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Thermophilic bacterial leaching of manganese dioxide

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SUMMARY

Thermophilic bacterial leaching of low-grade manganese oxide ore was demonstrated at 50 °C and 70 °C. A static culture system was used with cane molasses as the nutrient source. By employing batch cultures, with an acid wash to redissolve adsorbed Mn^{2+} at completion of each batch, the bacterial reduction could be driven to completion.

INTRODUCTION

Microbial leaching of manganese oxide tailings from Groote Eylandt, Northern Territory, Australia, has been the subject of many laboratory studies [1,3–5,10,13,15]. The aim of these investigations is to develop a low-cost treatment for dissolving manganese from low-grade process waste. The static leaching process is simple and effective, employing enrichment cultures of mixed microbes resident on the ore. A slurry of tailings is submerged in dilute aqueous molasses in a reactor with a restricted air/water interface. Microbes, mainly soil bacteria, are enriched with nutrients from molasses, grow rapidly and oxidize the sugars. Most of the oxygen is used up creating an oxygen-scarce environment adjacent to the sedimented tailings. Leaching bacteria, facultative aerobes, are then selected out at low oxygen and high levels of manganous ion leached by organic acids formed during the oxidative phase [9]. In the microaerobic conditions, remaining carbohydrate is fermentatively metabolised and manganese oxide is used as an electron acceptor, releasing manganous ion into solution (i.e., by direct leaching). It has been shown [3] that the direct leaching mechanism accounts for some 80% of bacterially formed Mn^{2+} in mixed static cultures. Indirect manganese leaching using bacterially formed H_2O_2 is also known [17].

Earlier, short-duration experiments (8-day batch) designed to detect high-temperature bacterial MnO_2 leaching (i.e., > 30 °C) were inconsistent [2]. Recent work [18] showed improvements in rate (1.25-times) could be reliably achieved at 50 and 60 °C over longer periods (8 weeks) using heavy inocula (approx. 10^8 cells/ml).

Apart from improving reaction rates, high-temperature bacterial leaching has several biotechnical advantages. Expensive cooling is not needed in large thermally insulated reactors and pathogenic or non-leaching microbes are suppressed. Furthermore, in the thermophilic range the increased temperature increases diffusion rates, solubility and ionisation, all of which are desirable improvements in a static microbial manganese-leaching reactor culture. A decrease in density and viscosity would also be of particular value under these conditions, where polysaccharide formation is common [15].

The following communication gives preliminary evidence for thermophilic bacterial leaching of manganese dioxide.

MATERIALS AND METHODS

Mineral composition

Manganese dioxide tailings were obtained from the Groote Eylandt Mining Company. The composition of this low-grade ore was: total Mn 17%, SiO_2 28%, Al_2O_3 22%, Fe 8% with minor amounts of Ti, B, K, Mg and Ca. Some 75% of particles in the slurries were approximately spherical with a diameter between 0.4–30 μm. The mineralogy of these ores has been described previously [14]. The ore contains a wide variety of essentially tetravalent manganese oxides (14 recorded oxides eg., Pyrolusite, Cryptomelane, Todorokite, Romanechite). The tailings gangue minerals are made up of kaolinite, quartz and lateritic ores.

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Thermophilic microorganisms

Viable counts were accomplished on solid medium with sucrose 1% (w/v), yeast extract 0.1% (w/v) and ball-milled manganese tailings solidified with agar (2.5%, w/v). The finely milled ore was prepared in a glass ball mill (a cylinder 12 × 12 cm diameter containing 20–50 g of 3-mm diameter glass beads). Thirty grams of tailings were ground for about a week at 30 rpm in 100 ml (reverse osmosis) water. The slurry was allowed to stand 10 min and 1 ml of supernatant fluid, containing the sub-micron particle size range was added per 20 ml agar nutrient mixture before autoclaving at 15 psi for 20 min [16]. Individual colonies which grew and removed MnO_2 on solid media within 48 h at 70 °C, were selected and purified by single colony streaking. Viable counts were made using spread plate technique with the above manganese/sucrose/yeast extract agar. Total cell counts were performed with a Thoma® cell counting chamber at 400 × using phase contrast microscopy. Cells adhering to manganese particles were not determined.

Culture methods

A non-sterile liquid medium was used throughout. The pH at inoculation was 5.9 ± 0.25. Sugar-cane molasses, 10% (w/v) used as the main nutritional source in liquid cultures, consisted of approx. 35% sucrose plus 1–12% glucose, fructose, starch and other minor carbohydrates. Molasses also contains 10% ash (K, Mg, Ca, Na, SO_4, Cl, P and Si) and 0.6% nitrogen, along with A and B vitamins. Potassium accounted for 30–50% of the inorganic elements in the ash. The stock viscous syrup contained about 20% moisture [12]. Reverse osmosis water was used throughout for dilutions. The effect of biotin addition to molasses was determined as a separate experiment.

