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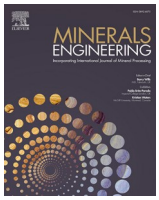

Continuous bioreactor leaching of nickel sulfide concentrates with moderately thermophilic bacteria and archaea

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ABSTRACT

A commercial process for bioreactor leaching of a nickel concentrate by-product of talc mining has been described previously. It was developed and operated (2016–2018) at about 45–46 °C. Further features of bioleaching that concentrate have now been investigated in laboratory-scale reactors with continuous feeds of up to 10% (w/v) solids and an emphasis on temperatures at and a few degrees above that of the commercial process. The sulfur-oxidizing *At. caldus* was more abundant than the sulfide mineral-oxidizing *S. thermosulfidooxidans* and *Atm. siderophilum* at 48 °C but was essentially lost with a 3 °C temperature rise, simultaneously with a rise in pH and in iron precipitation from solution, without adversely affecting nickel leaching. The relative abundance among bacteria was similar between two reactors operated in series but there was more than a fourfold increase in the relative abundance of the ferrous-iron oxidizing, heterotrophic archaeon *Ac. cupricumulans* in the secondary reactor, most likely in response to an increase in the acidity as the sulfide concentrate oxidation proceeded.

1. Introduction

A commercial process for bioreactor leaching of a pentlandite/pyrrhotite concentrate by-product of talc mining was developed and operated from 2016 to 2018 (Laukka et al., 2018) before the marginal economics of base-metal concentrate processing in industrial bioreactors resulted in its suspension before it reached the design criteria throughput. The operating temperature for the stirred-tank bioreactors was about 45 °C, with a requirement for it to remain below 49 °C to avoid culture harm (Neale et al., 2015). Previously, industrial bioreactor processing of a cobaltiferous pyrite concentrate operated at 42 °C (Morin and d'Hugues, 2007). Oxidation of refractory gold concentrates in most BIOX® reactors is also at about 40–45 °C (van Aswegen et al., 2007) with mesophilic, thermotolerant and moderately thermophilic species co-existing. Therefore, relatively small changes in the temperatures of all these processes could change the compositions of their mixed microbial populations. *Leptospirillum ferriphilum* is generally the key ferrous iron-oxidizing bacterium in the processes noted above although 45 °C appears to be close to the upper temperature limit for *Leptospirillum* species, with only one of four enrichment cultures of *Leptospirillum* sp. from mine sites able to grow at this temperature (Norris, 1983). Growth of *Leptospirillum* sp. up to 50 °C has been reported (Okibe et al., 2003) but the capacity to sustain growth at such temperatures has not been confirmed with isolated strains. This investigation focussed on a mixed population of moderately thermophilic organisms and the talc by-product nickel concentrate noted above at temperatures at and just above those recommended for the industrial process. A description of continuous leaching of a different nickel sulfide concentrate with the same culture included assessment of the bacterial species present (Cleaver et al., 2007) but not of the presence and some characteristics of an archaeal component of the microbial population, which are described here.

2. Materials and methods

2.1. Nickel concentrates

Two samples of a flotation by-product from a talc mining operation

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were used. These were blends of source materials, from two sites, comprising (w/w) 48–58% pyrrhotite, 37–38% pentlandite, 1–9% pyrite, 2–5% gersdorffite and approximately 1% magnesite and talc (Neale et al., 2015). The first sample, with a particle size D_{80} of 81 μm (see Results), contained 9.0% (w/v) Ni and 32.3% (w/v) Fe. The second sample with a particle size D_{80} of 46 μm contained 13.0% (w/v) Ni and 41.4% (w/v) Fe. Particle size analysis used a Malvern MasterSizer 3000 (Malvern Panalytical Ltd., Malvern, UK).

2.2. Microorganisms and culture media

A mixed culture of moderate thermophiles used previously in continuous nickel sulfide concentrate leaching (Cleaver et. al., 2007, Norris et al., 2017) was also used in this study. The principal bacteria in this culture are *Acidithiobacillus* (*At.*) *caldus*, *Sulfobacillus thermosulfidooxidans* and *Acidithiomicrobium* (*Atm.*) *siderophilum* (the proposed species name for what was previously known as *Acidithiomicrobium* strain P2; Davis-Belmar and Norris, 2009, Norris et al., 2011). The culture also contained at least two species of moderately thermophilic Euryarchaea.

