IN SITU ANAEROBIC MICROBIOLOGICAL TREATMENT OF A CHLOROBENZENE CONTAMINATED GROUNDWATER

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Abstract

A one-year site investigation was conducted in a contaminated area near a pharmaceutical industry. The main contaminants were chlorobenzene and benzene. Over time, concentration of the contaminants decreased below regulatory level under anaerobic conditions by biostimulation. To confirm the transformation processes and reveal the mechanisms, a corresponding laboratory microcosm study was set up which completed demonstrating the microbial degradation of these two main pollutants. Assumptions about the transformation processes inferred from field data were confirmed by microcosm studies, consequently necessary supplementary information were provided for bioremediation technologies.

Introduction

Accidental activities at a pharmaceutical factory resulted in contamination of groundwater with a mixture of organic pollutants. The primary pollutant of the zone was monochlorobenzene (up to 14 000 μg/l), and benzene (up to 1 300 μg/l) was detected as a secondary contaminant. Although other BTEX compounds, *cis*-1,2-dichloroethylene, vinyl chloride and *tert*-butyl alcohol were also detected, the concentration of these pollutants were in two orders of magnitude lower than chlorobenzene. Possible bioremediation methods were tested with conditions ideal for nitrate reduction. Since the biological degradation, worked excellently due to biostimulation regarding the main pollutants (data not shown), nitrate, phosphate and microelements enriched nutrient solution was applied for the *in situ* treatment of the polluted area on a regular basis. One and a half years after the start of field treatment, then another year later, we investigated groundwater from the contaminated area. The experimental results in summary can provide a guideline for planning biological treatment processes regarding areas with a similar pollution profile.

Experimental

In this work, we compared the results of two consecutive groundwater samplings from the contaminated area, which took place 1 year apart, one in May 2022 and the other in July 2023. We studied the microbiological activity in the affected area primarily by examining the effectiveness of possible bioremediation, which was determined by the biological degradation of pollutants. The *in situ* bioremediation process as well as our tests were carried out under nitrate-reducing conditions, because the most effective way of chlorobenzene degradation, the aerobic way, was not feasible due to the unique characters of the area. Some microcosm studies were set up to acquire knowledge about the microbiological activity of the polluted zone. Microcosm is an artificial and simplified ecosystem which can simulate the behaviour of natural ecosystems under defined and well-regulated conditions. This method is perfect for examining field conditions in laboratory on a small scale. The microcosms were set up using 5 groundwater samples extracted from 5 monitoring wells. The laboratory bottles used for microcosm tests were filled up with the groundwater samples collected from the chlorobenzene-contaminated aquifer, in N_2 (90%), CO_2 (5%), H_2 (5%) containing atmosphere, using an anaerobic workstation. The headspace was 10% of the total volume. Three types of microcosm systems were assembled using every groundwater sample, each in three parallels. Abiotic microcosms were supplemented with mercury(II) sulfate (240 mg/l) to rule out possibility of accidental biological activities, in order to evaluate abiotic degradation and volatilization. Biotic control systems were set up using untreated groundwater. Biostimulated microcosms were amended by adding nitrate, phosphate and microelements to the systems. Microcosms were incubated at 13-15 °C, which corresponds to natural environmental temperature conditions. The concentration of VOC (Volatile Organic Compounds, e.g.: the contaminants), concentration of nitrate, nitrite and phosphate were analyzed weekly by using GC-MS, and HPLC Ion chromatography.

Results and discussion

Analytical results

	2022	2023	2022	2023
	Nitrite (mg/l)		Nitrate (mg/l)	
MW-1	<2,50	<2,50	<2,50	<2,50
MW-2	<2,50	5,62	<2,50	76,55
MW-3	<2,50	9,85	115,40	273,34
MW-4	<2,50	6,86	<2,50	145,65
MW-5	<2,50	9,43	<2,50	413,33

As a result of the nutrient injections into the polluted area, the concentration of nitrite and nitrate increased in all areas except for MW-1 labelled groundwater.

Microcosms studies

MW-1

By 2023, the initial concentration of chlorobenzene (6 136 μg/l) and benzene (326 μg/l) in the MW-1 labelled groundwater decreased significantly compared to last year's data. In 2022, an 83.91% decrease in concentration of chlorobenzene was observed only in the biostimulated microcosm after six weeks of incubation, while in case of the biotic control, neither of the contaminants' concentration changed noticeably compared to the abiotic system. In contrast, in 2023, chlorobenzene was already almost completely degraded in both biological systems after two weeks of incubation. The concentration of benzene did not change significantly in 2022 compared to the initial values, but in 2023, the entire amount of benzene was completely degraded in both biotic and biostimulated microcosms.

