Tailored carrier/bacteria technology for rehabilitation of areas with pesticide-containing pollution – AQUAREHAB WP2

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INTRODUCTION

The overall objective of this part of AQUAREHAB was to develop a semi-passive rehabilitation technology to cope with aqueous pesticide pollution. The basic idea of the rehabilitation technology is to treat the pollutants in open-air trenches that drain contaminated groundwater from the riparian zone and that contain tailored materials as support for microbial biofilms that degrade the pesticides in the contaminated drainage water (Figure 1).

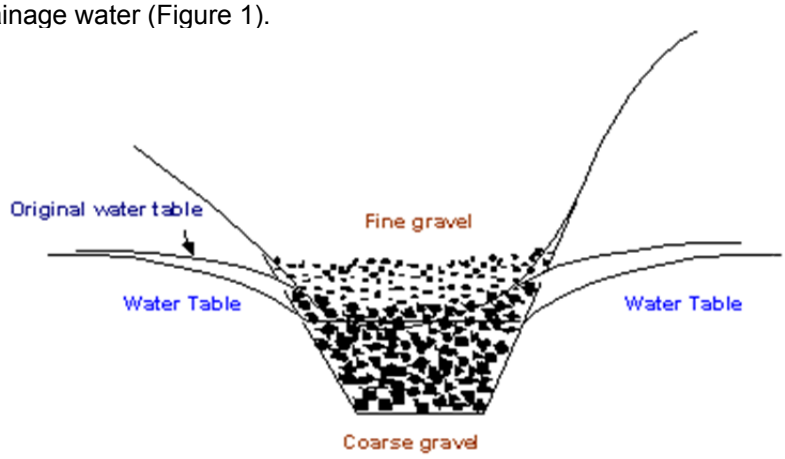

Figure 1: Cross-section of a drain with coarse carrier material, on which pollutant-degrading bacteria can form a biofilm that will degrade pollutants (pesticides) in the passing groundwater.

The research was carried out in three stages: (1) selecting the bacteria-carrier combination, (2) running lab-scale column experiments and (3) performing field-scale experiments.

SELECTION OF BACTERIA-CARRIER COMBINATIONS

We tested different bacterial strains for their interactions with both artificial and natural carrier material. Among the strains tested were the *Pseudomonas* sp. strain ADP that degrades atrazine, *Variovorax* sp. SRS16 that degrades linuron, *Chelatobacter heintzii* SR38 that degrades atrazine, *Aminobacter* sp. MSH1 that degrades BAM, *Sphingomonas* sp. KN65.2 that degrades carbofuran and *Rhodococcus* sp. KS1 that degrades metamitron. An atrazine-enrichment culture and carbofuran-degrading consortia were also isolated from the field test site in Israel and evaluated. Carrier materials tested were: white chalk (WC), gray chalk (GR), gravel (GR), sand (SA), activated carbon (AC), biosep beads (synthetic), XAD-7HP (synthetic), XAD-2 (synthetic), IRC-50 (synthetic), and synthetic material based on calcite and activated carbon. All of the tested material enabled the formation of a microbial biofilm, regardless of the specific surface area or hydrophobicity of the carriers. White chalk (WC), gray chalk (GR), gravel (GR), sand (SA), and activated carbon (AC), immersed in the contaminated groundwater, each attracted different native microorganisms forming

significant biofilms. The composition of the attached populations on these materials appeared to be related to the carrier properties (mineral vs. organic, for example; Figure 2).

