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ORIGINAL ARTICLE

Alginate from the macroalgae *Sargassum sinicola* as a novel source for microbial immobilization material in wastewater treatment and plant growth promotion

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Abstract Alginate extracted from the macroalgae *Sargassum sinicola* was used as the raw material for co-immobilization of the microalgae *Chlorella sorokiniana* and growth-promoting bacterium *Azospirillum brasilense* for wastewater treatment and as an inoculant carrier of *A. brasilense* for plant growth promotion. The composition, structure, viscosity, color, and phenolic compound content of the alginate were analyzed and compared with commercially available alginate produced from the macroalgae *Macrocystis pyrifera*. From ^{1}H NMR analysis of alginate, *S. sinicola* was found to have more guluronic acid ($F_G = 0.64$) than it had mannuronic acid ($F_M = 0.38$) and had a viscosity of 13.5 m Pa s compared to 50 m Pa s for *M. pyrifera*. The *S. sinicola* alginate had dark brown color, reducing light penetration, with more phenolic compounds than *M. pyrifera* alginate. Nonetheless, growth of *C. sorokiniana* and *A. brasilense* in *S. sinicola* alginate was not significantly different than the growth in *M. pyrifera* alginate beads. Nutrient removal from wastewater by the co-immobilized microorganisms was similar for both types of alginate beads, and so was the growth enhancement of tomato plants inoculated with microbeads containing *A. brasilense*. This study shows the potential use of *S. sinicola* alginate as a raw material for cell immobilization for wastewater treatment and plant growth promotion.

Keywords Alginate · Algae · Immobilization · *Macrocystis pyrifera* · Plant growth promoting bacteria · *Sargassum sinicola* · Wastewater treatment

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Introduction

Alginate is the most commonly used polymer for microbial cell encapsulation, also called immobilization (Chen & Huang, 1988; de-Bashan & Bashan, 2004; Fenice et al., 2000; Paul et al., 1993; Smidsrød & Skjåk-Bræk, 1990; Walker & Connick, 1983). Its source is mainly brown macroalgae, where it is the major structural component of the cell wall and intercellular matrix, providing mechanical properties to the

algae. The polymeric chain is made of mannuronic (M) and guluronic (G) acids in varying proportions and sequential arrangements. The monomers can be linked by calcium ions through binding consecutive blocks of guluronic acid to form gels. The relative amounts and occurrence for both monomers and their sequential arrangement in the polymeric chain are related to the origin of the alginate, both environmentally and genetically. This conformation dictates the properties of the alginate (McHugh, 1987; Skjåk-Bræk & Martinsen, 1991a) such as solubility, viscosity, and the ability to exchange ions with divalent metals (King, 1983; Shymali et al., 1984). The alginate produced from *Macrocystis pyrifera* (kelp), which is most frequently used for cell immobilization, yields gels with lower strength and stability than gels produced from other alginates (Skjåk-Bræk & Martinsen, 1991b).

During the last decade, several experimental microbial formulations based on natural polymers have been evaluated as inoculants for agriculture to improve plant productivity (Bashan, 1998) and as carriers for agents for wastewater treatment (de-Bashan et al., 2002; de-Bashan & Bashan, 2004). The plant growth promoting bacterium (PGPB) *A. brasilense* (Bashan et al., 2004) has been immobilized in macrobeads (1–4 mm diameter), together with the green microalga *Chlorella* species for environmental uses (Gonzalez & Bashan, 2000), and in microbeads (200 μm diameter) for agriculture purposes (Bashan, 1986, 1998; Bashan et al., 2002). These formulations encapsulate the living cells, protecting the microorganisms against many environmental stresses and allowing cells to continue their growth and metabolism. Environmental applications of microorganism immobilized in polymers for cleanup of hazardous materials are available commercially (Cassidy et al., 1996; Stormo & Crawford, 1992). However, these formulations are more expensive than peat and soil formulations commonly used in agriculture and require more biotechnical handling by the inoculants industry (Fages, 1990, 1992).

Sargassum species are common macroalgae occurring worldwide and a potential renewable marine resource because of its great abundance (Lee, 1980). In the Gulf of California, *Sargassum sinicola* dominates over other species (Hernández-Carmona et al., 1990; Rivera & Scrosati, 2006) with an estimated biomass yield of 200 000 t (fresh weight) per year near the Baja California Peninsula, Mexico (Pacheco-Ruiz et al., 1998; Hernández-Carmona et al., 1990; Casas-Valdez et al., 1993). The alginate content in this species ranges from 10 to 25% (Casas-Valdez, 1982), but neither its chemical composition and physical properties nor its possible applications have been determined.

