Growth and phosphorus removal by *Synechococcus elongatus* co-immobilized in alginate beads with *Azospirillum brasilense*

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Abstract This study examined the co-immobilization of the cyanobacterium *Synechococcus elongatus* with the plant growth-promoting bacterium *Azospirillum brasilense* in alginate beads and its potential application for the removal of phosphorus from aquaculture wastewater. Co-immobilization of both microorganisms significantly increased the cell density of *S. elongatus* (2852.5×10^4 cells mL^{-1}) compared with that of immobilization of cyanobacteria alone (1325.2×10^4 cells mL^{-1}). Chlorophyll *a* content was similar in co-immobilized (11.1 ± 3.5 pg cell^{-1}) and immobilized *S. elongatus* (14.5 ± 4.9 pg cell^{-1}). *Azospirillum brasilense* showed continuous growth until day 2, after which its cell concentration declined until the end of the assay. Co-immobilized *S. elongatus* removed more phosphorus (44.8 %) than immobilized cyanobacteria cells alone (32.0 %). In conclusion, phosphate removal was greater with free cells of *S. elongatus* but overlapped with the values that were obtained with the treatment of co-immobilization of cells. Our results demonstrate that *A. brasilense* enhances the growth of *S. elongatus* and improves its removal of phosphorus when they are co-immobilized in alginate beads compared with only immobilization of cyanobacteria cells alone.

Keywords *Synechococcus elongatus* · *Azospirillum brasilense* · Growth-promoting bacteria · Co-immobilization · Phosphorus removal

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Introduction

Wastewaters from aquaculture are rich in nutrients, such as phosphorus and nitrogen, which causes problems for receiving water bodies due to eutrophication, such as toxic algal blooms, reduced yields in desirable species, and water column transparency (Smith 2003). Nitrogen compounds are commonly removed from wastewater by physiochemical (e.g., ion exchange, reverse osmosis, and activated carbon adsorption), biological (e.g., bioflocs and wetlands), and electrochemical treatments (Mook et al. 2012).

The removal of phosphorus, the second most important nutrient in aquaculture wastewater, is less efficient because the principal method of eliminating phosphorus from wastewater effluent is chemical precipitation with metal ions such as iron, calcium, and aluminium (Donnert and Salecker 1999; Hernandez et al. 2006). Thus, this approach does not recycle phosphorus as a truly sustainable by-product, because it is removed with other waste by-products, some of which are toxic (de-Bashan and Bashan 2010). The levels of phosphorous in aquaculture wastewaters generally range from 4 to 10 mg L^{-1} (Dumas et al. 1998; Lananan et al. 2014; Lin et al. 2002; Nora'aini et al. 2005).

The biological removal of nutrients involves bacteria, cyanobacteria, and microalgae. The immobilization of cyanobacteria and microalgae has several advantages with regard to wastewater treatment over the use of free cell cultures, such as the ability to facilitate the recovery of biomass and maintain high biomass for further processing (Mallick 2002). Recent efforts have been made to improve the growth of microalgae that are used in such water treatment methods, such as co-immobilization. In the last decade, several studies have co-immobilized microalgae and the plant growth-promoting bacterium *Azospirillum brasilense* in alginate beads, which enhances the growth and nutrient removal

capacity of the microalgae, for which only microalgae of the genus *Chlorella* have been examined (de-Bashan et al. 2004a; González and Bashan 2000). Thus, we were interested in measuring the enhanced effects of *A. brasilense* on other species of microalgae and cyanobacteria, based on the high growth rate of the prokaryote *Synechococcus elongatus* versus eukaryotic *Chlorella* cells.

Synechococcus is a nontoxic cyanobacterium with excellent potential for biotechnological applications based on its physiological and biochemical characteristics (Waterbury et al. 1986), such as its high growth rate (Aguilar-May and Sánchez-Saavedra 2009) and ability to adapt to environmental variations in salinity, temperature, and pH (Billini et al. 2008; Ohto et al. 1999; Rosales et al. 2005). *S. elongatus* removes high percentages of nutrients as free and immobilized cells (Aguilar-May and Sánchez-Saavedra 2009).Thus, the aim of this study was to examine the growth of and phosphorus removal by *S. elongatus* that has been co-immobilized in alginate beads with *A. brasilense*.