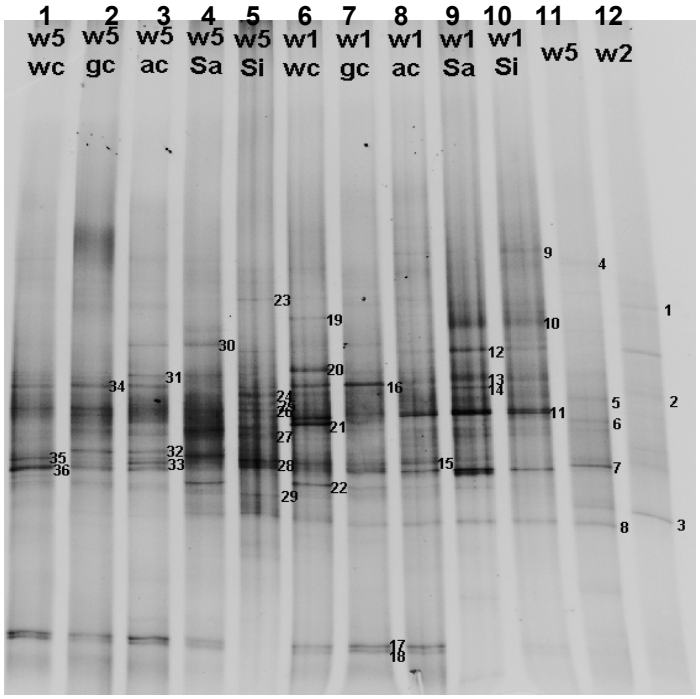

Figure 2: Photograph of the DGGE gel of 16SrRNA gene fragments extracted from the different incubated carriers' bags and the GR extracted from the site drainage channels. First row: lane number; second row: drain location; and third row: description of the carriers. Lanes 11 and 12 are of GR excavated from different locations along the drainage channels.

Although the attached biomass was active in mineralizing simple compounds, such as benzoic acid, the activity of these bacteria towards the different pesticides was very slow, with the exception of bacteria grown on sand and atrazine (Figure 3).

The biodegradation of the different pesticides by carrier bacteria combinations was extremely dependent on the strength at which the organic pollutants were sorbed onto the carrier. For example, the mineralization of ^{14}C-BAM by *Aminobacter* sp. MSH1, in the presence of different carriers, showed that it was able to mineralize 50% of the added compound in the presence of the XAD 7HP carrier and was not able to mineralize the compound when activated carbon was used as a carrier. These observations suggest that the dynamic of sorption/desorption from the carrier is the most important factor allowing degradation (Figure 4). Promising bacteria-carrier combination that were identified comprise gravel and the resin XAD-7HP.

