Biogeochemistry of a subterranean acidic mine water body at an abandoned copper mine

Kris Coupland, Owen Rowe, Kevin B. Hallberg and D. Barrie Johnson

School of Biological Sciences,
University of Wales, Bangor. LL57 2UW, U.K.
Tel: (44) 1248 382358; Fax: (44) 1248 37031
e-mail address: d.b.johnson@bangor.ac.uk

Abstract

Since the 1950's, a large body of acidic (pH ~ 2.5) metal-rich water has accumulated and been retained within the underground workings of the former Parys copper mine, Amlwch, north Wales. Concern that the failure of the concrete plug retaining this water body would result in a catastrophic release of acid water that could cause major flooding and pollution problems in the nearby town of Amlwch, resulted in the commissioning of a dewatering scheme at the abandoned mine. Over a ten week period (April to June, 2003) about 274,000 m^3 of water was pumped out of the old mine workings. The composition of the discharge water was monitored throughout this period, as were changes in chemistry of the water as it flowed down the relatively short distance (about 3 km) to the sea. The subterranean water body contained ca. 500 mg/l of iron (almost exclusively ferrous at source), 50 mg/l of zinc and 30 mg/l of copper and 10 mg/l of manganese. There was virtually no removal of zinc or copper as the water flowed to the sea, so that large quantities of these metals were discharged into the bay at Amlwch. Significant numbers ($>10^4$/ml) of acidophilic iron-oxidisers were present in the subterranean water. Perturbations in the microbial biodiversity of the water body during the pumping operation were assessed using combined cultivation-based (isolation on plates) and cultivation-free (T-RFLP and clone library) techniques. Dewatering of the mine allowed access to previously submerged lower depths within the Parys workings. Biofilms and "acid streamers" were sampled from these zones, and their microbial compositions were studied using T-RFLP.

1 Introduction

Mynydd Parys, located in the north-west corner of Anglesey, Wales (grid reference SH 440 095) is an area with a long history of copper mining, dating back to the Bronze Age (Southwood and Bevins 1995). In its zenith, in the late 18^{th} and early 19^{th} centuries, two adjacent mines were operating at the site (the Parys and Mona mines) and they rapidly developed into Europe's premier copper mines, governing the world price of the metal. The mines went into decline in the mid-1800's, and underground mining ceased in about 1880, though significant quantities of copper (ca. 60 tonnes/annum) were still recovered by leaching and cementation well into the 1950's (Southwood and Bevins 1995). The site itself continues to be a focus of interest for industrial archaeologists, geologists and biologists, due to its many interesting features (Jenkins et al 2000).

During the time it was operating, the deep mines were dewatered using a network of wooden pipes (the highly acidic nature of the water caused rapid corrosion of iron pipes) and was powered by a mix of windmills and steam engines. When deep mining was abandoned, dams were constructed to allow the build up of water in the underground chambers, allowing the mineral-rich mine walls to be subjected to bacterially-accelerated oxidative dissolution. The impounded water was periodically drained into precipitation ponds, where the dissolved copper was precipitated by adding scrap iron. The iron-enriched, copper-depleted mine water was then diverted to larger pits and treated with lime to form ochre, which was recovered as a low value by-product. However, when the precipitation operations were abandoned in the early 1950's, the valves in the concrete dam (at the Dyffryn Adda adit) that controlled the release of the subterranean water were closed, allowing, inadvertently, the water level within the mines to build up. The latter was limited by a second, open adit (the Mona adit) through which the acid mine drainage (AMD) disgorged, via a series of precipitation ponds, forming one source of a stream (the Afon Goch ("red river") south) that flowed in a south-eastern direction, joining the sea at Dulas.