For shaken flask batch culture with ferrous iron as substrate (50 mM; $FeSO_4{\cdot}7H_2O$, 13.9 g l^{-1}), the medium (100 ml) contained (g l^{-1}) K_2HPO_4 (0.1), $(NH_4)_2SO_4$ (0.2), $MgSO_4{\cdot}7H_2O$ (0.4) and yeast extract (0.2) and was initially adjusted with H_2SO_4 to a range of pH values (see Results). For shaken flask batch culture with pyrite (10 g l^{-1}, 40% w/w Fe, minus 75 um particle size), the medium (100 ml) was initially adjusted to pH 2 with H_2SO_4 and contained (g l^{-1}) K_2HPO_4 (0.2), $(NH_4)_2SO_4$ (0.4), $MgSO_4{\cdot}7H_2O$ (0.5) and, where indicated, yeast extract (0.2) or gassing with 5% v/v CO_2 in air. The culture medium feed (adjusted to pH 0.8–0.9) for continuous culture with nickel concentrates contained (g l^{-1}) K_2HPO_4 (0.3), $(NH_4)_2SO_4$ (0.6) and $MgSO_4{\cdot}7H_2O$ (0.5).

2.3. Bioreactors and assays

These were essentially as described previously for continuous nickel sulfide leaching in stirred reactors with essentially equal culture working volumes of approximately 550 ml, overflows through reactors' side arms and aeration with 1% v/v CO_2 in air (Norris, 2017). The medium feed flow rates, nickel concentrate concentrations and varied temperatures of operation are given in the Results. Either 5 or 10% w/v concentrate feeds were intended with the values in practice and the residence times also given in the Results. At the end of the longer term operation of reactors (217 days), 25 ml samples were taken (in quadruplicate) and washed twice with resuspension of pellets (leached residues and cells) in water, first at pH 1.7 and secondly at pH 3. Pellets were stored at −80 °C. DNA extraction was carried out using UltraClean™ Power Soil Microbial Kit (MoBio Laboratories) and DNA extract stored at −20 °C. DNA amplification was conducted for both bacterial and archaeal 16S rRNA genes as described in Santos (2018). Metagenomic analysis of V3-V4 variable regions of 16S rRNA genes was performed as described in Korzhenkov et al. (2019).

3. Results and discussion

3.1. Continuous leaching at 48–49 °C

A continuous reactor was operated at 48–49 °C with a three-day residence time and a feed of 10% (w/v) of the first concentrate which had 9.0% (w/v) nickel and a particle size D_{80} of 81 μm (Fig. 1).

Over short periods, for example a few weeks, the nickel concentration in solution appeared almost stable, but over a longer term a gradual increase in the concentration was observed. After 200 days of continuous operation, rather than the 70–80% nickel extraction expected from previous work with this culture (Cleaver et al. 2007), almost 100% extraction appeared to have been achieved, with the suspicion that this yield was inflated through some unintended retention of larger particles

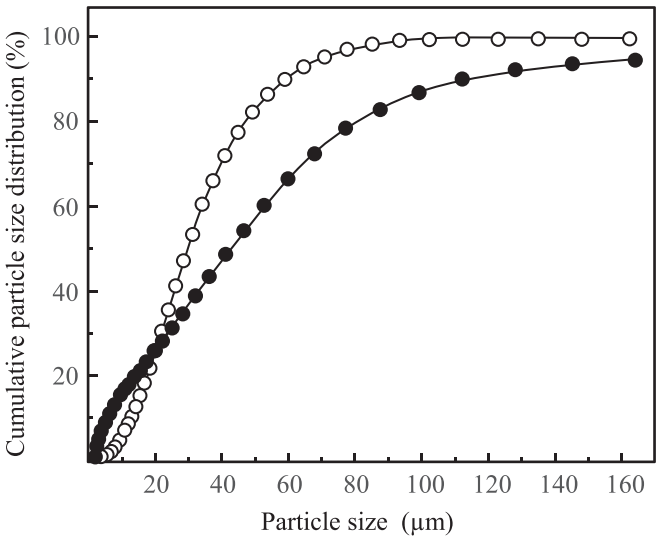

Fig. 1. Particle size distribution of concentrates 1 (closed symbols) and 2 (open symbols).

