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Do mature hydrocarbons have an influence on acid rock drainage generation?

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A B S T R A C T

The generation of acid rock drainage (ARD) is a biogeochemical process that causes severe ecological impacts, threatening human health worldwide. Microbes involved in acid drainage reactions are generally considered autotrophic but heterotrophic and mixotrophic microorganisms have often been identified at ARD sites. This raises questions about the role of organic matter naturally present at these sites, such as mature hydrocarbons, in promoting the microbial processes underpinning ARD generation. To investigate this, aerobic microcosm experiments were carried out using ARD samples collected at a well-characterised site in northern England (Mam Tor, Derbyshire). Organic analyses indicated the presence of substantial amounts of mature, petroleum-derived hydrocarbons and microbial analyses indicated that the sediment hosts acidophilic bacteria with the capability of degrading petroleum-derived compounds. However, the aerobic microcosm experiments indicated that these petroleum-derived hydrocarbons were not used by the bacterial community and, therefore, are not involved in the reactions that ultimately lead to ARD generation. These observations support a primary role for autotrophs in ARD generation.

1. Introduction

Acid Rock Drainage (ARD) is an environmental phenomenon characterised by waters of low pH with elevated concentrations of sulfur, iron and other dissolved metals and that commonly arises from the weathering of naturally exposed metal sulfides, especially pyrite. Acid Mine Drainage (AMD) is a similar phenomenon but associated with active or abandoned mine workings. These phenomena threaten human health (Chen et al., 2007; Zhou et al., 2007; Corkhill et al., 2008), have severe ecological effects (Grimalt et al., 1999; Solà et al., 2004) and require costly remediation (Zinck and Griffith, 2013).

The breakdown of pyrite during aqueous oxidation is a relatively complex multistage process (Rimstidt and Vaughan, 2003) which can be simplistically represented by the overall reaction (Singer and Stumm, 1970):

$$4FeS_2 + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_3 + 8SO_4^{-2} + 16H^+$$

The reaction is accelerated in the presence of acidophilic microorganisms such as *Acidithiobacillus ferrooxidans* (Singer and Stumm, 1970), which fixes atmospheric CO_2 as a carbon source and grows using energy obtained from the oxidation of reduced sulfur compounds and/or ferrous iron (Ingledew, 1982; Jones and Kelly, 1983). This chemoautotrophic metabolism can be considered a consequence of the stability and availability of ferrous iron (Roger et al., 2012) and the low carbon conditions of ARD sites (Johnson, 2012). However, heterotrophic and mixotrophic bacteria have been identified in close association with acidophilic chemoautotrophs (Wood and Kelly, 1983; Harrison, 1984; Johnson et al., 1992; Johnson and Bridge, 2002), raising questions about the potential use of other specific carbon sources to mediate microbial processes associated with ARD generation. Mature hydrocarbons have, for instance, been identified in sediments near ARD sites (Pering and Ponnamperuma, 1969; Nooner et al., 1973; Pering, 1973) while a study by Hamamura et al. (2005) detected a microorganism able to degrade alkanes under conditions similar to those in ARD sites. However, it remains unclear whether the occurrence

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of petroleum-derived compounds can be linked to the microbial generation of acidity. To explore these processes, sediments from an ARD site in the UK were collected and analysed to determine the relative importance of petroleum-derived hydrocarbons in these sediments. In addition, the potential role of these mature hydrocarbons in ARD generation was determined via aerobic microcosm experiments.

2. Materials and methods

2.1. Study site and sample collection

The Mam Tor landslide (Derbyshire, UK), formed over 3200 years ago (Donnelly, 2006; Rutter and Green, 2011), has exposed a sequence of shales, siltstones and fine-grained, sole-marked sandstones (the Mam Tor beds) overlying dark pyritic shales and dark grey mudstones (Edale Shales) with the high ground south of Mam Tor formed by limestones (Fig. 1) (Skempton et al., 1989; Waltham and Dixon, 2000). Some of these strata are fossiliferous (Jackson, 1925) and some have been associated with petroleum-derived hydrocarbons (Pering and Ponnamperuma, 1969; Nooner et al., 1973; Pering, 1973). The Mam Tor landslide is still moving at 7 cm/y at the edges and 0.5 m/y in the middle section (Skempton et al., 1989; Waltham and Dixon, 2000; Rutter and Green, 2011), leading to a continuing exposure and breakdown of the source rock and the ongoing generation of ARD (Vear and Curtis, 1981).

