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# Evidence of Ancient Microbial Activity on Mars

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## ABSTRACT

We report for the first time *in situ* observations of a relatively rare secondary iron arsenate-sulphate mineral named bukovskýite –  $\text{Fe}^{3+}_2(\text{As}^{5+}\text{O}_4)(\text{S}^{6+}\text{O}_4)(\text{OH})\cdot 7(\text{H}_2\text{O})$  - found in a shock melt vein of the Tissint Martian meteorite. It is hypothesised that the mineral formed when high concentrations of aqueous  $\text{H}^+$ , Fe(III),  $\text{SO}_4$  and  $\text{AsO}_4$  were maintained for long periods of time in microenvironments created within wet subsurface Martian clays. The aqueous  $\text{H}^+$ , Fe(III),  $\text{SO}_4$  and  $\text{AsO}_4$  species arose from the microbial oxidation of  $\text{FeS}_2$  with concurrent release of sequestered As. The availability of aqueous  $\text{AsO}_4$  would also be complemented by dissolution by-products of the microbial reduction of Feoxides influenced by dissolved organic matter that alters the redox state and the complexation of As, thus shifting As partitioning in favour of the solute phase. This hypothesis is substantially supported by SEM analysis of a 15 $\mu\text{m}$  spherical structure comprising of a carbonaceous outer coating with an inner core of  $\text{FeS}_2$  (pyrite) that showed the pyrite surface with spherical pits, and chains of pits, with morphologies distinct from abiotic alteration features. The pits and channels have a clustered, geometric distribution, typical of microbial activity, and are closely comparable to biologically mediated microstructures created by Fe- and S-oxidising microbes in the laboratory. These microstructures are interpreted as trace fossils resulting from the attachment of bacteria to the pyrite surfaces.

**Keywords:** Tissint, Meteorite, Mars, Carbonaceous, Pyrite, Bacterial Etch Pits, Bukovskýite

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## 1. INTRODUCTION

Speculation about the source and nature of the organic carbon component in Martian meteorites continues. Competing theories include terrestrial contamination (Jull *et al.*, 1998; Stephan *et al.*, 2003), abiotic geophysical processes (Treiman, 2003; Zolotov and Shock, 2000; Steele *et al.*, 2007) and the detritus of past life (McKay, 1996). The new Tissint Martian meteorite offers a rare opportunity to examine the carbon component of a fresh, relatively uncontaminated, Martian basalt. Stepped combustion mass spectrometry has confirmed that much of Tissint's organic material is of Martian origin with total carbon abundances of 173 ppm ( $\delta^{13}\text{C} = -26.6 \text{ ‰}$ ) and 12.7 ppm nitrogen ( $\delta^{15}\text{N} = -4.5 \text{ ‰}$ ) (Aoudjehane *et al.*, 2012).

An abiotic (igneous) origin for the reduced organic component in Tissint was initially proposed by Steel *et al.*, (2012). This was based on observations of macro molecular carbon inclusions within feldspathic glass phases in a number of

Martian meteorites. Subsequent investigations led the authors to revise this interpretation and current thinking now favours fluid precipitation (Steele *et al.*, 2013). An independent study by Lin *et al.*, (2013) found two petrographic settings for organic carbon in Tissint, characterised by a) the complete filling of fractures in olivine and pyroxene and b) an entrained component in shock-melt veins. Raman spectral analysis indicated a kerogen-like material with elemental ratios of H, N, O, S, P, F and Cl to C comparable to the coal reference and distinctly higher than graphite standards. C isotope analysis revealed the organic carbon component was characterised by light C isotopes ( $\delta^{13}\text{C} = -13.0$  to  $-33$  ‰) which accorded well with the earlier isotope data from Aoudjehane *et al.*, (2012). The clear petrographic settings for the organic carbon together with the C isotope results led the authors to conclude that a) the organic carbon component was deposited from organic-rich fluids and was not magmatic in origin, b) was unlikely to have originated from chondritic debris and c) was more likely of biogenic origin (Lin *et al.*, 2013).

Our earlier studies of the Tissint Martian meteorite are consistent with the latest findings of Lin *et al.*, (2013) and Steele *et al.*, (2013). We identified the presence of a number of 5-50 $\mu\text{m}$  carbonaceous globules both embedded in the rough pyroxene substrate but also in an unbound state, having been dislodged during the experimental fracturing process (Wallis *et al.*, 2012). SEM and EDS elemental spectra for 11 selected globules confirmed that they comprise of a carbonaceous outer coating with an inner core of  $\text{FeS}_2$  (pyrite) and are characterised as immiscible globules with curved boundaries (Wallis *et al.*, 2014a). Raman spectral characteristics imply a level of crystallinity and disorder of the carbon component consistent with previous reports of organic carbon in Tissint (Steele *et al.*, 2012). D-band spectral characteristics further imply a complex precursor carbon component comparable to the precursor component of materials of known biotic source (plants, algae, fungi, crustaceans, prokaryotes). Correlation between peak metamorphic temperatures and Raman D-band ( $\Gamma_{\text{D}}$ ) parameters further indicate that the carbonaceous component was subjected to a peak temperature of  $\sim 250$  °C suggesting a possible link with the hydrothermal precipitation processes responsible for the formation of remarkably similar structures observed in hydrothermal calcite veins in central Ireland (Lindgren *et al.*, 2011). This link was confirmed by the petrographic setting of the organic carbon component that showed examples of organic carbon completely occupying the cracks and cleavage around pyrite crystals, suggesting that pyrite had acted as an attractive substrate for the collection of organic material in a hydrothermal setting.

