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Immobilization of microorganisms in alginate beads as protection against hostile conditions when treating wastewater)

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INTRODUCTION AND OBJECTIVES

Immobilization of microalgae, as part of a global trend of immobilizing microorganisms in an assortment of matrices, is used for a wide variety of biotechnological applications, such as production of metabolites, removing metal from water, and tertiary treatment of wastewater. Immobilization is a 40-year-old, established technology that was tried for wastewater treatment about 20 years ago (de-Bashan and Bashan 2010).
Tertiary treatment of biological wastewater exposes treating agent(s) to competition and predation by large populations of microflora but mainly by microfauna residing in the wastewater during secondary wastewater treatment; this effectively reduces their efficiency. Therefore, protection against competition is vital. When microalgae are applied as cell suspensions to natural wastewater, the most competitive microorganism prevails, not necessarily the intended agent. It is assumed, yet without experimental proof, that immobilization in a polymer matrix, such as beads, will provide a physical barrier between the treating agents and the microbiologically-hostile wastewater environment, thus permitting the water to reach the agents yet restrict predators.
Our hypothesis is that immobilization in alginate protects the internal populations of wastewater treating agents of microalgae and bacteria from predation/herbivory by micro-predators by having a physical barrier between the two microenvironments yet allowing uninterrupted tertiary wastewater treatment.
Our objectives were to: (1) Demonstrate that the internal population of wastewater treating agents within the bead is not reduced upon expose to wastewater; (2) Determine if the population of microorganisms in the wastewater can also be found within the confines of the beads; and (3) Quantify how much tertiary treatment is performed. All of this has been done using wastewater from the municipal treatment plant of La Paz, B.C.S., Mexico.

MATERIALS AND METHODS

Microorganisms, axenic and non-axenic growth conditions, immobilization of *Chlorella sorokiniana* with *Azospirillum brasilense* in alginate beads

The unicellular microalgae *C. sorokiniana* (UTEX 2805) was used. Before immobilization in alginate beads, the microalgae were cultured in sterile mineral medium (C30) for five days, following a described method (Gonzalez and Bashan 2000). The plant growth-promoting bacterium (PGPB) *A. brasilense* Cd (DSM 1843) was combined with the microalgae in joint immobilization experiments. Microorganisms were immobilized following a described method (de-Bashan et al. 2004). For immobilization of the two microorganisms in the same bead, each was re-suspended in 10 mL 0.85% saline solution and then mixed in the alginate before the beads were formed. Microorganisms used in solitary or jointly immobilized treatments were grown under semi-continuous conditions where wastewater was changed every 48 h, but not the immobilized microorganism.

Bacteria and microalgae counts and detection by fluorescence in situ hybridization (FISH)

Counts were done as described by Gonzalez and Bashan (2000) using image analyzer. FISH employed two probes; one to the domain *Bacteria* and one specific for *A. brasilense* using modifications of the technique described by de-Bashan et al. (2010).

RESULTS AND DISCUSSION

When placed in wastewater, populations of *C. sorokiniana* in the beads increased over time. Joint immobilization with *A. brasilense* further increased the population size of the microalgae and mitigated the decline of *A. brasilense* populations over time (Figs. 1 a,b). Contaminants in the beads were low, within the range of 3×10^3 CFU ml^{-1} (after 48 h of incubation) to 8×10^3 CFU ml^{-1} (after 96 h) in jointly immobilized treatments and at a level of 2 to 6×10^3 CFU ml^{-1} when only *A. brasilense* was present.

Evaluation of bacterial population of jointly-immobilized beads, using FISH, revealed that exterior populations was made of unidentified bacteria (red colour in analysis), where the inside population was composed solely of *A. brasilense* (yellow colour in analysis) (Fig. 2).

About 49% to 65% of all phosphorus was removed from the wastewater by immobilized microalgae, and this is comparable to studies employing sterile, synthetic wastewater (de-Bashan et al. 2002, Hernandez et al. 2006) or sterile municipal wastewater (Perez-Garcia et al. 2010).