This study (i) determined the chemical composition and several physical properties of alginate derived from *S. sinicola*, (ii) tested the use of this alginate for microbial cell immobilization in producing macro- and microbead formulations, and (iii) evaluated these formulations for removal of nutrient from domestic wastewater and to enhance plant growth.

Materials and methods

Alginate sources

S. sinicola (Setchell and Gardner) macroalgae were collected from the sea at Sausozo, near the abandoned mining town of San Juan de la Costa (24°22′N, 110°40′W), Baja California Sur, Mexico, in May and June 2001. Collection of samples was done by cutting the thallus with a knife near the rhizoid. The algae were sun-dried (ambient temperature 35–45 °C), pulverized to 40 mesh using a mill (Hernández-Carmona, 1999a), and stored in aerated bags in a shaded and ventilated site until the alginate was extracted (see later). Alginate from *M. pyrifera* (a mixture of 14 000 and 3500 m Pa s forms at a 1:4 w/w ratio to create 6000 m Pa s, Sigma, USA) was prepared as described by Gonzalez & Bashan (2000) and used routinely as the alginate control.

Alginate composition, color, and phenol content

The alginate from *S. sinicola* was produced at a pilot facility for alginate production at CICIMAR-IPN using the methods described by Hernandez-Carmona et al. (1999a,b, 2002) and McHugh et al. (2001), but with the following minor modifications: (i) during hydration, the formalin concentration was increased to 0.4% for 12 h; (ii) the acid pre-extraction was carried out at pH 2, and the extraction occurred at 80 ± 2 °C for 2 h; and (iii) conversion to calcium alginate was done at 3:1 ratio ($CaCl_2$:alginate solution) without the bleaching procedure. Alginate chemical composition was determined by nuclear magnetic resonance (^{1}H NMR) at 300 MHz, including the frequencies of monads, diads, and triads of mannuronic (M) and guluronic (G) acids. The ^{1}H NMR spectroscopic technique is the most

reliable method for determining the composition and the block structure of alginate molecules (Grasdalen, 1983). Analyses were performed at the Norwegian Biopolymer Laboratory of the Norwegian University of Science and Technology in Trondheim. Color was determined in solution by a spectrophotometric method (Hardy, 1936), using a spectrophotometer (UV-2800, Unico, USA), measuring transmittance values using 10 different ordinates from 400 to 700 nm. The transmittance values corresponding to the 10 wavelength ordinates used to calculate the tri-chromatic coefficients from the tri-stimulus values X, Y, and Z, according to Hardy (1936). The color characteristics, hue, luminance, purity, and percent of luminance at the original pH, were expressed in terms of dominant wavelength (nanometer to the nearest whole unit). Additionally, alginate color was evaluated with powdered preparations (60 mesh) using the Munsell soil color charts (Anonymous, 2000). Total phenolic compounds was determined by the Folin-Ciocalteau colorimetric method (Waterhouse, 2002), using a spectrophotometer (DR/2000, HACH) and gallic acid (Sigma, USA) as the calibration standard.

Alginate viscosity

Alginate viscosity was measured with a viscometer (LVT Brookfield, Brookfield, MA) at 22 °C at 60 rpm with a built-in spindle. Viscosity of *M. pyrifera* and *S. sinicola* alginates vary at the same concentration. Therefore, the curve equation of the viscosity was measured at different concentrations (1.0, 1.5, 2.0, 2.5, and 3.0%) to reach a viscosity of 200 m Pa s in each alginate type after sterilization by standard autoclaving procedures. The viscosity was measured before and after sterilization. Additionally, the percentage of reduction in viscosity during sterilization was determined by the following equation:

$$\frac{(I\eta - F\eta)/I\eta}{100},$$

where $I\eta$ is the initial viscosity and $F\eta$ the viscosity after sterilization.

