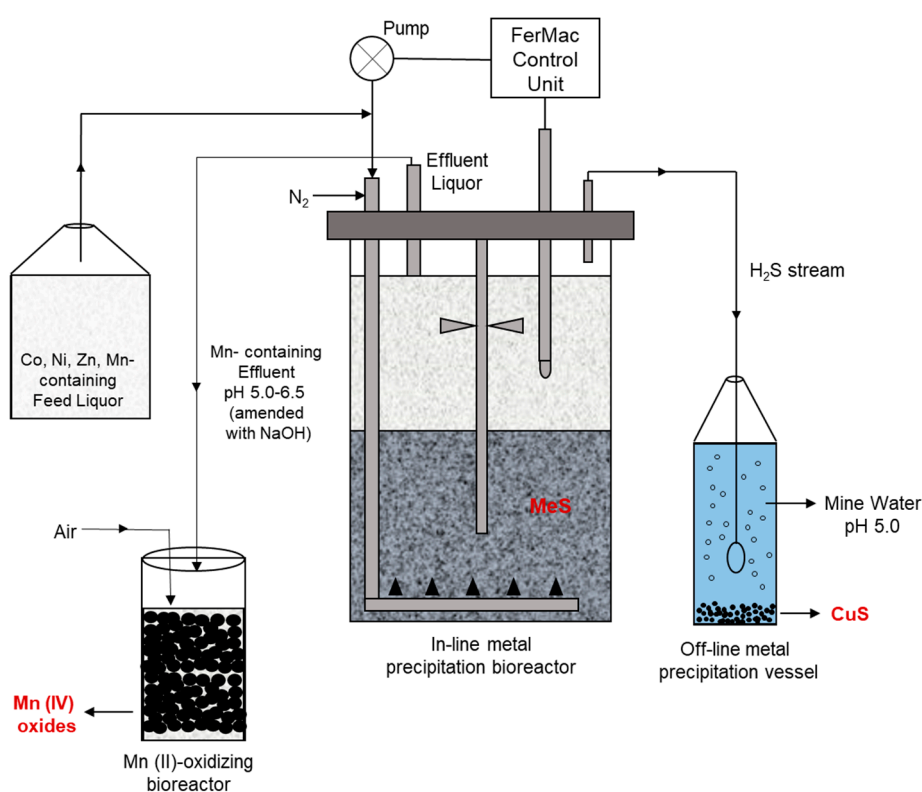
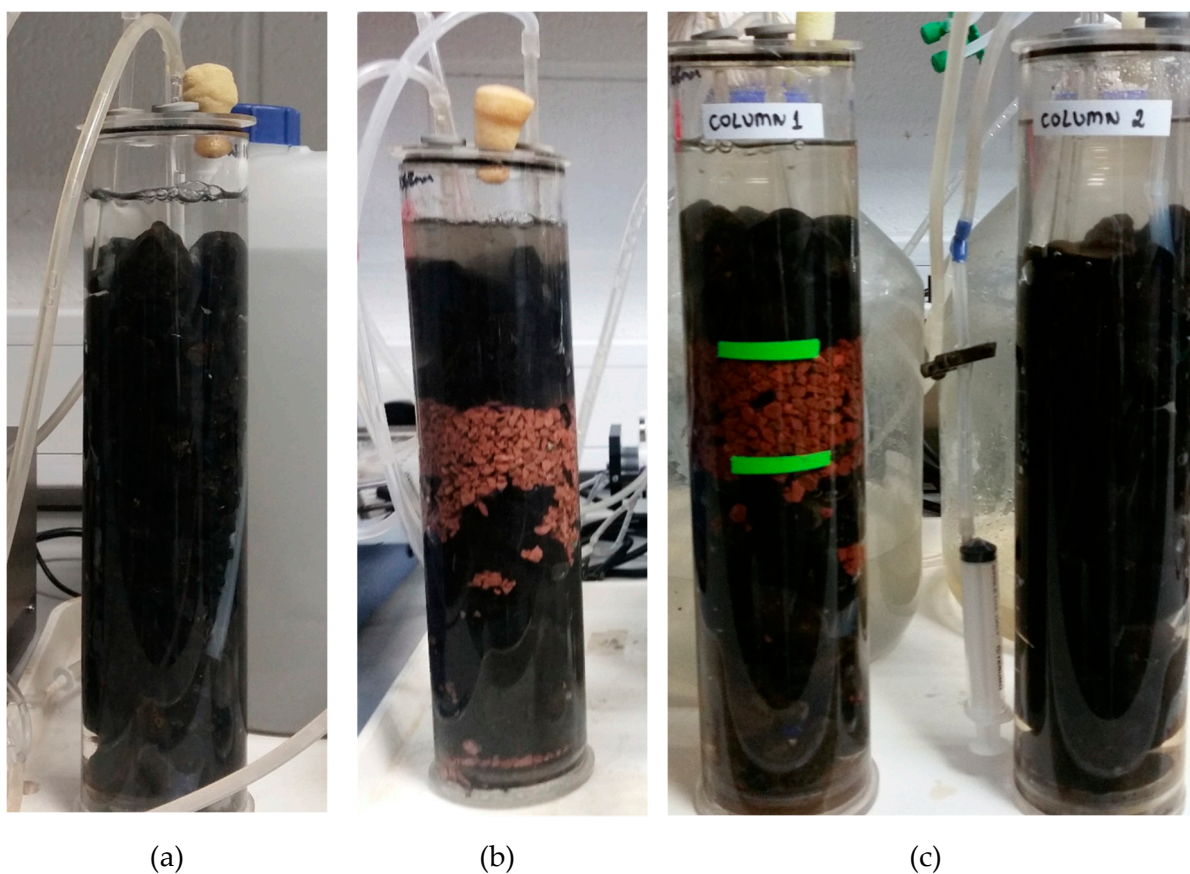