in the reactor while their dissolution was in progress. Nevertheless, the ferrous iron in solution remained below 0.5 g l^{-1} which indicated its sustained oxidation by microbial activity. After those 200 days of continuous operation, a change to the second concentrate (13.0% w/v nickel, D_{80} 46 μm) resulted in a relatively stable nickel concentration in solution (Fig. 2), presumably because of an improved distribution of particles between the two reactors with the relative absence of the larger particles of the first concentrate (Fig. 1). An increase in the overall residence time is shown after a secondary reactor was added in series after 141 days. There were some fluctuations with extreme nickel concentrations in solution of 6.3 and 9.6 g l^{-1} in the primary reactor and 8.3–12.1 g l^{-1} in the secondary reactor, which mainly resulted from unintended interruptions to aeration with consequent spikes in the ferrous iron concentrations, particularly at day 153 which was followed by one of the lowest concentrations of nickel in solution in the primary reactor (Fig. 2).

In summary (Table 1), the overall average nickel extractions for the two reactors were 66% for the primary reactor and 78% for the secondary reactor (calculated from the nickel concentrations in solution). The pulp density feed increased by just over 4% after the second reactor was added and the overall nickel solubilization increased by 18%. In contrast, the apparent iron extractions were similar in both reactors which, assuming proportional leaching of nickel and iron, suggested increased precipitation of iron hydroxysulfates with the extended residence time. The overall residence time with two reactors was slightly greater than double that of the single reactor because a slightly lower effluent volume was found with two reactors in series and a slightly greater solution working volume of the secondary reactor. In the commercial process with concentrate from the same source, nickel extraction was an average of 66% in the four commercial primary reactors in parallel with an increase to 76 to 88% through four secondary reactors in series, with a 17% w/v feed (D_{80} of 59 μm) and a residence time of 9.5 days (Laukka et al., 2018).

3.2. Microbial population composition at 48–49 °C

Compared to previous work with this mixed culture in continuous leaching of a different nickel concentrate (Cleaver et al., 2007), far fewer *S. thermosulfidooxidans* than *Atm. siderophilum* (then called *Acidimicrobium* species 2) were indicated, while *At. caldus* was again prominent (Fig. 3). The relative abundance (excluding archaea) of each bacterial species remained essentially the same in the primary and secondary reactors. Relatively few 16S RNA gene sequences were