In the late 1990's, a group of mining enthusiasts (the Parys Underground Group; PUG) raised concern about the state of the concrete dam within the Dyffryn Adda adit. There was evidence of severe corrosion and dissolution of the concrete, as would be predicted given the extremely acidic (pH 2.1-2.5) nature of the subterranean water body that it constrained. It was estimated that the water level within the mine was about 31 m above the floor of the dam, and that there was a danger of catastrophic failure of the dam which, if it did occur, would cause severe flooding and pollution of the nearby coastal town of Amlwch. Consequently, a major dewatering exercise was carried out to lower the water level within the interconnected

Parys and Mona mines to the level of the Dyffryn Adda adit. This would facilitate the controlled removal of the dam, and allow future drainage of the mine water through this lower adit. Water from this adit joined another stream, forming the Afon Goch north that flowed north westwards, entering the Irish Sea at Amlwch. The dewatering exercise was undertaken by personnel working for Cementation Skanska, U.K, under supervision of Ove Arup & Partners, U.K.. The operation commenced on April 7th, 2003, by accessing the impounded subterranean water body via an existing shaft (the "Gardd Daniel" shaft). A submersible pump, with a capacity of 50 l/s, coupled to a 100 mm diameter discharge pipe, was sunk into the shaft, and pipework connected to allow the water to flow into the roadside drainage ditch, from which it flowed over the wetland area adjacent to the Afon Goch north (Fig. 1). Pumping rates varied from 20-50 l/s, though the corrosive nature of the mine water caused intermittent problems with the pumps and steel hose connections. Pumping continued until July 12th, 2003, by which time about 274,000 m^3 of AMD had been removed and the water level in the interconnected Parys and Mona mines lowered by 36.5 m. Lowering of the water level within the mines allowed access to new underground areas within the Parys and Mona mines, some of which were noted to contain massive growths of biological materials. Since the completion of mine drainage, the chemistry and biology of AMD flowing out of the Dyffryn Adda adit has been monitored on a monthly basis.

This paper describes the geochemistry of the AMD that was discharged during the dewatering operation, and of the water that has flowed out of the mine (by gravity) since active dewatering ended. The microbiology of these waters, and molecular characterization of the massive microbial growth within the mines that were exposed by the dewatering exercise, are also described.

2 Materials and Methods

2.1 Sampling sites and protocols

Water was sampled at the Mona and Dyffryn Adda adits, prior to the mines being dewatered. During the pumping operation, water samples were taken close to the discharging hosepipe at the Gardd Daniel shaft and, on one occasion, within the mine itself. Since dewatering was completed, subterranean water flowing from the mines has been sampled at the Dyffryn Adda adit. Measurements of pH, redox potential, temperature and conductivity were carried out on site, using a Hanna Water Test field analyser (VWR, U.K.). Dissolved oxygen concentrations were measured using a YSI 95 dissolved oxygen meter (Yellow Springs Instruments, Ohio, U.S.A.).

Other water samples were filtered through 0.2 µm cellulose nitrate membrane filters into sterile bottles (a sub-set acidified for analysis of dissolved metals). Sulfate concentrations were determined turbidimetrically (as $BaSO_4$) using the Hydrocheck system (WPA, U.K.). Dissolved metals (iron, aluminium, copper, manganese and zinc) were analysed using atomic absorption spectrophotometry (SpectrAA Duo, Varian, U.K.). Ferrous iron was determined colormetrically using the FerroZine assay (Lovley and Phillips 1987).