Materials and methods

Synechococcus elongatus (SYE-1) was obtained from the Microalgae Culture Collection of Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE, Baja California, México) and maintained in monospecific, nonaxenic batch cultures in 1-L Erlenmeyer flasks that contained "f" medium (Guillard and Ryther 1962), using tap water to prepare the growth medium. The cultures were kept at 20±1 °C under continuous light at 100 μmol photons m^{-2} s^{-1}.

Azospirillum brasilense (CDBB-B-1229) was obtained from the Microorganisms Culture Collection of the Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV, Distrito Federal, México). The cultures of *A. brasilense* were monospecific, nonaxenic batch cultures in 500-mL Erlenmeyer flasks that contained mannitol medium (Asai et al. 1964) and maintained at 25±2 °C, oxygen at 1.70±0.05 mg L^{-1} and light at 0.10 μmol photons m^{-2} s^{-1}.

Evaluation of co-immobilization

All treatments were performed in 1-L Erlenmeyer flasks with 400-mL artificial wastewater (AWW) medium. The AWW medium, designed to simulate the nutrient concentrations in aquaculture wastewater (Aguilar-May and Sánchez-Saavedra 2009), was prepared as a variation of f medium, with double the concentration of nutrients—8.25 mg L^{-1} of phosphate and 22.38 mg L^{-1} of nitrate. The effects of co-immobilization of *S. elongatus* and *A. brasilense* were measured under the following five conditions: (1) AWW medium without cells and beads as control, (2) AWW medium that contained beads

without cells as control, (3) AWW medium that contained free cells of *S. elongatus*, (4) AWW medium that contained immobilized cells of *S. elongatus*, and (5) AWW medium that contained co-immobilized cells of both microorganisms. All treatments were performed in triplicate.

In the treatments with beads (blank beads, immobilized and co-immobilized cells), 400 beads were placed in each flask. For the treatment with free cells, an equivalent amount of *S. elongatus* cells—0.1 mL of centrifuged pellet, as described in the next section—was used for the immobilized cells. All treatment cultures were maintained under the same conditions as in the maintenance of *S. elongatus* cultures for 7 days.

Cell immobilization

Microorganisms were immobilized in alginate per Smidsrød and Skjåk-Bræk (1990). The cyanobacteria cells in alginate beads were entrapped as follows: 50 mL of nonaxenic *S. elongatus* culture was centrifuged at 4000 rpm for 25 min at 4 °C. Then, 0.1 mL of the pellet was withdrawn and mixed with 20 mL of sterilized alginate solution at 4 %. Alginate-immobilized cyanobacteria beads were obtained by dropping the mixture into 2 % CaCl$_2$ solution using a burette with a tip with a 0.5-mm opening. The beads were allowed to harden in the CaCl$_2$ solution for 60 min in 50 mL for every 100 beads. The beads were washed with sterilized distilled water using a strainer mesh.

To co-immobilize both microorganisms, we first determined the ratio of *S. elongatus* to *A. brasilense* cells at which the growth of cyanobacteria was greater. Based on several preliminary assays (results not shown), we used a cyanobacteria-bacteria ratio of 3:2. Accordingly, 35 mL of *A. brasilense* culture was centrifuged at 4000 rpm for 5 min at 4 °C. Then, 0.06 mL of bacterial pellet was mixed with the same volume of cyanobacterial cell pellet for immobilization to 20 mL alginate solution at 4 %. From this point, the co-immobilization steps were similar to those for the immobilization process described above. Blank alginate beads (without cells) were used as control treatments and were made using the same process of immobilization. Prior to the experiment, immobilized and co-immobilized cells in alginate beads were maintained in culture "f/2" culture medium at 20±1 °C under continuous light at 100 μmol photons m^{-2} s^{-1} for 12 h in order to reduce the stress effect in the cells due to immobilization.

