LABORATORY MICROCOSM STUDY OF A POLLUTED GROUNDWATER ZONE

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Abstract
Microcosm studies were set up to predict and evaluate the most effective conditions for the bioremediation of a chlorobenzene contaminated groundwater zone. In the literature there are only few studies for chlorobenzene degradation under nitrate reducing circumstances. In most of our microcosms the indigenous groundwater bacteria were able to degrade this widespread recalcitrant compound under the new conditions. We have isolated a chlorobenzene degrading bacterium, *Pseudomonas* sp. EM1 which is capable of the mineralisation under aerobic and anaerobic conditions.

Introduction
Chlorobenzene is primarily used as a solvent for paints and pharmaceutical products, as a heat transfer medium, and in the production of phenol, aniline and silicone. The production and usage of this organic compound has been declining due to the availability of more environmentally friendly replacements [1,2].
Chlorobenzene has a greater density than water and only slightly soluble in water (500 mg/l), it belongs to the DNAPLs (Dense Non-Aqueous Phase Liquids). It can be slightly accumulated at the bottom of an aquifer as a DNAPL pool which can serve as a continuous source of groundwater pollution [3,4]. Different methods have been developed to transform chlorobenzene into less toxic by-products, including adsorption [5], ozone oxidation [3], Fenton's reaction [6] and catalytic hydrodechlorination [7,8]. Biodegradation also can be an effective method. Even though chlorobenzene is a recalcitrant chemical, it can be mineralised with microorganisms under aerobic and anaerobic conditions.

Aerobic degradation
There are a couple of aerobic microbes that can use chlorobenzene as the sole energy and carbon source. The activation of the C-Cl bond requires high activation energy and a strong attacking nucleophile [9]. *Figure 1.* shows that the initial aerobic attack of chlorobenzene is catalysed by dioxygenases resulting in the formation of 3-chlorocatechol as an intermediate [10].

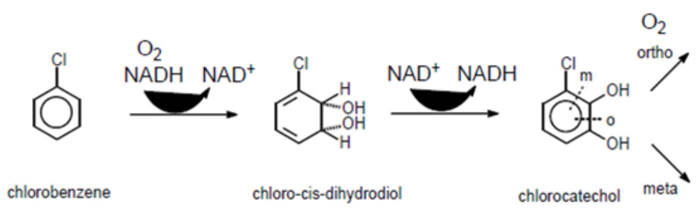


Figure 1: The aerobic initial attack of chlorobenzene

After the first step of the aerobic chlorobenzene degradation, there are two pathways: ortho and meta cleavage. The intermediates are metabolized further compounds of the citric acid cycle (CAC)[9].

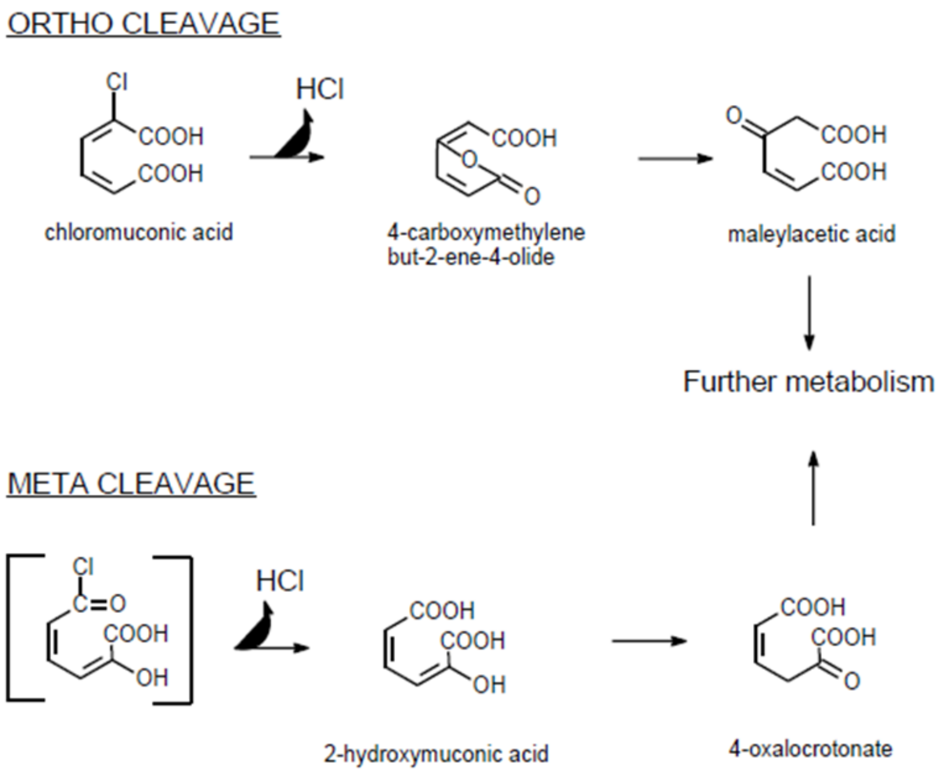


Figure 2: The ortho and meta cleavage of aerobic chlorobenzene degradation (9)