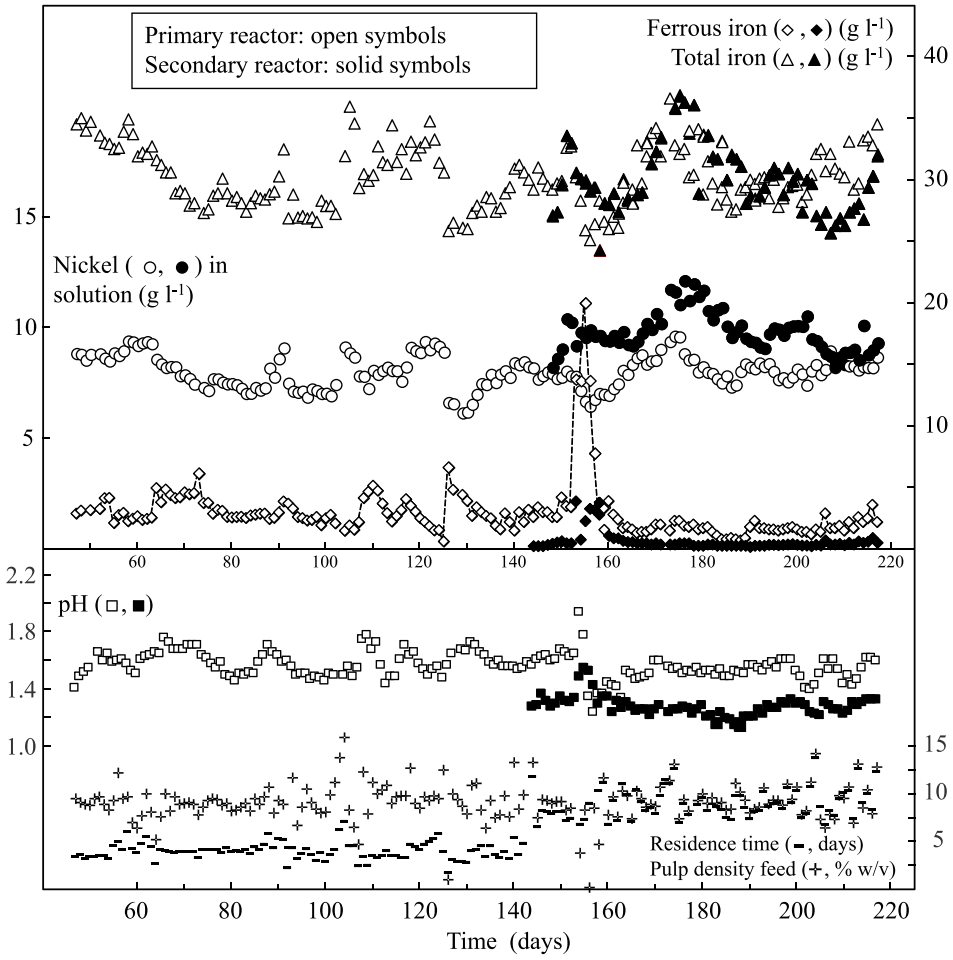

Fig. 2. Continuous leaching of a nickel concentrate at 48–49 °C, initially in a single reactor, with a secondary reactor added in series after 141 days.

Table 1
Mean concentrations of elements in solution and the pH over the indicated time periods of continuous nickel concentrate leaching at 48–49 °C.

	Reactor 1		Reactor 2
	0–142 days	143–217 days	143–217 days
Residence time (days)	4.3	–	9.4[a]
Concentrate feed (% w/v)	9.4	9.8	–
Nickel (g l^{-1})	8.1	8.1	9.9
Total iron (g l^{-1})	30.3	30.2	30.2
Ferrous iron (g l^{-1})	3.2	2.1	0.7
pH	1.6	1.6	1.3

[a] Overall residence time in two reactors.

detected that indicated either the acidophilic, anaerobic, sulfate-reducing *Desulfosporosinus acidiphilus* (Alazard et al., 2010) or *Desulfosporosinus acididurans* (Sánchez-Andrea et al., 2015); these appeared to represent fewer than 0.1% of the population. It is not known if these species could survive in oxygen-depleted accretions of concentrate on surfaces at the margins of the stirred suspension or if a DNA trace was added daily with the concentrate feed, with no growth of the bacteria in the reactors.

This community analysis has not taken account of different copy numbers of 16S RNA genes in the principal species: two in *At. caldus* and *Atm. siderophilum*, possibly four or five in *Sulfobacillus* sp. and one in the archaea described here.

The abundance of archaea in the secondary reactor increased to 50.5% from 9.8% in the primary reactor. The fewer of the two different archaeal, partial 16S RNA gene sequences were identical to only a few cloned sequences from unidentified archaea in The National Center for Biotechnology Information GenBank® nucleic acid sequence database: the highest identity (91%) to a named species was to *Thermogymnomonas acidicola*, for which iron and sulfur oxidations have not been described (Itoh et al., 2007). The predominant archaeal sequence was identical to those of *Acidiplasma cupricumulans* (*Ac. cupricumulans*) and *Acidiplasma aeolicum* (Golyshina et al., 2009) and in this case assumed to originate from an isolate (which was added to the mixed culture used here) after its isolation in 1997 from the Fairview (South Africa) refractory gold, arsenopyrite-leaching bioreactors (and then described as 'Sideroplasma' in Norris and Burton, Abstract BT-O5 of Thermophiles'98 Conference, Brest, France). The full 16S RNA gene sequence and the mol% GC content of this isolate are identical to those of *Ac. cupricumulans* and *Ac. aeolicum*. The ferrous iron-oxidizing isolate from the Fairview reactors grew with an organic medium supplement (yeast extract). However, in contrast to *Ac. aeolicum* (Golyshina et al., 2009), it grew well with ferrous iron and yeast extract without the need for an additional supplement of tetrathionate, suggesting the isolate was *Ac. cupricumulans*. Its growth and pyrite oxidation were more rapid at 48 than 55 °C (Fig. 4). The increased relative abundance of archaea in the secondary reactor (Fig. 3) could result from their greater tolerance of the acidity therein (Table 1). The effect of pH is shown, with the growth-associated ferrous iron oxidation rate measured at its most rapid at the beginning of exponential growth before acid consumption during ferrous iron oxidation increased the pH and before the presence of the ferric iron