S. elongatus growth

Changes in *S. elongatus* cell density were measured daily using a hemocytometer. The cell densities of *S. elongatus* were used to calculate their growth rate (μ) in the exponential growth phase—$\mu = \frac{\log_2 N_2 - \log_2 N_1}{t_2 - t_1}$, where N_2 is the cell density at the final time, N_1 is the cell density at the initial time, t_1 is

the initial time in days, and t_2 is the final time in days (Fogg and Thake 1987). To count immobilized and co-immobilized *S. elongatus* cells, two beads were collected from each treatment and dissolved in 1-mL 0.5-M sodium phosphate buffer for 30 min. For treatments that contained free cells, a 1-mL aliquot was taken from the culture medium.

Chlorophyll *a* content

To quantify the chlorophyll *a* content, we collected two beads from each immobilization and co-immobilization treatments and for free cell treatment passed 1 mL of culture through 0.1 μm GF/C Whatman filters. Chlorophyll *a* was measured at day 7 of the experiment per Parsons et al. (1984).

A. brasilense growth

To measure the growth of *A. brasilense* daily, two beads were collected from the co-immobilization treatment and dissolved in 1 mL 0.5-M sodium phosphate buffer for 30 min. Then, an aliquot from the dissolved beads was withdrawn to make serial dilutions in 1 % saline solution (de-Bashan et al. 2004a). Then, the serial dilutions were used to inoculate Petri dishes with mannitol agar and incubated at 25 ± 2 °C for 24 h. *Azospirillum brasilense* was counted and expressed in colony forming units (CFU) mL^{-1} (Tortora et al. 2007). To evaluate the effects of immobilization on the growth of *A. brasilense*, we established free cell cultures of *A. brasilense* in AWW medium, from which an aliquot was taken to generate serial dilutions in 1 % saline solution; *A. brasilense* growth was measured as in the co-immobilization treatments above.

Phosphorus removal and pH

Phosphate concentration was measured by ascorbic acid method (APHA et al. 1992) on a BioTek Microplate Spectrophotometer (Model Epoch). Phosphorous removal in each treatment was calculated from the remaining phosphate concentration in the medium at the end of the experiment (day 7) and expressed as a percentage. Also, the pH in the medium of each treatment was measured with a HANNA pH meter (model HI 98103B) at the beginning and end of the experiment.

Statistical analysis

Differences in growth, chlorophyll *a* concentration, phosphorus removal, and pH between treatments were analyzed by one-way analysis of variance (ANOVA). Statistical significance between treatments was determined by Tukey's a posteriori test, based on a significance level of $P < 0.05$. The statistical analysis was performed with Statistica, version 7.0 (Statsoft Inc., 2004).

Results

We observed significant differences in the growth of *S. elongatus* between treatments (Table 1). Immobilized cell cultures experienced a lag phase of 2 days, whereas free cells and co-immobilized cells had a lag phase of 1 day (Fig. 1). The initial cell concentration of free cell cultures was higher ($67.0 \pm 5.1 \times 10^4$ cells mL^{-1}) ($P < 0.05$) compared with immobilized ($15.0 \pm 2.5 \times 10^4$ cells mL^{-1}) and co-immobilized cells ($17.3 \pm 2.0 \times 10^4$ cells mL^{-1}). The final cell concentration of free cell cultures ($2537.5 \pm 265.1 \times 10^4$ cells mL^{-1}) exceeded ($P < 0.05$) that of immobilized cells ($1325.2 \pm 171.4 \times 10^4$ cells mL^{-1}) but was similar compared with co-immobilized cells ($2852.5 \pm 74.2 \times 10^4$ cells mL^{-1}). The growth rates (μ) were similar between treatments ($P > 0.05$) (Table 1).

Chlorophyll *a* concentration differed significantly between treatments ($P < 0.05$) (Table 1). Chlorophyll *a* content was similar in the immobilized (14.5 ± 4.9 pg cell^{-1}) and co-immobilized cell cultures (11.1 ± 3.5 pg cell^{-1}) and higher compared with the free cell cultures (2.0 ± 0.3 pg cell^{-1}).