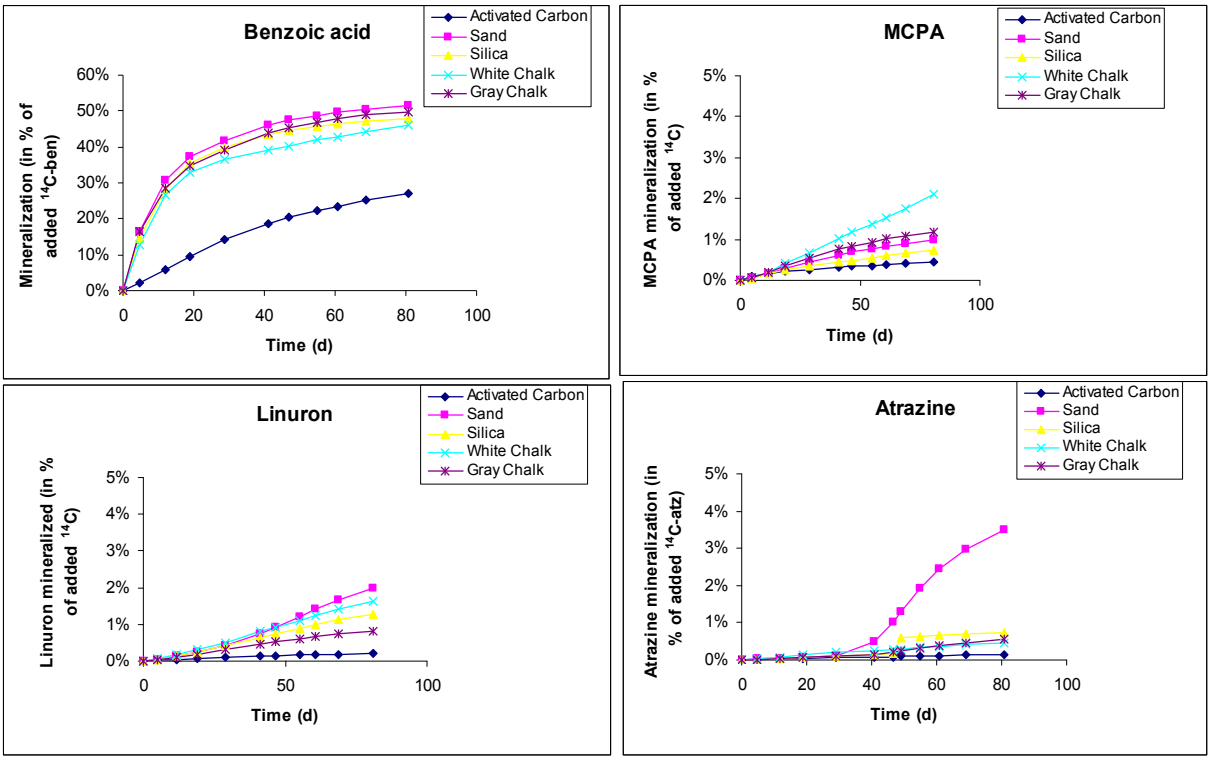

Figure 3: Mineralization of benzoic acid and selected pesticides by carrier incubated within the drained water in situ.

COLUMN EXPERIMENTS

Sand column experiments were set-up with different carrier materials (Figure 4). The results suggested that if organisms are very robust concerning carbon source concentration changes, all columns perform well and XAD-7HP is not necessarily needed (e.g., in the case of linuron degradation by *Variovorax* SRS16). However, the presence of the resin has a positive effect in cases where the strains are less robust. In these cases, the resin either acts as a buffer to absorb pesticides, when the organisms are not yet reactivated, or provides a continuous flow of pesticides during the period of pesticide absence in the inflow medium, thus maintaining the degradation activity. The latter effect was observed with the strains *Aminobacter* MSH1 and *Sphingomonas* KN65.2 after the medium had been left without the respective pesticide for a longer period of time. Switching back to the medium with the pesticide revealed that the columns with a XAD-7HP / sand ratio of 1:500 showed the most stable pesticide-degrading performance (Figure 5). The column experiment with a gravel carrier and natural bacteria from the test site with artificial groundwater, amended with a mixture of pesticides and background compounds, suggested that the site's natural microbial population is not able to degrade the target pesticides but is able to degrade background contaminants.

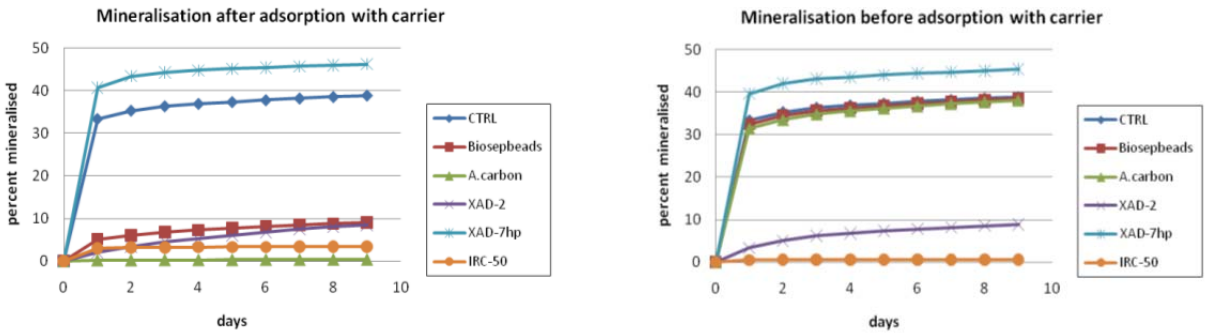

Figure 4: Cumulative mineralization of 14C-BAM by Aminobacter sp. MSH1 in the presence of different carriers. MSH1 was added either together with BAM (left-hand side) or after BAM had been sorbed to the carriers (right-hand side).

