Atrazine Degradation by *Pseudomonas* Strain ADP Entrapped in Sol-Gel Glass

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Received June 12, 1995; Accepted September 1, 1995

Abstract. Sol-gel entrapment was evaluated as a method for immobilization of an atrazine degrading *Pseudomonas*. It was found that the bacterium lost much of its atrazine degrading activity upon immobilization. However, partial activity could have been restored by amendment of nutrients. Bacteria immobilized using a prehydrolysis technique for the preparation of the sol-gel, retained better activity in comparison to bacteria immobilized using a composite calcium alginate/sol-gel procedure. Further study is underway to improve the activity of sol-gel entrapped bacteria.

Keywords: sol-gel, immobilization, entrapment, bacteria, atrazine, biodegradation, herbicide

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) has been the most widely used herbicide over the last 30 years for nonselective weed control on industrial and noncropped land and for selective weed control in such crops as corn, sorghum, and sugarcane [2]. It is not surprising though that contamination with herbicides at sites subjected to spills, discharge of industrial effluents from herbicide manufacturing plants, leakage from storage, mixing or disposal facilities, etc., caused major soil and ground-water pollution [7]. Since atrazine is only moderately biodegradable it was not possible to economically biodegrade atrazine [3]. However, recently a *Pseudomonas* sp. nominated strain *ADP*, that has the ability to rapidly metabolize atrazine, was isolated from a herbicide spill site [6]. Experiments with free cells, or Ca-alginate encapsulated cells of *P. ADP* indicated that this bacterium has the potential to be used for biodecontamination of water and soils [6, 8].

Using immobilized bacteria under field conditions has the advantages of avoiding random dispersal and providing protection from harsh environmental conditions prevailing in contaminated sites. Sol-gel entrapment has been explored in the last years as a novel immobilization method with significant advantages over conventional immobilization methods [1]. The aim of the present study was to evaluate the possible use of sol-gel glass to entrap live cells of *Pseudomonas ADP* for biodegradation of atrazine in aqueous environments. Only few studies on the entrapment of microbial whole cells using tetramethoxysilane (TMOS) sol-gel process were previously reported [5, 9]. Alkoxysilanes seem to be unsuitable for direct entrapment of live cells [9]. Thus, we have tested a
procedure that combines Ca-alginate encapsulation technique and the sol-gel process according to the method of Heichal et al. (this issue).

A fresh culture of *P. ADP* was grown according to Mandelbaum et al. [6], washed and resuspended in sterile saline. An aqueous (4% w/v) low viscosity Ca-alginate (Sigma chemicals, St. Louis) solution was mixed with equal volume of cell suspension in saline to yield $10^9$ bacteria ml$^{-1}$. The mixture of bacteria in Ca-alginate was added dropwise through a Pasteur pipette into a solution of $0.2M$ CaCl$_2$ to form beads. After a few minutes the beads were collected from the CaCl$_2$ solution, air dried on filter paper and placed in a beaker containing enough hexane to cover the beads. Beads formation was performed under non-sterile conditions. TMOS (Aldrich, Milwaukee) was added in excess to the beads (about 1.5 volumes of the beads). The flask was capped with a glass onion, sealed with parafilm and left for 1 day, at room temperature. Beads (~6 ml) were transferred to 100 ml of 200 ppb atrazine solution and incubated at $30^\circ$C, on a rotary shaker (150 RPM). After 12 h, the remaining atrazine was measured using a gas chromatographic method following the method of Wells et al. [10]. No reduction in atrazine concentration was observed.

In parallel experiments with calcium alginate encapsulated bacteria, over 50% degradation was observed after 12 h, and complete degradation in 24 h. Beads that were further incubated have shown swelling and eventually developed cracks.

In a second experiment a longer aging time (4 days) was allowed. Control consisting of beads that contained saline solution (0.8%) instead of cell suspension was prepared, in order to account for adsorption or for abiotic degradation of atrazine.

The experiment started by addition of the beads to the atrazine solution as before. After 48 h at $30^\circ$C, 150 RPM, only 8% reduction in atrazine concentration as compared to the control beads (not containing *P. ADP*), was observed. Also, significant adsorption of atrazine to the beads was recorded, with over 40% adsorption after 48 h. The absence of live free bacteria was certified by dilution plating on nutrient agar (Difco, Detroit).

It was concluded that a significant decrease in atrazine degrading ability upon entrapment occurred.

Ellerby et al. [4], reported that a major reason for decreased activity of entrapped proteins in conventional sol-gel procedure is the high acidity and high concentration of alcohol leading to the denaturation of proteins. Obviously, these harsh conditions could also reduce viability of bacteria. To avoid direct exposure of the bacteria to low pH conditions occurring during the hydrolysis of the TMOS, an experiment was conducted with bacteria added only after the HCl-catalyzed hydrolysis was buffered to above pH = 5.0. In this experiment we have generally followed the procedure suggested by Livage et al. [5], but the rapid gelation of the TMOS solution upon addition of the buffer was inconvenient. Increasing the HCl concentration retarded gelation and eased handling. Phosphate buffer (PBS) (2.5 ml, X2 concentration) was added to the pre-hydrolyzed TMOS, followed by 2.5 ml of cell suspension ($OD_{600} = 25$). The xerogel was left at 4°C overnight. Then, one-third of its volume was added to 20 ml of 100 ppm atrazine solution in a 100 ml capped glass flask. Radiolabeled atrazine was added to yield 2500 CPM ml$^{-1}$. The xerogel was incubated at $30^\circ$C, 150 RPM on a rotary shaker. At indicated times an aliquot was removed and partitioned with equal volume of ethyl acetate/hexane mixture (1:1 V/V). The undergrade atrazine was partitioned into the organic phase, while polar metabolites remained in the aqueous phase. Radioactivity in both phase was measured. It was found that radioactivity in the aqueous phase increased with time indicating biodegradation into polar metabolites. 45% and 60% of the radioactivity could be recovered in the aqueous phase after 69 h and 137 h respectively.

![Figure 1](image-url)