Light reduction through alginate gels

Light reduction in the two types of alginates was assessed with a light intensity gauge (Sper Scientific, Scottsdale, AZ). The light source was two 39-W fluorescent lamps (OSRAM, Mexico). Gel tubes made of *M. pyrifera* and *S. sinicola* alginates were constructed by adding 10 mL of each alginate solution (200 m Pa s) into standard cellulose dialysis tubes (Spectrapor, VWR Scientific, LA), which were sealed at the ends (length of tubes 10 cm, dry cylinder diameter 28.6 mm, and volume 6.4 mL cm^{-1}). Tubes were immersed in 2% $CaCl_2$ solution (500 mL), with slow stirring for 12 h at ambient temperature (~28 °C). Afterward, the gels were horizontally sliced into segments with a scalpel blade (~3 mm thick). Each segment was placed on a dark, hard plastic sheet (5 cm^2) with an aperture diameter of 2.5 cm and placed on the light intensity gauge. The gels were subjected to light at 23.65 μmol photons m^{-2} s^{-1}. The amount of light transmitted through the solidified alginate was calculated in 5 min measurement intervals. Light reduction was determined by subtracting the gel luminance from the initial luminance without the alginate cover. Twenty-one measurements were made from three gel tubes for each alginate type.

Organisms and growth conditions

Chlorella sorokiniana (Shih et Krauss, UTEX 1602, Austin, TX) and *Azospirillum brasilense* Cd (DSM 1843, Braunschweig, Germany) were used as the model organisms and the tomato (*Lycopersicon esculentum* Mill.) (cv Ace, Germinal, Mexico) was used as the model plant.

C. sorokiniana and *A. brasilense* Cd were immobilized and co-immobilized, as described by Bashan (1986) and de-Bashan et al. (2004). Macrobeads were prepared according to de-Bashan et al. (2004) and microbeads according to Bashan et al. (2002). Prior to immobilization, axenic *C. sorokiniana* was cultivated in a sterile mineral medium (C30) (Gonzalez et al., 1997) in 250 mL Erlenmeyer flasks at 28 ± 2 °C with constant illumination at 23.65 μmol photons m^{-2} s^{-1} for 5 days. *A. brasilense* was cultivated in liquid nutrient broth (Sigma, USA) at 30 ± 2 °C for 18 h in a rotary shaker at 150 rpm (Orbit Shaker, Lab-line, Melrose Park, IL). To obtain a higher number of bacteria required for tomato seed inoculation (described later), a trypton–yeast extract–glucose medium (TYG) was used (Bashan et al., 2002) under similar growth

conditions. Dried microbeads for plant inoculation were obtained by standard lyophilization.

Growth measurement of the microorganisms

Growth of *C. sorokiniana* was assessed by taking samples of beads in cycles of 48 h. At the end of the experiments, beads were solubilized (five beads were solubilized in 5 mL 4% bicarbonate solution, at ambient temperature for 30 min); counting of individual cells was done with light microscopy using a Neubauer hematocytometer, as described by Gonzalez & Bashan (2000) and de-Bashan et al. (2004), with minor modifications. Briefly, after bead solubilization, cell counts were expressed per milliliter of alginate and not per bead; and the initial amount of alginate solution used and the total number of beads formed were considered. This alternative was used because the number of beads formed from the different alginate solutions varied according to the source of the alginate, despite homogenizing viscosity to 200 m Pa s. *A. brasilense* was counted by the plate count method on nutrient agar plates (Sigma, USA) and expressed as colony-forming units (cfu) per milliliter of alginate. Synthetic wastewater used in the experiments on the removal of nutrients was prepared by the formula described by de-Bashan et al. (2002): NaCl (7 mg L^{-1}); $CaCl_2$ (4 mg L^{-1}); $MgSO_4 \cdot 7H_2O$ (2 mg L^{-1}); K_2HPO_4 (21.7 mg L^{-1}); KH_2PO_4 (8.5 mg L^{-1}); Na_2HPO_4 (33.4 mg L^{-1}); NH_4Cl (30 mg L^{-1}).

Water analysis

Standard water analyses were performed with a spectrophotometer (DR/2000, Hach Co., Loveland, CO) and analytical kits (Hach). Ammonium was analyzed by the salicylate method and phosphorus (orthophosphate) by the molybdovanadate method (APHA, AWWA, WPCF, 1992).

