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The oxidation, fate and effects of iron during on-site bioremediation of groundwater contaminated by a mixture of polychlorophenols

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Abstract

Kinetics of simultaneous iron and polychlorophenol (CP) oxidation by groundwater enriched cultures were studied in laboratory and during actual remediation in order to reveal the fate and effects of iron on aerobic on-site bioremediation of boreal groundwater. 2,4,6-tri- (TCP), 2,3,4,6-tetra- (TeCP) and pentachlorophenol (PCP) were degraded in fluidized-bed bioreactor (FBR) by over 99%, over 99%, and over 96%, respectively. The oxygen consumption rate for CP-biodegradation was 1.31 μmol DO L^{-1} min^{-1} and 0.29 μmol DO L^{-1} min^{-1} for iron oxidation, i.e. approximately 12% of the oxygen was consumed by iron oxidation during normal FBR operation. Mineralization of CPs was confirmed by DOC removal and chloride release of 158% and 78%, respectively. Excess DOC removal was due to partial degradation of the natural organic matter (NOM) (1.1 mg L^{-1} or 24% DOC removal) in the groundwater. Removal of NOM consumed 0.91 μmol DO L^{-1} min^{-1}. Iron oxidation in the FBR was over 94% of which chemical Fe(II) oxidation accounted for up to 10%. Fe(III) partially accumulated (58 to 69%) in the system. The TCP- and CP-biodegradation consumed DO at two times higher rates than the Fe(II)-oxidation in both, laboratory and full-scale, respectively. The batch assays at various TCP and Fe(II) ratios and DO concentrations showed simultaneous oxygen consumption by TCP and Fe-oxidizers and that increased Fe concentrations do not outcompete the bioremediation of CP's for available oxygen.

Abbreviations: DAPI: 4′,6′-Diamidino-phenylindole; DO: Dissolved oxygen; DOC: Dissolved organic carbon; FBR: Fluidized-bed bioreactor; HRT: Hydraulic retention time; NOM: Natural organic matter; PCP: Pentachlorophenol; SEM: Scanning-electron microscopy, TeCP: 2,3,4,6-Tetrachlorophenol; TCP: 2,4,6-Trichlorophenol

Introduction

Prior to aerobic on-site bioremediation of iron rich groundwater contaminated with organic compounds, iron is often oxidized and removed to avoid technical process failures by iron precipitates (Lüllmann & Schattney 1993; Czekalla & Wichmann 1994; Zettler 1996; Eble et al. 1999). Contamination of groundwater by organic compounds serving as electron donors for native microorganisms frequently results in anoxic groundwater (e.g., Chapelle 2001). Elevated ferrous iron concentrations are common in anaerobic aquifers (Appelo & Postma 1996). In such conditions, supplementation of dissolved oxygen as electron acceptor for bioremediation enhances iron oxidation. Iron oxidation is mainly biogenic (Hässelbarth & Lüdemann 1971; Czekalla et al. 1985; Czekalla & Kotulla 1990; Czekala 1997; Langwaldt & Puhakka 2002) and undesirable due to consumption of supplied oxygen and precipitate formation (e.g. Langwaldt & Puhakka 1999).

Boreal groundwater is cold and typically has an elevated concentration of NOM. The NOM constitutes mainly of humic substances originating from decay and re-formation of organic material of the topsoil (Kästner & Hofrichter 2001). In Finland, the boreal groundwater often contains high concentrations of

NOM (Kujala-Räty et al. 1998) and Fe(II) in oxygen depleted aquifers (Hatva 1989).

In this work an on-site bioremediation system for treatment of CP-contaminated groundwater containing high concentrations of Fe(II) and NOM was studied. The aquifer harbours a diverse microbial community, able to degrade CPs (Männistö et al. 1999, 2001a, b; Männistö & Puhakka 2002) and oxidize iron (Langwaldt & Puhakka 2002) at ambient environmental conditions. The competition for oxygen by Fe(II)- and CP-oxidizing bacteria was studied in laboratory and the full-scale bioremediation system.

Material and methods

Medium

Autoclaved, biologically treated groundwater of the FBR was used as low-CP groundwater for laboratory batch assays (Table 1).

Enrichment culture

In laboratory batch experiments, biomass attached onto carrier sand from the full-scale FBR was used as inoculum as described earlier (Langwaldt & Puhakka 2002). It was stored in the biologically treated groundwater for 3 days at 7–10 °C in the dark. This inoculum allowed direct comparison of oxygen consumption rates between laboratory assays and the full-scale process. The quantity of biomass attached onto the FBR-carrier sand was estimated as volatile solids (VS) (APHA 1992) and by enumeration of 4′,6′-diamidinophenylindole (DAPI)-stained cells with epifluorescence microscopy (Coleman 1980). For determination of the biomass, cells were detached from the sand by ultrasonication for 10 min in autoclaved, biologically treated groundwater.

Laboratory batch assays

Fe(II)-oxidation and TCP-biodegradation were studied in batch incubation vessels (Figure 1) at ambient groundwater temperatures 7–10 °C in the dark and by maintaining the gas phase composition constant during the experiments as described by Langwaldt & Puhakka (2002). The competition of Fe(II) and TCP oxidation for oxygen and the effect of different groundwater conditions and different TCP to Fe ratios was studied using the FBR culture as original inoculum.

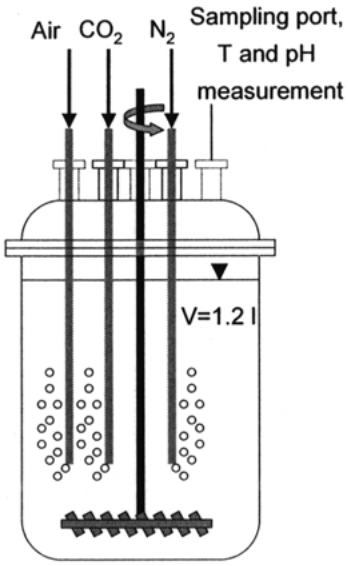

Figure 1. Batch vessel for biological oxygen consumption experiments.

