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A phenol-contaminated groundwater site: a microbiological perspective

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Abstract The microbiology of groundwater, contaminated with phenol and other tar acids, was analysed using a range of bacteriological, biogeochemical process-related and molecular techniques and the data was used to assess the microbial biodegradative potential in a contaminated plume (Lerner *et al*., 2000). The contaminant plume front was shown to be 500 m from the pollutant source and moving at 10 m year^{-1}. Two multilevel samplers (MLS) positioned in different regions within the plume (boreholes 59 and 60) were constructed (Thornton *et al*., 2001). Activity of the microbial community, as represented by phenol degradation potential and ability to utilise a range of substrates, was found to be influenced by the plume contaminant concentration. The highest phenol concentrations were shown to depress bacterial numbers, in some areas to <10 cells per ml (Pickup *et al.*, 2001). In both MLS, bacterial groups or biogeochemical processes (e.g. methanogenesis, sulphate reduction and denitrification) often associated with anaerobic degradation of contaminants were identified (Pickup *et al.*, 2001). Microbial activity measurements, combined with chemical analyses and hydrological data, allowed an assessment of the effects of the contaminant plume on the groundwater microbiology which could, in turn, be related to the potential for natural attenuation of the site (Pickup *et al.*, 2001).

Key words microbial ecology; natural attenuation; phenol

INTRODUCTION

Natural attenuation of polluted aquifers and monitored natural attenuation has been reported in a number of environments (Stapleton & Sayler, 1998; Thornton *et al.*, 2001; Williams *et al.*, 2001). Bacteriological, molecular analyses and biogeochemical process-related studies were combined with detailed hydrological data in order to assess the potential for attenuation in a contaminated aquifer (Pickup *et al*., 2001). This study focused on a tar acid-polluted aquifer (Lerner *et al*., 2000), which overlies a major UK aquifer of Triassic sandstone. It was chosen because a significant amount of site characterization was already available (Williams *et al*., 2001). Lerner *et al.* (2000) and Williams *et al*. (2001) characterized a major groundwater plume at this site comprising a complex mixture of phenolic compounds derived from the distillation of acidified coal tars.

This paper summarizes the microbial analysis of two areas within the plume sampled using vertical multilevel sampling (MLS) devices constructed within two

boreholes (boreholes 59 and 60; Thornton *et al*., 2001; Pickup *et al*., 2001) and highlights some useful microbiological tools for groundwater analysis.

Bacterial content of samples from MLS

Pumped groundwater samples containing no aquifer matrix material were collected from two MLS (boreholes 59 and 60) located at the contaminated site in May 1998 according to Thornton *et al*. (2001). Total bacterial cell counts were determined in acridine orange stained samples (as described by Miskin *et al*., 1998; Table 1) and 400 cells were counted. The precision at 95% confidence limits was ±10% (Jones, 1979). For borehole 59 (less contaminated than 60), bacterial numbers ranged from 2×10^5 to 7×10^6 cells ml^{-1} with no obvious trend with respect to sample depth or phenol concentration. Samples from borehole 60 contained between 7×10^4 and 6×10^6 cells ml^{-1} apart from samples from 23, 26 and 33 m depth where few cells were detected which coincided with the maximal concentrations of phenol. However, at these depths contaminant levels prevented accurate counting with acridine orange due to the formation of an opaque precipitate but further investigation showed consistently that few cells (<10 cells ml^{-1}) were actually present. Levels 23, 26 and 33 m were flanked by regions containing bacteria in concentrations in excess of 10^5 cells ml^{-1} which reflected lower phenol and total organic carbon concentrations.

Culturability of bacteria within the contaminant plume

In this particular survey culturability was not examined.

