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Research article

Intrinsic and bioaugmented aerobic trichloroethene degradation at seven sites

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ABSTRACT

Trichloroethene (TCE) is one of the most prevalent contaminants in groundwater pollution worldwide. Aerobic-metabolic degradation of TCE has only recently been discovered at one field site. It has significant advantages over aerobic co-metabolism because no auxiliary substrates are required, and the oxygen demand is considerably lower. This study investigated the intrinsic degradation potential as well as the stimulation potential by bioaugmentation in microcosm experiments with groundwater from seven different sites contaminated with chloroethenes. An enrichment culture metabolizing TCE aerobically served as inoculum. The groundwater samples were inoculated with liquid culture in mineral salts medium as well as with immobilized culture on silica sand. Additionally, some samples were inoculated with groundwater from the site where the enrichment culture originated. The microcosms without inoculum proved the occurrence of aerobic TCE-metabolizing bacteria stimulated by the supply of oxygen in 54% of the groundwater samples. TCE degradation started in most cases after adaptation times of up to 92 d. The doubling time of 24 d indicated comparatively slow growth of the aerobic TCE degrading microorganisms. Bioaugmentation triggered or accelerated TCE-degradation in all microcosms with chlorothene concentrations below 100 mg L^{-1}. All inoculation strategies (liquid and immobilized enrichment culture or addition of groundwater from the active field site) were successful. Our study demonstrates that aerobic-metabolic TCE degradation can occur and be stimulated across a broad hydrogeologic spectrum and should be considered as a viable option for groundwater remediation at TCE-contaminated sites.

1. Introduction

Volatile chlorinated hydrocarbons (CHC) are the most common organic groundwater contaminants. The highly chlorinated compounds tetrachloroethene (PCE) and trichloroethene (TCE) are prevalent solvents primarily used in dry cleaning and the metal industry. As a result of accidents and spills or improper use, they were released into the subsurface. They represent the most common contaminants in CHC groundwater pollution and are detected in the highest concentrations [1]. While chloroethenes have many advantages for industrial use, their persistence and ecotoxicity demonstrate potential environmental hazards and impact human

Abbreviations: TCE, trichlorethene.

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Table 1
Initial values of the untreated groundwater samples, aquifer information and degradation potential.

Site	Unit	LOD	1	2	2	3	4	4	4	4	5	5	5	6	7
Well			A	B	C	D	E	F	G	H	I	J	K	L	M
pH	–	–	7.1	7.1	6.7	7.2	6.8	7.0	6.9	6.9	6.7	6.9	6.4	6.6	6.4
O_2	mg L^{-1}	–	6.0	0.05	0.020	0.37	0.11	0.087	0.081	0.010	0.40	0.60	0.40	0.12	1.0
PCE	mg L^{-1}	0.0002	0.095	0.34	11	0.040	0.32	0.42	0.077	0.70	0.93	< LOD	1.3	0.042	< LOD
TCE	mg L^{-1}	0.0002	0.037	0.61	58	0.28	14	13	2.1	22	29	0.036	41	0.32	1.0
cDCE	mg L^{-1}	0.02	< LOD	0.02	81	0.38	8.2	11	2.4	11	0.34	0.068	3.5	0.062	0.045
VC	mg L^{-1}	0.12	< LOD	< LOD	12	< LOD	0.41	0.73	0.13	0.32	0.15	< LOD	0.66	< LOD	< LOD
Ethene	mg L^{-1}	0.06	< LOD	< LOD	1.3	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
DOC	mg L^{-1}	0.2	0.63	0.48	16	3.3	5.2	6.5	3.6	4.6	9.3	4.6	11	1.5	1.3
Cl^-	mg L^{-1}	2.0	112	214	1290	130	59	76	55	65	125	97	31	51	84
NO_2^-	mg L^{-1}	2.0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
NO_3^-	mg L^{-1}	2.0	51	< LOD	< LOD	37	< LOD	< LOD	< LOD	2.6	< LOD	< LOD	< LOD	27	14
SO_4^{2-}	mg L^{-1}	2.0	91	374	195	187	76	146	67	136	57	98	49	154	130
Aquifer	Fissure[a]	Fissure[a]	Fissure[b]	Pore[d]	Fissure[b,c]	Fissure[b,c]	Fissure[b,c]	Fissure[b,c]	Pore[e]	Pore[e]	Pore[e]	Pore[d]	Pore[d]		
Intrinsic TCE degradation potential	yes	yes	no	yes	no	no	yes	no	yes	no	yes	no	yes		
Bioaugmented TCE degradation	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes		

LOD Limit of determination.
[a,b,c,d,e] refer to geology.
a: Lettenkeuper, b: Gipskeuper, c: Muschelkalk, d: Quaternary, e: Sand channel in boulder clay.

health. TCE has been linked to an increased risk of breast cancer [2], autoimmune diseases [3], and Parkinson's disease [4].