Two incubation vessels were used to study the oxygen competition at low and high oxygen levels. The experimental set-up was as described in Table 2. Experiments on oxygen competition by Fe(II)- and TCP-oxidizing bacteria were performed at low DO, on average 1.3 mg L^{-1}, due to earlier reports on microaerophily of the Fe(II)-oxidizing bacteria (Ghiorse 1984), and the CP-oxidizing bacteria, when grown on rich organic media (Männistö et al. 2001a). Experiments at high DO concentration, on average 11.5 mg L^{-1}, were carried out to study the competition for oxygen by Fe(II)- and TCP-oxidizing bacteria under FBR-treatment conditions.

To study the effect of groundwater DO-level and various TCP to Fe ratios on the competition for oxygen by TCP and Fe-oxidizers the following batch experiments were carried out. In vessel 1, the effect of increasing Fe(II)-concentration on TCP-oxidation by TCP enriched culture was studied. In vessel 2, the effect of increasing TCP-concentrations on Fe(II)-oxidizer enriched culture was studied. After stable oxidation rates for the primary electron donor, Fe(II) or TCP, had been reached, a secondary, competing electron donor, TCP or Fe(II), respectively, was spiked to the vessel. In vessel 1, the initial TCP spike concentration was held constant to enrich for TCP oxidizing bacteria, followed later by spiking of increasing initial Fe(II)-concentrations. In vessel 2, the initial Fe(II)-spike concentration was kept constant to enrich for iron oxidizing bacteria. This was followed later by

Table 1. Chemical and physical characteristics of medium used in Fe(II)-oxidation and TCP-biodegradation experiments

TCP	TeCP	PCP	Fe(II)	n	DOC	Conductivity*	n
	[μmol L^{-1}]				[mg L^{-1}]	[μS cm^{-1}]	
<0.2	1.3	0.3	<0.5	3	5.1-7.1	260–285	2

*Conductivity was measured at 20 °C.

Table 2. Set-up for biological TCP- and Fe(II)-oxidation laboratory batch assays

Experimental conditions	Low DO-concentrations	High DO-concentrations		
Assay/Vessel	A/1	B/2	C/1	D/2
Average DO concentration [mg L^{-1}]	1.3	1.3	11.5	11.5
TCP-spike concentration [μmol L^{-1}]	18.0[a]	5.3–34.7[b]	19.8[a]	3.9–34.0[b]
Fe(II)-spike concentration [μmol $^{-1}$]	31.5–804.5[b]	105.1[a]	40.8–778.9[b]	92.4[a]
DO-demand by TCP-spikec [μmol L^{-1}]	100	30–193	110	22–189
DO-demand by Fe(II)-spiked [μmol L^{-1}]	8–201	26	10–195	23
Inoculum of enrichment culture [g]	9.1	9.4	9.3	8.3

[a] Re-spiked to give constant initial concentration.
[b] Re-spiked with increasing concentrations.
[c] Theoretical DO-demand for mineralization of TCP: $TCP + 5.5O_2 \Rightarrow 6CO_2 + 3HCl$.
[d] Theoretical DO-demand for oxidation of Fe(II): $Fe^{2+} + 0.25O_2 + 2.5H_2O \Rightarrow Fe(OH)_3 + 2H^+$.

stepwise increasing TCP-spike concentrations. Time between re-spikes was 10 to 12 h. The concentrations of Fe(II) and TCP during enrichment were close to those of the actual groundwater conditions. In biological Fe(II)-oxidation and TCP-degradation experiments FBR-carrier sand (8.3 to 9.4 g) and autoclaved, biologically treated groundwater (1.2 L) were added to the vessels.

On-site bioremediation system

The studied bioremediation system consisted of a full-scale FBR combined with a pre-storage tank (Figure 2) for treatment of CP-contaminated groundwater and is located in the municipality of Kärkölä in Southern Finland (Järvinen 2001; Järvinen & Puhakka 2002). The volume of the pre-storage tank and the bioreactor is 1.6 and 4.9 m^3, respectively. The HRT in the fluidized-bed and the FBR was 1.7 and 2.6 h, respectively, the feed rate was approximately 45 m^3 d^{-1} and the recycle ratio was 1:15. The FBR has successfully operated since 1995 (Järvinen 2001; Järvinen & Puhakka 2002). The source of contamination and the boreal aquifer has been described earlier (Nystén 1994). During this study, concentrations of CPs in the FBR-influent, i.e. in the contaminated untreated groundwater, were at a level of approximately 30 μmol L^{-1}. Ferrous iron content in the FBR-influent was stable at 120 μmol L^{-1}. The DO-concentration in the FBR groundwater influent remained below 1 mg L^{-1}.

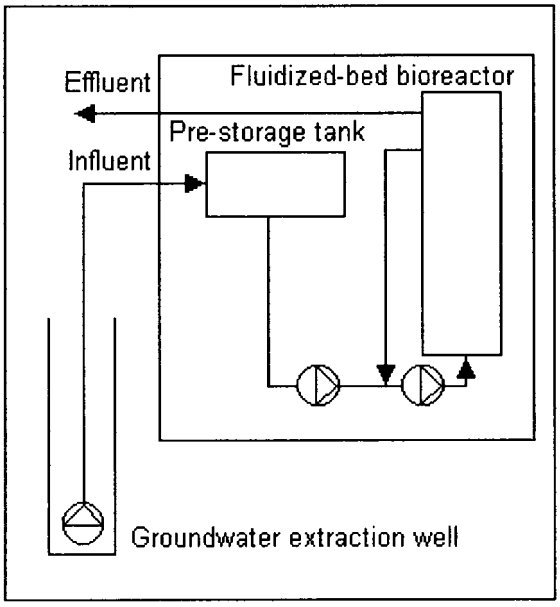

Figure 2. Schematic diagram of the on-site bioremediation system consisting of a FBR. Bioreactor volume 4.9 m^3, HRT in fluidized-bed 1.7 h, average flow rate 45 m^3 d^{-1}.

