Utilisation of native microbes from a spent chalcocite test heap

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Abstract

A variety of acidophilic iron and/or sulphur-oxidising microbes capable of growth on several substrates (chalcopyrite, pyrite, ferrous ion, sulphur, glucose) in the range 30–60 °C were recovered from a spent chalcocite/chalcopyrite/pyrite heap. Several isolates exhibited tolerance to salt, up to 5 g/L NaCl, and to the metals nickel, cobalt, zinc and copper at up to 50 g/L.

Leaching tests on chalcopyrite concentrate indicated higher copper yields when native isolates were employed, compared with the laboratory reference strains Acidithiobacillus ferrooxidans (DSM 583) and Sulfobacillus thermosulfidooxidans (DSM 9293). After 30 days, several native isolates had leached 20–30% more copper than the abiotic controls during experiments conducted at 45 °C. The results demonstrate that the native isolates are potential bioleaching candidates, adapted to diverse growth conditions.

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Keywords: Chalcopyrite; Microbial leaching; Kinetics

1. Introduction

Bioleaching involves the exploitation of microbes that thrive in high acid environments, require inorganic food sources and frequently display resistance to heavy metals. These unique chemolithoautotrophs are employed to leach sulphide ores such as chalcopyrite, an abundant but refractory copper sulphide mineral. Chalcopyrite leaching involves biooxidation of ferrous ion to ferric ion, the oxidation of chalcopyrite by ferric ion and the biooxidation of the sulphur thus generated (Eqs. (1)–(3)). Only 10–25% of the copper in chalcopyrite is released before the reaction slows or stops [1]. Eventually, products such as iron hydroxides, oxyhydroxides and jarosite form as a result of ferric ion hydrolysis (Eq. (4)).

\[
\begin{align*}
4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ & \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O} \quad \text{(bacterially mediated)} \\
\text{CuFeS}_2 + 4\text{Fe}^{3+} & \rightarrow 5\text{Fe}^{2+} + \text{Cu}^{2+} + 2\text{S}^0
\end{align*}
\]

(1)

(2)

\[
2\text{S}^0 + 2\text{H}_2\text{O} + 3\text{O}_2 \rightarrow 2\text{SO}_4^{2-} + 4\text{H}^+ \quad \text{(bacterially mediated)}
\]

(3)

\[
3\text{Fe}^{3+} + 2\text{SO}_4^{2-} + 6\text{H}_2\text{O} \rightarrow 2\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6^- + 6\text{H}^+
\]

(4)

The use of native microbes, acclimatised to Australian copper sulphide ores through many generations,
may result in increased copper extraction efficiency during bioleaching. In addition, the exploitation of such microbes would eliminate the need to use standard laboratory cultures, particularly for large-scale studies. However, the recovery and characterisation of environmental microbes presents significant challenges, requiring a detailed understanding of the mechanisms and microbial interactions present in the leaching environment. In this paper, methods developed and applied to the recovery of novel Australian bioleaching microbes, their phenotypic characterisations and their ability to enhance the leaching of chalcopyrite are described.

2. Materials and methods

2.1. Microbial enrichment

Samples of chalcocite/chalcopyrite/pyrite ore from a spent test heap were received from the Nifty Copper Operation (located about 460 km east of Port Hedland, WA). This ore contained about 1% copper and 0.01% zinc prior to leaching. Samples recovered from various locations on the heap were used to enrich bioleaching microbes. Mixed consortia were subsequently transferred to a variety of growth substrates before attempts to recover single isolates were made. For each chosen heap location, samples from 0.5 to 5.1 m depth were pooled, homogenised and added aseptically to 250-mL flasks containing 100 mL sterile basal salts medium (BSM), [1.5 g/L (NH₄)₂SO₄, 0.25 g/L KH₂PO₄, 0.25 g/L MgSO₄], pH 1.8. This procedure yielded a slurry with between 30% and 50% pulp density. Initially, flasks were placed in incubators at 30, 45 and 60 °C for 24 h. Growth was observed using phase contrast microscopy and cell types were recorded. Growth was allowed to continue for a further 96 h before sub-culturing into BSM, pH 1.8 (0.01% yeast extract) supplemented with the following: 1% w/v chalcopyrite concentrate; 1% w/v pyrite concentrate; 22 g/L FeSO₄·7H₂O; 5 g/L elemental sulphur; 0.05 mM K₂S₄O₆; 10 mM FeSO₄·7H₂O; 0.05 mM K₂S₄O₆ (Fetet solution), 5 g/L.