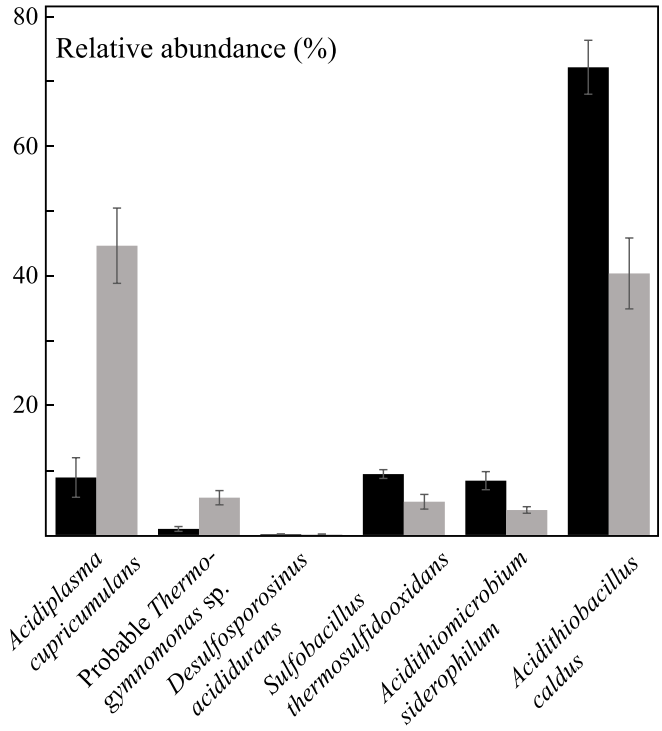

Fig. 3. Microbial population abundance analysis of samples (in quadruplicate) from two continuous, nickel concentrate reactors in series after 217 days operation at 48–49 °C. Primary reactor: black bars, secondary reactor: grey bars.

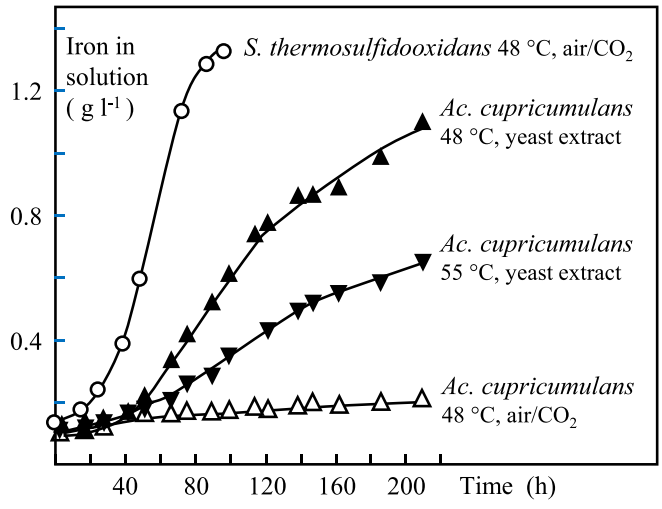

Fig. 4. Pyrite dissolution in shaken flasks during growth of *S. thermosulfidooxidans* and the *Ac. cupricumulans* isolate from the moderately thermophilic mixed culture with supplements of either CO_2 or yeast extract.

product reduced the oxidation rate (Fig. 5). Similar isolates to the *Ac. cupricumulans* described here have a pH 1 optimum for growth (Zhou et al., 2008). A potentially increasing concentration of organic substrates released from autotrophic bacteria could also be utilized and contribute to the relative increase in the archaea in the secondary reactor.