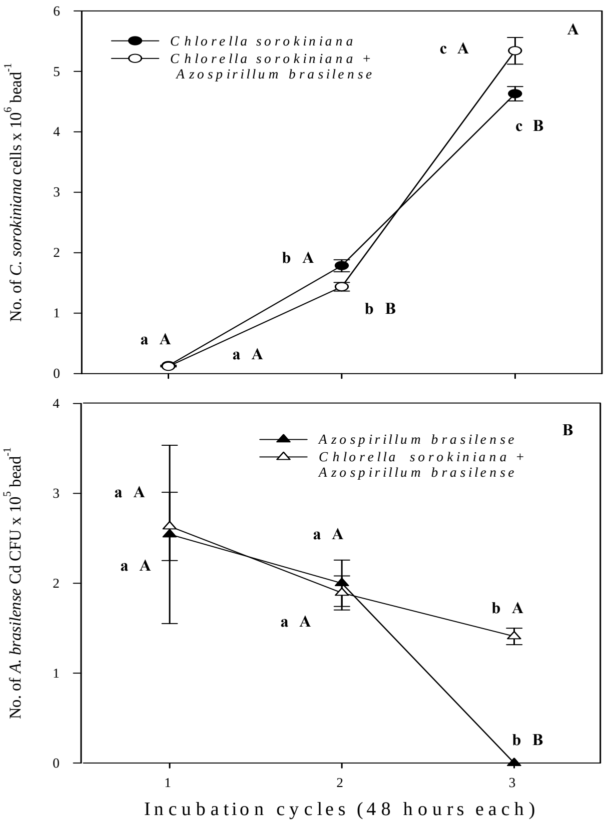

FIG 1.Multiplication of *C. sorokiniana* (A) and *A. brasilense* (B) inside of the alginate beads. Points on each curve denoted by a different lowercase letter differ significantly at $P \leq 0.05$ in one way ANOVA. Points denoted by different capital letters after each day of incubation differ significantly $P \leq 0.05$ at in Student´s *t* test.

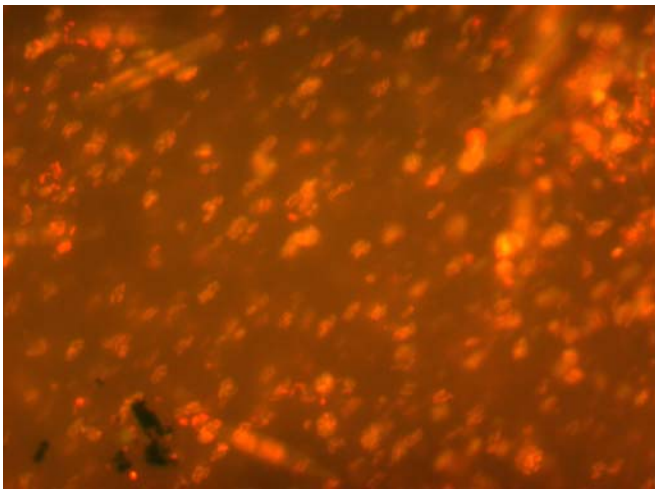

FIG 2. View of the interior of the bead, containing *Azospirillum* by FISH

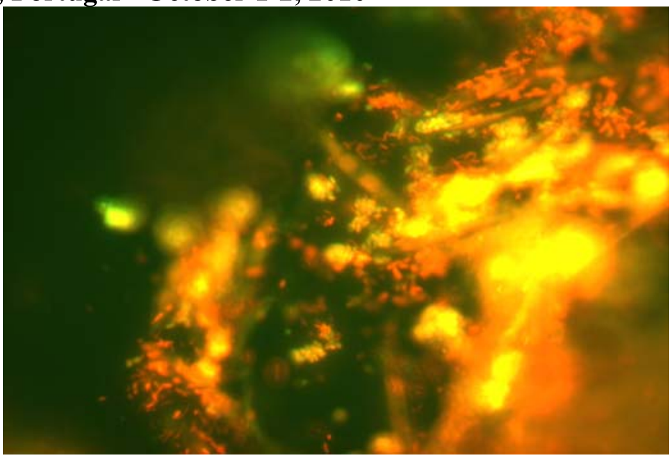

FIG. 3 View of the exterior of the bead. Organism in red are Bacteria, organism in orange are *Azospirillum*

CONCLUSIONS

Immobilization in alginate beads provides a separation barrier between wastewater treating agents and the micro- and macro-organisms inhabiting wastewater, which allows uninterrupted tertiary wastewater treatment.

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