from each of the different treatments (3 N levels, immobilized and co-immobilized) and for each cycle separately ($n = 12$ treatments) were analyzed by ANOVA. Linear regression analyses, correlation coefficient (r) and determination coefficient (r^2) were determined for 29 independent experiments. All analyses used Statistica software (StatSoft, Inc., Tulsa, OK) and CurveExpert 1.3 (D. Hyams, Hixson, TN).

3. Results

Analyses of experiments with immobilized and co-immobilized *C. vulgaris* showed that increased microalgal populations do not necessarily correspond to enhanced nitrogen uptake per cell. As a general trend, there are relatively low, but statistically significant negative correlations between the size of the developing population and the capacity of its cell to uptake nitrogen, with or without interaction with *A. brasilense* ($r = -0.417$ for *C. vulgaris* solitary immobilized and $r = -0.466$ for *C. vulgaris* co-immobilized, both statistically significant at $P \leqslant 0.05$; $n = 28$ independent experiments).

The detailed data of microalgal growth and nitrogen uptake (ammonium or nitrate) by microalgae are shown in Table 1. Controls containing only *Azospirillum* cultures did not take up significant amounts of nitrogen and therefore, those results are not presented. Factors that affect microalgal growth and nitrogen uptake can be segregated into two phenomena.

3.1. Factors that affect microalgal growth and nitrogen uptake

3.1.1. Factors that increase the abundance of C. vulgaris co-immobilized with A. brasilense, but do not affect the uptake of ammonium

When microalgae are grown in SGM at different concentrations (3 and 13 mg l^{-1} ammonium), ammonium uptake is similar in cultures of *C. vulgaris* immobilized alone or *C. vulgaris* co-immobilized with *A. brasilense* (Table 1). However, increase in *C. vulgaris* abundance is positively affected by *A. brasilense* and the population is larger than the population of *C. vulgaris* immobilized alone (Table 1). The pH of the SGM was 7 ± 0.2. At this pH, *A. brasilense* significantly enhanced growth of *C. vulgaris*, but to a moderate degree (Fig. 1(a)). However, at higher pH (8.0), populations of *C. vulgaris* co-immobilized with *A. brasilense* grew significantly larger compared to growth at lower pH (Fig. 1(b)). In any case, pH did not affect nitrogen uptake by *Chlorella*. Uptake of ammonium per cell of microalgae co-immobilized with *A. brasilense* is still lower than uptake per cell of *C. vulgaris* immobilized alone (Table 1).

3.1.2. Factors that increase the uptake of nitrogen by C. vulgaris when co-immobilized with A. brasilense, but not affect their abundance

When the SGM was supplemented with a source of carbon (either nutrient broth or arabinose), the populations of *A. brasilense* were higher than the populations obtained in SGM without a supplemental carbon source (2.3×10^6 and 1.7×10^6 cfu ml^{-1}, respectively). The larger population of *A. brasilense* did not enhance the population size of *C. vulgaris*; yet nitrogen metabolism of microalgae cells was affected. Adding nutrient broth (used by microalgae and bacteria) or arabinose (used only by *A. brasilense*) to the SGM increased the uptake of ammonium per cell (Table 1). However, when any source of carbon is added, a negative correlation

Table 1
Growth of the populations, uptake of N per cell and uptake of N per culture of *Chlorella vulgaris* in 17 independent experiments, solitary immobilized and co-immobilized with *A. brasilense*