3.3. Effect of temperature on iron precipitation

The effect of temperature on precipitation of iron leached from the first concentrate (9% w/v nickel) was investigated twice: (1) with

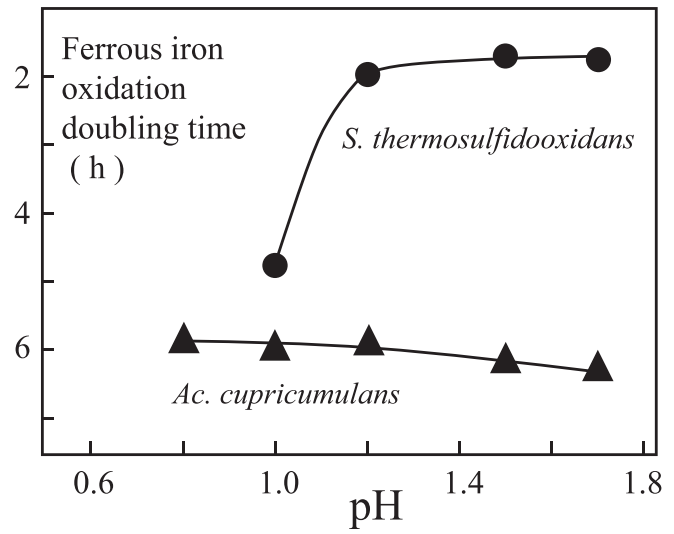

Fig. 5. The initial rates of ferrous iron oxidation by *S. thermosulfidooxidans* and *Ac. cupricumulans* in shaken flask batch cultures at 48 °C with yeast extract (0.2 g l^{-1}), the initial pH of the medium as indicated.

adjustment of the temperature in three reactors initially operating at 48–49 °C with a 5% w/v feed and (2) in a single reactor with a change in temperature during leaching of a 9–10% w/v feed (Fig. 6; Table 2).

The observed increases in precipitation of iron at the higher temperatures would be expected to lead to some loss of nickel from solution by co-precipitation but only a minor loss might be expected at these pH values (Kaksonen et al., 2014). The pH increased further to 1.7 after 10 more days at 50 °C probably because of inhibition of *At. caldus* as the temperature was raised further above its optimum of 45 °C. Microscopy showed a much greater total cell number at 45–46 °C than at the higher temperatures. The culture was less diverse and cells less numerous at 51.5 °C, with the absence of *At. caldus*. Previously, the presence of *At. caldus* was greatly reduced at 55 °C in continuous leaching of another nickel sulfide concentrate and its presence was likely not sustainable at that temperature (Cleaver et al., 2007). It was also present in another mixed culture at 48 °C but not 55 °C in batch leaching of a nickeliferous polysulfide where the higher temperature also resulted in increased precipitation of iron (Hubau et al., 2020). In this present study with the talc by-product concentrate, the relative numbers of *Atm. siderophilum* and *S. thermosulfidooxidans* were not determined at the higher temperatures. In previous work with this mixed culture in continuous culture, *S. thermosulfidooxidans* dominated at 55 °C (Cleaver et al., 2007). In contrast, at 55 °C in batch culture, *Acidithiomicrobium* P2 (*Atm. siderophilum*) was dominant (Hubau et al., 2020).

