In situ source zone sediment mixing coupled to groundwater biostimulation to enhance phenol natural attenuation

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ABSTRACT

Phenol is an industrially key compound that has a wide range of applications and also one of the most commonly found toxic pollutants in wastewaters and groundwater. This paper demonstrates the applicability of in situ remediation at a deactivated industrial site using source zone excavation and sediment mixing associated with nutrients delivery into groundwater. Sediment excavation and mixing displaced the entrapped source zone enhancing mass transfer into groundwater and contaminant bioavailability. A nutrient solution prepared with nitrate, phosphate, sodium hydroxide and hydrogen peroxide was continuously delivered into groundwater to stimulate biodegradation and restrict plume migration. The observed correlation between phenol-dependent Enterobacteriaceae concentrations throughout the remediation time frame supported circumstantial evidence of biodegradation. Phenol concentration in groundwater (up to 1,300 mg/L) was reduced >99% after 5 months following remediation and remained under the established site specific target level (4 mg/L). Nitrate and phosphate concentrations returned to background concentrations levels at the end of the remediation. Overall, the proposed in situ remediation scheme was effective to remediate this particular aquifer contaminated with phenol for over 20 years.

Key words | bioremediation, Enterobacteriaceae, groundwater, phenol, source zone

INTRODUCTION

Phenol is a compound widely used in many industrial processes for the production of polymeric resins, paints, explosives, inks, perfumes, textiles and antibacterial agents (Kiliç 2009) and also commonly found in industrial wastewaters (Marrot et al. 2006; Chakraborty et al. 2010). Phenolic compounds are toxic and carcinogenic by ingestion, contact or inhalation (Van Schie & Young 2000; Yang & Lee 2007). Based on toxicity tests, Brazilian drinking water standards have limited phenol concentration up to 0.14 mg/L (CONAMA 2009).

Typical remediation strategies to clean up soil and groundwater contaminated with phenol include physical–chemical (solvent extraction, adsorption, chemical oxidation, incineration, electronkinetics) and biological-mediated processes (Remediation Technologies Screening Matrix and Reference Guide, Version 4.0). Phenol in situ bioremediation holds high potential for site clean up due to its safety, cost-benefits and the unlikely capacity to produce secondary pollution in the process (Yeom & Ghosh 1998). Many microorganisms have been isolated and characterized as phenol-degrading organisms (for review see Kiliç 2009) including several members of the environment-ubiquitous Enterobacteriaceae family such as Klebsiella, Citrobacter, Shigella (Shawabkeh et al. 2007; Miller et al. 2004; Narde et al. 2004; Chen et al. 2010; Kafilzadeh et al. 2010; Selvakumaran et al. 2011), Enterobacter (Thomas et al. 2002), and Proteus (Miller et al. 2004). Therefore, whereas phenol rapid biodegradability in soil and groundwater has been documented (Vipulanandan et al. 1995; Hughes et al. 1997), phenol removal was not accomplished at this particular site investigated by this work even after 20 years of exposure to natural attenuation. Thus, as most microorganisms that degrade phenol are ubiquitous in soil, phenol persistence in aquifers might result...
primarily from its high concentrations and associated toxicity (Lowry et al. 2009; Nweke & Okpokwasili 2010), mass transfer limitations (Simoni et al. 2001) and/or, unavailability of adequate supply of electron acceptors and nutrients to support significant biodegradation (Son et al. 1998; Baek et al. 2003; Da Silva et al. 2005). This paper addresses the applicability of a full-scale in situ remediation based on the excavation and mixing of unsaturated soil encompassing the phenol source zone. Electron acceptors and nutrients were continuously delivered downgrading from the source zone during the remediation time frame to warrant groundwater phenol biodegradation and minimize the contamination plume. The main objective was to reduce phenol contamination to below the established contaminant specific target level (0.14 mg-phenol/L) using a low cost in situ remediation approach that could eliminate the need for off-site disposal and treatment and allow for site redevelopment.