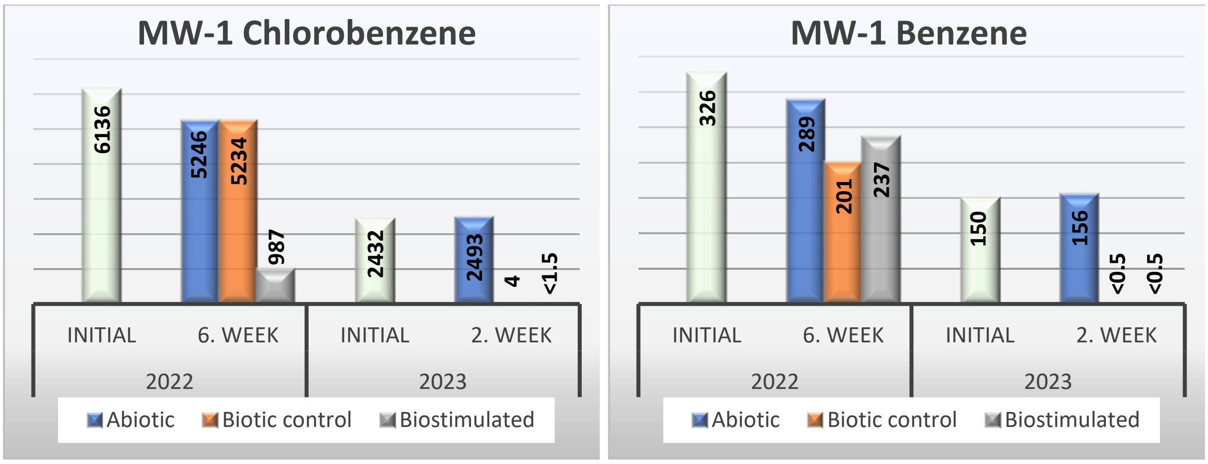

Figure 1. Evolution of concentration (µg/l) of chlorobenzene and benzene in the MW-1 groundwater area

MW-2

In 2023, the initial concentration of chlorobenzene (13 968 μg/l) and benzene (1 183 μg/l) in the groundwater area labelled MW-2 decreased significantly compared to the 2022 data. In 2022, during the 8-week incubation period, concentration of chlorobenzene decreased by only 60.42% in the biostimulated system, while in 2023, almost complete biodegradation occurred in just two weeks, both in case of biotic and biostimulated microcosm. The concentration of benzene did not decrease significantly in 2022 during the 8-week incubation period, however, in 2023, benzene was biodegraded in just two weeks in case of both biological systems.

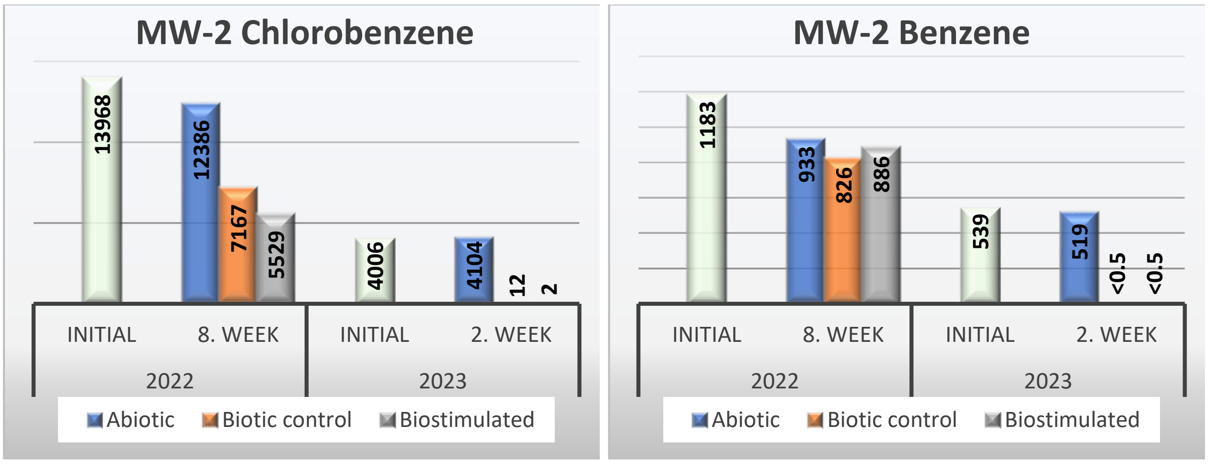

Figure 2. Evolution of concentration (μg/l) of chlorobenzene and benzene in the MW-2 groundwater area