Seed inoculation and seedling growth conditions

Tomato seeds were immersed in a solution of alginate (*M. pyrifera* or *S. sinicola* at 200 m Pa s) to cover the seeds and make a sticky surface. Then, dried microbeads (∼200) per seed were manually mixed with the sticky-surfaced seeds until all the seed were coated with the microbeads. All experiments were carried out in pots. Pot experiments for promotion of plant growth were conducted in a nutrient-poor desert soil passed through 500 mesh screens that were obtained from barren desert areas where perennial plants do not usually grow (Carrillo-Garcia et al., 1999). The detailed analysis of this soil was published earlier (Bashan et al., 2000; Carrillo-Garcia et al., 2000). The soil was placed in round, opaque 100 mL pots (5.5 cm diameter and 4.5 cm effective soil depth). The substrate was saturated with distilled water before sowing. The pots were immediately sown with five inoculated seeds. The level of inoculation was 200 (average) microbeads per seed, corresponding to 2.5×10^7 cfu per seed. Seeds, and later plants, were incubated in a growth chamber at $27 \pm 2\,^{\circ}C$ for 12 h under illumination at 200 μmol photons $m^{-2}\,s^{-1}$ (Conviron TC 16) for 30 days after sowing. Fertilizer was not applied; the plants did not show any deficiency during the course of the experiment. Distilled water was added only when needed to keep the pots moist without exceeding the water-holding capacity of the soil. After 30 days, standard plant growth parameters such as root length, plant height, and their respective dry weight were measured separately as responses to inoculation.

Dry weight of stems and roots was determined by placing each into small, pre-weighed aluminum foil and drying them in a forced-draught oven at $75 \pm 2\,^{\circ}C$ for 16 h (Bashan & de-Bashan, 2005).

Experimental design and statistical analysis

Each experiment was carried out in triplicate with three replicates per treatment, where one flask or one pot served as a replicate. Each chemical or physical analysis was done in triplicate. The setup for microalgae growth was in semi-continuous culture, where wastewater was replaced every 48 h but not the immobilized beads, as described by de-Bashan et al. (2004). Control beads of each type of alginate without microorganisms were routinely used. Wastewater samples (50 mL) were taken every 48 h for analyses, and then the culture medium was changed.

The growth cycles of the microalgae in wastewater were compared, or growth parameters of tomato plants were analyzed using one-way ANOVA and then by post-hoc analysis, using Tukey's test at $p \leq 0.05$. Growth of *C. sorokiniana* in different alginates and different immobilization treatments were analyzed by the K–W test and a post-hoc analysis by Nemenyi's test at $p \leq 0.05$. Growth of *A. brasilense* co-immobilized

Table 1 Chemical composition and sequence parameters of alginates by the ^{1}H NMR technique

Alginate source	F_G	F_M	F_{GG}	F_{MM}	F_{MG}	F_{GGG}	F_{MGG}	F_{MGM}	$N_G > 1$
Sargassum sinicola	0.64	0.36	0.54	0.25	0.11	0.50	0.04	0.08	14
Macrocystis pyrifera	0.38	0.62	0.18	0.42	0.20	0.13	0.05	0.18	4

in different alginates was compared by Student's *t*-test at $p \leq 0.05$. All analyses were carried out with Statistica software (Statsoft^{TM}, Tulsa, OK).

Results

Alginate properties

Composition of alginates, expressed as frequencies of mannuronic and guluronic acid in the polymeric chains, as well as diad and triad frequencies, was determined in *S. sinicola* and *M. pyrifera* alginates (Table 1). Although there were some differences between several different alginate fractions, the most important difference was that the G content is relatively higher in the *S. sinicola* alginate ($F_G = 0.64$, $F_{GG} = 0.54$, $F_{GGG} = 0.50$, NG $> 1 = 14$, pH 7.6) than in *M. pyrifera* alginate ($F_G = 0.38$, $F_{GG} = 0.18$, $F_{GGG} = 0.13$, NG $> 1 = 4$, pH 6.8).

Color characteristics varied greatly from one alginate to the other (Table 2). The *S. sinicola* alginate showed a distinctive brownish dark color (Table 2), with concentration of total phenolic compounds higher than the concentration in *M. pyrifera* alginate (58.83 ± 8.62 and 7.13 ± 3.83 mg kg^{-1} gallic acid equivalents), at 1% alginate concentration (significant at $p \leq 0.05$). Color intensity was reduced during the gelling process; nevertheless, some dark color remained in the gels. The reduction in light intensity was higher with *S. sinicola* alginate ($41.68 \pm 4.49\%$; 13.80 ± 1.06 μmol photons m^{-2} s^{-1}) than with the control of *M. pyrifera* alginate ($14.46 \pm 3.93\%$; 20.24 ± 0.93 μmol m^{-2} s^{-1}; $p \leq 0.05$, 23.65 μmol photons m^{-2} s^{-1} initial light intensity).

