

IDENTIFICATION OF A SUITABLE SOLID CARRIER AS A MATRIX FOR THE PERMEABLE REACTIVE BARRIER OF LABORATORY SCALE MODEL AQUIFER FOR ATRAZINE DEGRADATION

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ABSTRACT

Three different types of physical substrata such as sand, granular activated carbon (GAC) and plastic macaroni beads were selected to identify a suitable solid carrier as a coarse matrix for the permeable reactive barrier (PRB) in a sand filled two-dimensional laboratory-scale model aquifer. An adhesion experiment was performed and tested with *Pseudomonas* sp ADP (PADP) under agitated as well as static conditions. In static conditions, adhesion to GAC was the highest (80%) followed by that to beads (60%). No adhesion to sand was observed under static or agitated conditions. Since atrazine is adsorbed by GAC, its bioavailability for degradation by PADP may be affected. To test this, simultaneous loss of atrazine and release of chloride (dechlorination, the first step in the pathway of atrazine degradation by PADP) were determined in the presence and absence of GAC. Although cells remained viable, no significant chloride release was detected. Thus, plastic macaroni beads were identified and selected as the most suitable support for PADP in the biological permeable reactive barrier (BPRB) of the model aquifer.

INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) (Figure 1), one of the most commonly detected organic contaminants in groundwater, has been found more resistant to biodegradation in nature than other s-triazine ring compounds (Masaphy & Mandelbaum, 1997). When conditions in soils change from aerobic to anoxic, the rate of atrazine degradation slows to a large extent. For this reason, groundwater and poorly aerated surface waters have higher atrazine concentrations than well-aerated water (Ribaudó & Bouzaher, 1994).

Mandelbaum *et al.* (1995) isolated the first atrazine-degrading bacterium, *Pseudomonas* sp. strain ADP that is able to completely mineralize the compound while using it as its sole nitrogen source by metabolizing into ammonia, carbon dioxide and chloride (Seffernick *et al.*, 2000). The three main steps involved in the atrazine metabolic pathway are dechlorination, *N*-dealkylation and ring cleavage (Mandelbaum *et al.*, 1995).

In-situ groundwater bioremediation is a technology that stimulates growth and reproduction of indigenous microorganisms to enhance biodegradation of organic as well as inorganic constituents in the saturated zone. It can effectively degrade constituents, which are dissolved in groundwater and/or adsorbed onto the aquifer matrix (USEPA, 2002). Degradation alters the molecular structure of the organic compounds and either simplifies the compounds into daughter products or completely breaks down the organic compounds into cellular mass, carbon dioxide, water and inert inorganic residues. For *in-situ* groundwater bioremediation, the groundwater system must be reasonably well characterized in terms of direction of flow, width and depth of plume, concentrations of contaminants along the plume, flow velocity and hydraulic conductivity (Strietelmeier *et al.*, 2001). Although various types of laboratory scale fixed-film bioreactors have been studied for the remediation of atrazine from contaminated groundwater both under aerobic and anoxic conditions, *in-situ* biodegradation treatment of atrazine-contaminated groundwater by using biological permeable reactive barrier are not known. Therefore, the removal of organic pollutants from groundwater using permeable reactive barriers (PRB) is a novel technique for groundwater remediation (Meggyes & Simon, 2000). A biological permeable reactive barrier (BPRB) is an underground permeable wall with reactive materials and microorganisms that degrade or immobilize contaminants during groundwater flow (Scherer *et al.*, 2000). It is an innovative combination of two cost-effective *in-situ* remediation technologies: *in-situ* bioremediation and permeable barrier walls (Shirazi & Rodriguez, 2000). It is largely applicable to shallow, alluvial plumes (less than 20 feet deep), although PRBs have been placed at much greater depths, up to 70 feet deep (Strietelmeier *et al.*, 2001).

To date, no one has used PRB for the degradation of atrazine in atrazine-contaminated groundwater. Therefore, this research was designed to study degradation of atrazine in groundwater by using a laboratory scale model aquifer and also the identification of a suitable solid carrier to be used as a matrix for the PRB was made from among the three different physical substrata such as sand, plastic macaroni beads and granular activated carbon (GAC).

MATERIALS AND METHODS

ORGANISM:

Pseudomonas sp. strain ADP (PADP) was obtained as a gift from R. T. Mandelbaum. Bacterial cultures were incubated aerobically in an incubator shaker (150 rpm), at a temperature of 25 °C, in a mineral medium containing citrate (2 g/l) as a carbon source and atrazine (35 mg/l) as the sole nitrogen source in order to determine the growth characteristics of the bacterium, its atrazine degradation capacity, and its adhesion to sand, beads and GAC, respectively.

SOLID CARRIERS FOR THE BPRB

Plastic "macaroni" beads

The plastic "macaroni" beads used in the experiment (Aridal Plast Ltd, Kfar Hasidim, Israel) (Figure 2) were made of high-density polyethylene, had an average diameter of 1 cm and a specific weight of about 160 kg/m³. They were hollow, had a surface area of approximately 860 m²/m³ and a void space of more than 80% of the total volume.