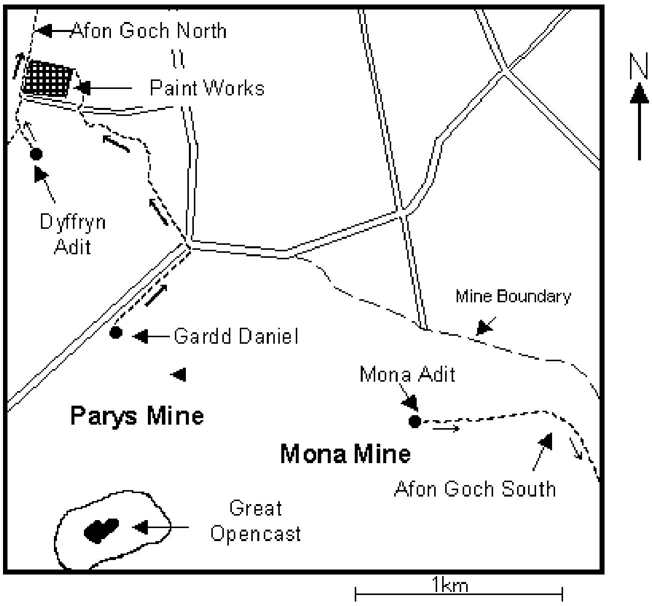

Fig. 1. Map of the Mynydd Parys site detailing the path of discharged AMD from pumping point at the Gardd Daniel shaft to its convergence with the Avon Goch North.

2.2 Microbiological and Biomolecular Techniques

Unfiltered water samples (in sterile polypropylene bottles) were taken for isolation and enumeration of acidophilic microorganisms on solid media. Colonies were enumerated and preliminary identification made on the basis of colony morphologies (Johnson, 1995). Biomolecular analysis of the bacterial populations of AMD and subterranean “acid streamer” growth was carried out using terminal restriction fragment length polymorphism (T-RFLP) analysis, following amplification of eubacterial 16S rRNA genes by Touchdown PCR. Full details of the procedure are given elsewhere (Coupland and Johnson, 2004). Planktonic microbes were harvested on site by filtering 500 ml of water through 0.2 μm cellulose nitrate membrane filters (Millipore, U.S.A.) and DNA extracted using the UltraClean™ Soil DNA Kit (MO BIO Laboratories Inc., USA). DNA was extracted from acid streamer and drapes using the same kit.

3 Results

Prior to the dewatering of the mines, the water body within Mynydd Parys was draining through the Mona adit, which was reflected by the fact that AMD at this point was more acidic (pH 2.7) and metal-rich (e.g. total dissolved iron ca. 490 mg/l) than at the Dyffryn adit (pH 3.2; ca. 13 mg/l iron). The AMD at the Mona adit was also essentially devoid of oxygen (<0.05 mg/l) had a low redox potential (Eh *ca*. +420 mV). Microbial growths at the Mona adit were very evident as macroscopic “acid streamers”, the microbial composition of which has been described elsewhere (Kimura et al. 2003).

Concentrations of iron, sulfate and the major dissolved heavy metals and aluminium in the water pumped out of the mine between April 10th and June 19th 2003, are shown in Figs. 2 and 3. Again, most of the soluble iron in the freshly-discharged AMD was present as ferrous iron, and E_h values were relatively low (+610 to +666 mV), though drainage waters were notably more aerated (41-85% oxygen saturated) than that in the gravity-fed Mona adit, probably as a result of air/water mixing during pumping. Concentrations of most metals (except copper) and of sulfate showed a general increase as time progressed, though there was notably less total and ferrous iron in AMD sampled on May 1st than on other occasions.