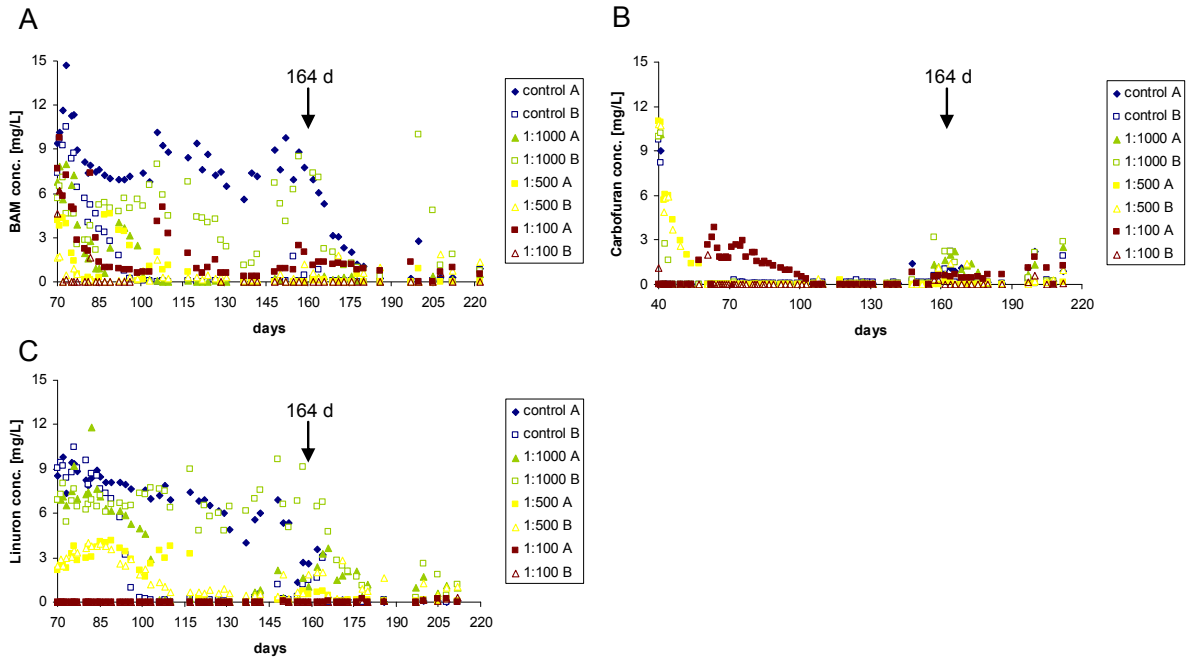

Figure 5: Effluent concentrations over time of (A) BAM, (B) carbofuran and (C) linuron in columns filled with sand (blue symbols, control) or Amberlite XAD-7HP and sand in a w/w ratio of 1:1000 (green symbols), 1:500 (yellow symbols) and 1:100 (brown symbols). Sphingomonas KN65.2 was injected on day 40, Aminobacter MSH1 on day 61 and Variovorax SRS16 on day 71. From day 164 on, only BAM and carbofuran were added with the influent medium (indicated by arrow). Figures show the two biological replicates.

This led to the enrichment of atrazine and carbofuran-degrading consortia from the site water. Additionally, the ability of pure cultures to degrade pesticides at the site water high salinities and in the filtered site water was evaluated; carbofuran degradation by indigenous bacteria was efficient in the presence of the gravel as a carrier. Atrazine mineralization, however, was dependent on the availability of sand as a carrier and an additional carbon source. From the tested pure cultures, *Sphingomonas* sp. KN65.2 that degraded carbofuran was active at high salinities, as well as in real groundwater (Figure 6). Importantly, we noted that abiotic reactions also took place. The *Pseudomonas* sp. strain ADP that degraded atrazine was adapted only for high salinities in a defined medium.

