Enhanced rates of iron oxidation in mine waters by the novel acidophilic bacterium "*Ferrovum myxofaciens*" immobilized in packed-bed bioreactors[1]

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Iron is often the most abundant dissolved metal present in acidic waters draining coal and metal mines and mine spoils. Ferric iron is highly insoluble in all but the most acidic mine waters, while ferrous iron is soluble over a much wider pH range. Abiotic (chemical) oxidation of ferrous iron is also pH-dependent, and proceeds very slowly, even in fully oxygenated waters, at pH <4. Many different species of acidophilic and acid-tolerant bacteria are now known to accelerate the oxidation of ferrous iron in acidic waters (Johnson and Hallberg, 2008), though only one of these (*Acidithiobacillus ferrooxidans*) has tended to be investigated in this context (e.g. Long et al., 2003). One of the more interesting of the novel isolates is a currently unclassified betaproteobacterium with the proposed name of "*Ferrovum myxofaciens*". This is a cold-tolerant bacterium that produces copious amounts of extracellular polymeric slime, which causes it to grow as macroscopic jelly-like streamers in flowing water (Johnson, 2009).

To elucidate the abilities of acidophilic bacteria other than *At. ferrooxidans* to oxidize ferrous iron in mine waters, we have compared the performances of four different species of acidophilic iron-oxidizing bacteria that are maintained in the *Acidophile Culture Collection* at Bangor University. These were: (i) *Acidithiobacillus ferrivorans,* an iron/sulfur-oxidizing bacterium that is closely related to *At. ferrooxidans*, (ii) *Leptospirillum ferrooxidans* (type strain), (iii) *Ferrimicrobium* sp. TSTR, and (iv) "*Fv. myxofaciens*", the proposed type strain isolated from acid rock drainage (ARD) at the abandoned Parys copper mine, North Wales. The bacteria were immobilized, separately onto an inert support matrix (Poraver porous glass beads) as described by Rowe and Johnson (2008) and in then put into cylindrical Perspex columns, to produce packed bed reactors (Fig. 1). The bioreactors were set up in a laboratory where the ambient temperature was between 18° and 22°C, and all media and test liquors used were sterilized. When operated in continuous-flow mode, flow rates varied between 100 ml and 2000 ml h^{-1}.

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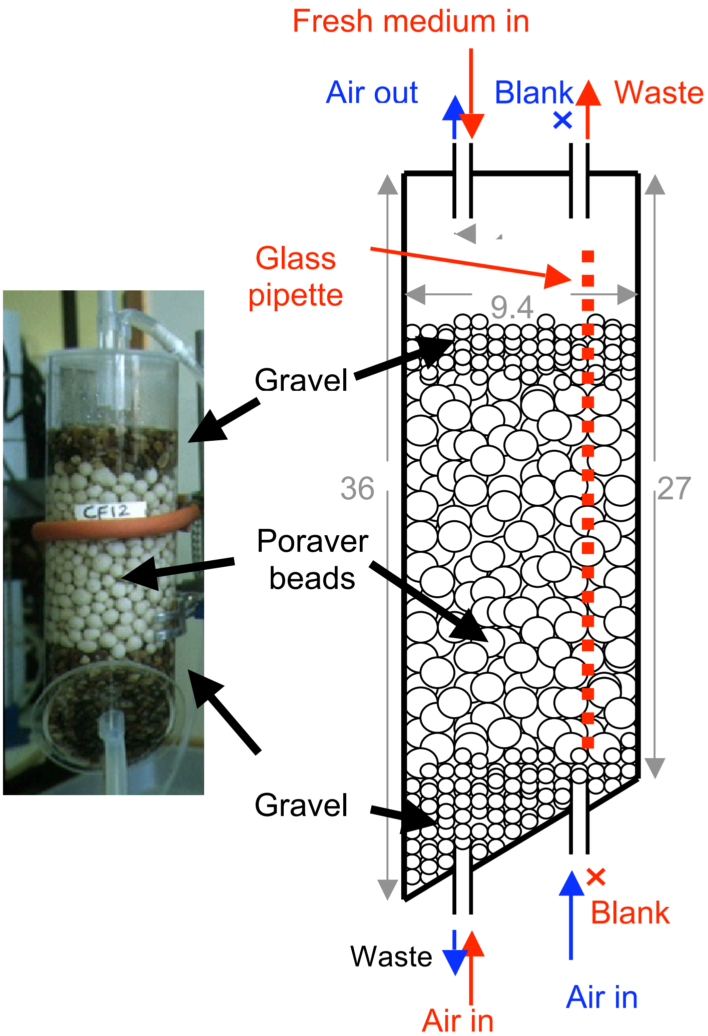