In this study we report the results of SEM, optical microscopy and Raman spectroscopy on the Tissint Martian meteorite. Our results show the presence of a relatively rare secondary iron arsenate-sulphate mineral found in a shock melt vein of the meteorite. The presence of this mineral is interpreted as arising from microbial activity, a position substantially supported by SEM images of a pyrite grain showing morphological alteration features distinct from abiotic alteration features, and closely comparable to biologically mediated microstructures created by Fe- and S-oxidising microbes in the laboratory. These results confirm the observations and interpretations of Steel *et al.*, (2013) and Lin *et al.*, (2013) insofar as Tissint's organic carbon component arose from fluid precipitation. More significantly, they support the conclusions of Lin *et al.*, (2013) that the precipitated organic carbon component had a biogenic origin. Direct evidence of the activity of Fe- and S-oxidising microbes on pyrite surfaces strongly supports the findings of McKay *et al.*, (1996).

## 2. EXPERIMENTAL METHODS

To further examine the viability of the hypothesis being considered, scanning electron microscopy was utilised to first identify the presence of enriched arsenic domains in one polished 30 $\mu\text{m}$  thin section (section A) and one polished thick section (Section C) of Tissint that were prepared at the School of Earth and Ocean Sciences, Cardiff University. Details of these sections are provided in Wallis *et al.*, (2014a). Once identified, Raman spectroscopy was then utilised to constrain the observed mineral species.

Further SEM studies were undertaken for the purpose of identifying the presence of bacterial etch pits in the surface of pyrite grains present in the meteorite. SEM studies were conducted using the FEI (Phillips) XL30 FEG ESEM (Environmental Scanning Electron Microscope) FEG (Field Emission Gun) at the School of Earth and Ocean Sciences at Cardiff University. The unit incorporates a secondary electron detector (SE), a back scatter electron detector (BSE) and a gaseous secondary electron detector (GSE). It also has an Oxford Instruments INCA ENERGY (EDX) x-ray analysis system. Image recording is via a SONY video graphics printer or digital by processing image frames in a 16 bit framestore computer for output to hard drive. Optical microscopy was conducted using an RM-1POL polarising light microscope with

360° rotating analyser. Image capture was via a 9M pixel digital trinocular camera unit processed by View7 PC software for output to hard drive.

Independent studies using a separately sourced sample were undertaken using a Hitachi S-3700N Field Emission Scanning Electron Microscope at the NASA Marshall Space Flight Centre. High resolution Raman spectra were acquired using a Renishaw inVia microRaman system at the School of Chemistry, Cardiff University, UK. Laser focusing of the sample was performed using a research grade Leica analytical microscope fitted with a 100x objective and a 514.5 nm Argon laser line. Data processing was via the Wire2 software application for data output to hard drive.

### 3. RESULTS

An arsenic enriched domain was observed in EDAX elemental maps in the vicinity of a ~10 µm wide glass shock vein. Black glass melt pockets and veins are characteristic of Tissint. Figure 1 (a) shows an optical light montage in cross polarised transmitted light of a typical glass shock vein cutting through the pyroxene groundmass and passing through an olivine crystal. The white grains are maskelynite while the glass shock veins show up as black. Figure 1 (b) shows a similar image of the shock vein containing the arsenic signature with the site of interest highlighted. Also shown beneath it is a typical olivine crystal with a melt inclusion and radiating cracks. Figure 1 (c) shows an enlarged image of the vein now seen to enclose a mineral inclusion that is again shown in higher magnification in figure 1 (d).

Raman spectroscopy provided an unambiguous identification of the glass melt inclusion as comprising of an iron arsenate sulphate mineral (bukovskýite). Figure 1 (e) shows Raman spectra of the grain compared with similar spectra (Ruff ID: R050630) for the iron arsenate sulfate mineral, bukovskýite –  $\text{Fe}_3+2(\text{As}_5+\text{O}_4)(\text{S}_6+\text{O}_4)(\text{OH})\cdot 7(\text{H}_2\text{O})$  – sourced from Kutná Hora, Bohemia, Czechoslovakia by the University of Arizona Mineral Museum. Comparison of the spectra provides an unambiguous identification with distinct bands in the 1000-1200  $\text{cm}^{-1}$  range - assigned to the  $\nu_3$  ( $\text{SO}_4$ )<sup>2-</sup> antisymmetric stretch ( $\delta$ -Fe-OH bend) - with corresponding coincident bands in the 900-1000  $\text{cm}^{-1}$  range (assigned to  $\nu_3$  ( $\text{AsO}_4$ )<sup>3-</sup> antisymmetric stretch or libration of water molecules and the  $\nu_1$  ( $\text{SO}_4$ )<sup>2-</sup> symmetric stretch) (Loun *et al.*, 2011).