Batch fermentation was carried out in static solution with resuspension of particles by mechanical agitation once every second day. Twenty grams of tailings was added to 100 ml of dilute molasses (10% w/v) solution. The 250-ml flask was screw-capped to exclude airborne debris and in long-term cultures no attempt was made to maintain sterile cultures. After batch growth (3 days) the supernatant medium and tailings were mixed and centrifuged (Beckman model J21B) at 8000 × *g* for 15 min. Fifteen milliliters of the supernatant medium (approx. 10^8 cells/ml) before centrifugation, was used to reinoculate subsequent cultures. Experiments were performed in duplicate over the full temperature range where leaching was taken to completion. Manganese ion adsorbs on MnO_2 at pH > 5 [11] and limits further leaching [1]. As manganese oxide has negligible solubility in dilute sulphuric acid at ambient temperature [6] the adsorbed manganous ion was re-dissolved in H_2SO_4 (0.03 M, pH 1.5, 2 × 100 ml). The acid-treated mineral was washed twice with 100 ml reverse osmosis water to bring the pH of the solids to pH 7. Mn^{2+} concentrations stated herein are the sum of acid desorbed and soluble Mn^{2+} at the end of each batch leach. After washing the acid-treated residues with water, the centrifuged pellet was resuspended in fresh molasses with the 15-ml inoculum from the previous batch. When the tailings were autoclaved with molasses a small amount of manganous ion was formed (approx. 90 μg Mn^{2+}/g tailings/g molasses), but there was no significant Mn^{2+} production in sterile cultures in the temperature range 4–60 °C [18].

Chemical analysis

Culture fluids were analysed for soluble manganese (Mn^{2+}) by atomic adsorption spectrometry using a Varian Techron® spectrometer model AA1475. An Activon® pH meter was used for pH determinations. Acid digests to determine residual Mn content after leaching were made with boiling HCl.

RESULTS

Fig. 1 shows the manganese batch leaching curves for static bacterial cultures degrading manganese oxide slurry tailings over a range of temperatures between 4–70 °C. Most Mn^{2+} was formed at 50 °C (average rate 3.3 g Mn^{2+}/l/day for the first 3-day batch) with least leaching activity at 4 °C and 20 °C. The highest average batch rates, for each temperature, were recorded in the first 3 days which was probably related to preferential attack on the smaller particles in this mixed size range. The decline in Mn^{2+} productivity at 30 °C in the final two batches was not understood but could relate to subtle changes in mineral composition and associated alterations in the bacte-

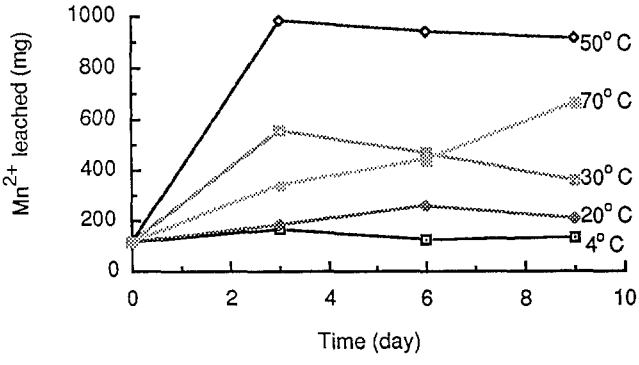

Fig. 1. Effect of temperature on static bacterial leaching of manganese dioxide tailings. Unpurified enrichment (100 ml) cultures, 10% (w/v) molasses, 20% (w/v) MnO_2 tailings.

rial populations (Srimekanond et al., unpublished data).

Fig. 2 shows the batch leaching curves at 50 °C and 70 °C where bacterial conversion of the Mn^{IV} to Mn^{2+} was taken to completion (> 95% reduction). The thermophilic culture at 50 °C leached MnO_2, on average, at 2.8-times the rate at 70 °C over the first 8 days of culture (i.e., two batches). Concentrations of Mn^{2+} reached 13.6 g/l at peak of recovery at 50 °C. Symmetrical leaching curves suggest that the gangue (i.e., 80% (w/v) tailings) does not impede bacterial attack on MnO_2. Despite better leach rates at 50 °C, the 70 °C system was chosen for further study because of the advantages of lower viscosity and flexibility of operation in terms of temperature tolerance.

Table 1 gives the response of the 70 °C mixed cultures of thermophiles to a range of molasses concentrations. The optimum level of molasses was 20% (w/v) (approx. 10% sucrose). Viable counts were many orders of magnitude less than direct microscopic counts. It is uncertain, at this stage, whether the viable counting method is reliable, as on occasions unpredictable zero counts were recorded (e.g., at 5% molasses). As noted previously [1–3,15], the recovery of Mn^{2+} in terms of molasses usage was better at low sugar concentrations (approx. 1% (w/v) molasses). The poor economy (Mn^{2+} formed/molasses used) at higher nutrient levels is thought to be due to non-leaching growth and metabolism wasting sugar where a surface-dependent reaction has become limiting.