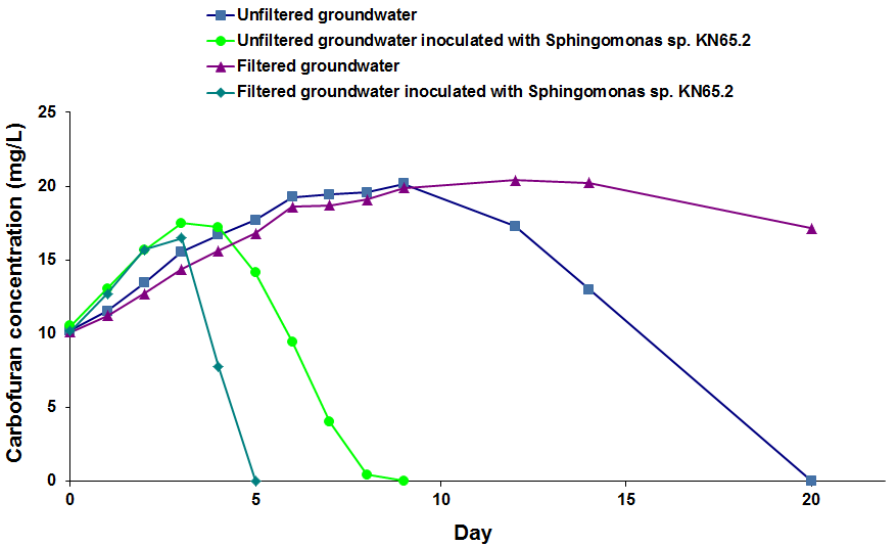

Figure 6: Biodegradation of carbofuran by Sphingomonas sp. KN65.2 with gravel as a carrier and with water from the site. The initial increase in concentrations is due to our inability to separate between carbofuran and degradation products.

PILOT TEST IN THE FIELD

A pilot field system that simulates flow conditions within the drainage channels was built in order to evaluate the introduction of bacteria/carrier combinations. The pilot system contains three columns filled with clean gravel, as well as with contaminated gravel from different sections of the site's drainage system (Figure 7).

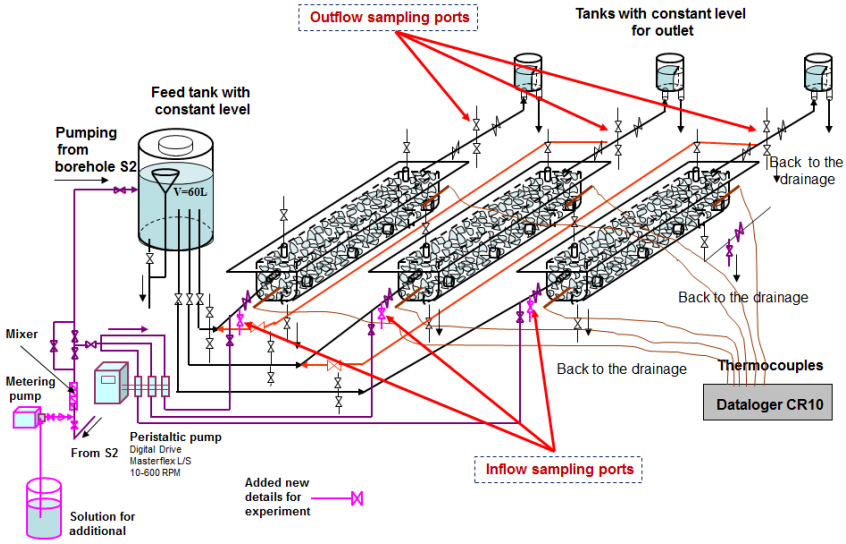

Figure 7: Scheme of the pilot system with the addition of the peristaltic pump and carbofuran container.