Azospirillum brasilense grew continuously until day 2 (Fig. 2). The bacterial concentration was higher in free cell cultures ($13.0 \pm 1.9 \times 10^3$ CFU mL^{-1}) ($P < 0.05$) compared with co-immobilized cells ($7.3 \pm 0.1 \times 10^3$ CFU mL^{-1}). After the second day until the last day of the experiment, the bacterial concentration declined equally in free cells ($0.5 \pm 0.1 \times 10^3$ CFU mL^{-1}) and co-immobilized cells ($0.5 \pm 0.1 \times 10^3$ CFU mL^{-1}) (Fig. 2).

Table 1 Initial and final cell concentrations ($\times 10^4$ cells mL^{-1}), growth rate (μ), and final chlorophyll *a* content of *Synechococcus elongatus* in the various treatments during 7 days of culture

Growth parameters	Free cells	Immobilized cells	Co-immobilized cells
Initial cell concentration	67.0±5.1a	15.0±2.5b	17.3±2.0b
Final cell concentration	2537.5±265.1a	1325.2±171.4b	2852.5±74.2a
μ (divisions day^{-1})	1.04±0.07a	1.25±0.36a	0.87±0.12a
Chlorophyll *a* (pg cell^{-1})	2.0±0.3b	14.5±4.9a	11.1±3.5a

Values are mean ±1 SE, $n=3$; letters indicate significant differences (by one-way ANOVA and Tukey's a posteriori test, $P < 0.05$; a>b)
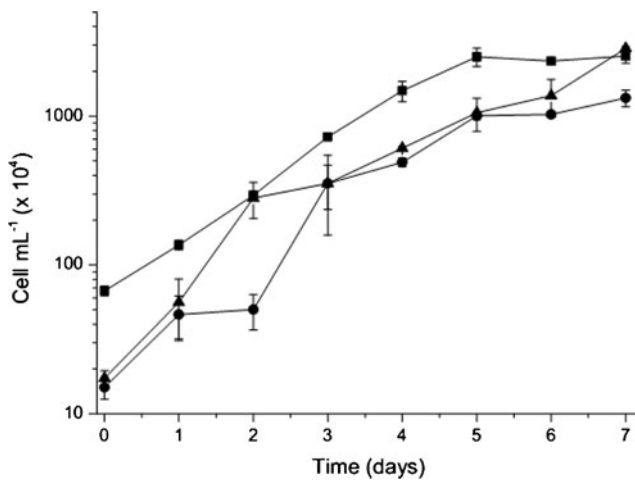
Fig. 1 Mean $\log_{10}$ values of *Synechococcus elongatus* (cell mL^{-1}) in the various treatments. Free cells of *S. elongatus* (*black square*), *S. elongatus* immobilized alone (*black circle*), and *S. elongatus* co-immobilized with *Azospirillum brasilense* (*black triangle*). *Bars* indicate ±1 SE; *n*=3

Fig. 2 Mean $\log_{10}$ values of *Azospirillum brasilense* (CFU mL^{-1}) in artificial wastewater medium. Free cells of *A. brasilense* (*black circle*) and *A. brasilense* co-immobilized with *Synechococcus elongatus* (*black square*). *Bars* indicate ±1 SE; *n*=3

Phosphorus removal

The initial concentration of phosphate in the AWW medium was 8.35 mg L^{-1} and decreased in all treatments. Phosphate removal differed between treatments ($P<0.05$). Phosphate removal by cyanobacteria was higher in the first several hours under all treatment conditions (Fig. 3). On day 7, phosphate removal was significantly higher (Table 2) in free cell treatments (52.93 %, from 8.35 to 3.93 mg L^{-1}) than in immobilized cell cultures (32.07 %, from 8.35 to 5.67 mg L^{-1}). Nevertheless, phosphate removal on day 7 was similar (Table 2) between treatments with free cells and co-immobilized cells (44.87 %, from 8.35 to 4.60 mg L^{-1}). The blank bead control treatment had a phosphate removal rate of 24.91 % (from 8.35 to 6.27 mg L^{-1}), which was similar to the *A. brasilense* cell treatment (25.86 %, from 8.35 to 6.19 mg L^{-1}) (Table 2).