Experiments	Growth (No. cells $\times 10^6$ bead)$^{-1}$		Uptake of N per cell (pg cells^{-1})		Uptake of N per culture (mg l^{-1})	
	Immobilized	Co-immobilized	Immobilized	Co-immobilized	Immobilized	Co-immobilized
SGM different concentrations of ammonium (3* and 13** mg l^{-1}), pH 7.0	0.16 ± 0.03a*	0.28 ± 0.04a	11.0 ± 0.22a	3.57 ± 0.12b	1.76 ± 0.08a	1.0 ± 0.033b
	1.048 ± 0.09a*	1.815 ± 0.17b	2.38 ± 0a	1.37 ± 0b	2.5 ± 0a	2.5 ± 0a
	8.95 ± 0.07a*	9.5 ± 0.04a	0.33 ± 0a	0.31 ± 0b	3.0 ± 0a	3.0 ± 0a
	0.53 ± 0.17a**	2.06 ± 0.09b	11.4 ± 0.99a	2.36 ± 0.2b	6.08 ± 0.52a	4.88 ± 0.41a
	0.37 ± 0.02a**	2.01 ± 0.06b	35 ± 0a	6.38 ± 0.02b	13 ± 0a	12.83 ± 0.04a
SGM with 13 mg l^{-1} ammonium, pH 8.0	1.02 ± 0.08a	1.58 ± 0.06b	10.2 ± 0.3a	6.0 ± 0.09 b	10.44 ± 0.14a	9.54 ± 0.28a
	0.59 ± 0.03a	1.27 ± 0.05b	15.9 ± 0.36a	7.37 ± 0.14b	9.4 ± 0.21a	9.37 ± 0.17a
	0.63 ± 0.04a	1.52 ± 0.05b	16.1 ± 0.52a	6.58 ± 0.04b	10.19 ± 0.31a	10.01 ± 0.06a
	1.0 ± 0.06a	2.2 ± 0.26b	10.1 ± 0.15a	4.58 ± 0.06b	10.01 ± 0.15a	10.09 ± 0.13a
SGM with NB, Arabinose, and 13 mg l^{-1} ammonium, pH 7.0	3.6 ± 0.2a	2.5 ± 0.12a	2.41 ± 0.05a	3.72 ± 0.03b	8.7 ± 0.18a	9.3 ± 0.07a
	0.68 ± 0.06a	0.62 ± 0.03a	16.2 ± 0.32a	16.7 ± 0.48a	10.7 ± 0.2a	10.4 ± 0.3a
	0.91 ± 0.03a	0.84 ± 0.03a	10 ± 0.08a	11.7 ± 0.3b	10.44 ± 0.2a	10 ± 0.3a
	1.0 ± 0.05a	0.88 ± 0.08a	10.2 ± 0.08a	10.64 ± 0.17b	10.2 ± 0.08a	10 ± 0.1a
SGM with 24 mg l^{-1} nitrate as nitrogen source	1.45 ± 0.05a	0.93 ± 0.08a	7.1 ± 0.1a	14.0 ± 1.6b	10.4 ± 1.46a	13.3 ± 1.46a
	1.48 ± 0.1a	1.42 ± 0.06a	7.9 ± 0a	9.36 ± 1.0b	11.8 ± 0a	13.3 ± 0a
	0.7 ± 0.04a	0.6 ± 0.05a	21.8 ± 0.29a	24.8 ± 0b	14.4 ± 0.2a	14.88 ± 0a
	0.8 ± 0.07a	0.7 ± 0.04b	17.3 ± 0.2a	19.8 ± 0b	14.0 ± 0a	13.7 ± 0.14a

NB = Nutrient broth.
±SE, $n = 3$ replicates.
Data (for pairs of immobilized and co-immobilized) denoted by different letter differ statistically at $P \leqslant 0.05$ by Student's *t*-test.
Data of additional similar experiments ($n = 11$) are not presented to save space.

between the number of microalgae and ammonium uptake per culture is significantly more pronounced ($r = -0.77$, $r^2 = 0.6$ for co-immobilized *C. vulgaris* and $r = -0.91$, $r^2 = 0.83$ for *C. vulgaris* immobilized alone, when $n = 5$ independent experiments, all significant at $P \leqslant 0.05$), than when there was no source of carbon ($r = -0.59$, $r^2 = 0.35$ for *C. vulgaris* immobilized alone and $r = -0.54$, $r^2 = 0.3$ when *C. vulgaris* and *A. brasilense* are co-immobilized and $n = 16$ independent experiments, all significant at $P \leqslant 0.05$).