Biological in-situ processes are becoming increasingly important for groundwater remediation as a viable alternative to conventional techniques such as soil excavation or pump-and-treat. Various mechanisms are known for microbiological chloroethene degradation in groundwater [5]. In anaerobic dechlorination, the chloroethenes serve as electron acceptors (halorespiration). Beginning from highly chlorinated PCE to TCE, *cis*- and to a comparatively small extent also *trans*- and 1,1-dichloroethene (cDCE, tDCE, 1,1DCE), vinyl chloride (VC), and finally ethene, one chlorine atom is removed progressively and replaced by a hydrogen atom. With a decreasing number of chlorine atoms, dechlorination decelerates and proceeds much slower than the rapid conversion of PCE to TCE and cDCE [6]. Many anaerobic halorespiring bacteria can transform PCE via TCE to cDCE. However, the only bacteria known for complete dechlorination beyond cDCE to VC and ethene are members of the genus *Dehalococcoides*. As a result, accumulation of low-chlorinated cDCE and VC is observed at many sites [7].

In the aerobic environment, chloroethenes can be degraded both co-metabolically and metabolically. So far co-metabolic degradation has been observed for VC, cDCE, tDCE and 1,1DCE as well as TCE. Other compounds such as ammonium, toluene, or methane are growth substrates and serve as auxiliary substrates stimulating co-metabolic degradation [8–12].

Aerobic productive metabolism uses chloroethenes as the only growth substrate for carbon and energy yield. Aerobic metabolic degradation is well known for VC [13] and cDCE [14,15]. For TCE, aerobic metabolic degradation has been demonstrated in microcosms of a TCE polluted German field site [16], and was investigated recently for different environmental conditions [17].

Naturally occurring biodegradation in the field is often insufficient for remediation purposes and can be stimulated by adding nutrients, and, in the case of aerobic processes, oxygen [18]. If the required organisms are not present, stimulation by adding a capable culture (bioaugmentation) can be a promising remediation option [19,20]. Biostimulation and bioaugmentation are already established for reductive dechlorination, the longest known degradation pathway for chloroethenes [20]. Numerous application examples with enrichments as well as commercially available cultures prove the efficacy in laboratory and field tests [21–24]. Bioaugmentation can stimulate complete dechlorination [25,26] or accelerate degradation processes [27]. However, site remediation by anaerobic dechlorination can also have some significant drawbacks. Environmental conditions must be strictly anaerobic, which favors competitive reactions such as sulfate reduction and methanogenesis and often leads to the formation of toxic metabolites, in particular VC [5].

Therefore, aerobic-metabolic degradation can have decisive advantages for remediation practice [16]. However, there is a lack of knowledge about the occurrence of aerobic TCE-metabolizing bacteria as well as the application range and potential implementation of bioaugmentation. Therefore, this study had the objective to evaluate both intrinsic and bioaugmentation potential at multiple sites and with different inoculation strategies in terms of its applicability as a remediation method. Building on the initial research [16,17], extensive microcosm studies were conducted with groundwater from a total of seven different sites contaminated with chloroethenes (six in Germany and one in France). An unamended groundwater sample was used to investigate intrinsic degradation potential to determine if aerobic TCE-metabolizing bacteria were present at contaminated sites other than the one previously reported. To evaluate bioaugmentation potential, the groundwater samples were inoculated with TCE metabolizing culture enriched in mineral salts medium and immobilized on silica sand, and with groundwater from the site where the enrichment culture originated.

2. Materials and methods

2.1. Groundwater microsoms

The groundwater samples were taken from thirteen wells at seven contaminated sites and show different chloroethene concentration levels and ratios (Table 1). Sites 1 to 4 are located in the southwest of Germany in the area of Stuttgart and Mannheim. Site 5 in Hamburg is located in the north and site 6 in the Rhine-Ruhr area in the west of Germany. Site 7 is near Strasbourg in France. The locations represent typical CHC contaminated sites such as former or still running chemical storage facilities, dry cleaners, or production sites. Due to their regional distribution, the samples also differ in their aquifer properties, which are also listed in Table 1.