In the field experiments, with gravel-packed columns, the carbofuran degradation by the native bacteria was affected by environmental factors, such as temperature and dissolved oxygen. In the pilot system, the influent DO was initially relatively low (~0.8 mg/L) and decreased in the effluents to less than 0. 5 mg/L. Temperature was as high as 37 C° in the summer and decreased to below 10 C° in the winter. Initially, some degradation of carbofuran was observed in the system, but the levels diminished with time (Figure 8).

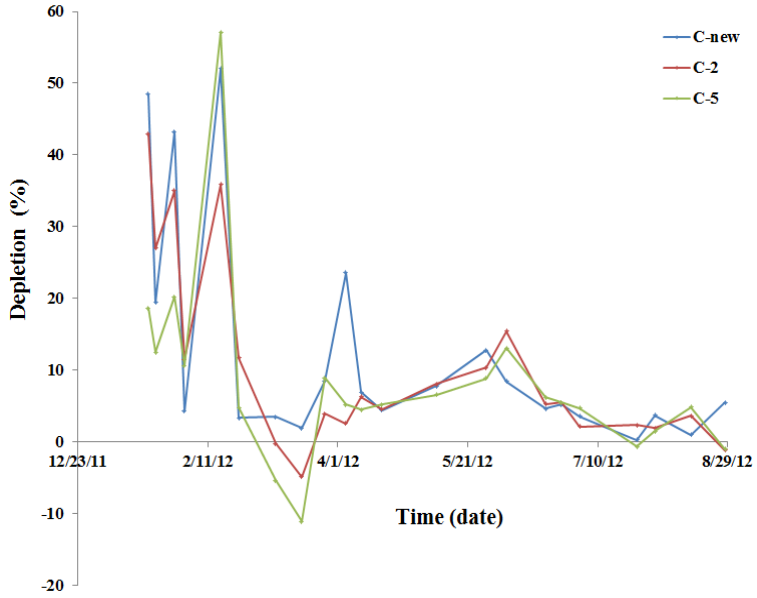

Figure 8: Carbofuran depletion percentage in the non-inoculated columns over time (from December 2011 until September 2012).

Introducing *Sphingomonas* sp. KN65.2 to the column resulted in a transient improvement in degradation (Figure 9). Attempts to oxygenate the water passing through the column failed because of clogging due to iron minerals within the contaminated water.

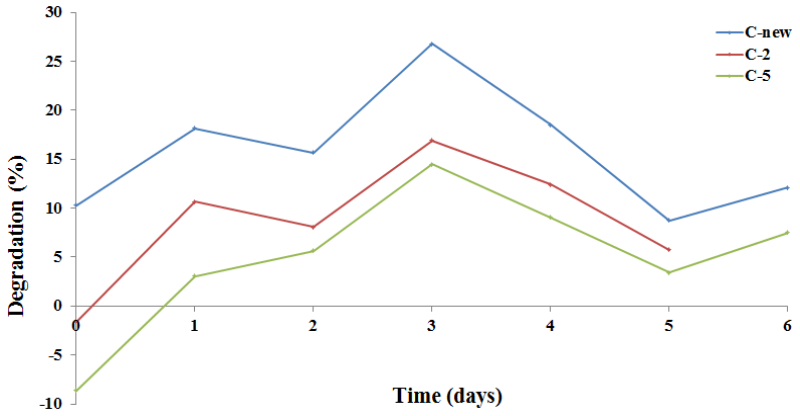

Figure 9: Carbofuran depletion (%) after inoculation with Sphingomonas sp. KN65.2

CONCLUSION

In conclusion, the carrier / bacteria technology requires that the carrier will not strongly adsorb the target pollutant, that the introduced bacteria will be active in the site water and that it will be possible to control the environmental conditions at the site to accommodate the physiological characteristics of the introduced bacteria.