Further strong bands assigned to the  $\nu_3$  and  $\nu_1$  ( $\text{AsO}_4$ )<sup>3-</sup> antisymmetric and symmetric stretch at 800-900  $\text{cm}^{-1}$  together with lower wavelength bands at ~400  $\text{cm}^{-1}$  assigned to the  $\nu_2$  ( $\text{SO}_4$ )<sup>2-</sup> bend, the  $\nu_4$  ( $\text{AsO}_4$ )<sup>3-</sup> bend or the Fe-OH and Fe-O stretch were similarly present. One prominent feature at ~ 1040  $\text{cm}^{-1}$  that is assigned to the  $\nu_3$  ( $\text{SO}_4$ )<sup>2-</sup> antisymmetric stretch ( $\delta$ -Fe-OH bend) (Loun *et al.*, 2011) was evident in the observed spectra but was only observed in weakened form.

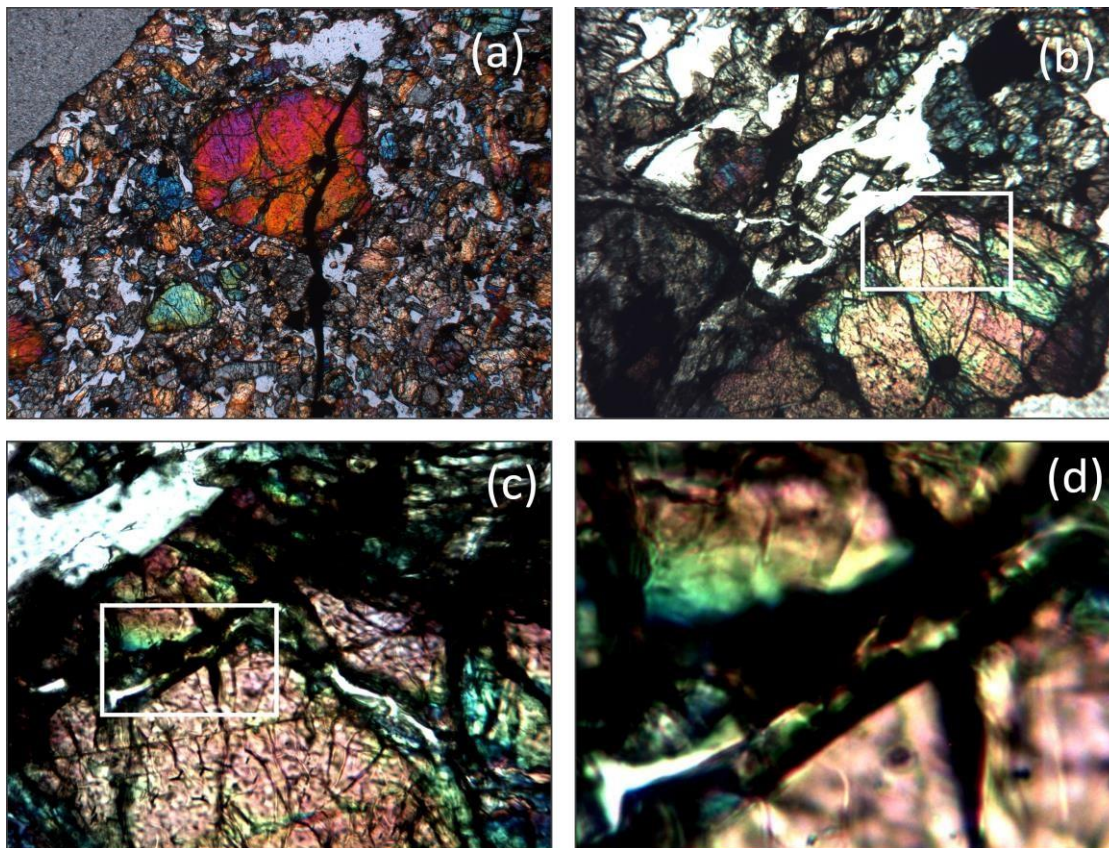
The presence of a secondary iron arsenate-sulphate in the Tissint Martian meteorite suggests an As mobilisation and sequestration process operating in the surface or near surface layers of Mars. The ecology of arsenic on early Mars could provide evidence of previous microbial activity or in the alternative constrain our understanding of early Martian surface conditions.