All evaluations of growth in varied pH, temperature, salinity and heavy metal concentrations were performed in BSM, pH 1.8 supplemented with Fetet solution and 0.01% yeast extract. Growth was evaluated at various pH (0.5, 1.0, 1.5, 2.0, 3), temperatures (30, 40, 50, 60 °C), NaCl concentrations (0, 1, 3, 5 g/L) and heavy metal concentrations up to 50 g/L (copper, nickel, zinc and cobalt).

2.2. Isolation and characterisation of isolates from Nifty ore

Characterisation of mixed consortia and growth in yeast extract amended media indicated that growth was significantly better than in the absence of yeast extract. Hence, the amended medium was used for all characterisation and leaching tests. Successive subcultures to all media were made in order to confirm growth. Following successful transfer, mixed cultures were serially diluted to extinction in 96-well microtitre plates containing BSM, pH 1.8 (0.01% yeast extract) supplemented with either 22 g/L ferrous sulphate or 0.05 mM potassium tetrathionate (sulphur source) using either doubling dilutions or ten-fold dilutions dependant on the initial starting concentration of cells. Mixed consortia were diluted to extinction repeatedly until pure isolates were recovered.

Pure isolates were screened for their energy substrate requirements, pH range, temperature profile, salt tolerance and heavy metal resistance. Five replicates were prepared for each isolate and the experiment repeated to confirm results. Isolates were evaluated for growth in BSM, pH 1.8 (0.01% yeast extract) supplemented with one of the following: 1% w/v chalcopyrite concentrate; 1% w/v pyrite concentrate; 22 g/L FeSO₄·7H₂O; 5 g/L elemental sulphur; 0.05 mM K₂S₄O₆; 10 mM FeSO₄·7H₂O; 0.05 mM K₂S₄O₆ (Fetet solution), 5 g/L.

2.3. Adaptation of microbes to chalcopyrite prior to leaching tests

Prior to commencing shake flask leaching tests, Nifty isolates and reference strains Acidithiobacillus ferrooxidans (DSM 583), used for 30 °C experiments, and Sulfobacillus thermosulfidooxidans (DSM 9293), used for 45 °C experiments, were sub-cultured onto BSM, pH 1.8 (0.01% yeast extract for DSM 9293 and Nifty isolates only) supplemented with 3% w/v chalcopyrite concentrate (24.2% Cu) and incubated in an orbital incubator shaker (180 rpm) at the appropriate temperature. Cultures were passaged at least twice on the concentrate before leaching tests commenced. Before leaching, cells were counted using a Petroff–Hausser counting chamber.

2.4. Chalcopyrite leaching tests

Erlenmeyer flasks were set up containing 100 mL BSM, pH 1.8 (0.01% yeast extract for DSM 9293 and Nifty isolates only) supplemented with 3% w/v chalcopyrite concentrate (sourced from Mount Isa Mines, see Table 1 for selected elemental analyses), and weighed. Flasks were autoclaved (30 min at 121 °C), allowed to
cool and re-weighed. Any water lost was replaced aseptically with sterile distilled water. Flasks were placed at the experimental temperature for 30 min prior to inoculation so that cells were not adversely affected by temperature change.