**MATERIALS AND METHODS**

**Site description**

This work was conducted in a deactivated industrial site manufacturer of synthetic resins located in southern Brazil. The climate in the region is mesothermic humid with an annual average precipitation of 2,205 mm and average temperature of 22 °C. Soil was composed primarily of unconsolidated sandy-clay sediments. Depth to the water table is approximately 0.2–1.0 m. Groundwater flow was unidirectional, with a seepage (pore) velocity of 56 m year⁻¹ [hydraulic conductivity (3.03 × 10⁻³ cm/s) hydraulic gradient (9.4 × 10⁻³ m/m)/effective porosity (0.16)] in the contaminated area (from MW-1 to MW-2; Figure 1). Phenol was detected in soil (up to 810 mg/kg at the source zone) and groundwater (up to 1,300 mg/L) 20 years after industrial closure. Physical–chemical characteristics of the groundwater were: temperature 16–25 °C, pH 4.5–5.7, dissolved oxygen (DO) 0.2–2.0 mg L⁻¹; nitrate 2.8–3.6 mg L⁻¹; phosphate <0.01 mg L⁻¹, sulfate <1 mg L⁻¹ and phenol 30–1,300 mg L⁻¹.

**Source zone**

An 8 m² portion of the unsaturated aquifer containing entrapped phenol (810 mg/kg detected at depths up to 0.7 m) was identified as the source zone (Figure 1). Based on soil bulk density of 1.6 kg/L, the total mass of phenol remaining at the source zone was estimated as ≈7.2 kg. An area of 52 m² encompassing the source zone was excavated up to a depth of 0.7 m (water table fluctuation between 0.2 and 1.0 m) and thoroughly mixed with the groundwater using a front end loader. Up to four excavations and in situ soil mixing were performed at the site at 6, 70, 111 and 167 days following the start of the nutrient injection (day 456) to promote contaminant mass transfer to groundwater and its bioavailability.

**Nutrient delivery**

A microbial nutrient solution was delivered continuously into the groundwater to enhance phenol biodegradability rates and sustain the increased water-phase contaminant load resultant from soil excavation and mixing. Groundwater monitoring conducted at the site indicated that the phenol contamination plume was limited to the MW-1 and MW-2 vicinity (Figure 1). Two systems were installed in situ (Figure 1). One system was installed 3 m downgradient from the source zone (MW-1). The second system was installed 26 m downgradient from the source zone (MW-2). The stock nutrient solution was prepared every other week by mixing NaNO₃ (188 g-NO₃), MgHPO₄·3H₂O (128 g-PO₄), and NaOH (193 g) in 50 L tap water and into a 60 L polypropylene container. The presence of low oxygen concentration in the groundwater (0.2–2 mg/L) suggested that oxidative conditions prevail in the aquifer even with the relatively high biological oxygen demand (BOD) exacerbated by the consumption of phenol by naturally occurring microorganisms. Therefore, whereas anaerobic phenol biodegradation potential has been demonstrated under sulfate- (Mort & Dean-Ross 1994), nitrate- (Schie & Young 1998; Baek et al. 2003), iron-reducing (Schleinitz et al. 2009) and methanogenic conditions (Béchard et al. 1990), the stimulation of aerobic processes at this particular site was reasonable. Hydrogen peroxide was delivered into groundwater in addition to the stock nutrients solution to aid aerobic biodegradation processes. Hydrogen peroxide was chosen because of its relatively low costs easy to handle, and it is not hazardous when properly used (Howsawkeng et al. 2001; Lin et al. 2004). Hydrogen peroxide (30% v/v) was continuously injected (130 mL/d) in line with the stock nutrient solution delivery hose using a peristaltic pump (Exatta, mod. EX00504).

To delineate the volume of contaminated groundwater, samples were taken from the contaminated areas to the depths at which analysis indicated no further contamination.
Groundwater flow (Q) at MW-1 (1.2 m³/d) and MW-2 (1.7 m³/d) was estimated based on the cross-sectional area of each treatment system (25 m²) × water table depth (1.9 and 2.8 m for MW-1 and MW-2, respectively) × hydraulic conductivity (K = 3.03 × 10⁻³ cm/s) × hydraulic gradient (9.4 × 10⁻³ m/m). This information was used to establish a nutrient delivery rate of 7 L/d for both systems.