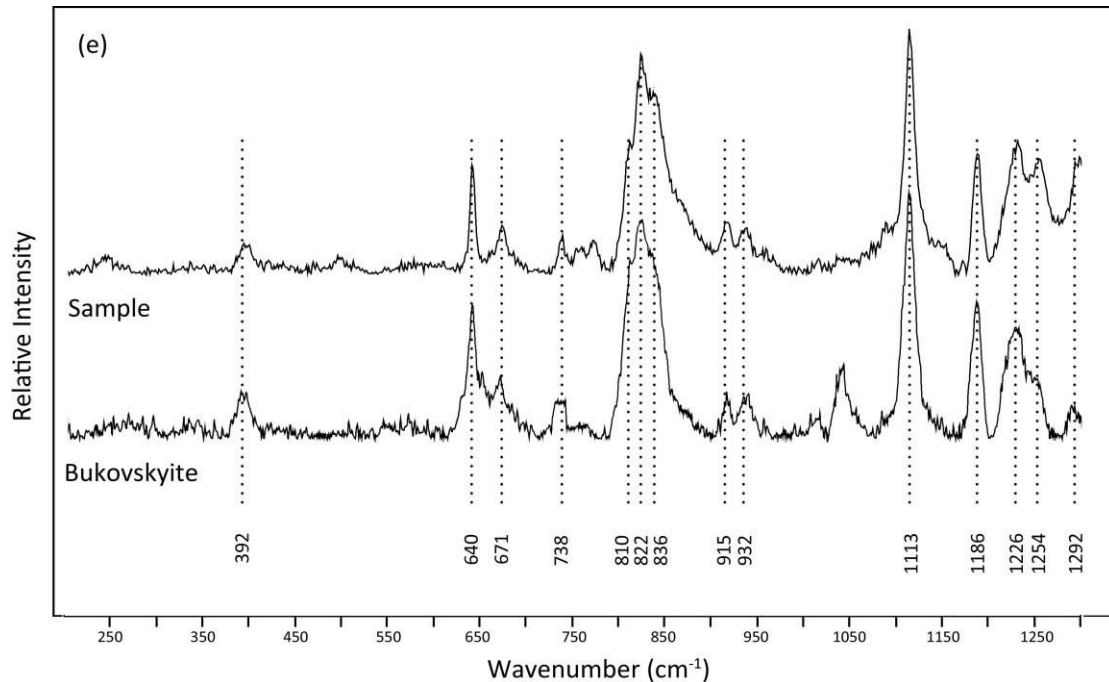
### 4. BUKOVSKÝITE

Bukovskýite is a relatively rare secondary iron arsenate-sulphate first analysed by Antonin Bukovský (Bukovský, 1915) and later by Slavik (1925) and Ulrich (1930). Its structure consists of octahedral-tetrahedral Fe-arsenate chains with sulphate tetrahedral bonded to the chains and free H<sub>2</sub>O molecules via a complicated network of hydrogen bonds (Majzlan *et al.*, 2012). It occurs in substantial quantities as the main secondary mineral in medieval dumps near the municipality of Kutná Hora, in the Czech Republic. While it has also been described at several other localities, Kutná Hora is the only site where the mineral occurs in substantial quantities. Novák *et al.*, (1967) conducted the first modern description of bukovskýite using X-ray diffraction, chemical analysis, TEM, and IR spectroscopy. At the time it was proposed that bukovskýite was the arsenate analogue of destinezite ( $\text{Fe}_2(\text{PO}_4)(\text{SO}_4)(\text{OH})\cdot 6\text{H}_2\text{O}$ ), but this has since been refuted by Johan (1986) and it is now considered an alteration product of arsenopyrite.

The oxidation of arsenopyrite has been the subject of several laboratory studies (e.g. Buckley and Walker, 1988; Richardson and Vaughan, 1989; Nesbit *et al.*, 1995; Nesbit and Muir, 1998). Further studies have concentrated on the precipitation mechanisms and stability of scorodite ( $\text{FeAsO}_4\cdot\text{H}_2\text{O}$ ) - the most common arsenopyrite oxidation product (e.g.

Dove and Rimstidt, 1985; Nordstrom and Parks, 1987; Krause and Ettl, 1988). Haffert *et al.*, (2010) reported the presence of bukovskýite at the Golden Port mine in New Zealand, where it occurs with scorodite and As-rich hydrous ferric oxides on the interior of waste dumps. These authors concluded that bukovskýite and zýkaite arose in the Golden Port mine as products of weathering resulting in elevated concentrations of sulphates that remained wet most of the time.





**Figure 1 (upper)** shows optical light images of a 30 $\mu\text{m}$  thin section of Tissint in cross polarised transmitted light. Black glass shock veins and pockets show up as black with maskelynite phases in white. (a) typical glass shock vein cutting through the pyroxene groundmass and olivine crystal (b) shock vein containing the arsenic signature with the site of interest highlighted, note also the presence of a typical olivine crystal with a melt inclusion and radiating cracks (c) enlarged image of the vein showing enclosed mineral inclusion and (d) same, in higher magnification. Field of view (a) 2.5mm (b) 1mm (c) 250  $\mu\text{m}$  (d) 100  $\mu\text{m}$ . **(Lower)** shows Raman spectra of mineral grain compared with similar spectra (Ruff ID: R050630) for the iron arsenate sulfate mineral, bukovskýite –  $\text{Fe}^{3+}_2(\text{As}^{5+}\text{O}_4)(\text{S}^{6+}\text{O}_4)(\text{OH})\cdot 7(\text{H}_2\text{O})$  – taken from Kutna Hora, Bohemia, Czechoslovakia. Sample courtesy of the University of Arizona Mineral Museum.

In contrast, Majzlan *et al.*, (2012) found that the Fe:As:S (2:1:1) ratio of bukovskýite precipitating gels varied widely and were depleted in S relative to the ideal 2:1:1 stoichiometry. They concluded that bukovskýite cannot originate by simple weathering and decomposition of pyrite or arsenopyrite and that some additional control over the chemical composition and final crystallisation mechanisms must exist (Majzlan *et al.*, 2012).