The flasks were inoculated aseptically to achieve a cell concentration of \( \sim 1 \times 10^6 \) cells/mL and were mixed before 3 mL was aseptically removed for analysis. Flasks were re-weighed and replaced at the appropriate temperature in the orbital incubator shaker (180 rpm). Prior to sampling, the flasks were weighed and water lost to evaporation was replaced with sterile distilled water. A sample of 3 mL was removed from each flask and weights recorded prior to re-incubating.

The pH and redox potential (Ag/AgCl) were measured for each sample. Following these measurements, 1 mL of sample was then transferred to a microfuge tube and centrifuged at 16,000 rpm for 10 min at room temperature. The resultant supernatant was removed and used for spectrophotometric ferrous ion determination using the Wilson method [2]. The remaining 2 mL of sample was filtered using a 0.2-μm nitrocellulose syringe filter and 1 mL of the filtered liquid was acidified using 0.5 mL concentrated HCl. Acidified samples were then analysed for total copper, iron and sulphur using inductively coupled plasma–atomic emission spectrometry (ICP–AES). Samples were recovered periodically over approximately 2000 h and monitored for bacterial growth using phase-contrast microscopy. The first shake flask trials tested all isolates and reference strains, whereas the second trial re-evaluated the most promising isolates.

3. Results and discussion

3.1. Isolation and characterisation of native isolates

Several isolates were recovered from two sites, designated 39 and 22, giving rise to the isolate nomenclature, e.g., N39-45-01 translates to N, Nifty; 39, location on heap; 45, temperature of enrichment; 01, first isolate for that group.

Phenotypic screening of the selected isolates revealed a diverse profile, but all could be categorised as mesophilic acidophiles. The observed improvement in growth when yeast extract amended medium was used prevents denoting the isolates as pure autotrophs. Experiments involving mixed consortia of Nifty organisms (data not shown) indicated comparable leaching without yeast extract, suggesting that carbon could be provided by organic compounds originating from other acidophiles. Hence, the isolates are more likely to be heterotrophs with a preference for inorganic compounds [3].

Both iron and sulphur oxidising bacteria with bacilli morphology were recovered. Each of the six isolates chosen for further study was able to utilise CuFeS₂, elemental sulphur and K₂S₄O₆ as energy sources. Additionally, several isolates displayed strong heterotrophic tendencies following successful sub-culturing in glucose. Results for isolates N39-30-02 and N39-45-02 indicated that they were primarily sulphur oxidisers, whereas the other isolates oxidised both iron and sulphur (Table 2). The failure of N39-30-02 to grow on pyrite or FeSO₄·7H₂O suggested that iron may be inhibitory to growth. However, growth on chalcopyrite counters this, as the pyrite concentrate contained similar percentages of iron (33%) and sulphur (37%) to the chalcopyrite concentrate (26% iron and 28% sulphur).

All Nifty isolates screened had a similar pH tolerance range (1.5–2.0) in which optimal growth could be achieved (Table 3), although this characteristic did not limit their leaching ability. The four isolates recovered at 45 °C (N39-45-01, N39-45-02, N22-45-01, N22-45-02) grew at 1 g/L NaCl, whereas the remaining 30 °C isolate

### Table 1
Selected elemental analyses for the chalcopyrite concentrate used in this work

<table>
<thead>
<tr>
<th></th>
<th>Cu (%)</th>
<th>Fe (%)</th>
<th>S (%)</th>
<th>Si (%)</th>
<th>Al (%)</th>
<th>Mg (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.5</td>
<td>25.9</td>
<td>28.6</td>
<td>5.19</td>
<td>0.32</td>
<td>0.95</td>
<td></td>
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</tbody>
</table>

### Table 2
Substrate utilisation of Nifty isolates

<table>
<thead>
<tr>
<th>Nifty isolate</th>
<th>CuFeS₂</th>
<th>FeS₂</th>
<th>S</th>
<th>FeSO₄</th>
<th>K₂S₄O₆</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>N39-45-01</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N39-45-02</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N22-45-01</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N22-45-02</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N39-30-02</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N39-30-03</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+*=growth; ‘-’=no growth.