The initial pH values (8.42±0.02) were similar between treatments ($P>0.05$). On day 7, the pH values differed between treatments ($P<0.05$). In cultures of free *S. elongatus* cells (9.00±0.04), free *A. brasilense* cells (8.73±0.50), immobilized cells (9.00±0.09), and co-immobilized cells (9.00±0.31), the pH values were very similar. The pH was lower in the control treatments with blank beads (8.00± 0.03) and AWW medium without cells or beads (8.00±0.01).

Discussion

S. elongatus growth

In our study, immobilized *S. elongatus* cells experienced a lag phase of 2 days, compared with 1 day for free cell treatments. Many studies have noted longer lag phases during
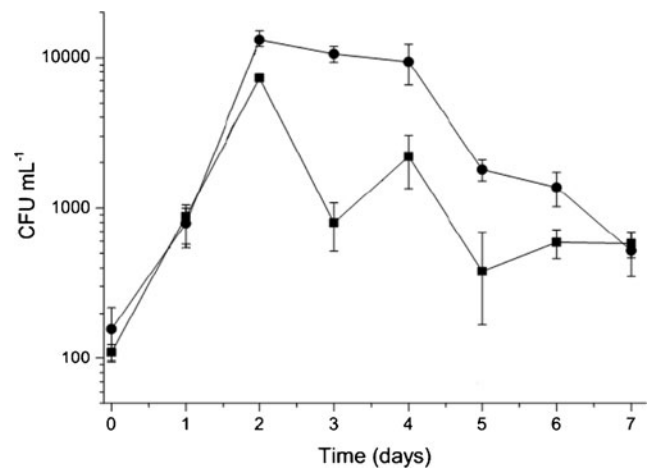
immobilized microalgae cell growth compared with free microalgae cells (Chevalier and de la Noue 1985; Lau et al. 1998; Fierro et al. 2008; Aguilar-May and Sánchez-Saavedra 2009). This effect has been attributed to acclimation of the cells to the new conditions that have been created by their immobilization inside of the polymer (Mallick 2002). Immobilization of microorganisms in polymers creates significant stress on the microorganisms due to the chemical process and the interactions between the immobilization matrix and cell wall (de-Bashan and Bashan 2010), increasing the lag phase. Co-immobilized cell treatments had a lag phase of 1 day, similar to the free *S. elongatus* cells. The negative effects of inmobilized cells of *Chlorella* sp. cells on growth
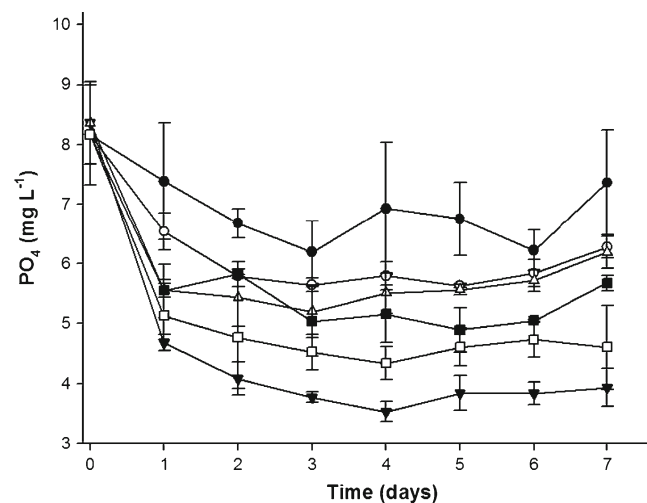

Fig. 3 Mean phosphate concentration values in the various treatments. Artificial wastewater medium without cells and beads (*black circle*), alginate beads without cells (*white circle*), free cells of *Synechococcus elongatus* (*black inverted triangle*), free cells of *Azospirillum brasilense* (*white triangle*), *S. elongatus* immobilized alone (*black square*), and *S. elongatus* co-immobilized with *Azospirillum brasilense* (*white square*). *Bars* indicate ±1 SE; *n*=3

Table 2 Initial and final phosphate concentrations (mg L^{-1}) and final percentage removal on day 7 in the various treatments