MW-3

By 2023, the concentration of chlorobenzene and benzene in the groundwater area marked MW-3 has reduced by a quarter compared to the data of 2022. In 2022, the initial concentration of chlorobenzene (9 995 μg/l) was reduced by almost half of the value after 8 weeks in both the biotic control and the biostimulated microcosm. On the other hand, in 2023, chlorobenzene was biodegraded in two weeks in both biological microcosms. The concentration of benzene did not change significantly during the 8-week incubation period in none of the biological microcosms compared to the initial value (723 μg/l). However, in 2023, almost 100% of the contamination biodegraded in just two weeks in case of both biotic control and biostimulated systems.

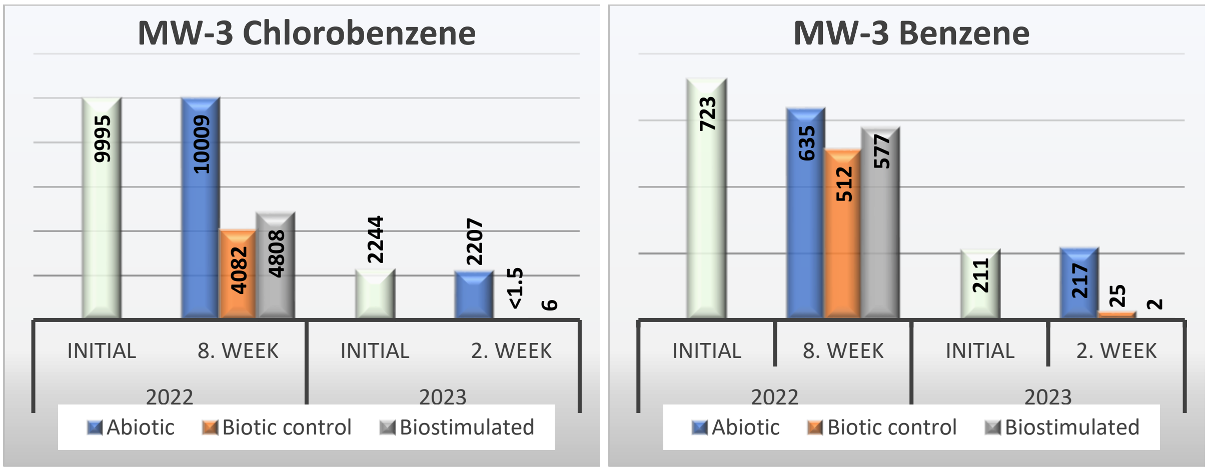

Figure 3. Evolution of concentration (μg/l) of chlorobenzene and benzene in the MW-3 groundwater area

MW-4

The initial concentration of chlorobenzene was ~25% lower in 2023 than in 2022, while the concentration of benzene did not differ significantly during the 1 year of the study. In 2022, after the 8-week incubation period, biodegradation rate of chlorobenzene in biostimulated microcosms was 44.51%, while its concentration in the biotic system did not decrease significantly compared to the initial value (10 562 µg/l). However, in 2023, we noticed an 84.52% decrease in concentration in case of the biotic microcosm within just two weeks. Similarly, also in case of the the biostimulated system, rate of biodegradation was high, almost 100%. In 2022, the initial concentration of benzene (1 278 µg/l) in biological systems decreased by only about 40% in 8 weeks. In contrast, in 2023, 61.56% biodegradation of the contaminant was observed in the biotic control microcosm and almost 100% in the biostimulated system during an incubation period of just 2 weeks.