### Table 3
pH, salt, temperature and heavy metal tolerances for Nifty isolates

<table>
<thead>
<tr>
<th>Nifty isolate</th>
<th>pH range</th>
<th>Salinity range (g/L NaCl)</th>
<th>Temperature range (°C)</th>
<th>Heavy metal tolerance (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cu</td>
</tr>
<tr>
<td>N39-45-01</td>
<td>1.5–2.0</td>
<td>0–1</td>
<td>30–50</td>
<td>45</td>
</tr>
<tr>
<td>N39-45-02</td>
<td>1.5–2.0</td>
<td>0–1</td>
<td>30–40</td>
<td>10</td>
</tr>
<tr>
<td>N22-45-01</td>
<td>1.5–2.0</td>
<td>0–1</td>
<td>30–50</td>
<td>50</td>
</tr>
<tr>
<td>N22-45-02</td>
<td>1.5–2.0</td>
<td>0–1</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>N39-30-02</td>
<td>1.5–2.0</td>
<td>0–3</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>N39-30-03</td>
<td>1.5–2.0</td>
<td>0</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>
(N39-30-03) did not tolerate saline conditions. Only N39-30-02 was able to sustain growth at 5 g/L NaCl, suggesting that this strain may be able to leach sulphide ores in the presence of chlorides, an advantage for deposits in areas of high salinity. The overall lack of salt tolerance suggested that microbes suitable for the bioleaching of mineral sulphides in areas of high salinity should be targeted specifically during the initial enrichment.

Temperature profiles of the six Nifty isolates indicated that the isolates recovered at 30 °C did not grow when the temperature was increased to 40 °C, whereas all of the isolates recovered at 45 °C grew at 30 °C. Additionally, isolates N22-45-01, N22-45-02 and N39-45-01 grew up to 50 °C but failed to sustain growth at 60 °C (Table 3). Bioleaching microbes recovered from Australian mine sites are likely to be mesophiles or moderate thermophiles, as temperatures at mine sites do not generally exceed 50 °C. Tolerance of a wide temperature range, 30–50 °C, makes them good candidates for heap bioleaching where temperature regulation is difficult and temperature fluctuations may occur depending on environmental or mineralogical conditions.

The soluble metals most often associated with bioleaching are cobalt, copper, nickel and zinc. The results of metal resistance tests demonstrated a high level of diversity among the isolates (Table 3). Resistance to high metal concentrations was expected to be seen only for copper due to the origin of the isolates. However, the results showed that the isolates expressed resistance to each of the metals, in particular zinc. Only isolate N39-30-03 was able to tolerate high concentrations of all four metals tested, with the other isolates being sensitive to one or more metals.

The survival of a microbe within a heap depends on the expression of metal resistance genes. The horizontal transfer of such genes within the environment has been well documented. Toxic concentrations of metals, made soluble by the metabolic activity of microbes, put

![Fig. 1. Percentage copper released during leaching at 45 °C.](image)