Samples of ARD sediment were collected from the surface of an acidic pond in the Mam Tor scarp zone (Fig. 1) using a stainless steel scoop, and transferred into clean (acid washed and prefurnaced) glass jars to minimise the possibility of contamination. Sub-samples for DNA isolation were frozen at −80 °C upon return to the laboratory. Other sub-samples were separated and dried anaerobically for Mössbauer analysis or freeze–dried for organic,

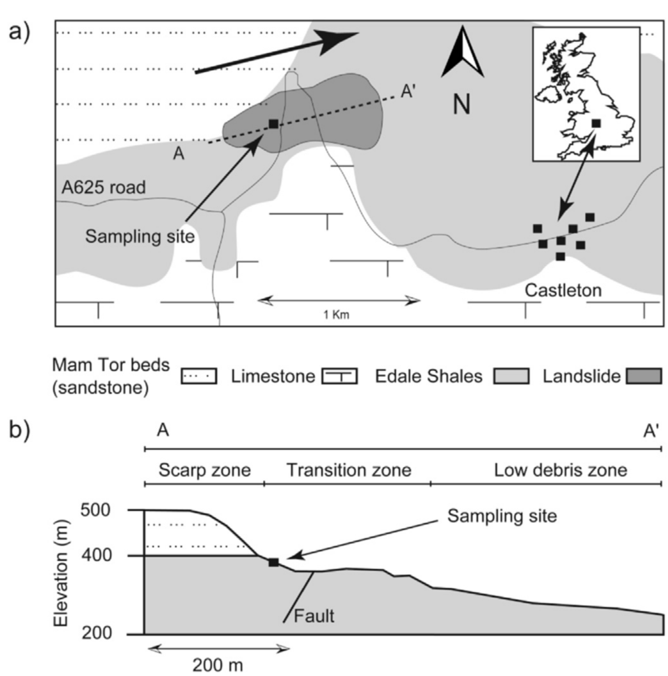

Fig. 1. (a) Location and relevant geology of the Mam Tor landslide, and (b) cross section along the A–A′ line, showing the Mam Tor beds and the Edale Shales; the scarp zone, transition zone, and low debris zones of the landslide are also indicated. The scarp zone comprises the head scar and the scree slope, and the general direction of the landslide is indicated by the bold arrow. Figure modified from Skempton et al. (1989) and Waltham and Dixon (2000).

X-Ray (powder) Diffraction (XRD) and X-Ray Fluorescence (XRF) analyses. The remaining ARD sediment was stored in the dark at 4 °C to minimise any microbial activity until its use in microcosm experiments.

2.2. Aerobic microcosms

To assess the potential role of the petroleum-derived hydrocarbons in the generation of ARD at Mam Tor, a series of aerobic microcosms were set up using appropriate sediment samples and a defined liquid medium (9K; Supplementary information, Table A.1) for acidophilic microorganisms (Silverman and Lundgren, 1959; Johnson, 1995). To distinguish abiotic processes occurring in the system, a series of control samples were also prepared using heat sterilised sediment (121 °C, 30 min). Both the aerobic microcosms and the control experiments were prepared in triplicate using 250 ml of the 9K medium and 2.5 g of either fresh ARD sediment or sterilised ARD sediment. All microcosms were incubated for 28 days at 20 °C using a shaking incubator at 100 rpm.

Slurry samples from the aerobic and control microcosms were taken at times t_0, t_7, t_{14} and t_{28} (0, 7, 14 and 28 incubation days, respectively) for pH, Eh and iron content measurements. The pH and Eh values were measured on a Basic Denver pH/ORP metre (Denver Instrument Company) with 3M KCl liquid-filled electrode Ag/AgCl reference. The rate of Fe(II) oxidation was monitored from the optical absorbance (at 562 nm) of the samples using the ferrozine method (Stookey, 1970) on a Jenway 6715 UV/visible spectrophotometer, with total iron concentration determined after reaction with hydroxylamine hydrochloride (Lovley and Phillips, 1987). Sub-samples taken at t_0 and t_{28} were immediately filtered (22 μm) and used for Volatile Organic Compounds (VOC) determination by Ion Chromatography. Trace amounts of lactate, formate and propionate (values below the typical routine detection limits, approximately 0.01–0.1 mg/l) were produced over incubation, but no individual compounds could be identified accurately due to the interference produced by high concentrations of dissolved sulfate (up to 5700 mg/l). However, it was possible to determine the approximate total VOC concentration based on their summed peak areas, at expected retention times in the IC-chromatograms. After incubation, the triplicates were mixed into clean glass jars and sub-samples were taken and stored at −80 °C for DNA analysis or dried anaerobically for Mössbauer analyses. The rest of the slurries were freeze–dried for organic compound and XRD analyses.

2.3. Mineralogical, geochemical and microbial analyses of Mam Tor sediment and microcosm slurries

The mineralogy and geochemistry of the Mam Tor ARD sediment (at time t_0) and the microcosm slurry samples after incubation (at time t_{28}) were studied using Mössbauer Spectroscopy along with XRF (ARD sediment only) and XRD analyses (see Supplementary information for details). Mössbauer data were collected on anaerobically dried samples at room and liquid nitrogen temperature, on a FAST ComTeC 1024-multichannel analyser system, using a constant acceleration drive with a ~25 mCi 57Co/Rh γ-ray source, and metallic iron foil for calibration. The spectra were fitted using the Lagarec/Rancourt Recoil software (Intelligent Scientific Applications Inc., Ottawa, ON, Canada; v. 1.0). The total organic matter content was quantified using the sequential loss on ignition method (Heiri et al., 2001; Beaudoin, 2003; see Supplementary information for details). To characterise the saturated hydrocarbon (*n*-alkane) fraction of the ARD sediment, a subsample of 40 g was extracted using a Soxhlet apparatus for 24 h with a solution of dichloromethane/methanol (2:1; v/v). In addition, from freeze–dried microcosm slurries (at time t_{28}) aliquots