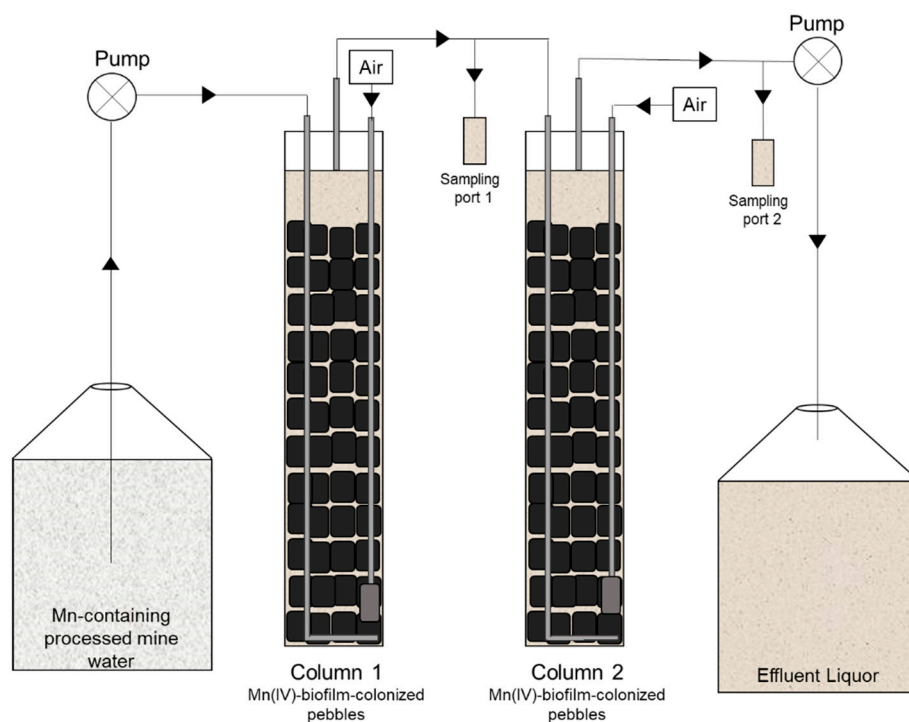
**Supplementary Figure S1.** Image of the Mn (IV)-coated pebbles collected from a catchment stream in North Wales, UK.