Granular activated charcoal (GAC)

The granular activated charcoal used was extra pure food grade (Merck, Germany), with a grain size of less than 1.5 mm and a density of approximately 2 g/cm³; prior to use, the granules were sieved through a 1190 μ mesh size to obtain a more uniform size distribution.

Sand

Three types of quarry quartz particles were mixed to achieve a matrix with granular size distribution and hydrological properties similar to those of the Israeli coastal aquifer (Table 1). The quartz particles (99.9% pure) were purchased from Negev Mineralim Industries Ltd (Omer, Israel).

EXPERIMENTS INVOLVING ADHESION OF PADP TO SAND, PLASTIC BEADS AND GAC

The use of PADP in a permeable reactive barrier requires the selection of an appropriate physical substratum (carrier) for the bacterium. For this reason, preliminary studies were carried out on the attachment of the cells onto sand, beads and GAC. Cellular adhesion experiments were carried out at 25 °C, in 250 ml Erlenmeyer flasks containing 100 ml mineral medium with citrate and atrazine added in the given amount as mentioned above, 1.5 ml bacterial suspension with 4.53 x 10⁶ CFU/ml and 10 g of sand, beads or GAC. All treatments were carried out in triplicate. The experiments lasted 48 h under static conditions or agitation in a rotary shaker at 140 rpm.

The number of bacteria adhered to each substratum was determined by subtracting the number of cells in the final suspension (CFU) from the number of cells in the initial suspension.

$$\% \text{ adhered cells} = (T_0 - T) - (C_0 - C) / T_0 * 100$$

where:

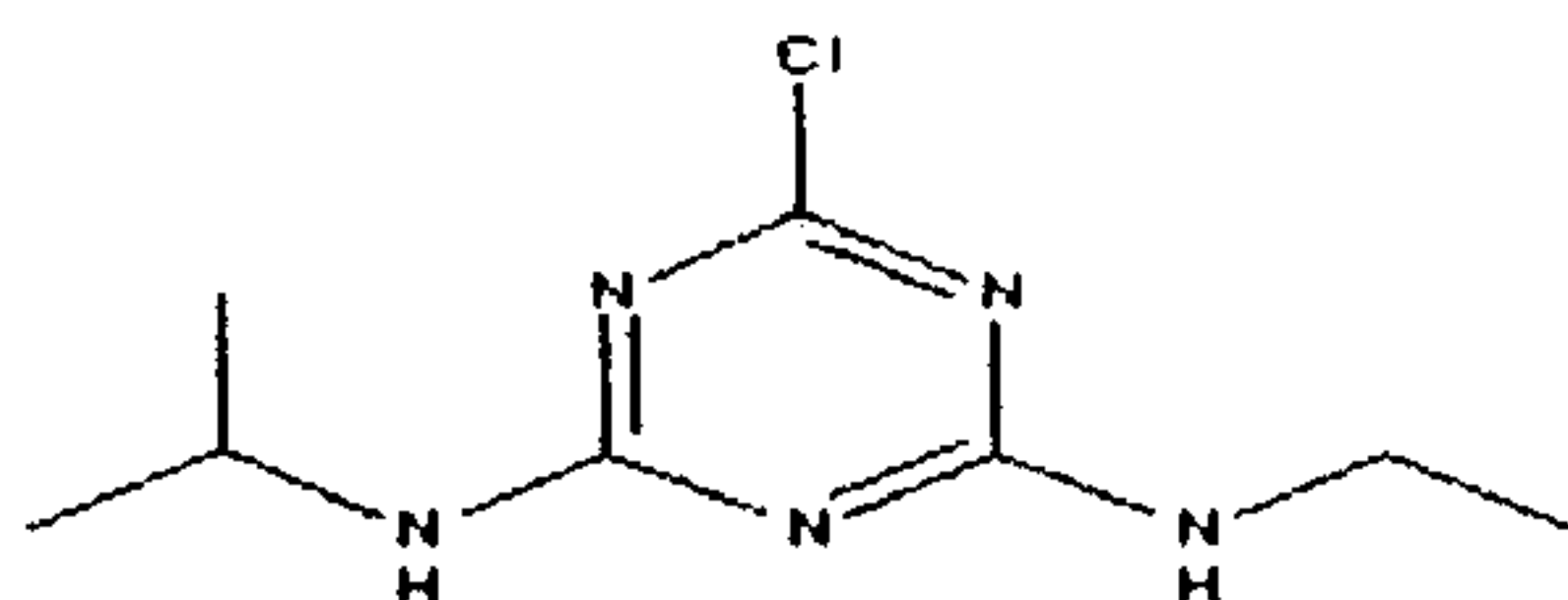
T₀ = number of cells in suspension at time 0,

T = number of cells in suspension at the end of the experiment

Controls consisted of bacterial suspensions without a carrier and were counted to correct for any loss of cells other than by adhesion.