There are a number of examples of the formation of trace bukovskýite occurring that involve biological processes, most notably biologically induced mineralisation. Márquez *et al.*, (2006) studied a two-step oxidation pre-treatment process involving a combination of bacterial and pressure oxidation of ores mined in the São Bento deposits in Brazil. They observed the presence of minor and trace amounts of zykaite ( $\text{Fe}_4(\text{AsO}_4)_3(\text{SO}_4)(\text{OH})\cdot 15 \text{H}_2\text{O}$ ) and bukovskýite as secondary products of the bacterial oxidation of gold ores. At the type locality of the Kaňk deposit in the northern part of Kutná Hora, two types of associations and dumps are distinguishable (Majzlan *et al.*, 2012). The first type are composed of large rock fragments with open space between them and are associated with scorodite, kaňkite and zykaite with nonarsenate secondary minerals including gypsum and jarosite. In the second type the rock fragments are completely filled by earthy to clayey material together with anthropogenic material including charcoal, timbers and burnt clay. These areas are associated with abundant bukovskýite and rare parascorodite ( $\text{FeAsO}_4\cdot \text{H}_2\text{O}$ ). This observation has led to the suggestion that clay minerals create sealed microenvironments where high concentrations of aqueous  $\text{H}^+$ , Fe(III),  $\text{SO}_4$  and  $\text{AsO}_4$  are maintained for long periods of time (Majzlan *et al.*, 2012).

While the precise processes responsible for the formation of bukovskýite remain unknown, it is not possible to unambiguously associate the formation of this mineral with biological activity. Nonetheless, the initial mobilisation of arsenic from As enriched Fe oxides, the subsequent sequestration of As by precipitating sulphides under anoxic conditions

followed by the oxidation to sulphide of sulphate species and the formation of relatively rare As secondary phases are all processes commonly associated with bacterial activity.

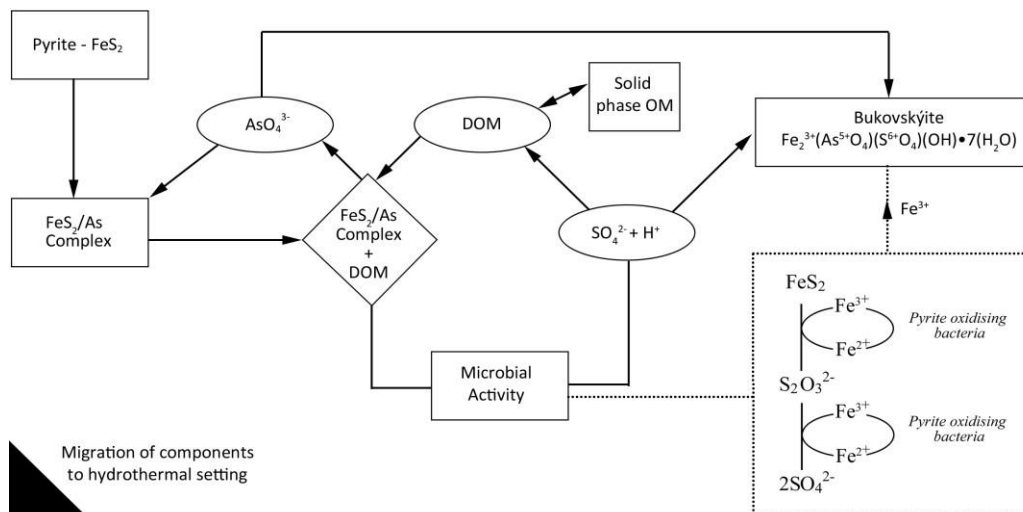
## 5. A MICROBIAL MODEL FOR BUKOVSKÝITE ON MARS

The working hypothesis considered here is that  $\text{Fe}^{3+}_2(\text{As}^{5+}_2\text{O}_4)(\text{S}^{6+}_2\text{O}_4)(\text{OH})\cdot 7(\text{H}_2\text{O})$  (bukovskýite) formed when high concentrations of aqueous  $\text{H}^+$ ,  $\text{Fe}(\text{III})$ ,  $\text{SO}_4$  and  $\text{AsO}_4$  were maintained for long periods of time in microenvironments created in wet surface and subsurface Martian clays. These ingredients arose as dissolution products of the microbial reduction of Fe-oxides and subsequent microbial oxidation of  $\text{FeS}_2$  with concurrent release of sequestered As to solute phase.

Arsenic (As) is a ubiquitous element known to have a high affinity for newly precipitating Fe oxides where it is both incorporated and subsequently stabilised within the mineral structure during aging and transformation (Jessen *et al.*, 2005; Roberts *et al.*, 2004). The sorption of As on Fe oxides occurs in both ionic outer, and specific inner, surface complexes (Sun and Doner, 1998; Waychunas *et al.*, 1993) resulting in solid phase As enrichment in Fe oxide precipitating sediments.

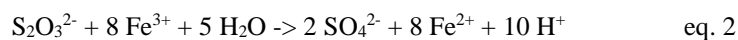
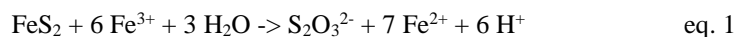
The potential for humic substances to influence the redox state and the complexation of As, thus shifting As partitioning in favour of the solute phase is commonly seen on Earth. In the sediments of South Asia, hot spots of As enriched aquifers are commonly observed in the vicinity of organic rich buried peat layers. These peat layers comprise of a complex mixture of polyfunctional organic molecules derived from the decomposition of plants, animals and microorganisms (Wang and Mulligan, 2006). While the precise composition of these mixtures varies, they all share common moieties such as polar carboxyl, hydroxyl, amino, sulfhydryl and phenol groups (Aiken *et al.*, 1985). Increasing concentrations of these common moieties were found to increase the chemical reduction of  $\text{Fe}(\text{III})$  complexes and oxidise  $\text{H}_2\text{S}$ , supporting the assumption that they are indeed the redox active moieties involved in the redox reactions (Bauer, 2008).