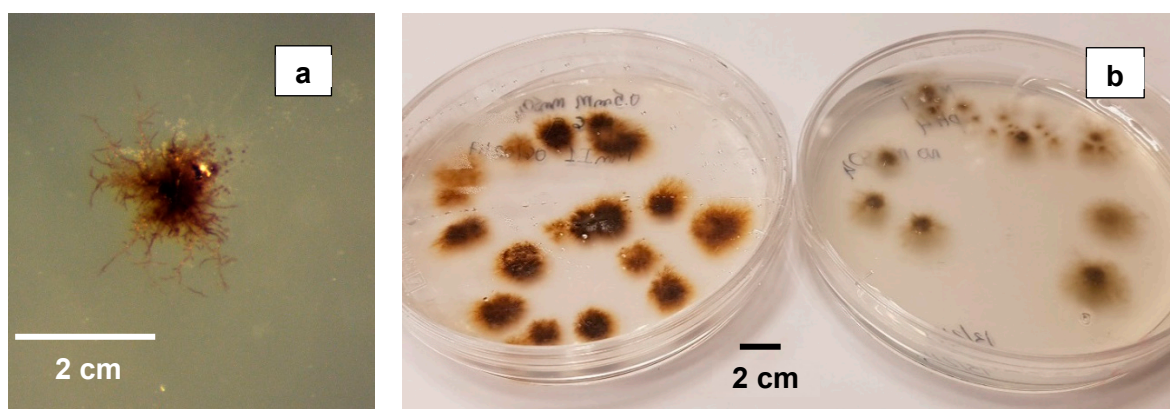
**Supplementary Figure S2.** Summary of the integrated system protocol. The synthetic acid mine drainage remediation process involved: (a) biosulfidogenesis, as (i) off-line precipitation of copper and recovery of CuS, (ii) in-line precipitation and accumulation of most other transition metals ( $\text{Me}^{2+}$ ) within the bioreactor, and (b) aerobic oxidation of soluble Mn (II) to Mn (IV) and precipitation of solid-phase Mn (IV) oxides.



**Supplementary Figure S3.** Fixed bed bioreactor packed with Mn (IV) biofilm-colonized pebbles. (a) 1-column system (C1: pH 5.0 and HRT ~ 11h), (b) 1-column system (C2a: pH 6.5 and HRT ~ 11h and C2b: pH 6.5 and HRT ~ 24h), where a thin layer of pale-colored inert gravel was placed between two layers of biofilm-coated pebbles in order to monitor Mn (IV) precipitation, and (c) 2-column system (C3: pH 6.5 and HRT ~ 45h).