DETERMINATION OF RELEASED CHLORIDE

A preliminary experiment was performed to determine whether chloride was sorbed by GAC. One gram of GAC (previously washed in distilled water until no trace of chloride could be detected) was added to a conical centrifuge tube containing 10 ml of a standard solution of 10 mg Cl⁻ as NaCl /l in distilled water. Tests were run in five replicates and with two different controls: chloride solution without GAC and GAC with distilled water. The tubes were maintained in a shaker (150 rpm at 25 °C) for 48 h. At 0, 24 and 48 h tubes were centrifuged for 10 min at 3000 rpm in a Sorval TC 6 at 25 °C, and 0.5 ml aliquots of the supernatant were analyzed for chloride after dilution with 0.5 ml distilled water.



2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine

Fig. 1: Chemical structure of atrazine

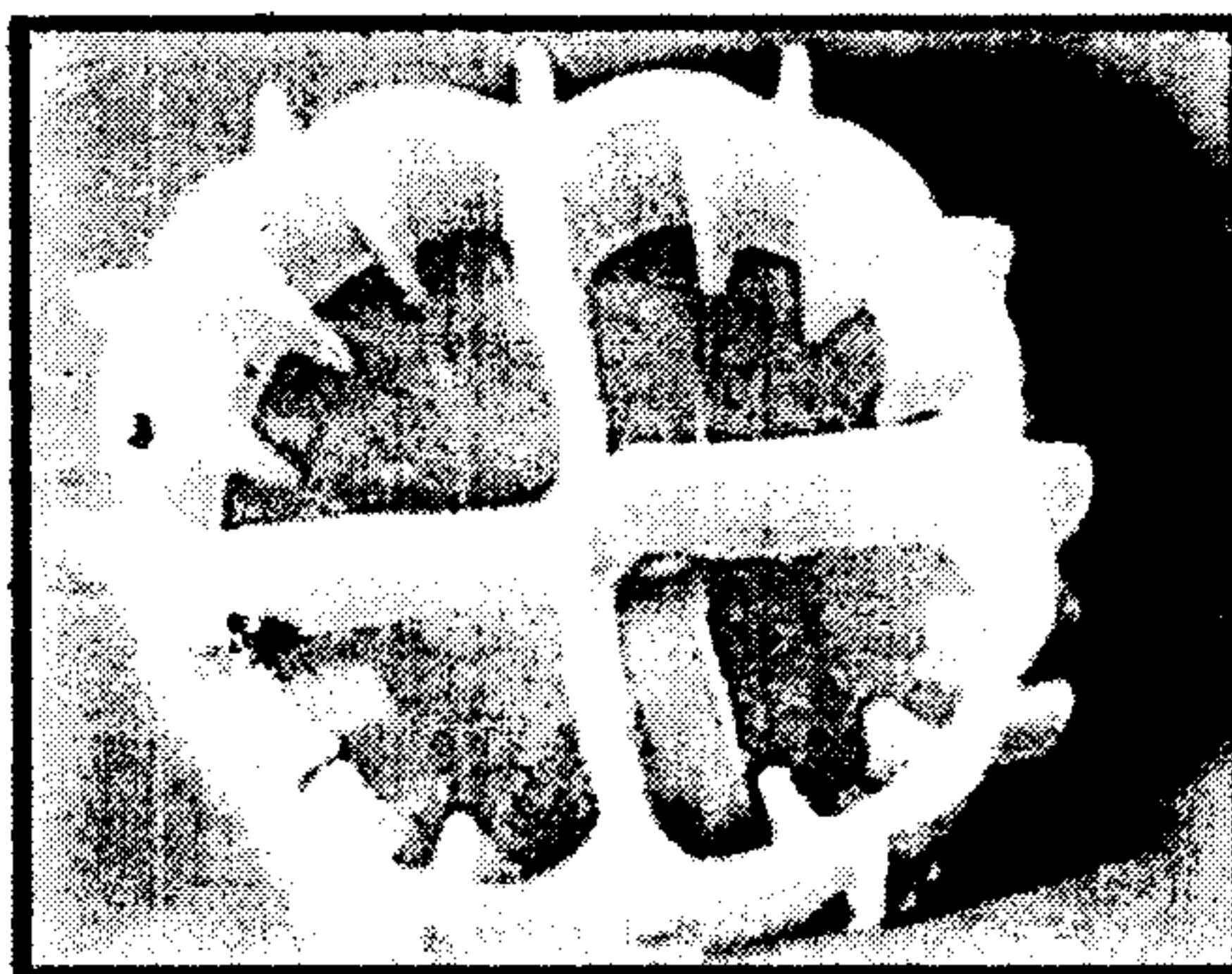


Fig. 2: Plastic "macaroni" bead (average diameter, 1 cm)