Assessment of bacterial enzyme activity

Bacterial enzyme activity was assessed using a modified approach involving the API 20NE system (Biomerieux, France), a commercial test kit, which produces a metabolic fingerprint for each sample. Briefly, this employs 22 independent substrate/enzyme tests arranged in separate wells in a single tray. The microbial community within each sample is therefore challenged with respect to its ability to utilise a range of carbon sources that in turn provides a metabolic fingerprint that reflects the metabolic versatility of the population. Dendogram/cluster analysis can be performed for each test sample and in this way the influence of the *in situ* conditions on metabolic diversity can be monitored (Morgan & Pickup, 1992; Pickup *et al*., 2001). More simply, the API index can be used to score growth on the individual substrates from a minimum value of zero (no growth) to a maximum 49 (growth on all substrates being positive). Using this latter approach, borehole 59 showed a depression of activity between 10 m and 30 m depth (Table 1). For borehole 60, no activity was detected at 23 m and 26 m, this region was flanked at 21 m and 33 m with depressed activity compared to higher activity detected outside this region (Table 1). In each case the depression of activity was associated with increasing phenol concentration. This indicates that the diversity of organisms that are active within the region of high phenol concentration has changed and reduced to a population of active bacteria that grow on a more limited substrate range.

Table 1 Detection of bacteria and associated reductive processes in MLS (Pickup *et al.*, 2001).

Depth below surface (m)	Phenol ($\mu g\ ml^{-1}$)	Direct count (10^4 cells ml^{-1})	API index (range 0–49)	Degradation ^{14}C-phenol (dpm × 10^3) [a]	SRB[b]	Methanogens present[c]	Denitrifying activity[d]
Borehole 59							
59/5	<1	65	46	40	-	+	+
59/8	<1	37	47	3.6	+	+	+
59/10	<1	660	47	79	+	+	+
59/17	50	29	19	2.9	+	+	–
59/23	95	21	20	0	+	+	–
59/30	56	240	43	35	+	+	+
Borehole 60							
60/5	<1	7.1	45	33	+	+	+
60/8	<1	14.6	45	41	+	-	+
60/17	<1	55	33	18	+	+	+
60/23	1007	0	3	2.5	-	+	–
60/26	1004	0	1	2.3	-	+	–
60/33	2665	0	1	0	+	–	–
60/42	73	580	2	2.1	+	+	+
250	52	250	47	5.5	+	+	+

[a] dpm = disintegrations per min determined from evolved $^{14}CO_2$ following degradation of ^{14}C-phenol under aerobic conditions.

[b] Determined using MPN count method (Miskin *et al*., 1998).

[c] Determined using PCR (Hales *et al*., 1996).

[d] Determined using denitrification test of API system (Biomerieux, France; manufacturer's instruction).

Phenol degradation potential

Biodegradation in MLS groundwater samples spiked with phenol-UL-^{14}C under aerobic and anaerobic conditions was used to show the phenol degradation potential of microbial populations at different depths within boreholes 59 and 60 through the production of $^{14}CO_2$ (Pickup *et al*., 2001). In general for both boreholes, the aerobic activity was greater than that obtained under anaerobic conditions (Pickup *et al*., 2001). Both boreholes showed a depression of aerobic and anaerobic activity between 10 m and 30 m (borehole 59) and 17 m and 42 m (borehole 60) (see Table 1 for aerobic values). Regions of higher activity flanked each depression. Similar profiles were obtained with the API data and both could be related directly to the increase in phenol concentration. It should be noted that the differences in degradation potential observed may be due either to depressed bacterial degradative activity caused by the concentration of phenol (plume effect) or a competition effect between the phenol present in the sample and the added radioactive substrate (experimental effect). In the case of the latter, high indigenous concentrations of phenol would result in lower phenol-UL-C^{14} assimilation. This would result in lower $^{14}CO_2$ production compared to less polluted samples and would suggest an apparent depression of activity where as, in reality, the samples may have the same activity. However, given the depression of substrate utilization, cell numbers and culturability, the depression of degradation potential is considered to be a true reflection of the effect of the phenol concentration in the plume rather than an artefact of the experimental system.