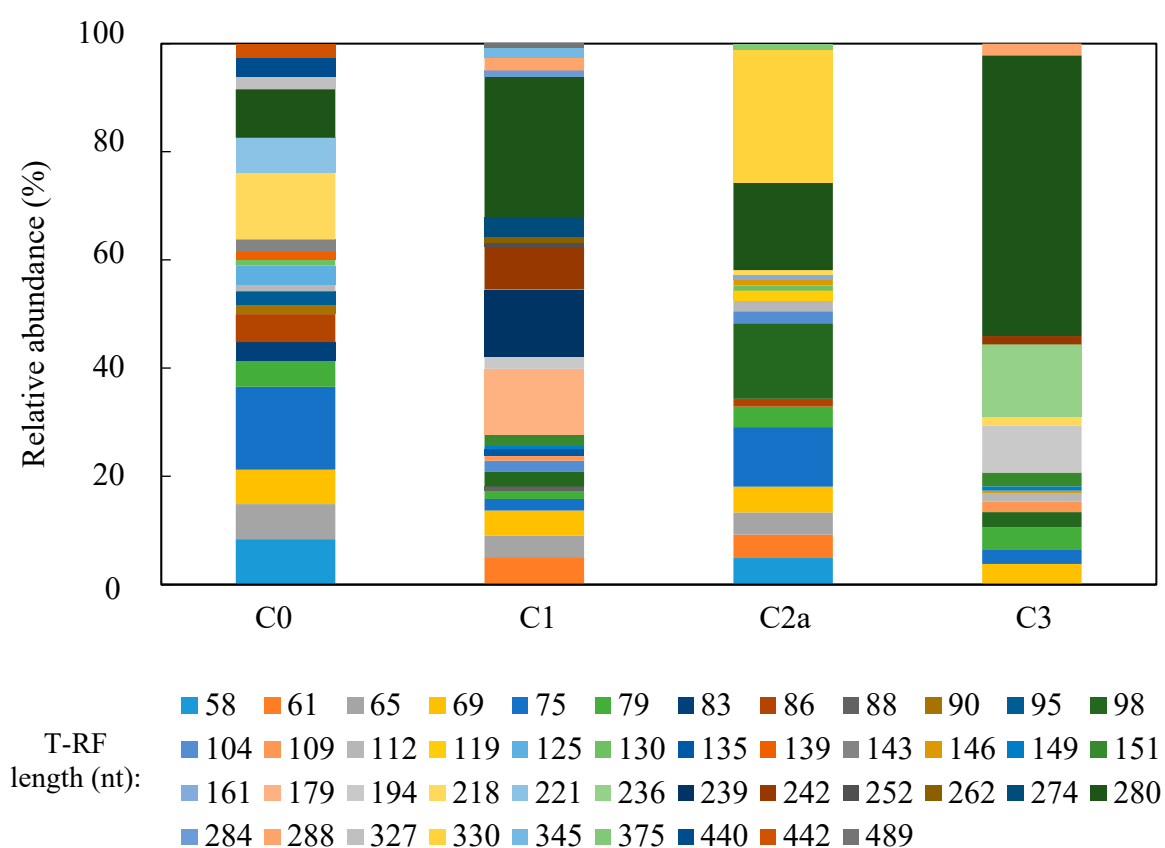


**Supplementary Figure S4.** Schematic representation of the in-line 2-column Mn (II)-oxidizing bioreactor. Effluent liquor from column 1 was pumped into the bottom of the column 2, drained at the top and collected into an effluent bottle. Sampling ports were added to the top of each column and analysis were carried out on both sampling points.

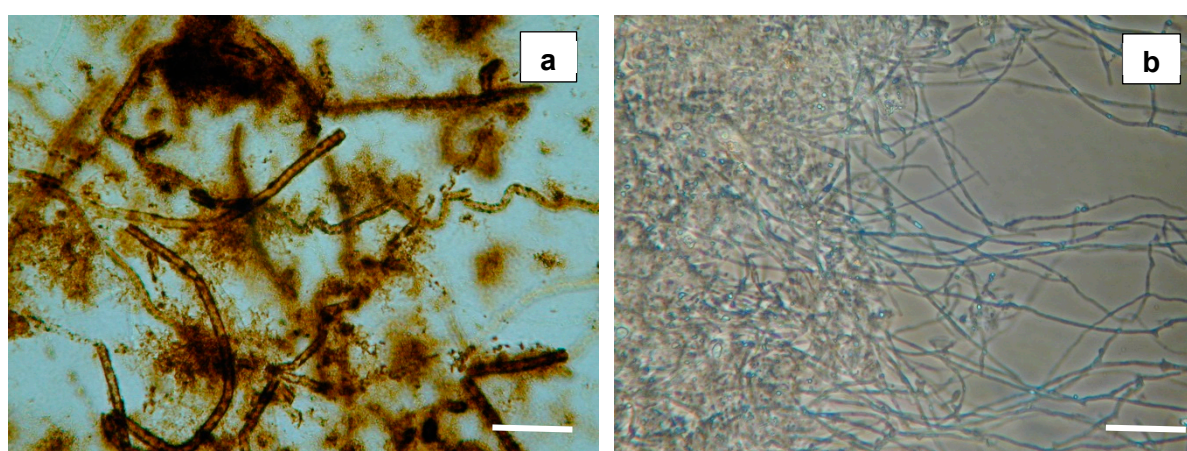


**Supplementary Figure S5.** Images of isolate MnI1 grown on solid media. (a) Rhizoidal colonies on Mn-containing plates with dark brown-coloured hyphae; (b) colonies growing on solid media at nominal pH 6.5 containing 0.5 mM Mn (II) (left) and no added Mn (II) (right).