Characterization of FBR biomass

The biomass in the FBR was measured as VS (APHA, 1992). Microorganisms were stained with 1 mg L^{-1} DAPI-solution (Coleman 1980). For scanning-electron microscopy (SEM), samples were fixed with 1% glutaraldehyde in 0.1 M KH_2PO_4 for 5 min. Samples were rinsed with MQ-water and dehydrated in an ethanol-series of 70, 85, 95 and 100% for 5 min each. The dehydrated samples were kept in hexamethyldisilazane for 5 min and air-dried prior to gold-covering. The used scanning-electron microscope was a Philips XL-3.

FBR spiking experiment

An over-saturated FeSO4-solution ($2 * 80$ L) made up with the FBR-effluent, was spiked to the contaminated groundwater in the pre-storage tank of the system to determine the oxygen-consumption rate by Fe(II)-oxidation in the FBR.

The pseudo first order rate constant k_1 of the chemical iron oxidation was calculated based on the kinetics (Equation (1)) as described by Stumm & Lee (1961) with following parameters: rate constant k as $2.2 * 10^{13}$ M^{-2} atm^{-1} min^{-1} (Langwaldt & Puhakka 2002), temperature of 8.5 C, pH of 6.8, DO-concentration of 11.5 mg L^{-1} and HRT in the fluidized-bed of 1.7 h.

$$\frac{-d[\mathrm{Fe(II)}]}{dt} = k[\mathrm{Fe(II)}]p_{O_2}[\mathrm{OH^-}]^2 \qquad (1)$$

Equation (1) integrated:

$$\ln[\mathrm{Fe(II)}_0/\mathrm{Fe(II)}] = \mathrm{HRT} * k * p_{O_2} * [\mathrm{OH^-}]^2$$

Analyses

Water samples were stored at 4 °C. Temperature, dissolved oxygen (DO) (WTW DO-electrode CellOx 325) and pH (WTW pH-electrode SenTix 41) were measured with a meter (WTW multiline P3). The conductivity was measured with a WTW meter (LF 95) equipped with a WTW electrode Con 96.

Ferrous iron was measured directly after sampling. In laboratory studies Fe(II) was analysed with a spectrophotometer by a standard method (APHA 1992) modified as follows: Samples of 3 ml were acidified with a drop of strong HCl and subsequently 2 ml of 1,10-phenanthroline (10 g L^{-1}) and 1 ml of acetic anhydride buffer were added to the samples. The detection limit for Fe(II) was 0.5 μmol L^{-1} at 510 nm.

In full-scale studies Fe(II) was determined following a standard protocol (APHA 1992) with a HACH-field spectrophotometer (DREL/5). Prior to Fe(II)-determination, groundwater was acidified with 0.5 ml strong HCl per 100 ml. 1,10-Phenanthroline (1 g L^{-1}, 5 ml) and ammonium acetate buffer (2.5 ml) were added to 0.3 to 15 ml of acidic groundwater. Sample volume was made up to 25 ml by addition of MQ-water. The detection range was 7.2 to 35.8 μmol L^{-1} at 510 nm.

Content of total iron in the samples was analysed by a standard method (SFS 1976). Dissolved organic carbon (DOC) was determined to account for NOM in the medium. CPs and DOC were analysed as described earlier (Langwaldt et al. 1998).

Results

The oxygen consumption rates in laboratory and full-scale studies were calculated based on the stoichiometry for Fe(II)-oxidation (Table 2) and for CP-mineralization (Table 4).

Competition for oxygen in batch laboratory assays

The original FBR biomass inoculum on the carrier sand used in batch assays contained on average 51 mg VS (g wet sand)$^{-1}$ or 1.2 to $3.2 * 10^9$ bacterial cells (g wet sand)$^{-1}$. Thus, the 8.3 to 9.4 g inoculum contained approximately 420 to 480 mg VS or 1 to $3 * 10^{10}$ cells.

The effects of different DO-levels and different TCP to Fe ratios on oxygen consumption were studied. The oxygen consumption rates during the enrichment of either Fe(II) or TCP oxidizing bacteria under low and saturated DO concentrations were as shown in the Figure 3.

In assay A, repeated TCP-spiking (18.0 μmol L^{-1}) at low DO concentration resulted in increasing rate of oxygen consumption by TCP-oxidizers. After 2 TCP respikings, Fe(II) (31.5–804.5 μmol L^{-1}) was included in the TCP spike. Repeated respiking with TCP-Fe(II) mixture resulted in slight decrease of oxygen consumption by TCP oxidation and simultaneous increase in oxygen consumption by Fe(II) oxidation. The finally reached oxygen consumption rates for TCP and Fe(II)-oxidation were 0.03 and 0.04 μmol DO L^{-1} min^{-1}, respectively (Figure 3A). This result shows that an excess of Fe(II) as compared to TCP