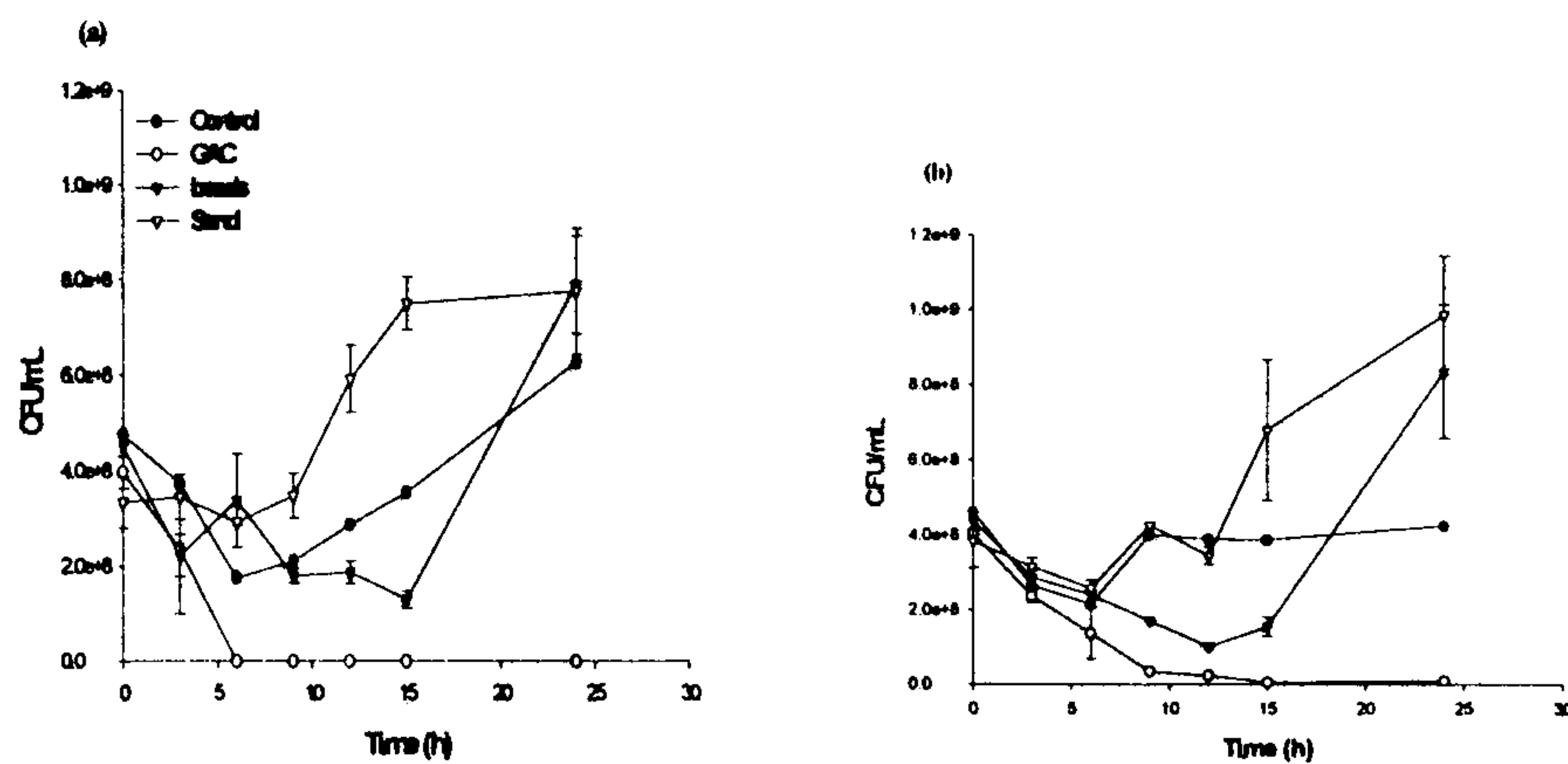


Fig. 3: Adhesion of PADP cells to sand, plastic beads and GAC under agitation (rotary shaker at 150 rpm) (a) and static conditions (b). Results represent cells remaining in suspension.