The groundwater samples were taken directly into gas-tight 2.3 L bottles (1.2 L bottles at site 6) prepared for the microcosm studies with polytetrafluoroethene-coated screw caps on the inside and a sampling port.

Groundwater samples were stored at 4 °C one to two days until microcosm setup with a volume of 2 L or 1 L (site 6). The 300 mL and 200 mL (site 6) headspace contained ambient air so that oxygen was sufficiently available for aerobic degradation at all times. The following microcosm series was set up for each monitoring well:

One sample was left unamended to investigate the intrinsic degradation potential. One sample each was inoculated with liquid and immobilized culture, respectively, to evaluate the bioaugmentation potential. Depending on the available culture volume at the time of preparation, 150–300 mL of liquid culture and 50–300 g of silica sand were added to the microcosms. In addition, one microcosm served as an abiotic control with 2 g L^{-1} of copper sulfate added for inactivation.

Groundwater from site 4 was also inoculated with 1000 mL of groundwater from which the TCE degrading culture was enriched (SF water, see 2.2). All inoculated microcosms from site 4 were prepared in duplicate with inorganic nutrients added to the duplicate microcosm at the beginning of the experiment. The same procedure was followed with the non-inoculated microcosms of sites 5 to 7. No duplicate was available for GWM2983 microcosm from site 5, so nutrients were added after 84 d and again after 210 d to rule out any nutrient deficiencies.

The inorganic N and P supplement solution contained 0.07 g L^{-1} K_2HPO_4 x $3H_2O$ and 0.26 g L^{-1} $NaNO_3$. Depending on the chloroethene content, 5–20 mL L^{-1}, as well as 1 mL L^{-1} trace element solution with the composition described in Ref. [16], were added to the above-listed microcosms.

For setting up the microcosms, the appropriate amount of groundwater was poured from the flasks to obtain a volume of 2 L or 1 L after the addition of the inoculum. To avoid degradation due to carry-over of the enrichment culture, the following order was always applied during preparation and sampling: From each well, the microcosm without inoculum was handled first, followed by the inoculated microcosms, and finally the sterile control. Sampling was performed by piercing the septum with a fresh sterile stainless needle for each microcosm. The glass syringe used was pre-rinsed with each sample and completely cleaned after completing a series. Therefore, a transfer of the degrading culture from an inoculated to an unamended microcosm can be ruled out. The pierced septum was quickly renewed after sampling, replacing the withdrawn volume with atmospheric oxygen, ensuring sufficient oxygen supply for aerobic degradation. To allow a faster observation of potential degradation, the microcosms were stored at room temperature between 18 and 25 °C and shaken by hand once a week and right before sampling. To obtain more distinct results, the low TCE amounts in the microcosms of well A from site 1 and well J from site 5 were also increased to 1 mg L^{-1} after 8 and 56 days, respectively. In order to test biodegradation reproducibility, TCE was spiked again to all TCE degrading microcosms.

2.2. Enrichment culture and media

The culture described as SF-culture in Ref. [17] was used for this study. The mixed culture was enriched from groundwater samples (SF water) obtained from the site where the aerobic-metabolic TCE degradation was first observed. Enrichment was performed in mineral salts medium without any auxiliary substrates and TCE as the sole carbon source, as described in Ref. [16]. The culture was maintained in batches as well as immobilized in recirculating columns on silica sand, and degradation was monitored based on TCE decrease and associated chloride formation [17]. Both the liquid culture and the biofilm on silica sand were used as inoculum for the microcosm experiments. Also, groundwater from the active TCE degrading site was used as inoculum exemplarily.

2.3. Chemicals

The following chemicals were used for the experiments: TCE: 99.9% (Fluka), cDCE: 97% (Sigma-Aldrich, St. Louis, USA), $CuSO_4$ x $5H_2O$ (Merck, Darmstadt, Germany), K_2HPO_4 x $3H_2O$ and $NaNO_3$ (Merck, Darmstadt, Germany).

2.4. Analytical methods

Analyses were performed as described in Ref. [17]. Chlorethenes were measured by headspace gas chromatography (GC) coupled with flame ionization (FID) and electron single detector (ECD). The model 7890A GC from Agilent Technologies (Waldbronn, Germany) with the autosampler G1888 was used. Duplicate measurements were performed. Chloride was determined with the 761 Compact IC ion chromatograph from Metrohm (Filderstadt, Germany) with a conductivity detector and a MetrosepA-Supp-5 column. DOC was analyzed with the Vario TOC Tube from Elementar Analysesysteme GmbH (Hanau, Germany). During sampling of the microcosms, the oxygen content, as well as the pH value, were measured with the Multi 350i and pH320 multimeters and the corresponding CellOx 325 and SenTix 41 electrodes from WTW (Weilheim, Germany).

