Biological deteriorated alginate beads containing bacteria and microalgae provide a beneficial physical barrier against native organisms during tertiary wastewater treatment.

de-Bashan, L.E., Cruz, I., Covarrubias, S.A., Bashan, Y.
[1]Env. Microbiol. Group, CIBNOR, La Paz, Mexico [2]The Bashan Foundation, Corvallis, Oregon, USA (e-mail: luzb@cals.arizona.edu)

INTRODUCTION

Biological tertiary wastewater treatment to eliminate nitrogen and phosphorus by free microalgae exposes these microorganisms to competition and predation by large populations of microflora but mainly by microfauna residing in the wastewater during the treatment. This effectively reduces their efficiency. Protection against competition is vital. Immobilization of these microorganisms in a polymer matrix, such as alginate beads, provides a physical barrier between the treating agents and the microbiologically-hostile wastewater environment. This will permit the wastewater to reach the agents, yet restricts accessibility for micro-predators. The working hypothesis is that immobilization in alginate protects the internal population of wastewater treating agents from predation by microfauna, by creating a physical barrier, allowing the uninterrupted tertiary wastewater treatment.

The objectives were to: 1. Demonstrate that the beads create a physical barrier where external micro and macro-organisms residing in the wastewater cannot penetrate, 2. Determine the mechanical stability of the alginate beads during wastewater treatment, and 3. Quantify the effectiveness of the immobilized microorganisms for elimination of nitrogen and phosphorus.

MATERIALS AND METHODS

Microorganisms and immobilization in alginate beads.
Unicellular microalgae *Chlorella* spp were 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 microalgae growth-promoting bacterium (MGPB) *Azospirillum brasilense* Cd 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 were grown in the wastewater for 48 h.

Bacteria and microalgae counts and detection by fluorescence in situ hybridization (FISH), and detection of microorganisms in the periphery of the bead
FISH was done following the technique described by de-Bashan et al. (2011). Detection of microorganisms in the periphery of the bead was done by scanning electron microscope (SEM), according to de-Bashan et al. (2011).

Analyses of degradation of beads
Beads were analyzed for textural strength using a texture analyzer (TA.XT plus).

Removal of nutrients (PO_4^{-3} and NH_4^+) from wastewater
Experiments were run in 60 L airlifting, autotrophic triangular bioreactor operating with 25 L of wastewater per run . The experimental conditions were: 10% beads (fresh weight/v), air 30 ml air ml^{-1}, temperature of 28 ± 1°C at light intensity of 90 μmol photons · m^{-2}·s^{-1}.

RESULTS AND DISCUSSION

When the microalgae *Chlorella* spp and the MGPB *A. brasilense* were deployed as free suspensions in unsterile, municipal wastewater for tertiary wastewater treatment their population were significantly lower compared with their populations in sterile wastewater. At the same time, the numbers of natural microfauna and wastewater bacteria increased.

However, when immobilized, the bead layer structure inhibited penetration of outside organisms into the beads. Even though the periphery of the bead is cover by different bacteria from the wastewater (Fig. 1), inside the

bead only the two immobilized microorganisms (*Chlorella* and *Azospirillum*) are detected (Fig. 2).

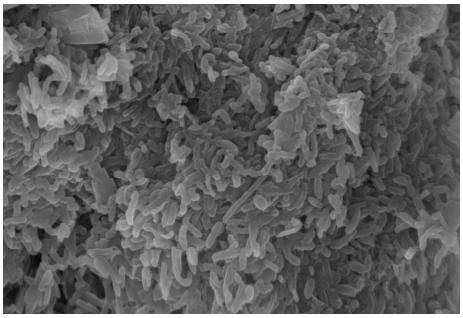

Figure 1. View of the periphery of the bead, covered by microorganisms.

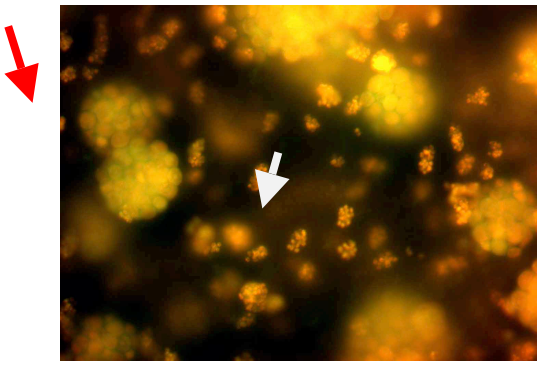

Figure 2. View of the interior of the bead, where only *Chlorella* spp. (red arrow) and *A. brasilense* (white arrow) are present.

After 48 hours of the beads being immerse in the wastewater, its stability declines (Fig. 3), as a result of a dense biofilm composed of wastewater bacteria and *A. brasilense* was created on the surface of the beads.

However, although beads lost their mechanical strength after 48 h of incubation their shape-integrity lasted for at least 96 h. This sustains tertiary successful wastewater treatment within 48 h.

Tertiary wastewater treatment in 25 L triangular, airlift, autotrophic bioreactors showed >90% of PO_4^{-3} and >50% of ammonium were removed. The decline in bead strength phenomena as in small bioreactors. Total bacteria during the wastewater treatment increased only in the presence of the immobilized treatment agents.

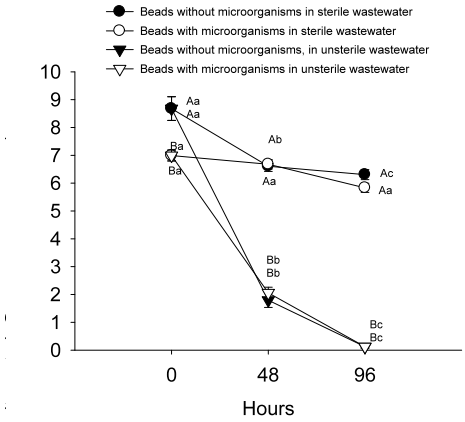

Figure 3. Reduction of gel strength of the bead during incubation of 96 hours in wastewater. Points on each curve denoted by a different letter differ significantly at $P < 0.05$ in one-way ANOVA.

CONCLUSIONS

This study demonstrates that partial biological degradation of alginate beads occurred during tertiary wastewater treatment but the beads survive long enough to permit efficient nutrient removal. The wastewater natural microbial populations are responsible for decreasing populations of microbial agents used for wastewater treatment and immobilization in alginate beads provided a protective environment for these agents to carry out uninterrupted tertiary wastewater treatment.

REFERENCES

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