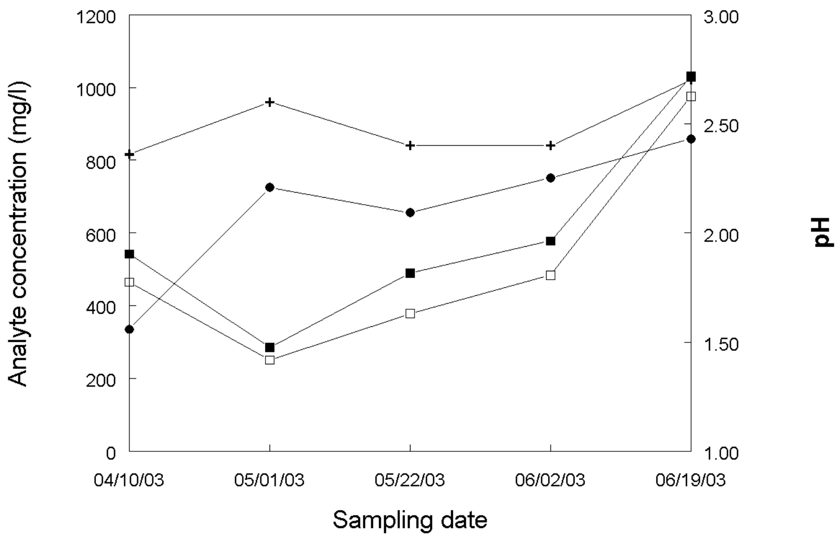

Fig. 2. Concentrations of total iron (■), ferrous iron (□), sulfate (●), and pH (+) of subterranean water discharged from Mynydd Parys during de-watering

Numbers of colony-forming iron-oxidising bacteria in the AMD pumped out of Mynydd Parys varied between 2.3 x 10^3/ml (on June 19th) to 2.7 x 10^4/ml (on May 22nd). Most of these isolates formed colonies that were typical of the iron- and sulfur-oxidiser, *Acidithiobacillus ferrooxidans*. Numbers of heterotrophic acidophiles (*Acidiphilium* and *Acidobacterium*-like colonies) showed a general decrease as pumping progressed, again peaking in the May 22nd sample (1.6 x 10^4/ml) and falling to 40/ml by June 19th. More detailed data on the bacterial diversity in pumped AMD came from analysis of 16S rRNA gene amplicons using T-RFLP. The dominant terminal restriction fragment (T-RF) was confirmed as corresponding to that of *At. ferrooxidans*. Other iron-oxidising bacteria were also detected

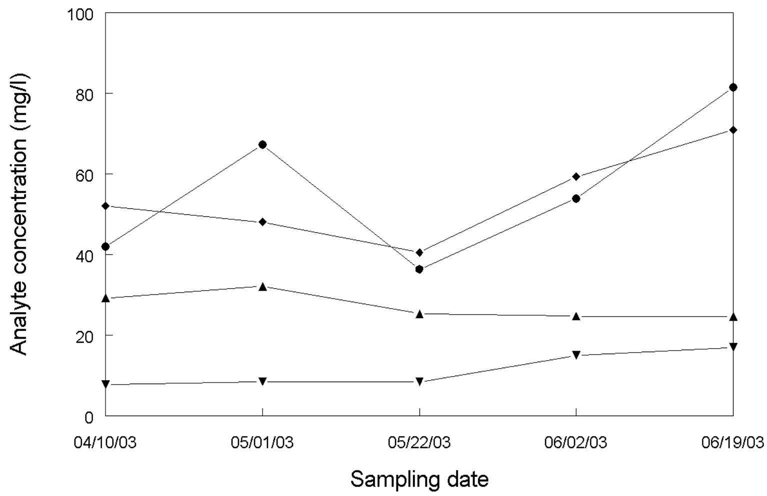

Fig. 3. Concentrations of copper (▲), zinc (◆), aluminium (●), and manganese (▼) of subterranean water discharged from Mynydd Parys during dewatering

from their T-RF's: *Leptospirillum ferrooxidans*, "*Ferrimicrobium acidiphilum*" and a *Gallionella*-like clone. Non-iron-oxidising heterotrophic bacteria detected from their signal T-RF's were an *Acidiphilium* sp., an *Acidocella* sp., and an *Acidobacterium* sp.. A few T-RF's were not identified.

Very large and extensive microbial growths, referred to by PUG members as "drapes", were found hanging from residual wooden structures within the underground chambers of the Parys mine as the water level fell. Amplification of 16S rRNA genes using eubacterial primers revealed that these were a complex community including iron-oxidizing bacteria (*At. ferrooxidans* and a γ-proteobacterium related to isolate WJ2, previously noted in AMD at the Wheal Jane tin mine, Cornwall (Hallberg and Johnson, 2003) heterotrophic bacteria (*Acidobacterium* and *Acidisphaera* spp.) and at least one actinomycete.