3. Results and discussion

3.1. Intrinsic aerobic TCE degradation potential

In the non-inoculated microcosms, aerobic conditions prevailed throughout the experiment with oxygen levels averaging 6.8 mg L^{-1}. TCE degradation was observed in the microcosms of seven of the 13 wells sampled, located at six of the seven sites investigated (see Table 1). The TCE profiles of all the microcosm series are found in the supplementary material (SM-Fig. 1, 2 and 4). Fig. 1 shows

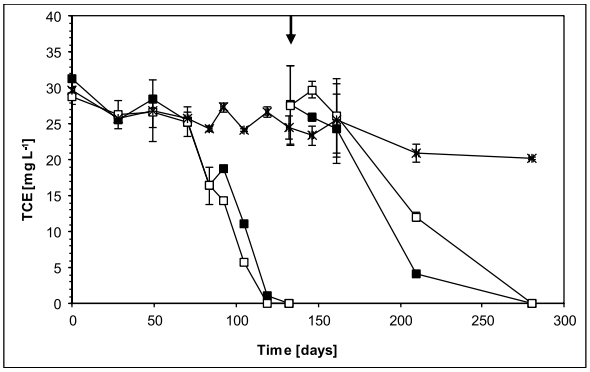

Fig. 1. TCE concentrations of a microcosm series from site 5 (well I) showing intrinsic degradation without inoculum (□) and without inoculum with mineral nutrient addition (■), compared to the sterile control (*). TCE was spiked on day 133 (↓). Error bars represent the standard deviation of duplicate measurements.

the TCE concentration of a microcosm with comparably high TCE concentrations. Significant TCE decrease compared to the sterile control is evident in both the non-inoculated microcosms with and without inorganic nutrient addition after an adaptation period of 70 d, and could be reproduced after spiking TCE at day 133.

Of the other non-inoculated microcosms, two showed a decrease in TCE concentration without delay (well A from site 1 and well B from site 2). The lag phase of the other microcosms without inoculum ranged from 43 to 92 d, which is considerably long compared to most reports for other chloroethenes in aerobic groundwater microcosm experiments. Aerobic degradation of VC and cDCE often was observed without delay [28,29] although adaptation times of up to 110 and 30 days, respectively, have also been observed [13,15, 28–30]. For other pollutants, reported lag times were mostly shorter, e.g., 4 d for BTEX compounds [31], 9 and 17 d for methane and ethane, respectively [30], and no lag time was observed for phenols [32] and PAH compounds [33].

Aerobic metabolic degradation of chloroethenes results in the stoichiometric formation of the degradation product chloride. During the TCE degradation in chloride-free medium, the correlation could be demonstrated with high accuracy [16,17]. In groundwater samples, chloride balancing is mostly not applicable due to high background concentrations. Nevertheless, in two microcosm series from site 5 (well I and well K), the amount of TCE degraded was high enough and the background concentration of chloride was low enough to observe significant and near stoichiometric chloride formation (Fig. 2). From the exponential increase in chloride formation evident in both well K microcosms, a doubling time of 24 d was determined using an exponential trend line fitting in Microsoft Excel 2016 (duplicate SM-Fig. 5). Obviously, the growth rate of the TCE degrading bacteria is considerably slower as compared to reports for other chloroethenes ranging from 0.71 to 1.4 d for aerobic VC degradation and 2.2–3.1 d reported for cDCE [13,14,28,34].

Exept for well B microcosm, which was the only one that fell below the limit of determination, TCE degradation stagnated after a substantial reduction at residual concentrations between 0.0050 and 0.34 mg L^{-1}. Inorganic nutrients were dosed into the degrading microcosms of the sites 1 to 3 approximately on day 275 to exclude nutrient limitation. On day 350, additionally TCE was dosed to test TCE degradation after the starvation periods beyond 200 days. The site 1 microcosm showed repeated TCE degradation. Microcosms from sites 2 and 3 did not show any TCE degradation after spiking, thus confirming that long starvation periods can be critical as was reported previously [17].