In our model, accumulated sedimentary organic material would react with Fe oxides resulting in the mobilisation of arsenic. Under anoxic conditions, sequestration of As may arise from As precipitation in sulphide compounds, the formation of thioarsenic aqueous species or As adsorption (and possibly co-precipitation) onto  $\text{Fe}(\text{II})$  sulphides (Henke, 2009) forming, for example,  $\text{FeS}_2/\text{As}$  complexes. Figure 3 provides a notional representation of pyrite surface sites and different kinds of interactions namely a) outer-sphere complexation of a non-specific nature and b) inner-sphere complexation of a metal. In this case the figures shows specific interaction with a sulphur surface site.

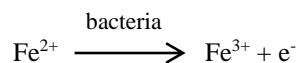


**Figure 2.** Schematic representation of the proposed chemical processes that result in the formation of bukovskýite in closed microenvironments. The mechanism proceeds via anoxic microbial pyrite oxidation and culminates in the accumulation of bukovskýite forming ingredients and conditions. Components of the model would then migrate from sedimentary layers to a hydrothermal environment where organic matter would precipitate on pyrite grains.

The availability of aqueous  $\text{AsO}_4$  would be complemented by dissolution by-products of pyrite oxidising bacteria giving rise to a ready supply of  $\text{H}^+$ ,  $\text{Fe(III)}$  and  $\text{SO}_4$  species as indicated by the following chemical reactions:



The iron (II) ions are oxidised by the bacteria:

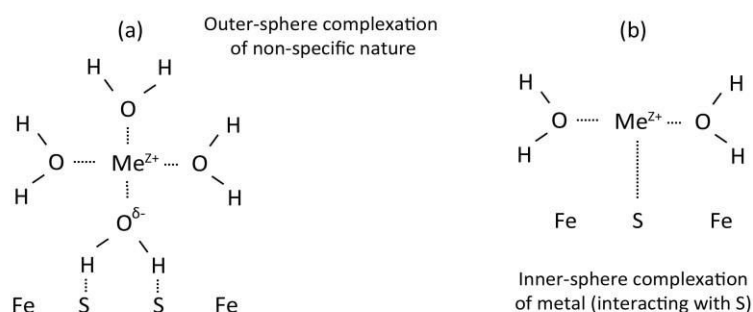


High concentrations of these species together with  $\text{AsO}_4$  would then be maintained for long periods of time in microenvironments supported in fine Martian clays. Figure 2 shows a schematic representation of the proposed chemical processes resulting in the formation of bukovskyite. Components of the model would then migrate from sedimentary layers to a hydrothermal environment where organic matter would precipitate on pyrite grains.

Leblanc *et al.*, (1996) reported the accumulation of iron-arsenate and arsenate-sulphate precipitates in the acidic stream (pH 2.2-4) of the Carnoulès Pb-(Zn) mine in Gard, France. Here, rod-shaped and sheathed bacterial forms were responsible for ferruginous bacterial accretions, morphologically similar to stromatolites.

Stromatolites represent the most ancient records of life on Earth by fossil remains which date from more than 3.5 billion years ago.

Also found within the accretions were living *Thiobacillus*-type bacteria. *Thiobacillus ferrooxidans* oxidise Fe (II) for energy for growth. Bennett and Tributsch, (1978) reported a strong correspondence between the attachment of *Thiobacillus ferrooxidans* to pyrite surfaces and the presence of residual etch pits during bacterial leaching experiments. The presence of some bacteria in the etch pits confirmed a causal link between the pits in the pyrite surface and the bacteria. Since the *T. ferrooxidans* microbes require oxygen as their terminal electron acceptor, it appears unlikely they would have been present on early Mars. However, purple and green sulphur bacteria (i.e. both Fe- and S-oxidisers) are anaerobic and potentially well suited to Martian conditions.



**Figure 3** a notional representation of pyrite surface sites and different kinds of interactions (a) Outer-sphere complexation of a nonspecific nature; (b) inner-sphere complexation of a metal, specifically interacting with a sulphur surface site



## 6. BACTERIAL ETCH PITS ON PYRITE

Oremland (2003) speculated on the role of dissimilatory arsenate-reducing prokaryotes in a hypothetical microbial 'biome' supported by arsenic cycling in the subsurface layers of Mars. Here As(III) would be recycled to As(V), possibly by aqueous nitrates permeating through the Martian surface. The activity of anaerobes that use As(V) as their respiratory oxidant is well documented (Switzer et al., 1998; Huber et al., 2000; Glhring and Banfield, 2001) and include several extremophiles adapted to high temperature, pH, and/or salinity. Furthermore, some arsenate-reducing bacterium (e.g. strain OREX-4) can use either arsenate or sulphate as the electron acceptor (Newman et al., 1997) enabling them to operate as both sulphate-reducing and arsenate-reducing bacterium.