The subterranean AMD discharge from Mynydd Parys since dewatering ceased and water flow diverted through the Dyffryn Adda adit (and from there to the Afon Goch north) has been monitored on a regular basis. The chemistry of the water in this adit is now similar to that found previously in the Mona adit; changes in the physico-chemistry of the discharging AMD are shown in Fig. 4. The stream outside of the adit entrance has become very rapidly colonised with macroscopic acid streamer growths. Analysis of these materials (T-RFLP analysis of amplified 16S rRNA

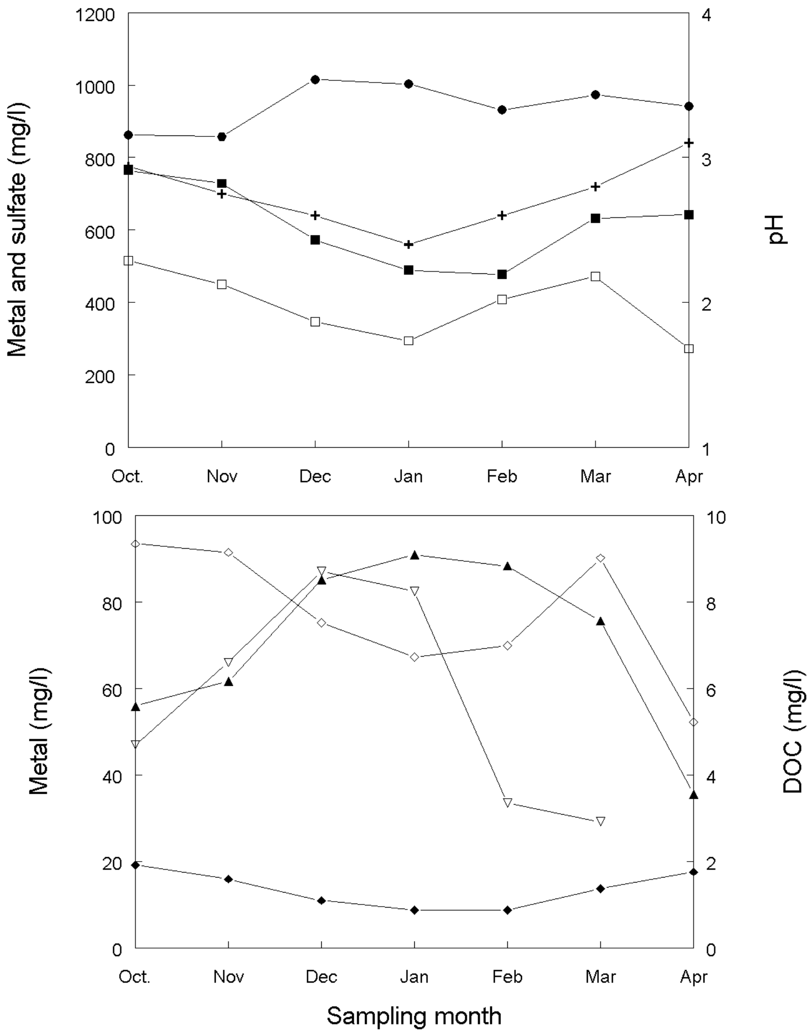

Fig. 4. Changes in pH, and concentrations of dissolved metals, sulfate and organic carbon in water draining Mynydd Parys (sampled at the Mynydd Adda adit) since the mines were dewatered. The period covered is from October 2003 until April 2004. Key: pH (+); total iron (■); ferrous iron (□); sulfate-S (●); copper (▲); zinc (◇); manganese (◆); DOC (▽).

genes) has indicated that the microbial communities that these growths represent have shown subtle changes over six months of monitoring. Initially (in October 2003), the streamers comprised a heterogeneous group of bacteria, including *At. ferrooxidans*, *Leptospirillum ferrooxidans, Gallionella* sp., *Thiomonas* sp., *"Ferrimicrobium"* sp. and a bacterium closely related to a clone (TRA3-20) identified in AMD within Iron Mountain, California (Edwards et al. 1999). Community profiling over the following months revealed the emergence of TRA3-20 as the dominant microbe, accounting for approx 70% of the streamer community by the December sampling occasion. All other microbes declined in relative abundance to very low levels, with the exception of *At. ferrooxidans* which maintained its abundance at around 10-15% of the microbial community.