The microcosms of sites 4 to 7 were set up later and have a shorter experimental period. In six microcosms from sites 4 to 7 aerobic TCE degradation was observed. After spiking, TCE removal increased to at least 98% with smaller residual concentrations between 0.012 and 0.018 mg L^{-1} than after the start. Initial lag phases were reduced from 70 to 28 d for site 5 microcosms and from 92 to 13 d for site 7 microcosm. Lower residual concentrations, and shorter lag times after spiking suggest increasing numbers and further adaptation of the aerobic TCE degrading microorganisms. However, also interactions of chloroethene mixtures have to be considered that might affect degradation kinetics [29,35].

In two microcosms, degradation was stimulated by nutrient addition (well G from site 4 and duplicate from site 7). Fig. 3 shows the TCE concentrations of well G microcosm, where degradation was observed after nutrient addition on day 210.

DOC values decreased only slightly in the microcosms over the experimental period (data not shown). Organic co-contaminants were not sufficient for co-metabolic degradation of TCE, taking into account the reported transformation yields [36]. Likewise, no significant changes in nitrate concentration were observed that could indicate co-metabolic conversion with ammonium. In addition, the repeated TCE degradation after spiking also suggests metabolic degradation.

Therefore, the results strongly indicate aerobic-metabolic TCE degradation in the groundwater of six of the seven sites studied (see Table 1).

3.2. Bioaugmentation potential

TCE degradation was observed under consistently aerobic conditions with oxygen levels averaging 6.7 mg L^{-1} in all microcosms with inoculum and TCE concentrations up to 41 mg L^{-1} (see Table 1, all TCE profiles in SM-Figs. 1–4). Fig. 4 shows the TCE content of a microcosm series from site 4 as an example. While TCE levels remain relatively constant over the experimental period in the sterile

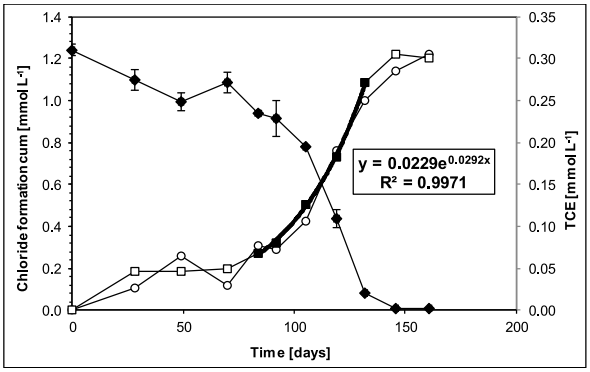

Fig. 2. Cumulative chloride formation in a microcosm without inoculation from site 5 (well K) measured (□) and stoichiometrically calculated from TCE decrease (◯) with determination of doubling time in exponential phase (■) during aerobic TCE degradation (◆). Error bars represent the standard deviation of duplicate measurements.

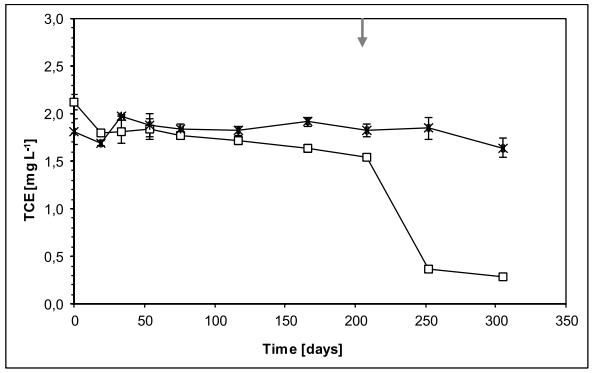

Fig. 3. TCE concentrations of a microcosm series from site 4 (well G) without inoculum showing stimulated TCE degradation (□) compared to the sterile control (*) after nutrient addition (↓). Error bars represent the standard deviation of duplicate measurements.

control and without inoculation, all inoculated microcosms show a significant TCE decrease, which was also observed in the duplicates (SM-Fig. 3) and could be reproduced after TCE spiking at day 210. As also seen here, degradation with immobilized culture started without delay in 63% of the cases, with liquid cultures in 25%. In the other cases and with SF water, lag times of up to 76 days were observed. For groundwater samples with intrinsic degradation potential, bioaugmentation resulted in accelerated degradation.

TCE degradation stagnated after a substantial decrease of averaged 94% at residual concentrations between 0.00093 and 1.7 mg L^{-1} As observed in the non-inoculated microcosms of sites 1 to 3, TCE degradation did not resume in every microcosm after long stagnation periods. Accordingly, the microcosms of sites 4 to 7 were spiked earlier. In all microcosms, degradation resumed and lower residual concentrations between 0.00045 and 0.79 mg L^{-1} were observed with a greater depletion of averaged 98%. Stagnation of degradation at low levels of inoculum was also found in microcosm experiments to stimulate aerobic cDCE degradation through bioaugmentation [37]. Obviously, a high level of active microorganisms contributes to fast chloroethene consumption and lower residual concentrations.