Viscosities of the two types of alginates were evaluated at various concentrations of raw alginates to reach a uniform viscosity of 200 m Pa s in both types. The fit of viscosity curves from *M. pyrifera* and *S. sinicola* alginates, before and after sterilization, were correlated with exponential equation ($y = 25.131x^3$, $r = 0.999$ and $y = 13.499x^3$, $r = 0.998$, respectively). *M. pyrifera* alginate showed far higher reduction in viscosity during the sterilization procedure ($600 \pm 95\%$ reduction) compared with *S. sinicola* alginate ($70 \pm 22\%$ reduction). Therefore, to establish alginate concentrations to reach a viscosity of 200 m Pa s, 2.45% alginate concentration for *S. sinicola* and 2.00% for *M. pyrifera* were used for the production of alginate beads.

Wastewater treatment

The growth of *C. sorokiniana* in semi-continuous treatments performed for five consecutive cycles was continuous when the immobilized microalgae were growing in the synthetic wastewater (Fig. 1a), at 4.0×10^7 to 12.6×10^7 cells mL^{-1} in *S. sinicola* alginate and 6.6×10^7 to 16.7×10^7 cells mL^{-1} in *M. pyrifera* alginate. No significant differences in microalgae counts were found between alginates in immobilized and co-immobilized treatments. *A. brasilense* continuously and significantly enhances the growth of *C. sorokiniana* when the co-immobilized microorganisms were grown in synthetic wastewater (Fig. 1b) at 5.1×10^7 to 19.5×10^7 cells mL^{-1} in *S. sinicola* alginate and 7.3×10^7

Table 2 Color characteristics of the two alginates

Color characteristics	*S. sinicola*	*M. pyrifera*
Alginate solution (Spectrophotometric method)		
Dominant wavelength	580	575
Purity[a]	80%	10%
Hue	Yellowish orange	Yellow
Luminance[b]	27.4	81.7
Alginate powder (Munsell charts)		
Color	Very dark brown	Pale yellow
Hue	7.5 YR (2.5/3)	2.5Y (7/4)