To define the optimal nitrate concentration for uptake by *C. vulgaris*, four initial nitrate concentrations were tested at 8, 12, 20, and 24 mg l^{-1} (data not shown). The highest uptake was obtained with 24 mg/l, and therefore, this was the concentration used for the experiments. Results of nitrate uptake per culture show no differences between the co-immobilized system and the system using only immobilized *C. vulgaris*. Although, similar to other cases, a high *A. brasilense* population (1.4×10^6 cfu ml^{-1}) did not support a larger population of *C. vulgaris*, there was an increase in nitrate uptake per cell of the microalgae (Table 1).

To confirm the above results, an additional experiment was performed. After combined analysis of population abundance, dry weight, cell size and total nitrogen content of immobilized and co-immobilized cultures growing on different NH_4^+ levels (1, 3, and 13 NH_4^+ mg l^{-1}) for three cycles, there was a positive linear correlation between abundance and weight of the microalgal population($Y = 0.18X - 0.005931$, $r = 0.803$, $r^2 = 0.64$). This signified that a larger number of cells, regardless of the six treatments, lead to a higher population weight for the population. However, the weights per cell of the microalgae are similar among all treatments (25.7 ± 0.28 pg; $P = 0.061$ ANOVA at a significance level of $P < 0.05$). Similarly, during the course of the experiment, the size of each cell did not increase, regardless the abundance of the population or the treatment (2.32 ± 0.05 μm; $P = 0.76$ ANOVA at a significance level of $P \leqslant 0.05$, when $n = 12{,}470$ cells measured). The total nitrogen content of the populations was related to the population size that developed in each ammonium concentration. At low ammonium level, the population size was 5.95×10^5 cells ml^{-1} and the total N concentration was 1.735 ± 0.35%. At mid ammonium concentration, the population size was 6.72×10^5 cells ml^{-1} and the total N concentration was 1.65 ± 0.1%. At high ammonium concentration, the population size was 4.48×10^5 cells ml^{-1} and the total N concentration was 0.95 ± 0.06%.

3.2. Correlations between population size of C. vulgaris and A. brasilense

A general analysis of the co-immobilized experiments showed that growth of *C. vulgaris* is linearly correlated to the population abundance of *A. brasilense* with or without carbon sources. Population abundances of *Azospirillum* lower than 1.18×10^6 cfu ml^{-1} (corresponds to 6.07 log cfu) reveal a better linear correlation with the population of *C. vulgaris* than higher *A. brasilense* populations (Fig. 2). Although both are statistically sig-