<table>
<thead>
<tr>
<th>Nifty isolates, reference strains and abiotic controls</th>
<th>Cu recovery % after 30 days</th>
<th>Cu recovery % after 90 days</th>
<th>Stabilised pH</th>
<th>Soluble S (mg/L) after 30 days</th>
<th>Soluble S (mg/L) after 90 days</th>
<th>Stabilised Eh (mV vs. Ag/AgCl) after 30 days</th>
<th>Total Fe (mg/L) after 30 days</th>
<th>Total Fe (mg/L) after 90 days</th>
<th>Stabilised Fe^{2+} (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N39-30-02</td>
<td>10.4</td>
<td>a</td>
<td>1.9</td>
<td>1643</td>
<td>a</td>
<td>380</td>
<td>350</td>
<td>a</td>
<td>50</td>
</tr>
<tr>
<td>A. ferrooxidans (30)</td>
<td>8.5</td>
<td>a</td>
<td>1.8</td>
<td>1902</td>
<td>a</td>
<td>600</td>
<td>947</td>
<td>a</td>
<td>10</td>
</tr>
<tr>
<td>Abiotic (30)</td>
<td>5.0</td>
<td>7.5</td>
<td>3.3</td>
<td>1313</td>
<td>1281</td>
<td>350</td>
<td>559</td>
<td>532</td>
<td>500</td>
</tr>
<tr>
<td>N39-45-01</td>
<td>19.7</td>
<td>b</td>
<td>1.7</td>
<td>2508</td>
<td>b</td>
<td>400</td>
<td>830</td>
<td>b</td>
<td>50</td>
</tr>
<tr>
<td>N39-45-02</td>
<td>26.3</td>
<td>75.7</td>
<td>1.6</td>
<td>2878</td>
<td>6403</td>
<td>600</td>
<td>1015</td>
<td>2367</td>
<td>15</td>
</tr>
<tr>
<td>N22-45-01</td>
<td>52.4</td>
<td>91.5</td>
<td>1.5</td>
<td>5054</td>
<td>7374</td>
<td>580</td>
<td>1850</td>
<td>2300</td>
<td>15</td>
</tr>
<tr>
<td>Mixed Nifty</td>
<td>48.4</td>
<td>90.0</td>
<td>1.6</td>
<td>4763</td>
<td>7145</td>
<td>590</td>
<td>1798</td>
<td>2596</td>
<td>10</td>
</tr>
<tr>
<td>S. thermosulfidooxidans (45)</td>
<td>20.4</td>
<td>b</td>
<td>2.7</td>
<td>1400</td>
<td>b</td>
<td>550</td>
<td>1000</td>
<td>b</td>
<td>20</td>
</tr>
<tr>
<td>Abiotic (45)</td>
<td>7.3</td>
<td>18.0</td>
<td>2.7</td>
<td>1380</td>
<td>1700</td>
<td>360</td>
<td>560</td>
<td>460</td>
<td>450</td>
</tr>
</tbody>
</table>

Table 4
Summary data for bioleaching screening tests

\(^a\) Leaching slow, experiment terminated.

\(^b\) Strain no longer viable; flasks terminated.
selective pressure on the cells to obtain and express genes coding for resistance mechanisms [4]. Cells possessing metal resistance are likely to play a more active role in mineral dissolution because they continue to grow when exposed to high metal concentrations. Analysis of the chalcopyrite ore from the Nifty heap showed it contained about 1% Cu and 0.01% Zn, which could explain, in part, why many of the isolates expressed resistance to both copper and zinc. The genes coding for resistance to cobalt and nickel can occur together on a plasmid that also codes for resistance to zinc. The existence of plasmids that code for a mechanism which gives multiple resistances is not uncommon. For example, among members of the *Ralstonia* sp. a plasmid, pMOL28, has been shown to code for cobalt and nickel resistance and, under selective pressure, also for zinc [5].

3.2. Bioleaching of chalcopyrite concentrate – screening of native isolates

The bioleaching capacity was evaluated for the Nifty isolates, except N39-30-03 which failed to grow in sufficient numbers for these tests, and N22-45-02, for which data are not yet available.

Isolates tested at 30 °C (mixed Nifty 30 °C isolates, N39-30-02 and *A. ferrooxidans* DSM 583) leached only 5% more copper than was recovered in the abiotic control (Table 3).

At 45 °C, greatest copper recovery was achieved with the mixed Nifty consortium of isolates (mixed Nifty) and strain N22-45-01 (Fig. 1). Strain N39-45-02 exhibited a delayed growth phase, after which copper recovery increased markedly. Strain N39-45-01 and, interestingly, *S. thermosulfidooxidans*, failed to grow after 20 and 30 days, respectively. The molecular characterisation of a number of isolates in this study was undertaken by analysis of 1400 bp of the 16S rRNA gene. Isolate N22-45-01 demonstrated a 99% homology with *S. thermosulfidooxidans* and isolate N39-45-02, 99% homology with *Acidithiobacillus caldus*. Further analysis is being undertaken.