**Supplementary Figure S6.** Terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis (HaeIII digests) of amplified 18S rRNA eukaryotic genes from the biofilm-colonized pebbles collected from the stream (C0) and at different phases (C1, C2a and C3) of operating MnOBs. The T-RFs are stacked upwards in each column in increasing fragment lengths.



**Supplementary Figure S7.** Micrographs of the isolated MnI1. (a) putative Mn oxides deposited onto the hyphae and (b) growth on Mn-free plates. Bars—10  $\mu$ m.

**Supplementary Table S1.** Summary of the forward and reverse primer sequences used in this study.

Gene	Primer name	Sequence (5' – 3')
<b>Bacterial 16S rRNA</b>	27F	AGAGTTTGATCMTGGCTCAG
	1387R	GGGCGGWTGTACAAGGC
<b>Archaeal 16S rRNA</b>	Arch 20F	TCCGGTTGATCCYGCCRG
	Arch 915R	GTGCTCCCCCGCCAATTCCT
<b>Eukaryote 18S rRNA</b>	Euk F	ACCTGGTTGATCDCTGCCAG
	Euk R	TGATCCTTCYGCAGGTTAC

M = A/C, W = A/T, Y = C/T, R = A/G, D = A/G/T.

**Supplementary Table S2.** Identity of cloned archaeal genes (16S rRNA gene) obtained from the biofilm-colonized pebbles collected from the stream in Snowdonia ( $n = 32$ ).

Clone designation	Closest Relative	% identity (16S rRNA gene)
<b>MNPARCH1</b> ( $n = 2$ )	<i>Candidatus Nitrocosmicus oleophilus</i> strain MY3 (CP012850.1)	96
<b>MNPARCH3</b> ( $n = 6$ )	Anaerobic methanogenic archaeon ET1-8 (AJ244284.1)	94
<b>MNPARCH5</b> ( $n = 8$ )	<i>Nitrososphaera viennensis</i> strain EN76 (CP007536.1)	93
	Uncultured archaeon clone Elev_16S_arch_651 16S ribosomal RNA gene (EF022795.1)	98
<b>MNPARCH8</b> ( $n = 3$ )	<i>Candidatus Nitrosocosmicus exaquare</i> strain G61 (CP017922.1)	98
<b>MNPARCH13</b> ( $n = 7$ )	<i>Candidatus Nitrosopumilus adriaticus</i> strain NF5 (CP011070.1)	95
	Uncultured thaumarchaeote clone LM_8Mar11_CL_41 16S ribosomal RNA gene (KP866399.1)	98
<b>MNPARCH17</b> ( $n = 1$ )	Anaerobic methanogenic archaeon ET1-10 (AJ244286.1)	90
	Uncultured archaeon clone LCDARCH18 16S ribosomal RNA gene (EU247270.1)	93
<b>MNPARCH32</b> ( $n = 5$ )	<i>Candidatus Nitrosoarchaeum koreensis</i> MY1 (HQ331116.1)	96
	Uncultured archaeon 16S rRNA gene, clone N4-F10 (FN691645.1)	98

**Supplementary Table S3.** Identity of cloned bacterial genes (16S rRNA gene) obtained from the biofilm-colonized pebbles collected from the stream in Snowdonia ( $n = 40$ ).