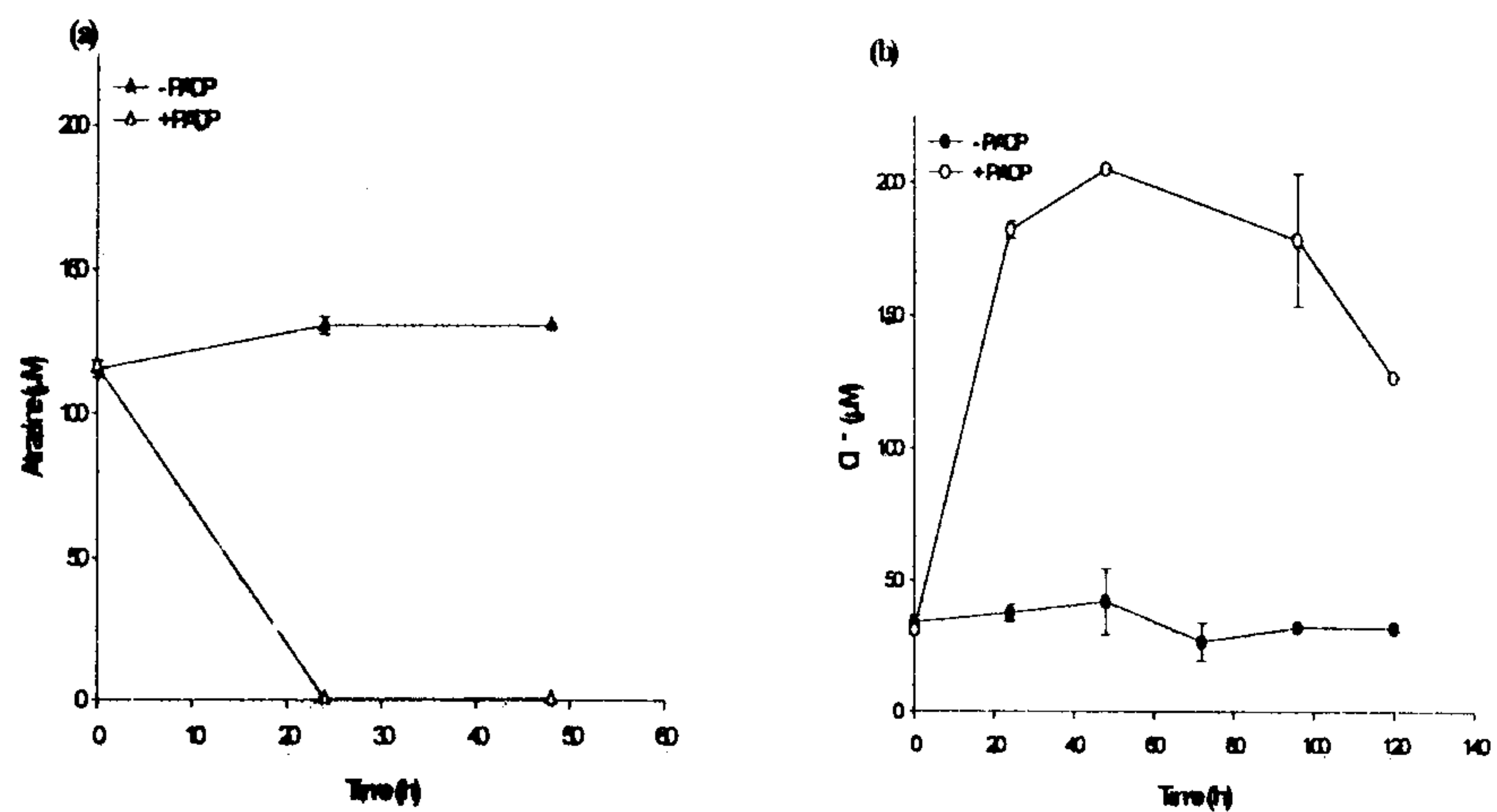


Fig. 4: Atrazine loss (a) and chloride release (b) in the absence of GAC.