Each nutrients delivery system consisted of two rows of five PVC pipes (ø i.d. 30 mm and 2 m length) distanced 1 m from each other and aligned in parallel (Figure 1). The systems were installed perpendicularly to the groundwater flow. The PVC pipes were slot cut at the bottom end (at the last 0.6 m) and capped. The wells were filled with coarse quartz sand to facilitate the distribution of the injected solution. The nutrient solution was transferred from the 60 L container to each of the delivering PVC pipes via silicon hoses (ø i.d. 5 mm). Solution was fed by gravity and proportionally distributed and controlled using adjustable flow restrictors installed at the end of each hose. The solution was delivered continuously into groundwater for 190 days.

First-order decay

Phenol decay rate (k; d⁻¹) at MW-1 and MW-2 was estimated using a simplified first-order kinetic model (Alvarez & Ilman 2005):

\[
\frac{dC}{dt} = -k'C
\]

where \( C \) = phenol concentration (mg/L), \( t \) = time (d) and \( k' \) = (d⁻¹) first-order rate coefficient for phenol removal. This simplified model was chosen because mass transfer limitations that could be rate-limiting at this particular aquifer follow first-order Fickian processes (Simoni et al. 2001).

Chemical analysis

Soil and groundwater samples were collected over time from MW-1 and MW-2 wells and analyzed by a subcontracted laboratory (Natrium Química: www.natriumquimica.com.br). Groundwater sampling was collected according to the Brazilian association of technical rules (ABNT) NBR 9898/87.

Figure 1 | Groundwater phenol concentration at MW-1 and MW-2 ranged from 30 up to 1,300 mg/L. Phenol was found entrapped into the unsaturated aquifer at concentrations from 10 up to 810 mg/kg. Dashed line delineates the sediment mixing boundaries enclosing the contaminant source. Nutrient solution was delivered in the vicinity of MW-1 and MW-2 perpendicular to groundwater flow direction and contaminant plume migration.
Briefly, groundwater samples were collected using Teflon type Bailer and transferred directly into flasks (400 mL) for nitrate and phosphate, (200 mL) for phenol, and (100 mL – sterile flasks) for microbial analyses. Samples for the physical–chemical analyses were immediately preserved at pH < 2 using sulfuric acid solution, stored on ice and transported to the laboratory. pH was measured in situ by potentiometric method using a Digimed pH meter model Dm 20. DO concentration was measured in situ using a portable oxygen meter (YSI 55). Nitrate and phosphate concentrations were determined by colorimetric assays utilizing a Hach DR2800 spectrophotometer by the phenol-disulfonic and 1-amino-2-naphthol-4-sulfonic acid methods, respectively (Standard Methods, APHA 1998). Groundwater phenol concentrations were determined analytically by liquid–liquid extraction and subsequent analysis on a gas chromatography mass/mass (GC/MS Varian 220-MS connected to a Varian 450 GC) (Standard Methods, APHA 1998).

**Microbial analysis**

The most probable number (MPN) culturing method with the non-selective presence–absence broth (Acumedia®) (Standard Methods, APHA 1998) was used to estimate the concentration of Enterobacteriaceae. This family encompasses several bacteria ubiquitous in soil and associated with degradation of phenol and/or its metabolites, including *Klebsiella*, *Citrobacter*, *Shigella* (Shawabkeh et al. 2007; Miller et al. 2004; Narde et al. 2004; Chen et al. 2010; Kafizadeh et al. 2010; Selvakumaran et al. 2011), *Enterobacter* (Thomas et al. 2002), and *Proteus* (Miller et al. 2004). Samples were incubated at 35 ± 0.5 °C for 24 h prior to colony formation unity (Enterobacteriaceae CFU/100 mL) enumeration.