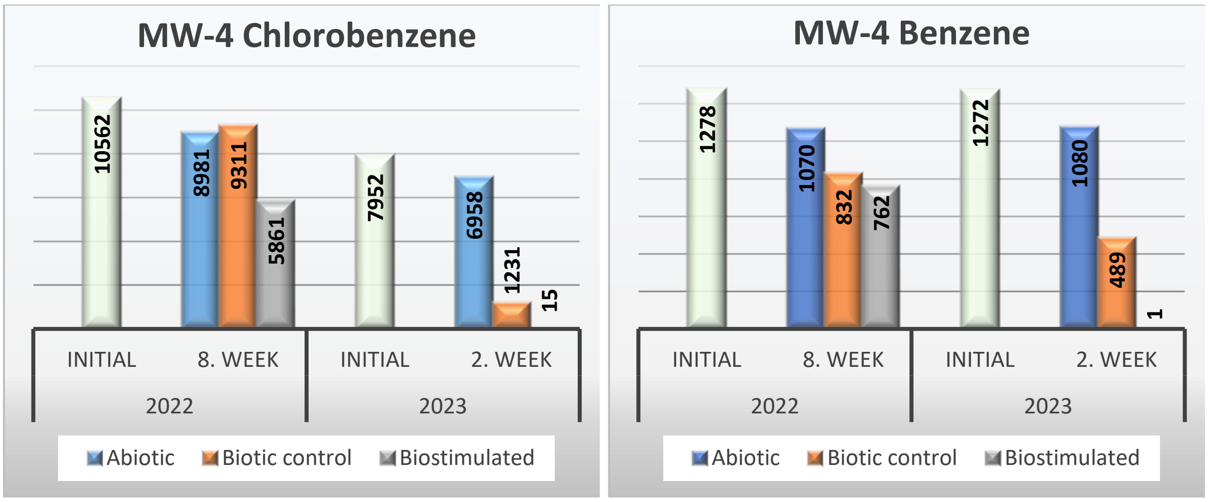

Figure 4. Evolution of concentration (µg/l) of chlorobenzene and benzene in the MW-4 groundwater

MW-5

Based on the microcosm tests compiled from the MW-5 groundwater sample, it was observed that the initial concentration of chlorobenzene in 2023 was significantly lower than in 2022. In 2022, the rate of biodegradation in the biotic microcosm was 65.40%, while in the biostimulated system it was 80.93% during the 8-week incubation period. In contrast, in 2023, chlorobenzene was almost completely biodegraded, both in the biotic and in the biostimulated systems. A significant decrease in the initial concentration of benzene was also observed in 2023. While in 2022, the concentration of benzene decreased by 46.59% in the biostimulated microcosm in 8 weeks, in 2023, the pollutant was completely degraded in both biological systems in just two weeks.

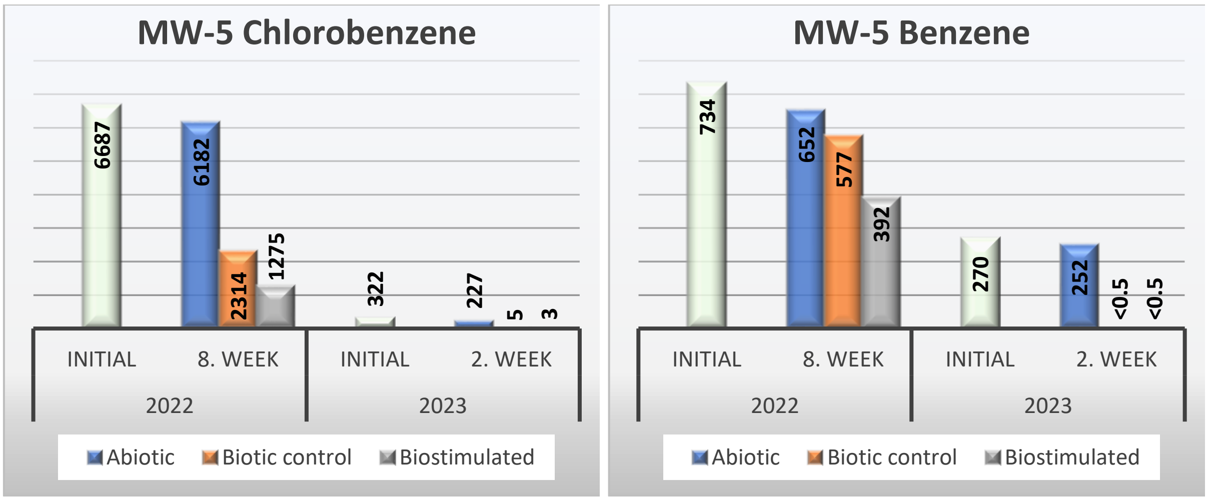

Figure 5. Evolution of concentration (µg/l) of chlorobenzene and benzene in the MW-5 groundwater area

Conclusion

The data gained from field investigations and groundwater analytical results suggest that intrinsic bioremediation process is occurring at the pharmaceutical factory site. The indigenous microbial community at the contaminated site is able to degrade chlorobenzene and benzene if appropriate conditions are provided. We managed to isolate a novel microorganism (*Pseudomonas* sp. EM1) [1] from the groundwater, that is capable of mineralizing chlorobenzene under both aerobic and anaerobic (nitrate reducing) circumstances. The microorganism has been approved as an inoculum. In the future, we intend to use *Pseudomonas* sp. EM1 in chlorobenzene-polluted areas to enhance bioremediation.

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