[a] Saturation
[b] In percent, degree of brightness

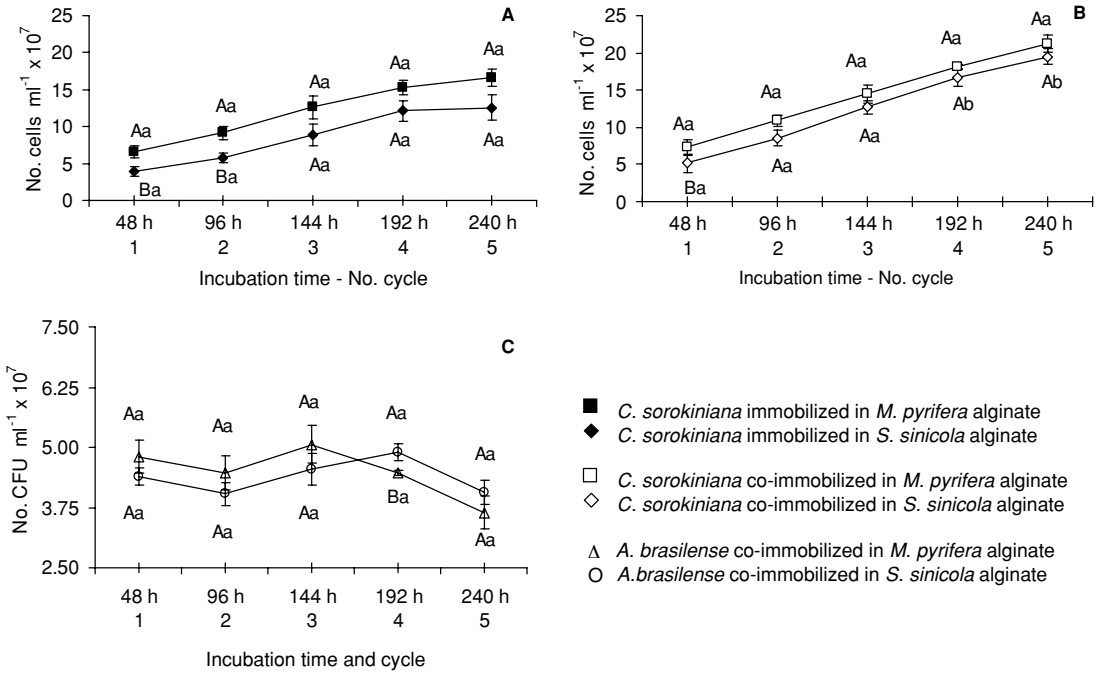

Fig. 1 Growth of *C. sorokiniana* and *A. brasilense* immobilized and co-immobilized in *S. sinicola* and *M. pyrifera* alginate beads. (A) *C. sorokiniana* immobilized alone. (B) Growth of *C. sorokiniana* co-immobilized with the PGPB, *A. brasilense*. (C) Growth of *A. brasilense* co-immobilized with *C. sorokiniana*. Points on each curves denoted by different lower case letter differ significantly by one-way ANOVA ($p \leq 0.05$). Points at each cycle of *C. sorokiniana* denoted by a different capital letter differ significantly by K–W test ($p \leq 0.05$), with post-hoc Nemenyi's test. Points at each cycle for *A. brasilense* denoted by a different capital letter differ significantly with Student's *t*-test ($p \leq 0.05$). Bars represent standard error

to 21.3×10^7 cells mL^{-1} in *M. pyrifera* alginate. This bacterium remains constant within the beads over time (Fig. 1c), which differs, as previously described (de-Bashan et al., 2002). Nevertheless, in this study, the bacterial concentration was higher from the beginning of the experiments ($\sim 4.5 \times 10^7$ cfu mL^{-1}).

The rate of removal of ammonium was very efficient and similar for the different formulations of alginate beads (Fig. 2). In immobilized and co-immobilized cultures of both alginates, ammonium was completely removed after the first cycle (48 h). In *M. pyrifera* alginate beads, but not in *S. sinicola* beads, removal of ammonium was less in the first cycle of the experiment (48 h) when co-immobilized. In later cycles, removal of ammonium was complete. The alginate source did not have an effect on ammonium removal ($p \leq 0.05$, data not presented). Removal of phosphate from the wastewater was similar in immobilized and co-immobilized beads (Fig. 3) and between alginate sources ($p \leq 0.05$, data not presented), with significant differences in the initial amount of phosphates and with control treatment of beads without microorganisms.

Plant growth promotion

The dried microbeads used for seed inoculation contained similar and high bacterial populations in *S. sinicola* and *M. pyrifera* alginate microbeads ($2.3 \pm 0.5 \times 10^9$ cfu g^{-1} and $2.5 \times 0.4 \times 10^9$ cfu g^{-1}, respectively). In both alginates, microbeads by themselves did not improve the plant growth compared with the non-inoculated control. However, inoculation of tomato plants with *A. brasilense* Cd immobilized in either *S. sinicola* or *M. pyrifera* alginate significantly increased the length of the roots but not the height of the plants (Fig. 4a) and the dry weight (Fig. 4b) of leaves and roots, without significant differences in the increased height or dry weight of the different plant parts from either alginate source.

Discussion

Production of alginate from *S. sinicola* has four main advantages. First, it is a renewable marine resource

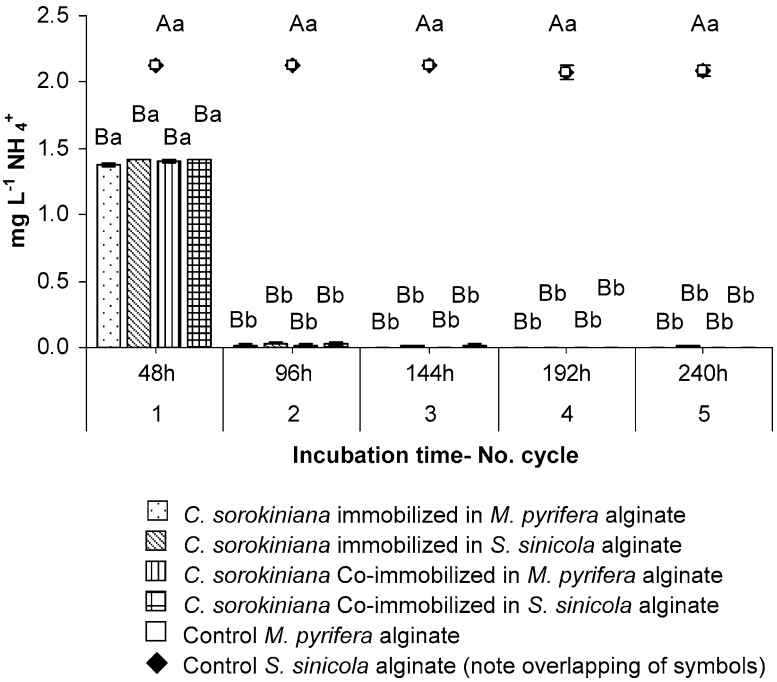

Fig. 2 Removal of ammonium ions from wastewater by *C. sorokiniana* immobilized and co-immobilized with the PGPB, *A. brasilense* in *S. sinicola* and *M. pyrifera* alginate beads. Columns denoted by a different lower case letter differ significantly by K–W test ($p \leq 0.05$) with post-hoc Nemenyi's test. Columns for each cycle denoted by a different capital letter differ significantly with its control at each incubation cycle. Bars represent standard error