Figure 1. Diagram of the fixed bed bioreactor system used for accelerating ferrous iron oxidation in synthetic mine water (right) and a picture of one of the reactors (containing immobilized *L. ferrooxidans* strain CF12; left). Numbers refer to bioreactor dimensions (in cm). Blue lettering refers to the configuration of reactors operated in batch mode, and red lettering when the reactors were operated as continuous flow systems.

The packed bed bioreactors displayed significantly different performances when operated as either batch or continuous-flow systems. The *L. ferrooxidans* reactor was particularly effective in batch mode (Table 1) while, when operated in continuous flow mode, the reactor containing immobilized "*Fv. myxofaciens*" was superior to the other three when challenged with either synthetic (pH 2.0; 275 mg Fe^{2+}/l) or actual (pH 2.40; 300 mg Fe^{2+}/l) acid rock drainage at high flow rates (Table 2). This was thought to be related, at least in part, to its superior biofilm development on the support matrix. The least effective bacterium overall was *At. ferrivorans*, which is interesting since the majority of previous reports of ferric iron-generating bioreactors for mitigating ferruginous waters have used only the closely-related *At. ferrooxidans*.

Table 1. Comparison of maximum rates of iron oxidation and residual ferrous iron concentrations in the packed-bed bioreactors operated in batch mode (mean values, +/- standard errors). Data showing maximum rates of iron oxidation and ferrous iron half-lives are from the final ten batch run tests of the bioreactors, prior to testing as continuous flow systems (Rowe and Johnson, 2008)

Bioreactor system	Maximum rate of Fe^{2+} oxidation (mmoles l^{-1} h^{-1})	Residual Fe^{2+} at 30 mins (μM)**
At. ferrivorans	18.1 +/- 0.47[a*]	642 +/- 24[a]
L. ferrooxidans	19.2 +/- 0.55[a]	394 +/- 26[b]
"*Fv. myxofaciens*"	17.3 +/- 0.44[a]	625 +/- 23[a]
Ferrimicrobium TSTR	17.2 +/- 0.56[a]	233 +/- 27[b]

*values denoted by different suffixes (a, b) in the same column are significantly different ($p < 0.05$)
** n = 7-11 replicates +/- standard errors;

Data from these experiments have shown that some hitherto unknown bacteria may be superior to more characterized iron-oxidizers for passive oxidation/precipitation of iron in acidic mine waters. A "hybrid" reactor combining the greater efficiency of "*Fv. myxofaciens"* for rapid oxidation of most the bulk of dissolved iron from mine waters, and the ability of *L. ferrooxidans* to lower the concentration of ferrous iron in treated water to very low levels, is proposed as a potentially highly effective system.

Table 2. Percentages of ferrous iron oxidized at flow rates of 396 ml/h (*) and 1593 ml/h (**) by bioreactors fed with synthetic (syn) AMD or filter-sterilized (fs) AMD and operated in continuous flow mode. Data from Rowe and Johnson (2008)

Isolate	Ferrous iron oxidized (as % of feed liquor)	
	synAMD	fsAMD
At. ferrivorans	87** / 96*	83** / 98*
L. ferrooxidans	89** / 98*	86** / 98*
"Fv. myxofaciens"	92** / 98*	92** / 99*
Ferrimicrobium TSTR	75** / 96*	84** / 98*

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