4 Discussion

Mynydd Parys was one of the earliest sites in the world where mineral bioleaching was practiced, long before the potential of microorganisms to accelerate the oxidative dissolution of sulfidic mineral was realised. Production of cement copper by precipitating the microbially-solubilised metal from acidic waters started very early in the modern phase (late 19th century onwards) of the mines' history. It was the abandonment of this practice, and permanent closure of the valves in the Dyffryn Adda adit, that led to the flooding and pollution hazard that was recognized some 50 years later. Dewatering of the mines proceeded rapidly, and there was no serious attempt to remediate the AMD that was pumped out. As a consequence, an estimated 7.5 t of copper, 3.1 t of manganese and 14.8 t of zinc was released into the Irish Sea from Mynydd Parys during the 14 weeks when the mines were dewatered (Coupland and Johnson 2004).

Concentrations of dissolved metals and of sulfate in the water pumped out of Mynydd Parys changed during the dewatering period, possibly due to stratification of the subterranean water body and also to temporary contributions from geochemically-variable adits and larger chambers that later became isolated as the water level continued to fall. The bottom of the pipe through which the subterranean water was evacuated was maintained a meter or so below the water table, so as to minimise disturbing any sediment, so the AMD pumped out at any time was always close to the surface of the impounded water body. The water pumped out of Mynydd Parys remained extremely acidic (pH <3) and metal- and sulfate-rich throughout the period of dewatering.

The dominant iron-oxidising bacterium isolated on solid media and also detected by a cultivation-independent approach (T-RFLP) was *At. fer-*

rooxidans. In a earlier study of the Afon Goch south, another iron-oxidiser (*L. ferrooxidans)* was found to be more abundant than *At. ferrooxidans* (Walton and Johnson 1992). One reason for this difference is that *L. ferrooxidans* is an obligate aerobe, whilst *At. ferrooxidans* is a facultative anaerobe, and can couple the oxidation of a range of electron donors (e.g. sulfur compounds and hydrogen) to the reduction of ferric iron. The dominance of *At. ferrooxidans* in the subterranean water body within Mynydd Parys which contained little or no dissolved oxygen (the water at the Gardd Daniel sampling station being aerated by the pumping process) suggests that mostly it was growing anaerobically in the underground AMD. The other iron-oxidising acidophile detected, "*Ferrimicrobium acidiphilum*", can also grow by ferric iron reduction though this bacterium is heterotrophic, in contrast to both *At. ferrooxidans* and *L. ferrooxidans* which are both autotrophs (Johnson and Hallberg 2003).

Other acidophilic bacteria detected in the pumped water and in microbial "drapes" exposed by the falling water level within the mine were obligate heterotrophs (*Acidiphilium*, *Acidisphaera* and *Acidobacterium* spp.) that do not oxidise ferrous iron, though some are able to reduce ferric iron under oxygen-limiting conditions. The presence of these bacteria at Mynydd Parys was not unexpected as similar bacteria have been detected in other metal-rich mine waters (Johnson and Hallberg 2003). A particularly interesting discovery was the presence of *Gallionella*-like clones in the pumped water and "acid streamers" in the Afon Goch north. The only characterized species of this genus is G. *ferruginea*, a neutrophilic iron-oxidiser that grows optimally in microaerobic environments (Hanert 1989). It is notable that this organism became less abundant in the streamers over the period of study, possibly reflecting a relatively greater sensitivity to extreme acidity than other members of the microbial community. The dominant organism in the newly emerging acid streamers is very closely related to the TRA3-20 clone from Iron Mountain, California, and has yet to be isolated and characterized.

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