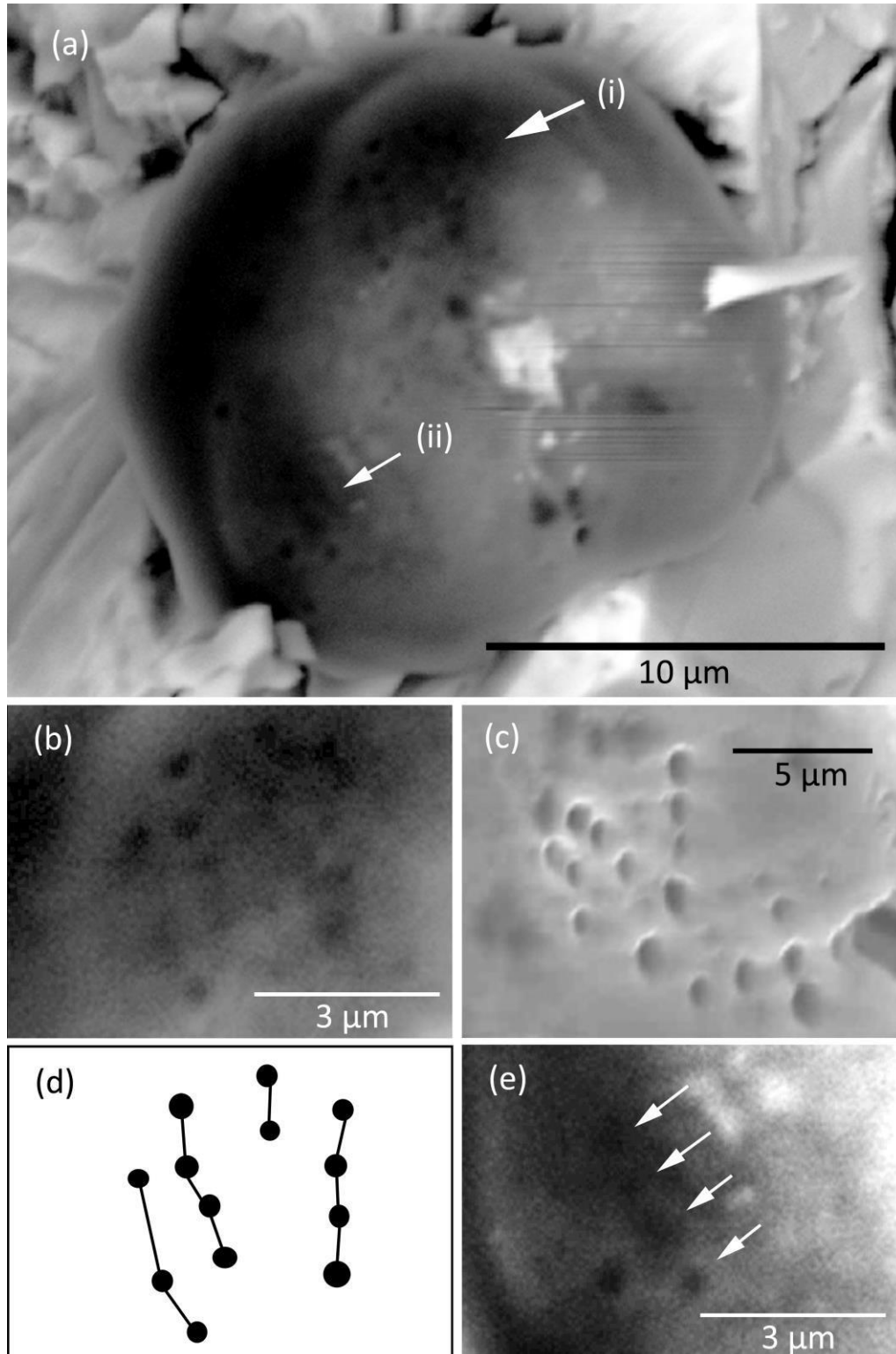
Evidence of the previous activity of bacteria was detected in the form of spherical etch pits and chains of pits on the surface of pyrite grains coated with carbonaceous material that were previously identified in the pyroxene substrate of Tissint. These etch pits occurred in clusters, and were comparable to biologically mediated microstructures created by Fe and S-oxidising microbes in the laboratory.

A correspondence between the attachment of some bacteria to pyrite surfaces and the presence of residual etch pits has been reported in bacterial leaching experiments using *Thiobacillus ferrooxidans* by Bennett and Tributsch, (1978). These authors reported the presence of rounded etch pits, typically between 1-2  $\mu\text{m}$  in diameter, often occurring as pairs or chains with individual pits linearly aligned, or as significant clustered accumulations. In particular, the presence of residual bacteria in some of the pits was observed, establishing a causal between the two. Similarly, Edwards et al., (1998, 1999) reported pitting in experiments using natural populations of microbes from acid mine drainage sites, while Etzel et al. (2007) showed that the shape of etch pits created by thermophilic Archaea was strongly related to crystallographic orientation.

Significantly, Wacey *et al.*, (2010) reported on pyrite grains with laminated carbonaceous coatings of early Archean age from the basal quartz arenite member of the 3.43–3.35 Ga Strelley Pool Formation (SPF) in Western Australia. Here, pyrite surfaces at the interface with the carbonaceous coatings exhibited spherical pits, chains of pits and channels that were widespread and had a clustered distribution typical of microbial colonisation. This author interpreted this as trace fossils formed by the attachment of bacteria to the pyrite surfaces.