It is well established that an increase in temperature through use of moderate thermophiles rather than mesophiles increases the rate and extent of nickel leaching from concentrates (Norris et al., 1986; Dew et al., 1999; Cruz et al., 2010). However, in contrast to the changes in iron concentrations in solution and in pH with the small temperature rises (Table 2), the time courses here were too short to confirm an expected slight increase in nickel extraction at the higher temperatures. The described nickel extraction with the moderate thermophiles did not improve on the results of the commercial process at 46 °C (Neale et al., 2015; Laukka et al., 2018) so higher temperatures with thermophilic archaea would be required to show a clear increase in leaching rate, as demonstrated previously (Norris, 2017). Shear sensitivity of thermoacidophilic archaea at high solids concentrations has limited the feed concentration to industrial bioreactors, with a 12.5% w/v concentrate feed in the high temperature BioCOP™ process (Batty and Rorke, 2006) compared to a 20% w/v feed to BIOX® reactors (van Aswegen et al., 2007) and the Kasese cobalt concentrate reactors (Morin and d'Hugues, 2007) at lower temperatures: so the high temperature could be used in

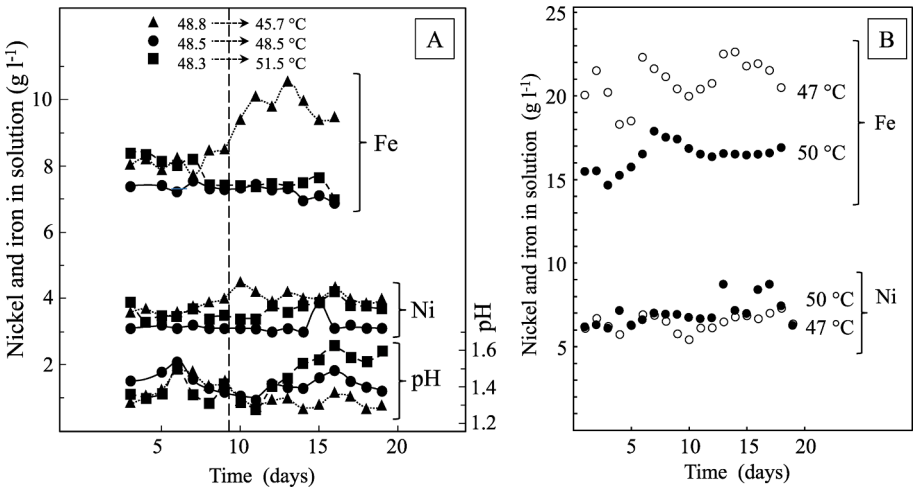

Fig. 6. Effect of temperature shifts (A) at the vertical dashed line during continuous leaching of nickel concentrate 1 with operation of three reactors simultaneously (5% w/v concentrate feeds) and (B) during continuous leaching at 47 °C for 20 days before and at 50 °C for 20 days after a temperature shift in a single reactor (with a 10% w/v feed).

Table 2
Features of continuous, nickel concentrate leaching and effects of temperature shifts.

Temperature (°C)	Residence time (days)	Feed (% w/v)	solution Fe concentration (% change)	pH*
47	2.81	9.5		1.37
50 (from 47)	2.91	9.2	−21	1.48
45.5 (from 48.8)	2.88	5.1	+20	1.31
48.5 (from 48.5)	2.96	5.2	+0.2	1.42
51.5 (from 48.3)	2.86	4.9	−8	1.57

* mean pH over final six days.

secondary reactors, where the solids concentration has been sufficiently reduced for the thermophiles to flourish and increase the rate and efficiency of target metal extraction and allow fewer bioreactors in series than generally proposed for commercial plants at lower temperatures.

4. Conclusions

1. The ferrous iron-oxidizing *S. thermosulfidooxidans* and *Atm. siderophilum* were outnumbered by the sulfur-oxidizing *At. caldus* in continuous leaching of a sulfide concentrate by-product of a talc mining operation at 48 °C, but a rise in pH was seen with restriction of *At. caldus* at and above 50 °C.
2. A small increase in temperature was insufficient to improve significantly the nickel leaching but, together with some consequent reduction in acidity, resulted in significant loss of iron from solution.
3. The relative abundances among bacteria was similar between two reactors operated in series but there was a fourfold increase in the relative abundance of the ferrous-iron oxidizing, lithoheterotrophic archaeon *Ac. cupricumulans* in the secondary reactor, most likely through a competitive advantage at the increased acidity.

CRediT authorship contribution statement

Paul R. Norris: Investigation, Writing - original draft, Writing - review and editing. **Robert Fitzpatrick:** Investigation. **Ana L. Santos:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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