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Comparison of ferric iron generation by different species of acidophilic bacteria immobilized in packed-bed reactors

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Abstract

Flooded packed-bed bioreactors, prepared by immobilizing four different species of acidophilic iron-oxidizing bacteria on porous glass beads, were compared for their ferric iron-generating capacities when operated in batch and continuous flow modes over a period of up to 9 months, using a ferrous iron-rich synthetic liquor and acid mine drainage (AMD) water. The bacteria used were strains of *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, a *Ferrimicrobium*-like isolate (TSTR) and a novel Betaproteobacterium (isolate PSTR), which were all isolated from relatively low-temperature mine waters. Three of the bacteria used were chemoautotrophs, while the *Ferrimicrobium* isolate was an obligate heterotroph. Greater biomass yields achievable with the *Ferrimicrobium* isolate resulted in greater iron oxidation efficiency in the newly commissioned bioreactor containing this bacterium, though long-term batch testing with organic carbon-free solution resulted in similar maximum iron oxidation rates in all four bioreactors. Two of the bioreactors (those containing immobilized *L. ferrooxidans* and *Ferrimicrobium* TSTR) were able to generate significantly lower concentrations of ferrous iron than the others when operated in batch mode. In contrast, when operated as continuous flow systems, the bioreactor containing immobilized PSTR was superior to the other three when challenged with either synthetic or actual AMD at high flow rates. The least effective bacterium overall was *At. ferrooxidans*, which has previously been the only iron-oxidizer used in the majority of reports describing ferric iron-generating bioreactors. The results of these experiments showed that different species of iron-oxidizing acidophiles have varying capacities to oxidize ferrous iron when immobilized in packed-bed bioreactors, and that novel isolates may be superior to well-known species.

Keywords: Acidophiles; Bioreactors; Bioremediation; Iron oxidation

Introduction

Iron, the most abundant heavy metal in the lithosphere, can exist in two ionic forms, ferrous iron (iron (II)) and ferric iron (iron (III)), which have very different solubility and stability properties [2]. Whilst iron is required in relatively small amounts by virtually all known life-forms, ferric iron metabolism plays a central role in the energetics of prokaryotes that use

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Non-standard abbreviations: AMD, acid mine drainage; synAMD, synthetic acid mine drainage; fsAMD, filter-sterilized acid mine drainage.

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either ferrous iron as an electron donor (as a sole or contributory energy source) or ferric iron as an electron acceptor (some facultative and obligate anaerobes). Some acidophilic bacteria and archaea (usually defined as those with pH growth optima of <3) can use both ionic forms of iron, depending on the availability of oxygen [8].

Soluble ferric iron is a powerful oxidizing agent, and is used commercially for a number of purposes, one of which is to accelerate the dissolution of sulfide minerals to enhance metal extraction and recovery ("biomining" [21]). Most commercial biomining operations use *in situ* generation of ferric iron by acidophilic iron-oxidizers, although an alternative arrangement is to separate the (biological) iron oxidation and (abiotic) ferric iron mineral oxidation in an indirect bioleaching process. With the latter, a bioreactor housing the iron-oxidizing bacteria is used to generate ferric iron-rich liquor which oxidizes the target mineral(s) and, in so doing, becomes reduced to ferrous iron, and is subsequently re-oxidized by the immobilized bacteria. Acidophilic iron-oxidizing bacteria are required since the ferric iron has to be maintained in solution, which requires a pH of less than ~2. Indirect mineral oxidation has two advantages: (i) conditions for the biological and abiotic reactions can be optimized independently of each other (e.g. different temperatures can be used for mineral oxidation and regeneration of ferric iron), and (ii) exposure of the ferrous iron-oxidizing prokaryotes to potentially toxic metals in mineral leachates can be minimized.

A second scenario in which iron-oxidizing bioreactors have potential application is in the remediation of acidic mine waters. Abiotic oxidation of ferrous iron, which is often the dominant soluble metal present in waters draining working and abandoned metal and coal mines, proceeds very slowly in acidic ($\mathrm{pH}<3.5$) mine waters [23]. Water discharging from abandoned mines and mine spoils is frequently devoid of oxygen, and ferrous iron tends to be the dominant soluble iron species present [9]. In contrast to indirect mineral bioleaching, a key objective in remediating ferruginous mine waters is to remove iron from solution, and this is more readily achieved with ferric than with ferrous iron. Ferric iron hydrolysis in acidic waters produces a variety of amorphous and crystalline solid phases, including various jarosites, schwertmannite and (at higher pH) ferrihydrite [22].

Most reports of ferric iron-generating bioreactors have focused solely on *Acidithiobacillus ferrooxidans*, and on how different approaches used to immobilize this acidophile impact the performances of bioreactors (e.g. [4,17,19,24]). However, although *At. ferrooxidans* is, by far, the most well-known and widely studied of all iron-oxidizing bacteria, more recent research has shown that many other species of bacteria and archaea can also catalyse the dissimilatory oxidation of ferrous iron at low pH. These include prokaryotes that, like *At. ferrooxidans*, are obligate autotrophs, others which are obligate heterotrophs and some that can utilize both organic and inorganic carbon [8]. Variations in substrate (ferrous iron) affinities, product (ferric iron) inhibition, temperature response and pH sensitivity greatly impact relative rates of ferrous iron oxidation by different prokaryotic species. In addition, different species, and strains of a single species, vary in how readily they attach to solid surfaces and form biofilms [3,6]. The physiological characteristics of a selected acidophilic prokaryote could therefore, in theory, have a major impact on the efficiency and versatility of a ferric iron-generating bioreactor.

Here, we report the comparative performances of flooded packed-bed bioreactors containing four different species of iron-oxidizing acidophiles, immobilized onto a single-solid support matrix (porous glass beads), and operated in batch and continuous flow modes.

Materials and methods

Bacteria

The four species of iron-oxidizing bacteria used were all originally isolated from relatively low-temperature mine waters, as the bioreactors were developed, primarily, for the purpose of remediating acid mine drainage (AMD) in temperate zones. These were: (i) *At. ferrooxidans* (strain NO37), isolated from AMD (pH 2.75) at an abandoned copper mine in Norway [13]; (ii) *Leptospirillum ferrooxidans* (strain CF12), isolated from AMD (pH 3.1, 14 °C) at the Blackbird mine, Cobalt, Idaho [12]; (iii) a novel Betaproteobacterium (PSTR) isolated from acid streamer growths in a stream (pH 2.5, 8–12 °C) draining the abandoned Mynydd Parys copper mines, Wales [5]; (iv) a *Ferrimicrobium*-like bacterium (TSTR) isolated from a chalybeate stream (pH 2.9, 9 °C) at Trefriw Wells spa, Wales [5].

Bacteria (i)–(iii) are autotrophic, while *Ferrimicrobium* TSTR (iv) is an obligate heterotroph. The autotrophic isolates were grown in an "inorganic" mineral salts liquid medium containing 5 mM ferrous sulfate (at pH 2.0 for *At. ferrooxidans* NO37 and *L. ferrooxidans* CF12, and pH 2.2 for the more acid-sensitive isolate PSTR), while *Ferrimicrobium* TSTR was grown in the same liquid medium (initial pH 2.0) supplemented with 0.02% (w/v) yeast extract.

The PSTR isolate (iii) has yet to be fully characterized, although it is known to be an obligate autotroph that appears to use only ferrous iron as an energy source, and it is more acid-sensitive than both *Acidithiobacillus* and *Leptospirillum* spp. (D.B. Johnson, unpublished data). Its most obvious physiological trait,