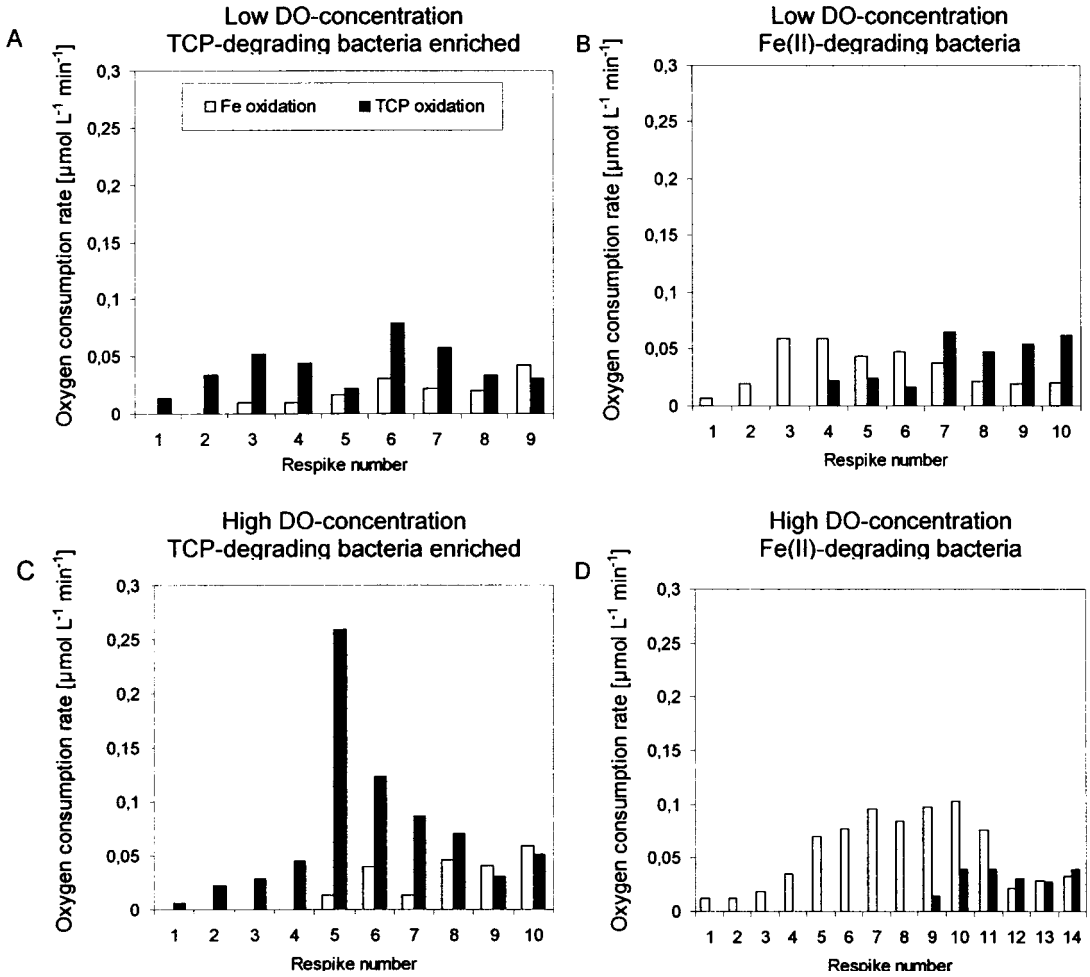

Figure 3. Competition for DO by Fe(II)- and TCP-oxidizing bacteria at pH of 6.3 at low (assays A and B) and high (assays C and D) DO-concentration. The batch cultures were repeatedly respiked in the following way: In assays A and C enrichment of TCP-oxidizing bacteria by repeated TCP-spikes (A: 18.0 and C: 19.8 μmol TCP L^{-1}). In A and C after 2nd and 4th TCP-spikings, respectively, Fe(II) was spiked in increasing concentrations, from 31.5 to 804.5 and 40.8 to 778.9 μmol Fe(II) L^{-1}, respectively. In assays B and D enrichment of Fe(II)-oxidizing bacteria by repeated Fe(II)-spikes (B: 105.1 and D: 92.4 μmol Fe(II) L^{-1}). In B and D after 3rd and 8th Fe(II)-spikings, respectively, TCP was spiked in increasing concentrations, from 5.3 to 34.7 and 3.9 to 34.0 μmol TCP L^{-1}, respectively.

(theoretical oxygen consumption ratio for last spiking 2:1) results in similar DO-consumption rates by both biooxidations.

In a second assay (B) at low DO concentration, repeated Fe(II) spiking (105.1 μmol L^{-1}) resulted in increasing rate of oxygen consumption by Fe(II) oxidizers. After 3 Fe(II) respikings, TCP (5.3 to 34.7 μmol L^{-1}) was included in the Fe (II) spike. Repeated respiking with Fe(II)-TCP mixture resulted in gradual decrease of oxygen consumption by Fe(II) oxidation and simultaneous increase in oxygen consumption by TCP oxidizers. The finally reached oxygen consumption rates for TCP and Fe(II)- oxidation were 0.06 and 0.04 μmol DO L^{-1} min^{-1}, respectively (Figure 3B).

At high DO concentration (assay C), increasing rates of oxygen consumption by TCP-oxidizers resulted from repeated TCP-spiking (19.8 μmol L^{-1}). After 4 TCP respikings, Fe(II) (40.8 to 778.9 μmol L^{-1}) was included in the TCP spike. Repeated respiking with TCP-Fe(II) mixture resulted in gradual decrease of oxygen consumption by TCP oxidation and simultaneous increase in oxygen consumption by Fe(II) oxidizers. The finally reached oxygen consumption rates for TCP and Fe(II)-oxidation were 0.05 and 0.06 μmol DO L^{-1} min^{-1}, respectively (Figure 3C).

In the fourth experiment (assay D) at high DO concentration repeated Fe(II) spiking (92.4 μmol L^{-1}) resulted in increasing rate of oxygen consumption by

Fe(II) oxidizers. After 8 Fe(II) respikings, TCP (3.9 to 34.0 μmol L^{-1}) was included in the Fe(II) spike. Repeated respiking with Fe(II)-TCP mixture resulted in gradual decrease of oxygen consumption by Fe(II) oxidation and simultaneous increase in oxygen consumption by TCP oxidizers. The finally reached oxygen consumption rates for TCP and Fe(II)-oxidation were 0.04 and 0.03 μmol DO L^{-1} min^{-1}, respectively (Figure 3D).

In all four batch assays the oxygen consumption rates accelerated in the beginning of enrichment and then gradually decreased during addition of the competing electron donor. Fe(II) and TCP-oxidizing bacteria reached higher oxygen consumption rates under conditions of oxygen saturation than at low DO concentration. The final oxygen consumption rates were similar in all four batch assays, on average 0.04 $\pm$ 0.01 μmol DO L^{-1} min^{-1} and the final total oxygen consumption rate was on average 0.08 $\pm$ 0.02 μmol DO L^{-1} min^{-1}.

Extend of the iron oxidation was highest under oxygen saturation conditions with up to 0.10 compared to 0.06 μmol DO L^{-1} min^{-1} at low DO-concentrations. The highest oxygen consumption rate in all assays was 0.26 μmol DO L^{-1} min^{-1} for TCP-oxidation at high DO-concentration. The batch assay results show that under different DO-levels and different TCP to Fe ratios both biological oxidations proceed simultaneously. These results demonstrate that the bioremediation of CP's will not be outcompeted for oxygen by Fe-oxidizers.