Clone designation	Closest Relative	% identity (16S rRNA gene)	Theoretical T-RF (HaeIII)
<b>MNPBAC4</b> ( $n = 6$ )	<i>Burkholderia</i> sp. strain WSM4678 (MF949049.1)	91	221/321/406
	Uncultured bacterium clone FCPN480 16S ribosomal RNA gene (EF515927.1)	99	221/321/406
<b>MNPBAC6</b> ( $n = 1$ )	<i>Dasania</i> sp. strain OUC005 (KY416514.1)	91	39
	Uncultured gamma proteobacterium clone MP-R100 16S ribosomal RNA gene (JN038769.1)	96	39/401
<b>MNPBAC9</b> ( $n = 8$ )	<i>Sphingopyxis rigui</i> strain HME8676 (KC157048.1)	97	293/379
<b>MNPBAC13</b> ( $n = 5$ )	<i>Novosphingobium tardaugens</i> strain ARI-1 (NR_028630.1)	95	253/319/404
<b>MNPBAC14</b> ( $n = 4$ )	<i>Rhodocyclus</i> sp. HOD 5 (AY691423.1)	92	224/324/409
<b>MNPBAC16</b> ( $n = 11$ )	<i>Leptothrix discophora</i> strain SP-6 (L33974.1)	96	223/231/406
<b>MNPBAC19</b> ( $n = 5$ )	<i>Brevifollis gellanilyticus</i> strain DC2c-G4 (NR_113149.1)	83	225/251
	Uncultured bacterium clone 3BH-4B 16S ribosomal RNA gene (EU937926.1)	96	223/250/434

**Supplementary Table S4.** Identity of cloned eukaryotic genes (18S rRNA gene) obtained from the biofilm-colonized pebbles collected from the stream in Snowdonia ( $n = 80$ ).

Clone designation	Closest Relative	% identity (16S rRNA gene)	Theoretical T-RF (HaeIII)
<b>MnE1</b> ( $n = 6$ )	<i>Micronuclearia podoventralis</i> (AY268038.1)	99	496
<b>MnE7</b> ( $n = 12$ )	<i>Gibbula magnus</i> (AY145375.1)	98	281
<b>MnE9</b> ( $n = 9$ )	<i>Chaetonotus aemilianus</i> voucher TK132 (JQ798556.1)	96	180/276
<b>MnE26</b> ( $n = 16$ )	<i>Serpulorbis imbricatus</i> isolate LSGB21001 (HQ833992.1)	98	282
<b>MnE27</b> ( $n = 17$ )	<i>Duplicaria dussumieri</i> isolate LSGB24501 (HQ834049.1)	97	287
<b>MnE30</b> ( $n = 6$ )	<i>Chaetonotus neptuni</i> (AM231774.1)	95	281
	Uncultured eukaryote gene for 18S rRNA, clone: AD_S13clone24 (LC109078.1)	95	282
<b>MnE79</b> ( $n = 13$ )	<i>Nassarius festivus</i> isolate LSGB2340102 (HQ834036.1)	98	281
<b>MnE80</b> ( $n = 1$ )	<i>Apicomplexa</i> sp. 1 KCW-2013 (KC890798.1)	90	342
	<i>Heterocapsaceae</i> environmental sample clone Elev_18S_1214 (EF024723.1)	92	333

**Supplementary Table S5.** Utilization of organic substrates by MnI1. Key: (-) no growth observed; (+) growth observed.

<b>Substrate</b>	<b>Concentration (mM)</b>	<b>Growth</b>
Mannose	5	+
Galactose	5	-
Glucose	5	-
Sucrose	5	+
Fructose	5	+
Trehalose	5	-
Maltose	5	+
Fucose	5	-
Lactose	5	+
Arabinose	5	+
Cellobiose	5	-
Ribose	5	+
Rhamnose	5	+
Xylose	5	+
Lactose	5	+
Sorbose	5	-
Na-pyruvate	5	+
Mannitol	5	+
Sorbitol	5	-
Glycerol	10	+
Methanol	20	-
Ethanol	15	+
Benzyl Alcohol	5	+
Phenol	5	-
1,3-propanediol	5	-
Glycolic Acid	15	-
Na-acetate	15	+
Citric Acid	5	+
Alanine	10	-
Leucine	5	-
Glycine	5	-