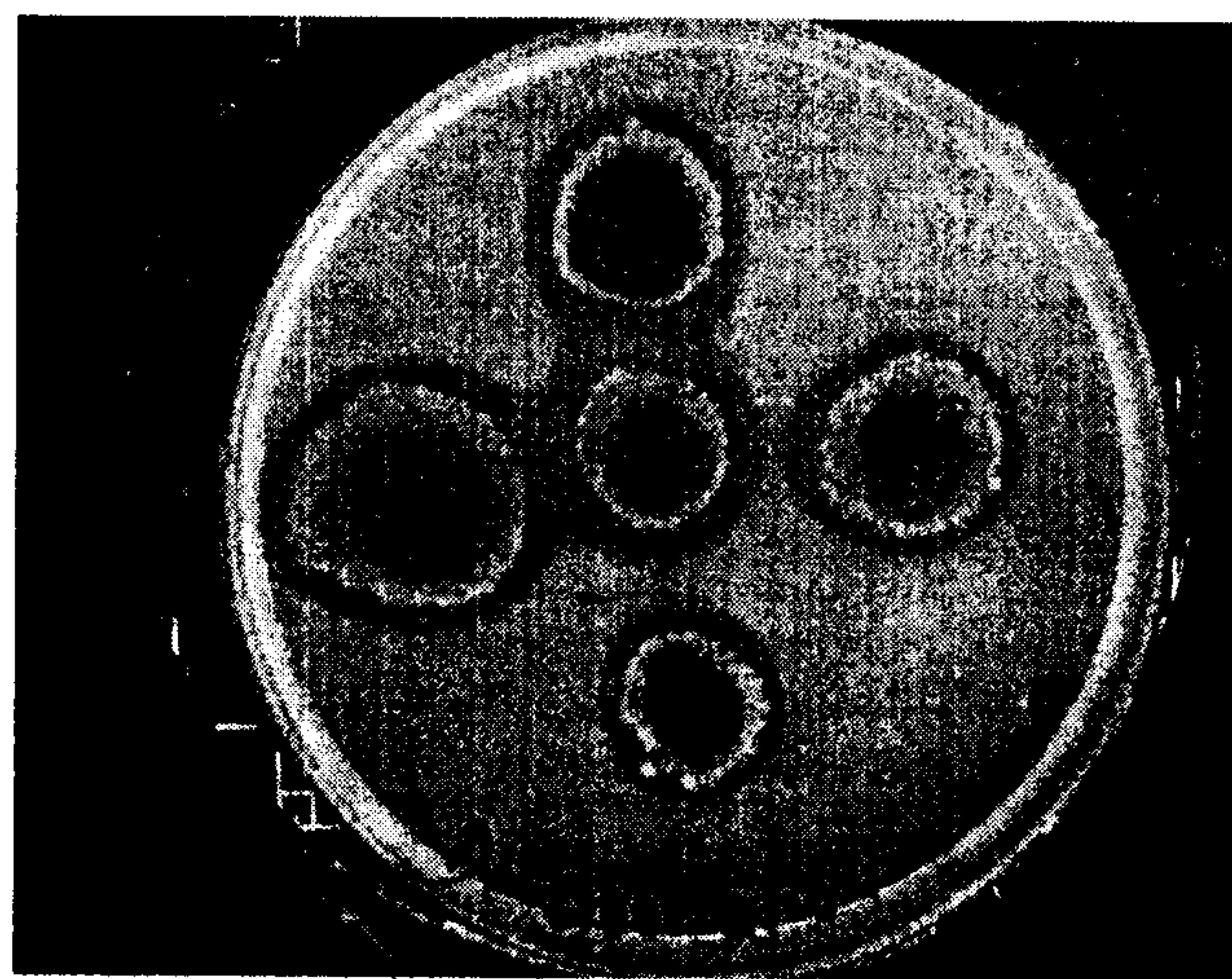


Fig. 5: Solidified atrazine (500 mg/l) plates showing clearing zones of atrazine degradation by PADP on GAC particles.