Removal PO$_4$$^{-3}$ parameters	AWW	Blank beads	FCA	FCS	Immobilized cells	Co-immobilized cells
Initial concentration	8.35±0.83	8.35±0.83	8.35±0.83	8.35±0.83	8.35±0.83	8.35±0.83
Final concentration	7.36±0.87a	6.27±0.18ab	6.19±0.26ab	3.93±0.31d	5.67±0.13bc	4.60±0.69cd
Final removal (%)	11.85	24.91	25.86	52.93	32.07	44.87

Values are mean±1 SE, $n=3$; letters indicate significant differences (by one-way ANOVA and Tukey's a posteriori test, $P<0.05$; a>b>c>d)

AWW artificial wastewater medium, *FCS* free cells of *Synechococcus* elongatus, *FCA* free cells of *Azospirillum brasilense*

can be mitigated by co-immobilization of the microalgae cells with *A. brasilense* (Hernández et al. 2006). This finding might explain why the lag phase in the co-immobilization treatment was 1 day, as in treatment with free cells.

The initial cell concentration was higher in the free cell treatment versus immobilized and co-immobilized cells. Usually, through immobilization, cells slow their growth because they become embedded in a matrix and are introduced to new chemical and physical conditions inside of the polymer matrix (Mallick 2002). After immobilization, cell viability depends on several factors, such as the method of immobilization; the type, concentration, and size of the polymer; and the characteristics of the microalgae species (Kaya and Picard 1995). In our study, immobilized cells had the lowest final cell concentration. Conversely, the final cell concentration in the co-immobilized cell treatments approached that of free cells, which was twofold higher compared with the immobilized cells. These results are consistent with the findings of González and Bashan (2000), who co-immobilized *Chlorella vulgaris* with *A. brasilense* in alginate beads for 6 days, at which time the growth of co-immobilized microalgae was threefold higher than that of immobilized microalgae alone. de-Bashan and Bashan (2008) reported that the most notable effect of co-immobilization was that the microalgae population increased significantly twofold or threefold compared with immobilized microalgae alone. The mechanisms by which *A. brasilense* affects microalgal growth have been examined recently and are presumably related to the ability of *A. brasilense* to produce the phytohormone indoleacetic acid (de-Bashan et al. 2008). The effects of indoleacetic acid have been studied less extensively in microalgae compared with higher plants. However, the mode of action of indoleacetic acid in plants is stimulation of cell division or cell elongation (Brummell and Hall 1987). Our study demonstrates that the growth-promoting effects of *A. brasilense* are not exclusive to microalgae of the genus *Chlorella*.

Furthermore, the growth rates were similar between treatments, consistent with findings that immobilized organisms have greater metabolic activity and lower generation times compared with organisms maintained in free form in culture (Kolot 1988). These results agree with Aguilar-May and Sánchez-Saavedra (2009), who obtained similar growth rates

in their treatments with free cells and immobilized cells of *S. elongatus*. Although the growth rate was similar between treatments, the final cell concentration was twofold higher in the co-immobilized cell treatment versus immobilized cells. Perhaps the immobilized cell treatment reached the stationary phase on day 5, while the co-immobilized cells continued to grow in the exponential phase until end of the experiment.

Chlorophyll *a* content

The chlorophyll *a* concentration in immobilized *S. elongatus* cells rose sevenfold compared with free cells. This increase, as discussed in other studies (Lau et al. 1998; Pane et al. 1998), was possibly caused by the shading effect of the immobilization matrix. Greater chlorophyll *a* production is commonly observed in immobilized cells, as a mechanism to increase the number of antenna units that collect light that is diminished by the shading effect of the matrix. Thus, the shading effect should be taken into account when estimating biomass by chlorophyll (Moreno-Garrido 2008).

Chlorophyll *a* production in this study was nearly equal in co-immobilized cells compared with immobilized cells, in contrast to the findings of González and Bashan (2000), who co-immobilized *C. vulgaris* and *A. brasilense* in alginate beads and reported 35 % greater chlorophyll *a* production in co-immobilized cells compared with immobilized microalgae alone. This difference might be attributed to the disparate units of chlorophyll *a* content between studies.