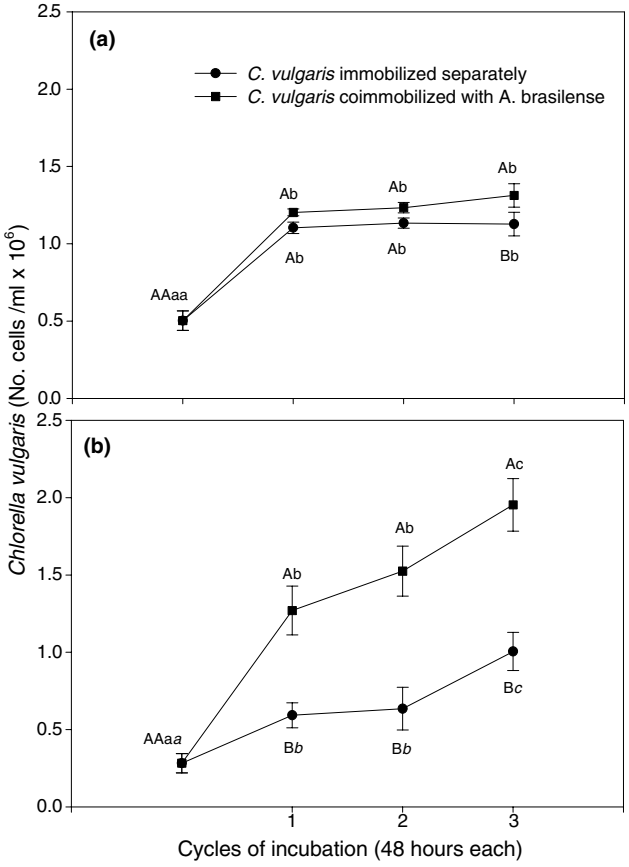

Fig. 1. Growth of *Chlorella vulgaris* immobilized alone and co-immobilized with *A. brasilense* in SGM under different pH conditions. (a) Initial pH: 7.0. (b) Initial pH: 8.0. Points denoted by a different lower case letter or italics lower case letter, in each subfigure separately, differ significantly by ANOVA and Tukey post-hoc analysis at $P \leqslant 0.05$. Points for each incubation cycle, in each subfigure separately, denoted by a different capital letter differ significantly by Student's *t*-test at $P \leqslant 0.05$. Bars represent standard errors. Absence of bars indicates negligible SE.

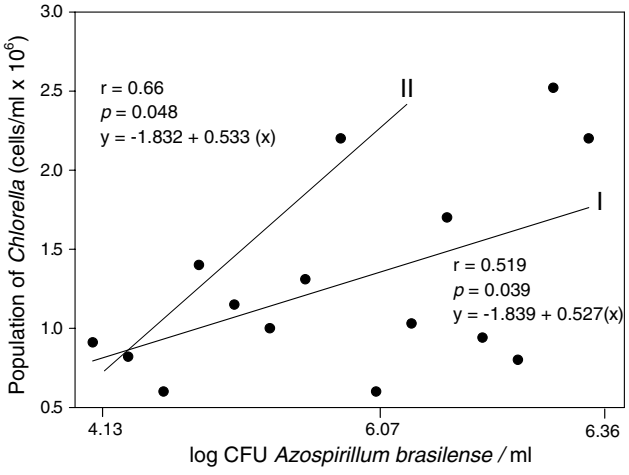

Fig. 2. Correlations between the populations of *Chlorella vulgaris* and *Azospirillum brasilense* Cd.: Line I = Population of *A. brasilense* Cd $\leqslant 2.3 \times 10^6$ cfu ml^{-1}, and Line II = Population of *A. brasilense* $\leqslant 1.2 \times 10^6$ cfu ml^{-1}.

nificant, the correlations between the two populations are relatively low ($r = 0.519$, $r^2 = 0.27$ for Line I; $r = 0.66$, $r^2 = 0.43$ for Line II, in Fig. 2).

4. Discussion

Analyzing the 29 independent experiments of this study together, our general conclusion is that the effect of the MGPB *A. brasilense* Cd on cultures of *C. vulgaris* appears in two distinct ways. (1) Most commonly, it enhances abundance of the microalgal population, as reported earlier [14,21,23], although in these cases, and as a novel result of this study, nitrogen uptake (ammonium or nitrate) per cell was not always enhanced. (2) The current experiments showed that, less commonly, the MGPB enhanced the microalgal cells' capacity to take up nitrogen (ammonium or nitrate) without increasing abundance of the microalgal population, that is, with fewer microalgal cells, there was more uptake of nitrogen per cell. This phenomenon usually results in no increase in uptake by the entire population. Both processes are affected by the cultivation factors of the microorganisms.

4.1. Influence of the nitrogen source

C. vulgaris can grow on a vast variety of organic and inorganic nitrogen compounds. The main nitrogen sources for growth of *Chlorella* spp. are ammonium and nitrate salts, and to a lesser extent, urea [27]. When ammonium and nitrate are supplied together, the microalgae preferentially assimilate ammonium–nitrogen first, which is incorporated into the organic compounds produced by the microalgae [27].