Anaerobic reductive dechlorination

The reduction of chlorobenzene under anaerobic conditions generates benzene and HCl. In this pathway the microorganisms use hydrogen as an electron donor and use the chlorobenzene as an electron acceptor [11]. This pathway requires the presence of an electron donor and it can result in the accumulation of benzene [10].

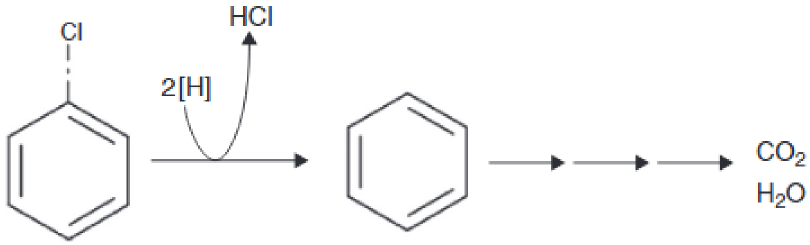


Figure 3: Pathway of anaerobic reductive dechlorination of chlorobenzene (12)

Anaerobic nitrate reducing degradation of chlorobenzene

There are quite a few experimental studies about chlorobenzene degradation under nitrate reducing circumstances [12–14]. In the contaminated area the pollution is combined, it contains BTEX compounds, *cis*-1,2-dichloroethylene, vinyl chloride and *tert*-butyl alcohol. Some studies showed that at low dissolved oxygen concentrations there is an enhanced biodegradation of BTEX in the presence of nitrate [15,16].

Experimental

In the contaminated area the primary contamination is chlorobeneze (> 25,000 µg/l), the secondary is BTEX (> 3,000 µg/l), and the tertiary is *cis*-1,2-dichloroethylene, vinyl chloride and *tert*-butyl alcohol. The polluted groundwater also contains acetic acid and ethanol which can serve as carbon and energy sources for the microbes. The pollution was originated from a drug company. The unique character of the area and the nature of DNAPLs, the most effective chlorobenzene degradation way, the aerobic degradation was unaccomplishable, hence we tested the nitrate reducing circumstances.

First of all we made some microcosm studies to know more about the groundwater. Microcosm is like a tiny, simplified ecosystem which can simulate the behaviour of natural ecosystems under different conditions. It is an excellent method to predict whether the indigenous microflora is capable of the degradation of the pollution and if it is which the best circumstances for the bioremediation are.

The microcosms were set up using 8 groundwater samples from 8 monitoring wells from the contaminated area. Three different types of microcosms were set up from every groundwater sample. There was an abiotic microcosm supplemented with mercury (II) sulphate, to evaluate abiotic degradation and volatilization. There was a biotic microcosm which contained only the untreated groundwater, it was used for modelling the natural conditions in the

1űarea. The third type of the microcosms, the biostimulated microcosm, was supplemented with nitrate, phosphate and microelements. Microcosms were incubated at 13-15 °C which is characteristic of the natural area. Every fourth week samples were taken to analyse the concentration of VOCs, nitrate, nitrite, phosphate, chloride, organic acids and ethanol. Nitrate or phosphate was spiked when it was necessary to maintain the appropriated circumstances in the microcosms.