Addition of microgram levels of biotin-enhanced leaching of MnO_2 3–4-times (Table 2). Similar improvements in thermophiles' response to biotin have been recorded previously [8] and are thought to be due either to increased pyruvate carboxylase activity, replenishing spent tricarboxylic acid cycle intermediates or to radical alteration in the microbial community.

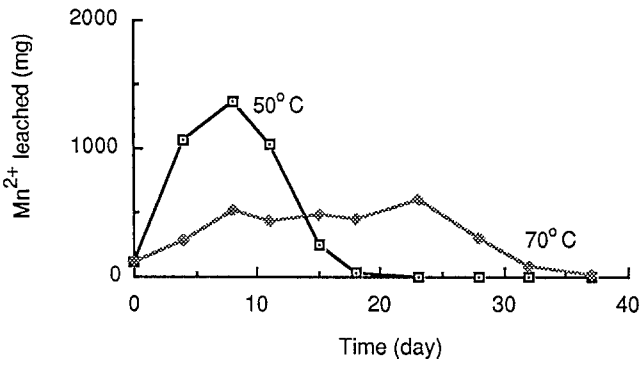

Fig. 2. Effect of static, bacterial, batch leaching (to completion) of manganese dioxide tailings at 50 °C and 70 °C. Unpurified enrichment cultures (100 ml), 10% (w/v) molasses, 20% (w/v) MnO_2 tailings.

TABLE 1

The effect of molasses concentration of microaerophilic bacterial leaching of manganese dioxide at 70 °C

Molasses (% w/v)	Microorganisms		pH	Mn^{2+} leached (g/batch)
	microscopic count (log_{10} cells/ml)	viable count (log_{10} cells/ml)		
1	7.7	3.6	6.31	0.38
5	9.0	0	6.80	0.29
10	8.2	4.0	6.99	0.79
15	8.5	4.0	6.90	0.90
20	8.7	2.8	6.29	1.18
25	9.0	2.5	6.57	0.97

Batch static leach, 10 days, inoculation 10^7 cells/ml (viable 10^2 cells/ml) mixed every second day, 20% (w/v) MnO_2 tailings, culture volume 100 ml.

TABLE 2

The effect of added biotin on microaerophilic bacterial leaching of manganese dioxide at 70 °C

Biotin (μg/l)	Microorganisms		pH	Mn^{2+} leached (g/batch)
	microscopic count (log_{10} cells/ml)	viable count (log_{10} cells/ml)		
0	8.5	3.5	6.74	0.31
0.1	8.5	3.3	6.95	0.29
0.5	8.1	6.0	6.95	0.28
1.0	9.1	5.4	6.47	1.14
2.0	8.7	3.6	6.49	1.27

Batch static leach, 8 days, inoculation 10^8 cells/ml (viable 10^4 cells/ml) mixed every second day, 20% (w/v) MnO_2 tailings, nutrient 10% (w/v) molasses, culture volume 100 ml.

Spread plates of crude mixed culture microbes at 70 °C showed two of five isolates were able to reduce MnO_2. Of these two colony types, one was found regularly, whereas the other was only detected once. The commonly appearing colony was opaque and flat (0.5 cm ± 0.3 mm) having an ill-defined circular boundary with a clear center, due to the reductive removal of black MnO_2, leaving the orange-colored lateritic gangue minerals. The atypical colony type at 70 °C had a circular shape with a raised chalky white centre (0.5 cm diameter) above the top surface and formed a cone-shaped MnO_2 clearing zone growing larger toward the plate base. The bacterium found frequently at 70 °C was Gram positive. Microscopically, rods of various sizes from 0.8–2.4 to 4.8 μ were seen with a racket shaped protrusion, possibly a spore, formed terminally (about 0.5 × 5.6 μ).

DISCUSSION

The demonstration of a thermophilic reduction of manganese dioxide has important implications for future bacterial processing of the mineral. Previous work [3,15] has dwelt upon use of *Enterobacter* species which predominate at 30 °C. The efficiency of the system depends upon static conditions with minimal mechanical mixing. One of the technical difficulties to be overcome at lower temperatures is formation of extracellular slimes that quickly reduce permeability in a static reactor. Operation at a higher temperature should help to overcome this problem. Evidence for viable activity at 70 °C encourages further study, particularly in terms of nutrient manipulation. An example of the type of improvements that might be expected from better nutrition, comes from the demonstration of the beneficial effect of added biotin.

An organism was purified from cultures incubated at 70 °C which appears to be the causative leaching agent, i.e., judging from the leaching ability of single colonies on MnO_2 agar. However, the quantitative contribution of this microbe to Mn^{2+} production in mixed cultures has yet to be confirmed. The best leaching cultures are often those that develop naturally from consortia rather than those from pure cultures [3]. Future work in this laboratory will involve purification and optimisation of thermophilic manganese oxide degrading microorganisms. In either pure or mixed culture the feasibility of using bacterial leaching on an industrial scale becomes greater at higher temperatures because of the ease of process management. Reduced residence times, greater latitude in the upper limits of heat control and the advantage of using non-pathogenic organisms are sound reasons for using high temperature microbes in biohydrometallurgy.

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