With inoculum, significant chloride formation was observed in the microcosms, and often exceeded the stoichiometrically calculated amount based on water analysis, as shown by the example in Fig. 5. This can be accounted for by the growing headspace over the experimental period, which results in lower TCE values in the liquid phase and therefore an underestimation of the amount of chloroethenes degraded.

Hence, aerobic-metabolic TCE degradation was successfully stimulated by bioaugmentation with all inoculation variants (liquid and immobilized culture, SF-Water) and in all groundwater samples with concentrations up to 41 mg L^{-1}. Only microcosms of well C near the contamination source at site 2 with TCE levels of approximately 58 mg L^{-1} and total chloroethenes beyond 100 mg L^{-1} showed no decline in TCE concentration. This is consistent with the studies on the range of aerobic-metabolic TCE degradation, which was observed up to a concentration of 53 mg L^{-1} [17]. Therefore, toxic concentrations for the responsible microorganisms can be assumed in the groundwater of well C.

4. Conclusion

This study strongly indicates aerobic-metabolic TCE degradation for six more sites following the initial report [16]. The long adaptation times and slow growth rates may be reasons why this process has not been observed more often. In addition, it was shown

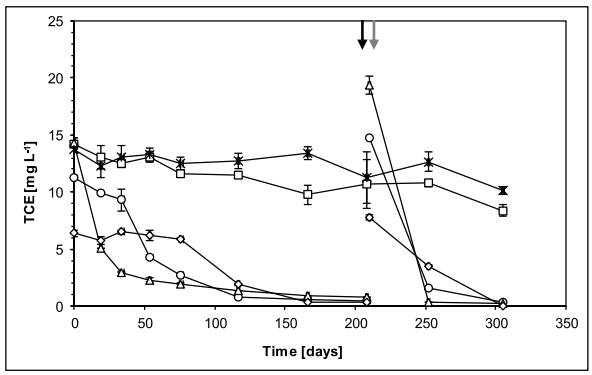

Fig. 4. TCE concentrations of a microcosm series from site 4 (well E) without intrinsic degradation (□) and stimulated degradation with liquid (◯) and immobilized culture on silica sand (△) as well as with SF water (◇) compared to sterile control (*), nutrients were added (↓) with spiking TCE (↓) on day 210. Error bars represent the standard deviation of duplicate measurements.

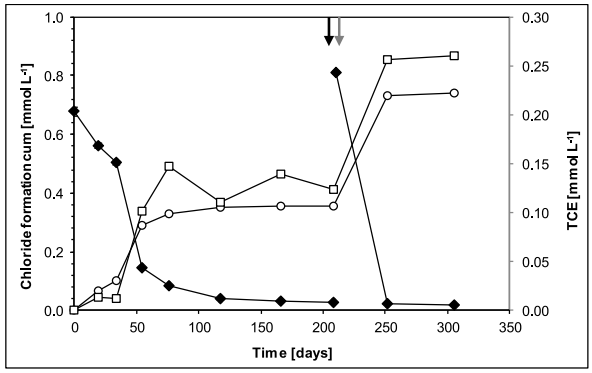

Fig. 5. Cumulative Chloride formation (□) of a microcosm with liquid culture and additional nutrients from site 4 (well E) during aerobic TCE degradation (◆) and cumulative chloride formation calculated stoichiometrically from the TCE decrease (◯). Nutrients were added (↓) with spiking TCE (↓) on day 210. Error bars represent the standard deviation of duplicate measurements.

that consideration of multiple wells at a site may be useful for discovering intrinsic aerobic TCE degradation potential.

Successful stimulation in laboratory experiments with all tested inoculation variants and groundwater samples from all sites investigated confirmed that bioaugmentation is a promising approach for site remediation. Field studies are highly recommended to test this new remediation option in larger scale and to evaluate the applicability of the different inoculation strategies under groundwater flow conditions. Further studies to identify the organisms and enzymes involved in the aerobic degradation pathway are emphasized for the development of specific monitoring methods.

Author contribution statement

Anna Willmann: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anna-Lena Trautmann: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ariel Kushmaro: Conceived and designed the experiments.

Andreas Tiehm: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13485.

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