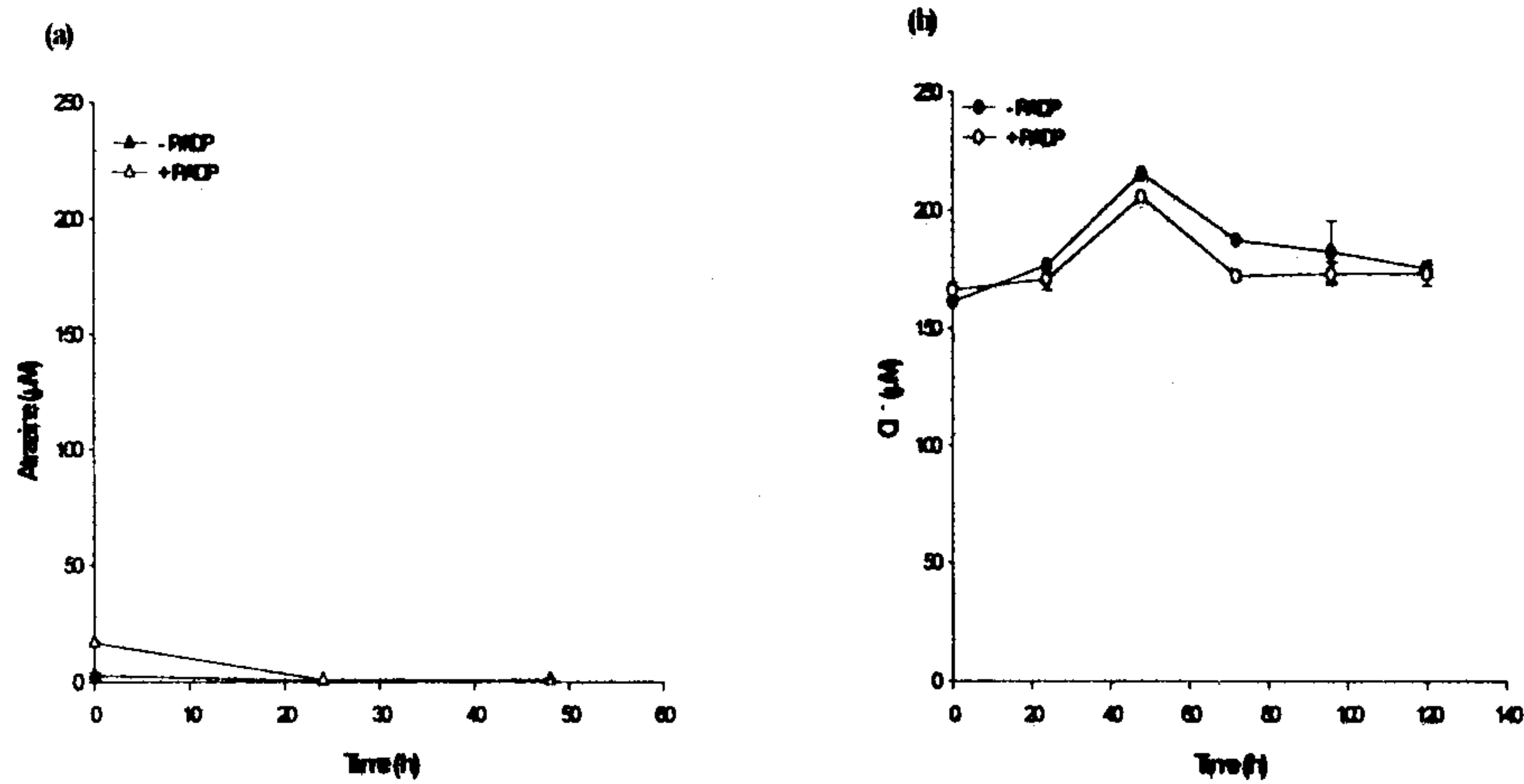


Fig. 6: Atrazine loss (a) and chloride release (b) in the presence of GAC

Table 1: Size distribution of sand mixture and mean grain size of the Israeli coastal plain aquifer.

Grain size (µm)	Sand mixture		Israeli coastal plain aquifer Mean grain size distribution
0-74	19.8%	} 67.16 µm	68 µm
74-125	6.4%		
125-150	27.4%		
150-250	13.6%		
250-300	0.7%	} 32.76 µm	32 µm
300-400	9.6%		
400-600	2.5%		

Table 2: Sorption of chloride to GAC over 48 hours. Results are averages and standard errors of 5 replicates.

Time (h)	GAC with standard chloride solution	GAC without Standard chloride solution	GAC without chloride addition
0	14.13 (2.17)	10.1 (0.25)	3.15 (0.09)
24	9.57 (0.34)	10.8 (0.35)	3.31 (0.07)
48	12.43 (2.31)	11.79 (1.36)	3.18 (0.06)

Chloride release from atrazine degradation was determined in 250 ml Erlenmeyer flasks containing 100 ml medium as mentioned above modified by substituting NaCl and CaCl₂ with Na₂SO₄ (0.124 g/l as Na) and Ca SO₄ (0.031 g/l as Ca), and 1 g of washed GAC. An inoculum of 1 ml of a suspension with 6 x 10¹⁰ CFU/ml was added and the cultures were transferred to a rotary shaker (120 rpm), at 30 °C; 4 h later, atrazine (at the usual final concentration of 35 mg/l) was added. Media samples for chloride and atrazine analyses were taken at 0, 24, 48, 72, 96, 120 and 168 h. Treatments were carried out in duplicate batches and with controls without GAC.

ANALYTICAL AND MICROBIAL METHODS

Atrazine determination

Concentrations of atrazine were determined in samples diluted 1:1 in methanol. The samples were centrifuged at 13,000 rpm for 10 min in a micro-centrifuge 5415 D (Eppendorf, Germany), prior to the addition of methanol, and analyzed by high performance liquid chromatography. Separation was carried out on a reverse phase 25-cm long Supelcosil LC 18 column (Supelco, Bellefonte PA, USA). The mobile phase was at a flow rate of 1.5 ml/min and consisted of 50% (v/v) acetonitrile. Atrazine was detected at 225 nm by a Kontron 440 diode array detector (Kontron Instruments, Milan, Italy) and its concentration was calculated using the external standard method.

Chloride determination

Chloride was determined Colorimetrically (Shochat *et al.*, 1993) in the supernatant of culture aliquots centrifuged at 13,000 rpm for 10 min.

Bacterial counts

Bacterial numbers were estimated by the number of CFU on plates of solid atrazine medium, prepared by adding 15 g/l agar (Difco) to ALM; plates were inoculated in triplicate and incubated at 30° C.

RESULTS AND DISCUSSION

ADHESION OF PADP TO SAND, PLASTIC BEADS AND GAC

Under agitation, adhesion was approximately 14% for GAC, 44% for beads, and 0% for sand. Under static conditions however, 80% of the cells adhered to GAC, 60% cells to beads and 0% to sand. With GAC, cells were not detected in suspension after 6 h under agitation whereas under static conditions, the lowest number of cells was observed in suspension at 15 h. Under static conditions, adhesion of cells to beads was faster than adhesion to GAC, although, as mentioned above, a higher percentage of cells adhered to GAC (Figure 3). The results showing a higher percentage of cells adhered to GAC are in agreement with Selim and Wang (1994) and Herzberg *et al.* (2003). A possible reason for the higher adhesion of PADP cells to GAC is its porous nature, which provides a large surface area for cell adhesion. No cells were detected in the suspension with GAC after 6 h of incubation, whereas increased cell numbers were detected with plastic beads and sand (Figures 3a and b). This increase in the number of cells in suspension was due to further growth of PADP.