**RESULTS AND DISCUSSION**

Phenol is commonly reported as an easily biodegradable contaminant in soil and groundwater (Mackay et al. 2006). In this study, however, phenol (810 mg/kg-soil) was found entrapped in the unsaturated aquifer and remained persistent to natural attenuation for over 20 years. Phenol persistence was likely associated with high concentrations at the source zone (810 mg/kg or equivalent 1,296 mg/L considering the soil bulk density of 1.6 kg/L), which are reported to exceed toxicity effects to most soil bacteria (i.e. above 600–1,100 mg/L) (Lowry et al. 2009; Nweke & Okpokwasili 2010). The low groundwater pH could also improve phenol’s sorption coefficient ($K_d$) thus contributing to its persistence (e.g.: $K_d$ of 1.76 at pH 4; 1.19 at pH 7; 1.05 at pH 10) (Fiore & Zanetti 2009). Therefore, the entrapped phenol in the unsaturated soil most likely restricted contaminant mass-flux to groundwater (Dragun 1998) and ultimately its bioavailability required for biodegradation.

Although ex situ phenol remediation using land farming soil mixing with addition of nutrients (N-P) has already been demonstrated for phenols (Guerin 1999), this work focused on the *in situ* remediation. *In situ* remediation through source zone sediment mixing coupled to nutrients injection into groundwater was proposed to avoid off-site disposal and treatment. The system was planned to offer simplicity and relatively low costs when compared with others complex physical–chemical strategies. Background groundwater concentrations of phosphate (<0.01 mg/L), nitrate (<4 mg/L), and DO (<2 mg/L) were relatively low to appropriately support significant phenol biodegradation (Figure 1). Therefore, to avoid electron-accepting and/or nutrient limiting conditions, biostimulation was encouraged downgrading from the source zone excavation and sediment mixing to warrant contaminant biodegradation and minimize the potential for plume migration. The high values of hydraulic conductivity ($10^{-4}–10^{-3}$ cm/s) and shallow water table (0.2–1.0 m below soil surface) at the site were also favorable to homogeneous distribution of nutrient solution into the aquifer (Sharma & Reddy 2004). The remediation goal was to achieve phenol concentration below the established specific target level (SSTL) of 4 mg/L.

Groundwater monitoring was conducted for almost 2 years (Figure 2). Prior to nutrient amendment, the median phenol concentration was 131 mg/L at MW-1 and 632 mg/L at MW-2. These concentrations were reduced to 9 mg/L at the MW-1 and <0.01 mg/L at the MW-2 after 150 and 50 days following the start of remediation, respectively (Figure 2). The total mass of nitrate added into groundwater (10.5 kg-N) could account for only 60% of the required mass needed for complete denitrification; i.e. assuming the total mass of phenol at the source ($\approx$7.2 kg) and the stoichiometry ($C_6H_5O_2 + 0.5NH_3 + 3.6NO_3^- + 3.6H^+ \rightarrow 3.5CO_2 + 0.5C_3H_5O_2N + 1.8N_2 + 3.8H_2O$; Schie & Young 1998). With the exception of two data points showing concentrations of DO above 12 mg/L (Figure 2), DO concentrations in groundwater did not exceed solubility levels (8 mg/L) throughout the remediation time frame. This could be attributable to the hydrogen peroxide chemical instability and tendency to rapidly decompose into free and gaseous oxygen. Thus, from the total mass of oxygen added into the aquifer (18.7 kg-O$_2$)
as 30% v/v hydrogen peroxide (theoretical concentration expected in groundwater $\approx$ 31.2 mg/L), only 52% was available as soluble oxygen. Thus, oxygen as the primary source of electron acceptor to aerobic microbial respiration could contribute only 51% of the total phenol removal (i.e. 19 kg-O$_2$ required for complete phenol mineralization according to the half-reaction: C$_6$H$_6$O + 7O$_2$ + 3H$^+$ $\rightarrow$ 6CO$_2$ + 3H$_2$O). Therefore, complete phenol mineralization could only be achieved if both oxygen and nitrate could be simultaneously utilized as a source of electron-acceptors by native bacteria.