FBR performance

Quantity and SEM characterization of biomass. The quantity of biomass attached onto the FBR carrier sand was 80 mg VS (g dry sand) $^{-1}$. The total volume of the sand-bed was estimated to be 2400 to 2600 L, resulting in the total biomass of 124 to 202 kg VS in the FBR. On the FBR-carrier sand, small rod shaped bacteria dominated (Figure 4).

In the pre-storage tank of the FBR-system, filamentous bacteria dominated as shown in Figure 5. Filamentous bacteria were not seen on the carrier sand (Figure 4). The numbers of DAPI-stained bacteria in the groundwater and in the FBR-effluent were $5 * 10^4$ and $4 * 10^5$ cells ml^{-1}, respectively.

CP-biodegradation performance. The FBR-process performance was analyzed 5 months before and 2 months after the spiking experiment and the process performance is summarized in Table 3. CP-biodegradation and Fe(II)-oxidation were over 99 and 95%, respectively. Accumulation of ferric iron was up to 69% during normal FBR-operation (Figure 6c).

Table 3. Typical FBR-process performance before and after the Fe(II)-spiking experiment

	CP	Fe(II)	Fe_{total}
5 months before experiment	[μmol L^{-1}]		
FBR-influent	27	136.1	150.4
FBR-effluent	0.02	<7.2	53.7
Change (%)	99.9	>94.7	64.3
2 months after experiment	[μmol $^{-1}$]		
FBR-influent	26.3	134.3	145.0
FBR-effluent	0.17	<7.2	44.8
Change (%)	99.3	>94.6	69.1

Fe(II)-spiking experiment. Chemical Fe(II)-oxidation using Equation (1) was estimated to have been up to 10% during FBR-treatment. The Fe(II)-concentrations and %-oxidation during the Fe(II)-spiking experiment were as shown in Figure 6. The oxygen consumption rates for CP-oxidation were calculated based on the HRT of 1.7 h in the fluidized-bed for CP-biodegradation and for Fe(II)-oxidation based on the difference between Fe(II)-concentrations in the FBR-influent and -effluent. The oxygen consumption rates for Fe(II)-oxidation were 0.64 to 0.92 μmol DO L^{-1} min^{-1}. During Fe(II)-spiking the iron oxidation efficiency was 54 to 99% and on average, 58% of the ferric iron accumulated in the on-site bioremediation system (Figure 6c). The washout of accumulated iron after the Fe(II)-spiking period is also shown in Figure 6c.

During the spike experiment, biodegradation of TCP, TeCP and PCP remained stable at over 99% for TCP and TeCP, and over 96% for PCP. The total CP-biodegradation was 99% (Figure 7). The DO-consumption rate for CP-biodegradation was 1.31 μmol DO L^{-1} min^{-1}.

Based on the stoichiometries for aerobic CP-mineralization, the mass balance for CP-biodegradation based on CPs, DOC, chloride concentrations was as presented in Table 4. The chloride release and DOC removal results indicate CP-mineralization.

Further, the fate of DOC was studied to account for degradation of the labile NOM. The DOC-content de-

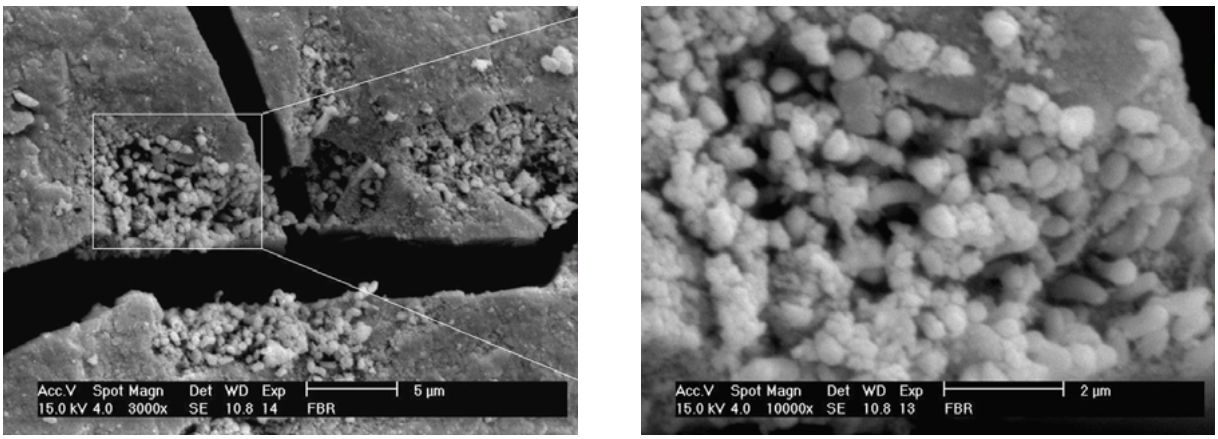

Figure 4. Scanning electron micrographs of biofilms developed on the FBR-carrier sand.

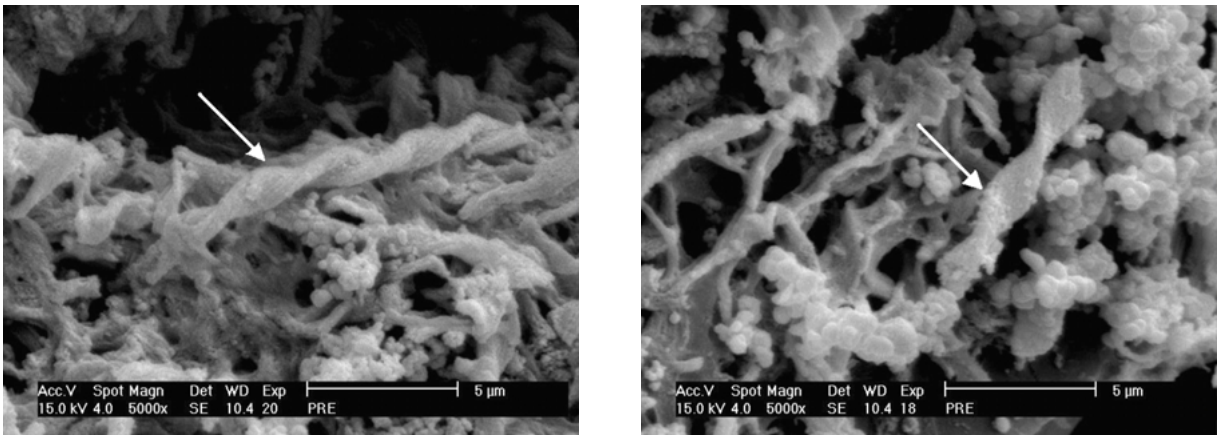

Figure 5. Scanning electron micrographs of the pre-storage tank biomass of the FBR-system. Twisted bands (indicated with arrows) similar to stalks excreted by *Gallionella*.