When considering a solid carrier for a reactive barrier, its effect on contaminant bioavailability must be taken into account. Since higher adhesion of PADP was observed on GAC and also atrazine adsorption by GAC was reported by Selim & Wang (1994) and Herzberg *et al.* (2003), it was assumed that bioavailability of atrazine for degradation in GAC by PADP would be affected. Thus, dechlorination test of sorbed atrazine in GAC by PADP was performed (Figure 6 and Table 2) before decided to use GAC as a suitable matrix to PRB.

DECHLORINATION OF SORBED ATRAZINE IN GAC

Since, dechlorination is the first step of atrazine degradation by PADP (Mandelbaum *et al.*, 1995), accumulation of chloride was taken as an indicator of atrazine degradation. To determine whether released chloride is sorbed or not by GAC a preliminary test was performed with standard chloride solution and with or without GAC. The result showed that after 24 h of shaking, about 30% of the initial chloride had disappeared from the solution, indicating sorption of chloride to GAC (Table 2). This finding is supported from the results obtained by Yanze-Kontchou and Gschwind, (1994); and Abdelhafid *et al.*, (2000). However, after 48 h the concentration of chloride was not significantly different from the concentration at the beginning of the experiment. This confirmed that released chloride after degradation of atrazine should not be adsorbed by GAC (as can be deduced from Table 2, chloride was not significantly adsorbed by GAC). Thus, chloride release and atrazine disappearance were monitored in the presence and absence of GAC (Figures 4a, b and 6a, b), in cultures in low chloride medium (34.14 μM) containing 115.41 μM atrazine. In the absence of GAC, atrazine disappeared within 24 h, with a chloride accumulation of 182.2 μM , corresponding to 158% of the expected value (Figure 4), this discrepancy was attributed to experimental error.

The same experiment was then carried out in the presence of GAC. In order to achieve the best adhesion of PADP, cells were first inoculated in the medium as mentioned above without atrazine. Atrazine was added only after 4 h, when maximal adhesion was expected (Figure 3a); cultures were then incubated for 6 days. The results in Figure 5 show that cells adhered to GAC remained viable, and that colonies emerging from GAC caused clearing zones on solidified atrazine plates. In the presence of GAC, atrazine concentrations in solution were very low, although the amount added was identical to that in the experiment without GAC (Figure 6a). No significant chloride release was observed, with practically identical concentrations of chloride being detected in the presence and in the absence of PADP (Figure 6b).

The results presented in Figure 6b show that the concentration of chloride in the control treatment (without PADP) remained constant from the beginning of the experiment. It was expected that the amount of chloride released from the degradation of the added atrazine would be 161.43 μM ; however, no significant change in chloride concentration was observed throughout the experiment. This suggests that atrazine was not available to the PADP cells adhered to GAC. The concentration of atrazine present in the solution in the

presence of GAC was very low from the beginning of the experiment, due to the rapid adsorption of atrazine (Figure 6a).

The above results contrast with reports by various authors. In a comparative study between a non-adsorbing granular carbon carrier and GAC, Herzberg *et al.* (2003) found higher biodegradation of atrazine by PADP cells on GAC. Selim and Wang (1994) also reported 98.10 to 98.67% of atrazine disappearance in their GAC-packed reactors compared to 42.10% in sand reactors, and Feakin *et al.* (1995) obtained reduced concentrations of atrazine by inoculating GAC with *Rhodococcus rhodochrous*.

CONCLUSIONS

In the face of the results obtained in the experiment on atrazine dechlorination by cells adsorbed to GAC, and considering the relatively high adhesion of PADP to the plastic beads, these were identified and selected as the matrix for the reactive barrier. Besides their low cost, the plastic beads presented a number of characteristics that were expected to enable free water flow through the BPRB and prevent "clogging" by bacteria and organic residues, thus ensuring direct water flow and minimizing water "channeling", which would decrease treatment efficiency. These characteristics are: high surface area (approximately $860 \text{ m}^2/\text{m}^3$) and a void space of more than 80% of the total volume. Furthermore, the plastic beads should not reduce the bioavailability of atrazine; they are a medium especially designed for use in various mechanical and biological filters for the removal of nitrogen and organic carbon in wastewater treatment processes and in the aquaculture industry. The presence of void spaces increases the biofilm surface area and the efficiency with which nutrients and gases are transferred between the biofilm and the surrounding water (Maier *et al.*, 2000). Hence, plastic beads appeared to form as an adequate matrix for a BPRB with PADP for the degradation of atrazine of a simulated model aquifer.

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