Denitrification, sulphate reduction and methanogenic potential

The potential activity of several geochemical cycles was assessed in a number of ways along the vertical transects of each borehole (Table 1). The presence of sulphate-reducing bacteria (SRB) was determined by a culture-dependent MPN method using standard procedures (Pickup *et al.*, 2001). For borehole 59, SRB were detected throughout the MLS apart from the 5 m sample. SRB reached maximum numbers (60 cells ml^{-1}) at 17 m depth, below which they were detectable but in very low numbers (2–15 cells ml^{-1}; Table 1). In borehole 60, SRB were not detected at 23 m and 26 m but, in the flanking regions, their numbers increased the further they were from this region of inactivity to a maximum of 60 cells ml^{-1} (Table 1). Methanogenesis was problematic to measure with methane being released from the control samples even after formaldehyde or oxygen treatment. Therefore no evidence of the active process could be provided unlike the samples in the general site survey which showed regions where methanogenesis was active (Williams *et al*., 2001). However, PCR using methanogen-specific primers (Hales *et al*., 1996) indicated that methanogens were present at all depths in both boreholes, apart from the sample obtained from 33 m in borehole 60 (Table 1). Bacteria capable of reducing nitrate to nitrite and nitrite to nitrogen gas were detected in both boreholes but not at depths 17 and 23 m (borehole 59) and 23, 26 and 33 m (borehole 60; Table 1). This indicated that denitrification, as a process, was potentially active at these depths.

DISCUSSION

Pickup *et al*. (2001) showed, with the results of Lerner *et al.* (2000) and Williams *et al*. (2001), that simple microbiological tools (that ask basic questions such as direct count (what is there in total?), multi-substrate analysis (are they different?) and assessing degradative potential with radiolabelled compounds (what is their activity?)), provide a means of assessing whether a plume is microbiologically "dead", or "inactivated" with the potential for stimulation either by dilution (Lerner *et al.*, 2000) or intervention (Head, 1998). Furthermore, this study showed that in isolation, single microbiological tests could produce ambiguous results (e.g. ^{14}C-phenol). However, taken as a "package", with other microbiological tests, more accurate assessments are possible. These studies (e.g. Pickup *et al*., 2001) could be used to direct such an approach to suitable areas in the plume (Power *et al*., 1998).

This study was directly able to show that in regions where bacteria were present at approximately 10^4 cells ml^{-1} both the anaerobic and aerobic degradation potential and bacteria associated with anaerobic processes such as sulphate-reduction, methanogenesis and denitrification, known to drive degradative processes under anaerobic conditions (Anderson & Lovely, 1997), were present. Chemical analysis showed that the phenol concentrations in borehole 60 were greater than borehole 59 (Thornton *et al*., 2001). The chemical profile of the samples from the MLS showed that the phenol (and total organic carbon) reached a peak between 17 m and 23 m depth for borehole 59, and between 23 m and 33 m depth for borehole 60. The rise in phenol concentration was related to a decrease in degradative activity, catabolic activity and a depression in total bacterial numbers and culturability (Pickup *et al*., 2001). Where

phenol levels were the highest in borehole 60 bacterial numbers were depressed to $<10^2$ cells ml^{-1} and little or no activity was detected in the samples.

Although our data show natural attenuation to be slow or non-existent (Lerner *et al*., 2000; Williams *et al*., 2001), there is potential outside the central core of pollutants for degradation to occur whilst inactivity of the central plume region was shown to be a result of high carbon loading under anoxic conditions (Lerner *et al*., 2000). There is ample evidence of degradation being coupled to oxidative processes such as denitrification (Edwards *et al*., 1992, 1994; Anderson & Lovely, 1998; Rooney-Varga *et al*., 1999). Conditions for coupling degradation to oxidative processes are present and above 25 m are active given, for example, the sulphide:sulphate ratios within the plume (Thornton *et al*., 2001). Furthermore, although sampling procedures excluded measuring methanogenesis as a process due to potential ingress of oxygen (Hall *et al*., 1996), the presence of methanogens was confirmed by PCR (Pickup *et al*., 2001).

The conclusions drawn from these studies were that if the conditions were favourable then the biological components are in place for attenuation to occur apart from in the region of very high total organic carbon (Thornton *et al*., 2001). However, the ratio of plume components (e.g. phenol:cresol) suggested that natural attenuation is not a feasible process to remove the plume contaminants in the timescale required (Williams *et al*., 2001).

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