Table 4. Mass balance for aerobic CP mineralization in the FBR based on theoretical ratios of inorganic chloride release and DOC removal to degraded CPs

	DOC-decrease (μmol L^{-1})	Chloride-release (μmol L^{-1})
Measured	250.0	79.0
Theoretical	158.3	101.5
Fit (%)	158	78

Stoichiometry for aerobic CP-mineralization:
TCP: $C_6Cl_3H_2OH + 5.5O_2 \Rightarrow 6CO_2 + 3HCl$, 0.54 mg Cl^- (mg CP)$^{-1}$; 0.36 mg TOC (mg CP)$^{-1}$.
TeCP: $C_6Cl_4HOH + 5.0O_2 + H_2O \Rightarrow 6CO_2 + 4HCl$, 0.61 mg Cl^- (mg CP)$^{-1}$; 0.31 mg TOC (mg CP)$^{-1}$.
PCP: $C_6Cl_5OH + 4.5O_2 + 2H_2O \Rightarrow 6CO_2 + 5\ HCl$, 0.66 mg Cl^- (mg CP)$^{-1}$; 0.27 mg TOC (mg CP)$^{-1}$.

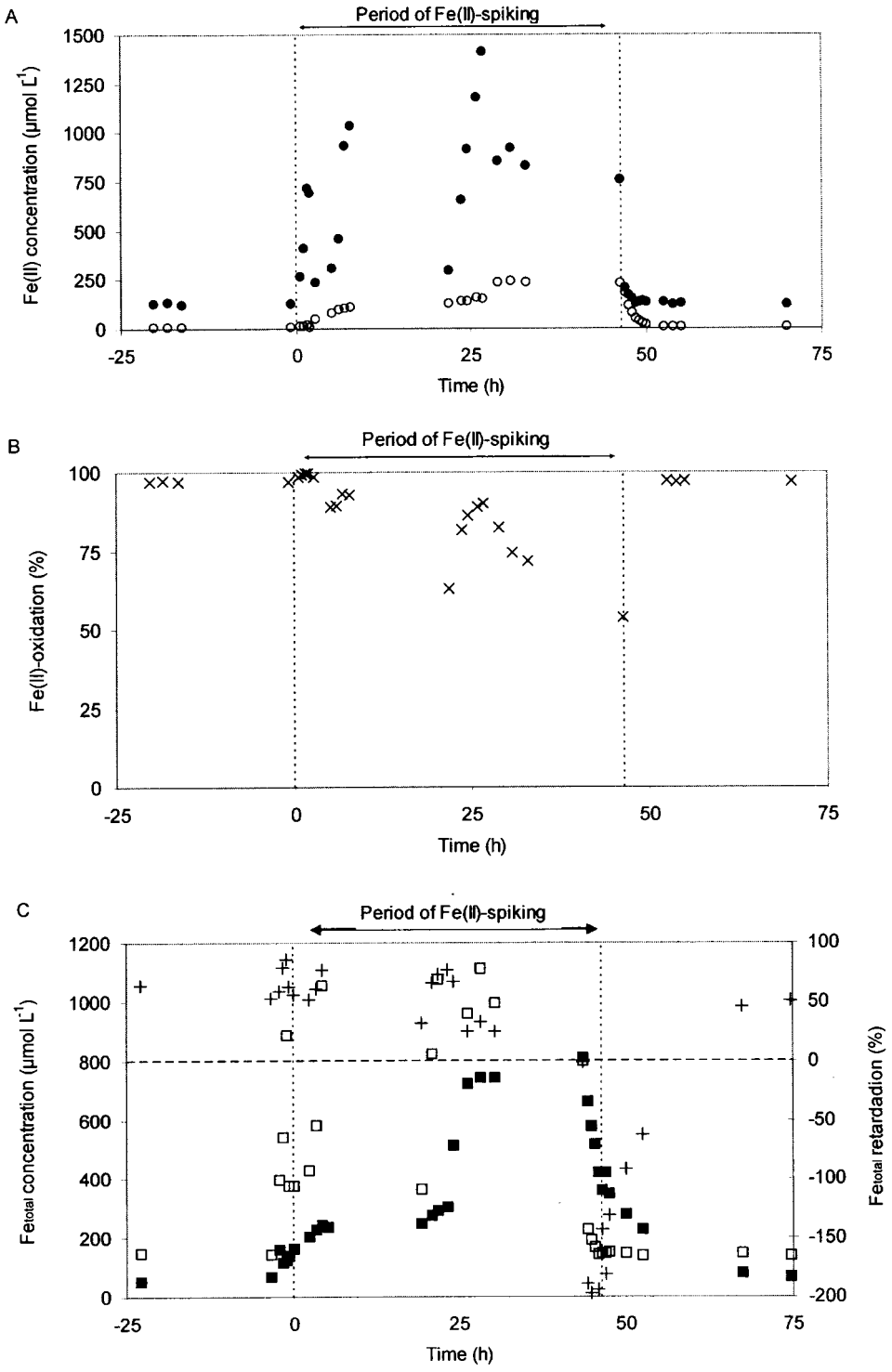

Figure 6. The fate of Fe(II) in the FBR-spike experiment. (a) Fe(II)-concentration in FBR-influent (●) and -effluent (○). (b) Fe(II)-oxidation (x) was calculated from the difference between Fe(II)-concentrations in the FBR-influent and -effluent. (c) The fate of Fe_{total} in the FBR-spike experiment. Concentrations of Fe_{total} in FBR-influent (■) and -effluent (□). Retardation of Fe_{total} (+) was calculated from the difference between concentrations of Fe_{total} in the FBR-influent and -effluent.