Results and discussion

Microcosms studies

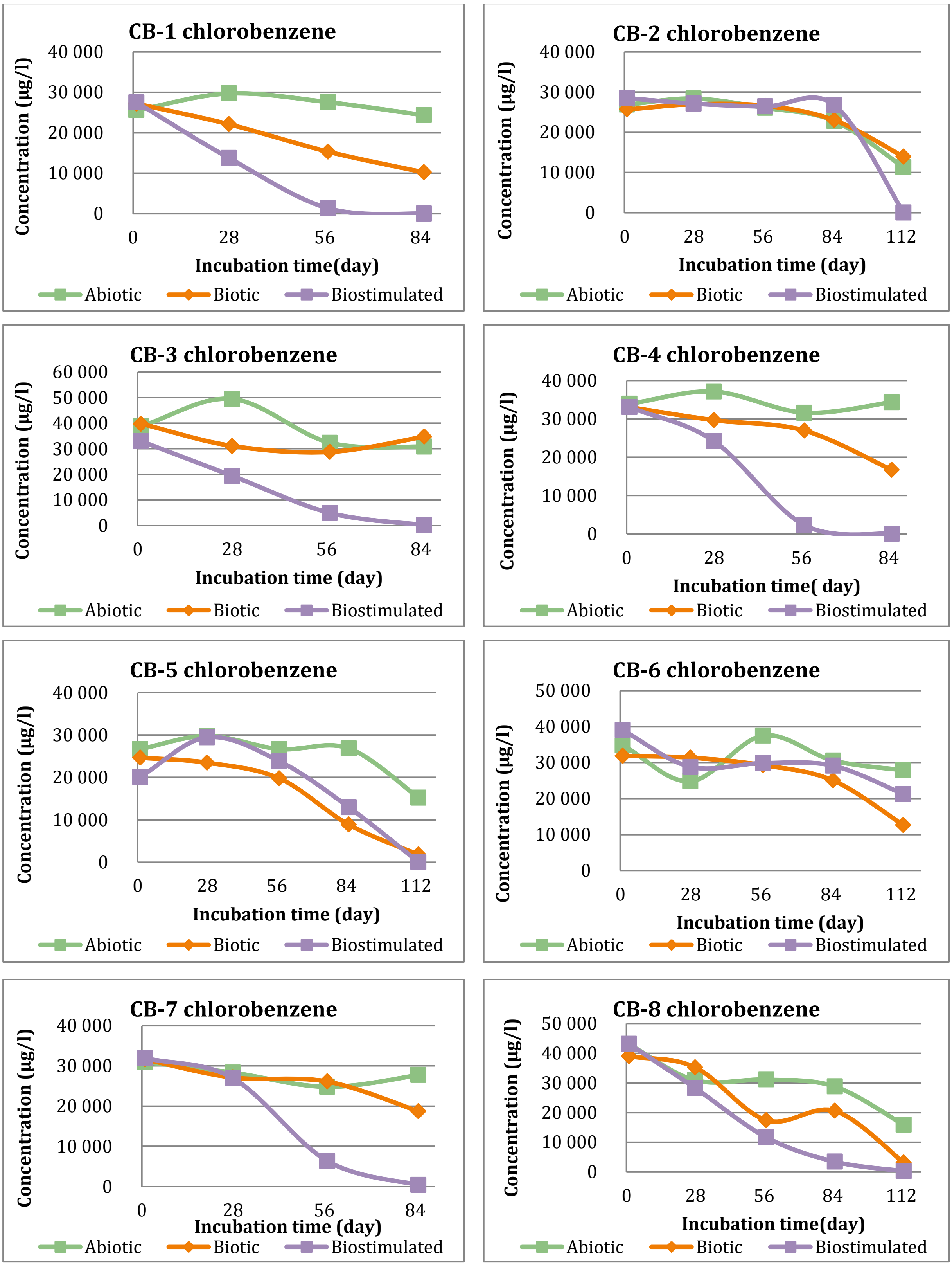
Figure 4. shows the overall results of the various chlorobenzene degradation experiments. In the groundwater samples from CB-1, CB-2, CB-3, CB-4, CB-7 and CB-8 monitoring wells the nitrate reducing circumstances promoted the chlorobenzene degradation. Additional microbiological activity (increased acetic acid degradation) was noticed in the biostimulated microcosm from CB-5 well, but the biostimulation did not have any positive effects on VOC degradation. The nitrate reducing conditions had no additional impact on the groundwater from CB-6 monitoring well. Increased BTEX degradation was observed in the microcosms from CB-1, CB-2, CB-3, CB-4 and CB-7 monitoring wells. The injection of nitrate, sulphate, phosphate and microelements to the contaminated area is recommended due to their positive effect on the degradation of chlorobenzene and BTEX compounds.

Isolation of chlorobenzene degrading bacteria

We have managed to isolate a novel microorganism (*Pseudomonas* sp. EM1) which is capable of chlorobenzene mineralisation under aerobic and anaerobic (nitrate reducing) circumstances. Now this microbe is under licensing procedures. In the future we intend to inject *Pseudomonas* sp. EM1 to the contaminated area.

Conclusion

Our goal was to determine the most effective conditions for chlorobenzene biodegradation in the contaminated aquifer. Microcosm studies were set up to evaluate and predict the effect of nitrate reducing circumstances in the polluted samples. In 6 out of 8 cases the biostimulation had really positive results and the recalcitrant chlorobenzene was biodegraded by the indigenous microflora. BTEX degradation was also contributed thanks to the nitrate reducing medium. Pseudomonas sp. EM1 was isolated from the groundwater. This microorganism is capable of the degradation of chlorobenzene both in aerobic and anaerobic conditions.



5. Figure: The change of chlorobenzene concentrations (µg/l) in the microcosm studies

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