A. brasilense growth

With regard to the co-immobilization of microalgae and bacteria, many studies have reported the growth of the microalgae but few have recorded bacterial growth. Hernández et al. (2009) co-immobilized *Bacillus pumilus* and *C. vulgaris* in alginate beads in "artificial wastewater" medium and noted continuous growth of bacteria until day 3, after which the bacterial concentration declined progressively. In this study, *A. brasilense* underwent exponential growth until the second day and then decreased in concentration until the end of the experiment. The bacterial concentration was higher in free cell cultures compared with co-immobilization treatments, possibly because the

immobilization of *A. brasilense* in alginate reduces its concentration. Thus, according to some studies, it is advisable to leave the beads in nutrient broth overnight prior to the experiment to increase cell viability (Bashan 1986; Covarrubias et al. 2012; Cruz et al. 2013).

The decrease in concentration of *A. brasilense* might be attributed to the rise in pH in AWW medium, because optimal *A. brasilense* growth occurs at a pH of 6.8 (Bashan et al. 1993). Another reason for bacterial concentration decline possibly is the lack of carbon sources that bacteria use as nutrients for growth. Although *A. brasilense* consumes inorganic salts (de-Bashan and Bashan 2008), the culture media of this bacterium contain carbon sources, such as malic acid (Bashan et al. 1993). In our study, the culture medium did not harbor carbon sources, because we assumed that the metabolic products of *S. elongatus* could be used by the bacteria for growth. In future studies, the addition of organic nitrogen sources (e.g., urea) to inorganic AWW medium could maintain the growth of *A. brasilense*.

Phosphorus removal

Nutrient removal is more efficient in free cell cultures than in immobilized cells, possibly due to the additional resistance in mass transfer of nutrients that is caused by the immobilization matrix (Pires et al. 2013). Co-immobilized cell treatments showed higher phosphate removal compared with immobilized cells. de-Bashan et al. (2004a) examined nutrient removal from wastewater by *C. vulgaris* and *C. sorokiniana* that were immobilized with and without *A. brasilense*. After 6 days, phosphorus removal was higher in co-immobilized microorganisms (36 %) compared with immobilized microalgae alone (19 %); the percentages of which are lower than the percentages that we obtained. The efficiency of nutrients removal from wastewater is linked to the bead concentration and microalgal biomass in the system (Tam and Wong 2000).

Phosphate removal by alginate beads without cells (blank beads) approximated the values that were obtained by Zamani et al. (2012) in similar alginate control treatments (25 %). The phosphate removal in blank beads treatment is presumably due to the continual precipitation of phosphate, as reported in other studies (Fierro et al. 2008; Aguilar-May and Sánchez-Saavedra 2009; Zamani et al. 2012). Phosphates have affinity for calcium ion; thus, phosphates sequester calcium ions from the alginate matrix and precipitate as calcium phosphate (de-Bashan and Bashan 2004b). This finding is attributed primarily to elevated pH values, approximately 8 or higher (Fierro et al. 2008), which is consistent with the values that we recorded. *Azospirillum brasilense* is unable to remove nutrients from wastewater (de-Bashan et al. 2004a; Moreno-Garrido 2008); thus, the phosphate removal in *A. brasilense* cell treatment also is presumably due to the continual precipitation by the alkaline values of the medium used.

pH of the medium

Several studies have reported that pH between 6 and 8 stimulates efficient nutrient removal (Mallick and Rai 1993). However, each species has specific requirements with regard to pH. Cyanobacteria require alkaline pH values for better growth and, thus, greater metabolic activity, increasing nutrient removal (Fogg et al. 1973). The rise in pH is due primarily mainly to photosynthesis and nitrate consumption (Fogg and Thake 1987; Verduin 1964) and is consistent with the findings of other studies on immobilization with *S. elongatus* (Aguilar-May and Sánchez-Saavedra 2009; Castro-Ceseña and Sánchez-Saavedra 2015).

In conclusion, phosphate removal was greater with free *S. elongatus* cells and overlapped with the values that were obtained in the treatments with co-immobilized cells. Our novel data demonstrated that the artificial association between *S. elongatus* and *A. brasilense* in alginate beads increases cyanobacterial growth and phosphorus removal compared with immobilized cyanobacteria cells alone.

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