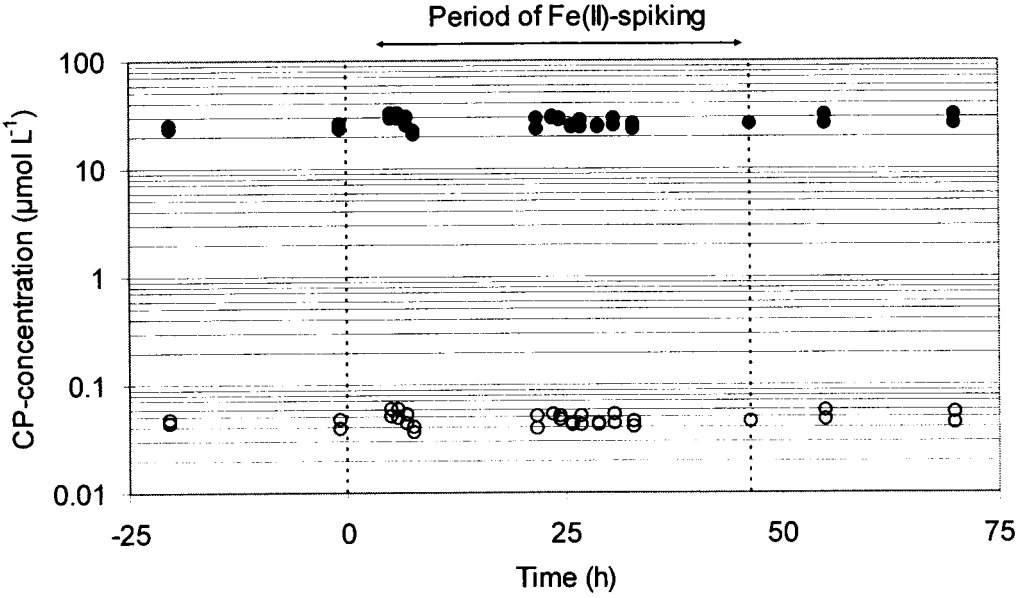

Figure 7. CP-removal performance in the FBR-groundwater treatment. FBR-influent (●) and -effluent (○).

creased by 3.0 mg L^{-1} in the FBR-system of which 1.9 mg L^{-1} was accounted for CP-mineralization. This result shows that the labile fraction of the NOM was 1.1 mg L^{-1} or 24% of removed DOC.

Discussion

The on-site, high-rate FBR bioremediation of groundwater contaminated with a mixture of polychlorophenols including TCP, TeCP and PCP resulted in 99% removal of the contaminants as reported earlier (Järvinen 2001; Järvinen & Puhakka 2002). In this work, the mineralization of CPs was confirmed based on the stoichiometry of chloride release (78%) and DOC removal (158%). The excess DOC removal was due to the NOM degradation (24%) in the FBR-treatment showing the partial recalcitrance of the boreal groundwater NOM. Approximately 12% of the oxygen consumed in the FBR was due to Fe oxidation and resulted in the accumulation of ferric precipitates in the FBR. This did not compromise the CP-mineralization performance. The batch assays with low and high DO-levels and with varying TCP to Fe ratios demonstrated that at all conditions the Fe-oxidation does not outcompete CP bioremediation for available oxygen.

During steady-state operation of the FBR the removals of CPs, Fe(II) and NOM were 99, 95 and 24%, respectively. The oxygen consumption for CPs, Fe(II) and NOM oxidation were 52, 12 and 36%, respectively. Thus during the CP-bioremediation process half of the oxygen was consumed by undesirable reactions.

Fe(II) oxidation was mainly biogenic and abiotic Fe(II) oxidation accounted only by 10% for Fe(II)-removal. Our earlier study showed that CPs and NOM in the contaminated groundwater had no effect on chemical Fe(II) oxidation (Langwaldt & Puhakka 2002). In the presence of iron oxidizing bacteria and at circumneutral pH, the Fe(II) removal in the groundwater was mainly biogenic as also shown in other studies (e.g. Czekalla et al. 1985; Sogaard et al. 2000, 2001; Langwaldt & Puhakka 2002). The oxygen consumption rate for iron oxidation during normal FBR-operation was 0.29 μmol L^{-1} min^{-1} this is lower than rates reported by others for biological iron oxidation, 2.7 to 5.5 μmol L^{-1} min^{-1} in laboratory (Emerson & Revsbech 1994) and 0.7 to 4.1 μmol L^{-1} min^{-1} in full-scale freshwater treatment systems (Sogaard 2000). In both referred studies, the process were designed for Fe(II) removal and Fe(II) served as the only electron donor.

During Fe(II)-spiking of FBR, CP-biodegradation remained stable even at tenfold increase of Fe(II)-concentration in the influent, demonstrating that availability of DO was not limiting the CP-biodegradation and that the elevated Fe(II) concentration was not inhibitory to the CP-degrading bacteria. At the highest spiked Fe(II) concentration, the oxygen consumption

rate for CP-biodegradation was twice of that for Fe(II) oxidation. The same ratio was observed in the batch assays for the highest measured rates and in an earlier study (Langwaldt & Puhakka 2002). This indicates that the long-term enrichment in the FBR-process has resulted in a microbial community with physiologically stability of CP degradation. The stability and low diversity of the microbial community were earlier shown by different 16S ribosomal DNA-based methods (Tiirola et al. 2002).

Batch assay results demonstrated that the fate and consumption of oxygen by the FBR-enrichment culture depended in some extend on the share of the Fe(II) and TCP supplied. The final oxygen consumption rates, however, were in all four batch assays on average 0.04 μmol L^{-1} min^{-1} for Fe(II) and TCP oxidation. Similar oxygen consumption rates (0.01 to 0.03 and 0.02 to 0.08 μmol L^{-1} min^{-1} for Fe(II) and TCP oxidation, respectively) were obtained when Fe(II) and TCP were simultaneously supplied (Langwaldt & Puhakka 2002). In this study, the highest measured oxygen consumption rate for TCP oxidation was two times higher than for Fe oxidation as also observed during simultaneous spikings of Fe(II) and TCP (Langwaldt & Puhakka 2002) and in the FBR-experiment. Even at the highest TCP-concentration of 34 μmol L^{-1} Fe(II) was oxidized at similar rates than without TCP in solution. This indicates that the applied TCP-spiking concentrations were below the toxicity level for Fe(II)- oxidizing bacteria.