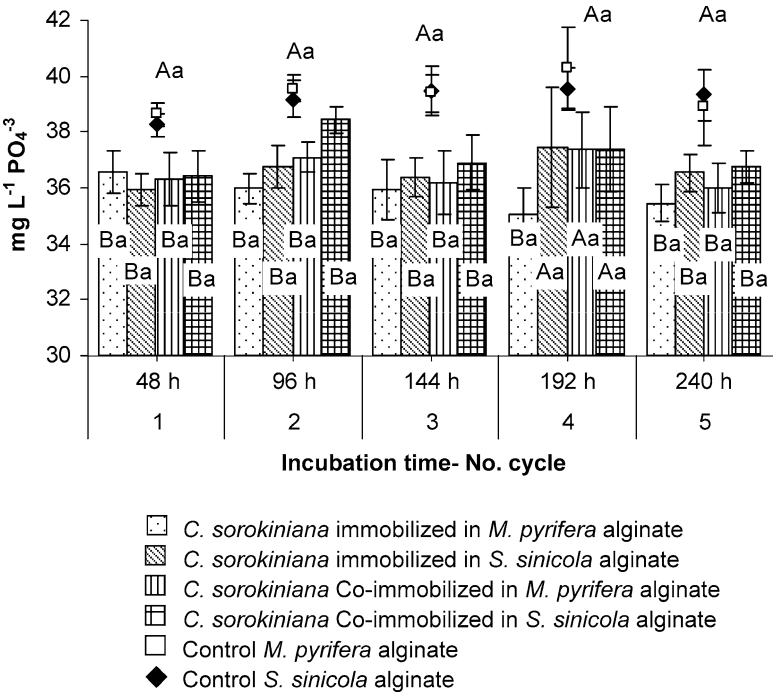

Fig. 3 Removal of phosphorous ions from wastewater by *C. sorokiniana* immobilized and co-immobilized with the PGPB, *A. brasilense* in *S. sinicola* and *M. pyrifera* alginate beads. Columns denoted by a similar lower case letter do not differ significantly by K–W test ($p \leq 0.05$), with post-hoc Nemenyi's test. Columns for each cycle denoted by a different capital letter differ significantly with its control. Bars represent standard error

of major quantities, sometimes considered as a contamination of tourist beaches or a plague that limits its growth of other marine resources of economic importance (Ochieng & Erftemeijer, 1999; Staehr et al., 2000; Boudouresque & Verlaque, 2002). Second, this macroalgae species grows in moderately protected areas, near the shore; therefore, its collection is far easier compared with other macroalgae. Third,

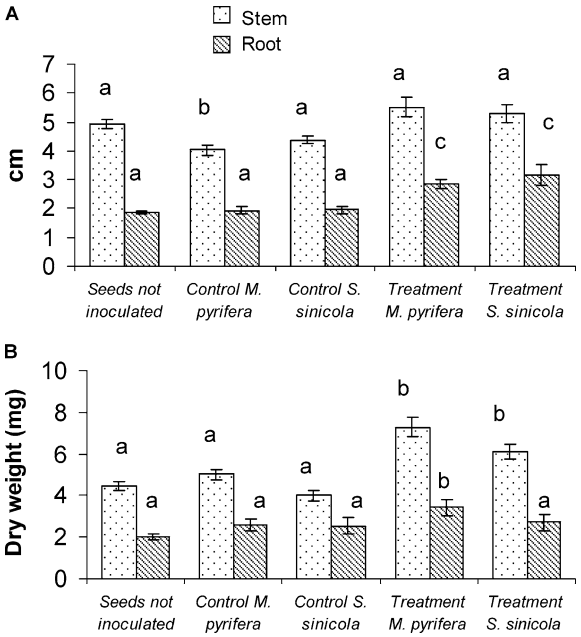

Fig. 4 Growth of tomato plants, cv. ACE inoculated with the PGPB *A. brasilense* immobilized in *S. sinicola* and *M. pyrifera* microbeads. (A) Height of plants and length of roots. (B) Dry weight. Columns denoted by a different letter for each plant part differ significantly by ANOVA ($p \leq 0.05$) and Tukey's as post-hoc test. Bars represent standard error

production costs for alginate from this macroalgae is currently lower than other sources of alginate (Yabur et al., unpublished) because there is no market demand or price for alginate from this source. Finally, alginate from *S. sinicola* has different chemical compositions that *may* provide other potential biotechnological applications. This variation in composition is related to the species and different environmental requirements of the macroalgae (Panikkar & Brasch, 1996), making alginate from different sources unequal and unique. Our study explored the use of this new alginate for two applications, which normally use more expensive alginate for wastewater treatment and plant growth promotion.