When the source of nitrogen in the medium was ammonium, *C. vulgaris* co-immobilized with *A. brasilense* Cd occasionally was able to take up more ammonium than *C. vulgaris* immobilized alone. Enhanced cellular nitrogen uptake was notable when the medium was supplemented with a source of carbon. These results can be explained by the microalgal need for carbon for assimilating more ammonium. However, it is not clear what the fate of nitrogen in the culture is, since there is no evidence of increase in the microalgal population and ammonium does not seem to be transformed to nitrate, since the concentration of nitrates in the cultures was negligible.

When the source of nitrogen was changed from ammonium to nitrate, *C. vulgaris* co-immobilized with *A. brasilense* Cd did not grow better than *C. vulgaris* alone, but the nitrate uptake per cell increased. The initial concentration of ammonium in the growth medium has an effect on the uptake by *C. vulgaris* cells immobilized alone, but not when *C. vulgaris* is co-immobilized with *A. brasilense* Cd. When

co-immobilized, nitrogen uptake depends more on the number of *Chlorella* cells than on the initial amount of nitrogen.

These results indicate that an increased abundance of microalgal population cultures does not correspond to an increased uptake of nitrogen per cell in immobilized or co-immobilized cultures. At least two plausible explanations can be envisaged. First, the higher the number of cells the older the culture physiologically. In this case, cells are less metabolically active and therefore uptake of nitrogen is reduced. Alternatively, results related to the size and weight of individual cells showed no increase in either parameter, regardless of the level of initial nitrogen in the medium or whether the cultures were co-immobilized or not. The total nitrogen content of the cells was related to the size of the population: higher population sizes resulted in higher N contents. This supports the general conclusion that the abundance of the population controls the uptake of nitrogen within *Chlorella* cells; the higher the population (regardless the experimental parameters), the less nitrogen each cell uptakes.

4.2. Influence of the pH

A co-immobilized system of *C. pyrenoidosa* and activated sludge appeared to be most efficient in removing phosphates and nitrates at pH close to neutral [16]. The maximum intracellular uptake of Cu and Ni by *C. vulgaris* is at pH 6.5–7.5, whereas absorption reached a maximum at pH 3.5 for Cu and 3.5 and 6.5 for Ni [28]. Guckert and Cooksey [29] found that a higher pH of the medium substrate for microalgae interferes with the cell cycle and decreases microalgal population growth. In our study, a high pH had no effect on ammonium uptake per culture or per cell, whereas a higher pH of the medium significantly enhanced the growth of co-immobilized *C. vulgaris* and yielded a higher microalgal population. This may show an ameliorating effect of the bacteria on the microalgae. Similar to the mitigation of the pH effect by co-immobilized bacteria, mitigation of environmental stress by *Azospirillum* spp. is reported in higher plants for drought [30], salt [31–33], humic acid [34] and heavy metals [35]; however, it was never reported for microalgae.

In summary, this study showed the effects of cultivation factors together with the MGPB *A. brasilense* Cd on populations of *C. vulgaris* and their capacity to take up ammonium or nitrate from the growth medium when both microorganisms are co-immobilized in alginate beads. Depending on the cultivation conditions, *A. brasilense* Cd may increase abundance of the microalgal population or enhance uptake of nitrogen by the cells. However, the developing microalgal population apparently controls uptake of nitrogen by individual cells.

Acknowledgements

Yoav Bashan participated in this study in memory of the late Messrs. Avner Bashan and Uzi Bashan from Israel. The editor at CIBNOR improved the English text and Sonia Rocha analyzed total nitrogen. This study was supported by Consejo Nacional de Ciencia y Tecnologia of Mexico (SEMARNAT-CONACYT contract 2002-C01-005) and by The Bashan Foundation, USA.

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