The concentration of Enterobacteriaceae present in groundwater samples from the MW-1 and MW-2 were assessed throughout the remediation time frame to provide evidences of phenol biodegradation (Figure 2). Several bacteria belonging to Enterobacteriaceae are ubiquitous in nature (Kafilzadeh et al. 2010) and capable of biodegrading phenol aerobically [e.g. Klebsiella, Citrobacter, Shigella, Proteus (Shawabkeh et al. 2007; Miller et al. 2004; Narde et al. 2004; Chen et al. 2010; Kafilzadeh et al. 2010; Selvakumaran et al. 2011)] or using nitrate as facultative terminal electron acceptor [e.g. Enterobacter (Thomas et al. 2002)]. Background concentration of Enterobacteriaceae in non-contaminated groundwater was 3.7 ± 2.1 CFU/100 mL (data not shown). The highest groundwater bacteria concentration in MW-1 (94.8 CFU/100 mL) and MW-2 (178.8 CFU/100 mL) measured after 40 days following biostimulation coincided with the highest concentrations of phenol (Figure 2). Bacteria concentration decreased over time simultaneously with the depletion of phenol as the main carbon source required to support their growth. We recognize that the low bacteria concentration measured was likely underestimated.
because most subsurface microorganisms [including Enterobacteriaceae (Kolmos et al. 2005)] are attached to surfaces rather than suspended in groundwater samples (Lehman et al. 2001). Nonetheless, these groundwater microbial analyses provided acceptable levels of information regarding the most relevant processes carried out during phenol removal at this site.

Identification of the dominant metabolic processes and by-products (Etchebehere & Tiedje 2005; Schleinitz et al. 2009; Iwai et al. 2011) were beyond the scope of this study. Nonetheless, based on the observed correlation between phenol and Enterobacteriaceae concentrations, two metabolic pathways were postulated: aerobically, through the formation of catechol by phenol-hydroxylase activity (Nair et al. 2008; Heesche-Wagner et al. 1999; Zeyaullah et al. 2009) leading to further oxidative ring cleavage to either muconic acid or 2-hydroxymuconic semialdehyde; or under facultative denitrifying conditions through phenol ring carboxylation to p-hydroxybenzoate (Thomas et al. 2002).

The estimated first-order decay obtained for MW-1 ($k' = 0.01 \text{ d}^{-1}$) and MW-2 ($k' = 0.06 \text{ d}^{-1}$) (Figure 3) were within typical biodegradation rates determined for phenolic hydrocarbons (e.g., 0.01 and 0.9 d$^{-1}$) (Nielsen et al. 1996; Antizar-Ladislao & Galil 2006).

Groundwater pH increased over the remediation time frame from 4.5 to 5.7 to a steady pH of 7 (data now shown). The supply of sodium hydroxide and phosphate buffer (pH 7.2) associated with the consumption of hydrogen ions by denitrification most likely contributed to the observed increase in groundwater pH.

The addition of nutrients into groundwater was discontinued 5 months after reaching the remediation goals (SSTL). Nitrate, oxygen and phosphate concentrations decreased to background levels just after remediation closure. Phenol concentrations in MW-1 and MW-2 did not rebound and remained below SSTL indicating that the remediation was robust (Figure 2).

**CONCLUSIONS**

From a bioremediation perspective, the results obtained from this field scale case-study served to provide supplementary information on phenol behavior, transport and removal rates in contaminated groundwater. Despite the fact that phenol is widely reported as an easily biodegradable compound, its natural attenuation has not been significantly observed over the years without human intervention. Phenol concentration at the source zone exceeded the toxicity levels supported by most soil microbial communities. The entrapped source of contamination into unsaturated soil affected mass-transfer to groundwater and bioavailability. Source zone dislodgment was achieved through sediment excavation and mixing. The application of biostimulation downgrading from the source zone supplied the groundwater with additional (non-limiting) electron acceptor and nutrient concentrations required to warrant complete biodegradation and restrict plume migration. Both the aerobic and facultative nitrate-reducing bacterial respiration mode were likely responsible for the complete phenol biodegradation as suggested by stoichiometric mass balances. Circumstantial evidence to further support aerobic and nitrate-reducing biodegradation activity was demonstrated by the presence of phenol-dependent Enterobacteriaceae in groundwater samples. Overall, in nutrient-limited groundwater, biostimulation served to effectively support the growth of soil bacteria associated with phenol biodegradation (e.g. Enterobacteriaceae) and thus minimize the remediation clean up times associated with natural attenuation alone.

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