Alginate properties, like structure, mechanical strength, porosity, and gelling force, are important variables to consider in selecting alginate for an application (Skjåk-Bræk & Martinsen, 1991a). A high content of guluronic acid provides greater mechanical strength, volume stability, and swelling properties and also lower shrinkage of alginate beads when combined with monovalent cations (Martinsen et al., 1989; Skjåk-Bræk & Martinsen, 1991b). Alginate from *M. pyrifera* has shown higher reduction in viscosity during autoclaving. Alginate with a higher degree of polymerization, like those of *M. pyrifera*, are generally less stable than those with a low degree of polymerization (McHugh, 1987). Consequently, the longest alginate chains in *M. pyrifera* are more susceptible to breakage by heat than the alginate chains of *S. sinicola*. The average of the G-block length larger than 1 ($N_G > 1$) has been related to gelling properties (Smidrød et al., 1992; Draget et al., 2002). We showed that these values were greater for *S. sinicola* alginate, as well as higher content of guluronic acid diads and triads than for *M. pyrifera* alginate. This suggests that alginates gels from *S. sinicola* are stronger than gels from *M. pyrifera*. Additionally, these characteristics affect the diffusion properties in the beads (Martinsen et al., 1989, 1992), an important parameter in wastewater treatment. Diffusion increased in beads with high guluronic acid content. Species of *Sargassum* from the Red Sea coast show similar content of guluronic acid, including *S. dentifolium*, *S. asperifolium*, and *S. latifolium*, $F_G = 0.66$, $F_G = 0.59$, and $F_G = 0.55$, respectively (Larsen et al., 2003). Alginate from two other *Sargassum* species from the Philippines, *S. ilicifolium* and *S. polycystum*, showed a lower viscosity compared to *S. sinicola* (Calumpong et al., 1999).

Some properties of the alginates such as molecular weight, color, and purity can be modified during the extraction procedure (McHugh, 1987, 2003). The production process at the facility for alginate extraction from *S. sinicola* was limited to the reduction of alginate costs (Yabur et al., unpublished data), leaving some impurities such as darker color and phenolic compounds. These factors may inhibit the growth of microorganisms, especially phototrophic ones, like the microalgae used in this study. Despite these theoretical limitations, the multiplication of microorganisms was similar in both alginates and without differences in the removal of nutrients in wastewater treatment. This may provide an advantage for using the cheaper alginate source as a carrier for agents of wastewater treatment. Regardless, *C. sorokiniana* is a photosynthetic microorganism, and light reduction imposed by *S. sinicola* alginate did not appear to be a limiting factor for growth. Robinson et al. (1985) suggested that growth of *Chlorella* in alginate beads is affected by the diffusion of CO_2. Also, there is a possibility that growth of *C. sorokiniana* in *S. sinicola* alginate beads may be heterotrophic in response to the reduction of light (Karlander & Krauss, 1966).

Inoculation of crop plants with PGPBs is a relatively novel agricultural technique. The material used as carriers are mainly peat and soil fractions. In recent years, some researchers proposed that peat and other materials can be replaced by "synthetic" materials, with alginate playing a major role (Bashan, 1998). Some formulations that use alginate were proposed (Bashan, 1998; Bashan et al., 2002; Fages, 1990, 1992). One of the main limitations to widespread use of this technology is the cost, where the commonly used alginate from *M. pyrifera* has a cost that is unacceptable in agricultural practices. This study demonstrate that low-cost alginate, such as that from *S. sinicola*, can be substituted for higher-cost alginates, since the response of a commercial plant to inoculation was identical in both alginate carriers.

Conclusions

This study is the first report describing the composition of *S. sinicola* alginate. This alginate is a new and cheaper raw material for immobilization of microorganisms employed in removing nutrients from wastewater and for inoculating agricultural plants with PGPB. At the current state of knowledge, this alginate can be readily used when color is not a limiting factor.

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