Figure 3(a) shows an SEM image displaying the presence of etch pits on the surface of a pyrite grain coated with carbonaceous material in the Tissint Martian meteorite. This image was previously reported in Wallis et al (2014) and was taken using a Hitachi S-3700N Field Emission Scanning Electron Microscope at the Marshall Space Flight Centre by RBH. It is shown here with the contrast improved for the purpose of highlighting surface features present at the interface of the pyrite and carbonaceous mantle. Figure 3(b) shows the area marked with arrow (i) in the upper image. Here a cluster of equally spaced spherical etch pits, arranged in linear order, become visible. Figure 3(d) shows an overlay diagram of their position and spacing. Figure 3(e) shows a similar set of four rounded pits equally spaced in a straight line as indicated by the four white arrows. For comparison, figure 3 (c) shows a cluster of spherical etch pits in the surface of pyrite taken from the Strelley Pool Formation (SPF) in Western Australia (Wacey *et al.*, 2010). These pits can be seen to be equally sized and spaced and linearly aligned.

The significance of the etch pit shape and geometric distribution cannot be overstated since pitting and channelling of pyrite surfaces can result from the non-biological oxidation of pyrite. For example, pits of approximately 200nm deep  $\times$  10 $\mu\text{m}$  wide have been observed in pyrite under aqueous, O<sub>2</sub> saturated, acidic conditions (Asta *et al.*, 2008). Though these significantly differ from those observed in microbial experiments, they do demonstrate that abiotic process can also affect surface features. Similarly, the random distribution of pits of size 30-250nm have been observed during HCl and H<sub>2</sub>SO<sub>4</sub> reaction with pyrite (Edwards *et al.*, 1999) and in the radiolytic oxidation of pyrite (Leticariu *et al.*, 2010). Furthermore, abiotic oxidation by H<sub>2</sub>O<sub>2</sub> has also been shown to cause pitting (Leticariu et al. 2006) though here the pits were larger (~10 $\mu\text{m}$  diameter) but did not cluster or form chains of pits. Consequently, the presence of pits alone on pyrite surfaces is not diagnostic of a biological reaction (Wacey *et al.*, 2010). However, the pits in the pyrite of Tissint are rounded and clustered, form chains with equal pit spacing, have dimensions within the range typical of microbes and are distinct from the abiotic features discussed above.



**Figure 3** (a) shows an SEM image of a pyrite grain coated with carbonaceous material located in the substrate of Tissint (Wallis *et al.*, 2012). The area marked with arrow (i) in shown in higher resolution (b) where a cluster of equally spaced spherical etch pits, arranged in linear order, become visible. (d) shows an overlay diagram of their position and spacing, while (e) shows a similar set of four rounded pits equally spaced in a straight line as indicated by the four white arrows. For comparison, figure 3 (c) shows a cluster of spherical etch pits in the surface of pyrite taken from the Strelley Pool Formation (SPF) in Western Australia (Brasier, 2010). These pits can be seen to be equally sized and spaced and linearly aligned.

## 7. DISCUSSION

The mobilisation and sequestration cycle of arsenic (As) is neither purely chemical nor purely microbial. However, the process is greatly enhanced by the activity of microorganisms that increase reductive Fe oxide dissolution with concurrent release of As to solute phase. The subsequent sequestration of As under anoxic conditions can then take place via precipitation in sulphide compounds. The presence of relatively rare secondary iron arsenate-sulphates does however tentatively suggest microbial activity. Iron arsenate and arsenate-sulphates, together with the alteration product, bukovskýite, have all been reported in ferruginous bacterial accretions in acidic mine waters in France. (Leblanc *et al.*, 1996).

Reliance on microfossil morphologies as conclusive indicators of relic life has a controversial history. Even in terrestrial settings the unambiguous identification of ancient biology has not always been easy to establish. Pflug, (1967) reported the presence of 'globular-type A microfossils' in acid macerated shales of the Fig Tree Group, but this has since been questioned by Altermann (2001). Walsh (1992) found ~90 µm Archaean carbonaceous spheroidal microstructures in the carbonate cherts of the Kromberg Formation, though Brazier *et al.*, (2006) have since reinterpreted these as selforganised abiotic structures. Buik (2001) also examined these structures and found that hollow kerogenous filaments associated with them may indeed be biological. Sugitani (2007) interpreted similar-sized morphological structures in the black chert beds of the Gorge Creek Group as 'probable microfossils'.

Significantly however, Javaux, Marshall and Bekker (2010) examined organic-walled microfossils in Mesoarchaeon shales and siltstones in the Earth's oldest (3.2 billion years) siliclastic alluvial to tidal estuarine deposits. They found unambiguous evidence in the wall ultrastructure of flattened hollow organic-walled vesicles with visible lumen between the compressed walls and concluded they must be of biological origin. Of significance, these investigators also reported the presence of pyrite and arsenopyrite grains in the walls of the microstructures observed during SEM energy dispersive X-ray analysis. Spheroidal carbonaceous microstructures with sulphide cores have also been observed in Proterozoic and Ordovician sandstones in Canada (Nardi, 1994) and hydrothermal calcite veins in Carboniferous limestones in central Ireland. Here the origin of the organic matter cannot be confirmed but is thought to have derived from the surrounding sedimentary terrain, where the hot hydrothermal fluids generated the migration of organics. (Lindgren *et al.*, 2011).

Nonetheless, the presence of spherical pits, and chains of pits, with a morphology and geometric distribution that is distinct from abiotic alteration features, and is closely comparable to biologically mediated microstructures created by Fe- and S-oxidising microbes in the laboratory requires further attention. At present, these features are interpreted as trace fossils resulting from the attachment of bacteria to the pyrite surfaces.

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