Fe(II) oxidation rates increased with DO concentration demonstrating that the iron bacteria were not microaerophilic. This was also shown in earlier studies (Czekalla 1997; Sogaard et al. 2000, 2001; Langwaldt & Puhakka 2002). The same applies for the non-microaerophily of TCP oxidizers, as TCP degraded faster at high than low DO, as also observed in our other studies (Männistö et al. 2001a; Langwaldt & Puhakka 2002).

The measured retardation of ferric iron of 58 to 69%, confirms observations by the system maintenance personnel that iron precipitates accumulating in the system outside of the fully mixed FBR resulted occasional failures in on-line flow and DO measurements and in increased maintenance frequence to prevent clogging of effluent-pipelines.

The biomass measured from the carrier material was high compared to CP-biodegradation in laboratory FBRs (Mäkinen et al. 1993; Melin et al. 1998). This difference is most likely due to the biomass growth on groundwater NOM in the full-scale FBR, whereas in laboratory studies synthetic CP-solutions were fed. The biomass attached onto the FBR carrier sand was estimated to be approximately $2 * 10^{12}$ cells (kg carrier)$^{-1}$. Incomplete detachment of cells from solids and biofilms by ultra-sonication (Ramsay 1983; Petermann-Herman 1992) may have underestimated the cell numbers from biofilm of the carrier. However, the biomass in the FBR was mainly on the carrier-sand. The fully mixed liquid phase of the FBR contained approximately $4 * 10^{12}$ cells m^{-3}. Similar suspended cell numbers (10^{13} cells m^{-3}) were reported by Omura et al. (1991) for an iron oxidizing FBR. Omura et al. (1991) measured higher attached cell numbers (approximately 10^{16} cells kg^{-1}) due to a larger porosity of the carrier material compared to the sand used in this study. DAPI-enumerated bacteria showed a washout of cells from the FBR of approximately $2 * 10^{13}$ cells d^{-1} due to particle-to-particle attrition and shear force. Calculations of the cell washout based on earlier cell enumerations of groundwater (Männistö et al. 2001b) and FBR-effluent (Tiirola et al. 2002) result in approximately 10^{13} to 10^{14} cells d^{-1}, showing that the groundwater bacteria only partially colonize the sand-bed and that they are mainly washed out. Tiirola et al. (2002) showed that the FBR-biofilm has a low microbial diversity. The SE-micrographs of the carrier sand showed bacteria mainly in the fractures of the grains and that exposed surfaces were not colonized.

The twisted bands in the pre-storage tank seen in the SE-micrographs were morphologically similar to stalks excreted by *Gallionella* (e.g. Lütters-Czekalla 1990). This is conformity with the conditions in the pre-storage tank, microaerobic (DO below 1 mg L^{-1}), slightly acidic pH of 6.3 and Fe(II)-concentration above 89 μmol L^{-1} (5 mg L^{-1}), that are beneficial for growth of *Gallionella* (Hanert 1981; Hallbeck & Pedersen 1990). The bean-shaped cells of *Gallionella* were not seen in the SE-micrograph. The FBR conditions (high DO, high shear force) may limit the enrichment of stalking iron oxidizing bacteria. Fe(II) may have been oxidized in the FBR by other iron oxidizing bacteria than *Gallionella*.

The CP-biodegradation in the FBR was 99% and was not affected by Fe(II)-spiking. Mineralization of CPs was confirmed by increase of chloride and decrease of DOC in the treated groundwater. The somewhat lower than predicted chloride increase (78%) by CP-mineralization was within the range of earlier studies (Langwaldt et al. 1998; Männistö et al. 2001a). The higher observed than estimated DOC removal

of 158% compared to theoretical DOC decrease by CP-mineralization shows that NOM was partially biodegraded (24%) in the FBR; thus, the major fraction of the NOM in boreal groundwater is recalcitrant to biodegradation. Earlier measurements indicated a 10 to 15% biodegradable fraction of groundwater NOM (as DOC) in the contaminated groundwater (Sjölund 1999). The NOM in the subsurface and groundwater represents the remaining refractory fraction of the NOM after migration through the biologically active topsoil (Ranville & Macalady 1997). Therefore, boreal aquifers are oligotrophic in respect to readily biodegradable substrates (Morita 1997). The NOM biodegradation is likely due to the CP-oxidizing bacteria and other oligotrophic groundwater bacteria of the aquifer (Männistö et al. 1999; 2001a, b; Männistö & Puhakka 2002). Similarly, partial removal of freshwater NOM in biological iron removal processes of drinking water works was related to heterotrophic iron bacteria (Mouchet 1992; Czekalla 1997). The role of iron bacteria on DOC-removal is limited due to the high ratio of Fe(II) oxidation to carbon fixation (Mouchet 1992).

Conclusions

The following conclusions can be drawn from this study:

1. On-site aerobic bioremediation of TCP, TeCP and PCP-contaminated anoxic groundwater remained stable at high Fe(II)-concentrations (120 to 1407 μmol L^{-1}) and resulted in over 99% removal of the CP congeners and 94% removal of Fe(II). Approximately 12 and 36% of the FBR-oxygen consumption was due to iron and NOM oxidation, respectively, but did not interfere with the long-term performance of CP bioremediation. The full-scale CP-bioremediation is not affected by Fe(II) and NOM oxidation, regardless that these reactions consume half of the oxygen.
2. Fe(II) and TCP-oxidation occurred simultaneously under microaerobic and aerobic conditions and at a variety of TCP to Fe ratios.
3. Oxygen consumption rates for TCP biodegradation were twice as high as for Fe(II)-oxidation in both laboratory experiments and full-scale treatment.
4. Iron oxidation in the FBR was mainly biological (90%) and 55 to 69% of the iron precipitates accumulated in the FBR-system.
5. The major fraction (76%) of the boreal groundwater NOM was recalcitrant in the FBR bioremediation system.

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