Solution chemistry parameters were monitored during the test (summarised in Table 4). After an initial rise from pH 1.8 to about 2.5, pH levels dropped and

![Fig. 2. Copper recovery during leaching at 45 °C.](image1)

![Fig. 3. pH profiles during leaching tests conducted at 45 °C.](image2)
then stabilised at about pH 1.6 for Nifty strains. The decreased pH is indicative of bacterial sulphur oxidation (Eq. (3)), and results in a steady increase in soluble sulphur (sulphate) in leachate. Total iron, ferrous ion and solution potential correlate well and together show that ferrous ion biooxidation is rapid and efficient in biotic tests (low [Fe$^{2+}$] and high Eh).

The results clearly demonstrate that some Nifty (45 °C) isolates have greater leaching capabilities than the laboratory control strain, S. thermosulfidooxidans DSM 9293. After 30 days, several Nifty isolates had leached between 20% and 30% more copper. Additionally, the laboratory control strain failed to grow after 30 days and was deemed non-viable, whereas the majority of Nifty isolates continued to sustain growth without additional nutrients or growth supplements for over 90 days. Nifty isolates, with the exception of N39-45-01, recovered over 70% of the available copper, with N22-45-01 and the Mixed Nifty consortium recovering 90% of the available copper.

3.3. Bioleaching of chalcopyrite concentrate – testing prospective strains

The leaching of chalcopyrite using the most prospective strains, N22-45-01, N39-45-02 and the mixed Nifty consortium, was examined in detail (Figs. 2–7).

During the screening tests, N39-45-02, N22-45-01 and the Mixed Nifty consortium recovered 75%, 91% and 90% copper, respectively, after 90 days (Table 4). Copper recoveries of the same order, 81%, 80% and 86% copper, respectively, were achieved in the repeated tests (Fig. 2). Strain N39-45-02 appeared to have adapted to bioleaching under these conditions and did not exhibit any lag time in growth and activity.

The solution pH stabilised at about 1.7 for the biotic tests, and this, together with the increase in soluble sulphur (sulphate) during leaching confirms the ability to oxidise sulphur (Figs. 3 and 4).

Ferrous ion and total iron concentrations, together with the high solution potential (Figs. 5–7) are consistent with there being good iron biooxidation in
biotic tests. The break in slope for both soluble sulphur (Fig. 4) and in total iron concentrations (Fig. 7) at about 5 days is indicative of jarosite formation, which removes some iron and sulphate from solution (Eq. (4)).

The shortened lag time observed for N39-45-02 suggests that during the screening tests, the inoculum may have lacked a healthy cell population. Alternatively, repeated sub-culturing on the chalcopyrite concentrate increased the level of adaptation, resulting in improved leaching kinetics.

Although variations were observed in final copper recoveries between the first and second experiments, the percentage of copper recovered was still significantly better than those for the abiotic control and the reference strain. Overall, these results indicated that the Nifty environmental isolates have significant potential for scaled up applications such as stirred tanks and column leaching. However, as with all bacterial strains, it is essential to understand the selective pressures that are required to maintain isolates or strains in their original form. This challenge is even greater when an isolate is recovered from an environmental system within which there are so many unknown variables that may interact with the microbe.

The time between initial enrichment and isolation is crucial to maintain the original genetic characteristics of the isolate. For example, removing an isolate from an environment where selective pressures such as high metal concentrations exist, may result in spontaneous mutations of genes that would otherwise provide resistance to particular heavy metals. The continuous sub-culturing of environmental isolates for biotechnological applications such as stirred tanks will inevitably tailor the isolate to that particular application, reducing its potential to adapt to a heap leach environment. So whilst environmental isolates could be successfully applied for multiple biotechnological applications, they may become more like laboratory reference strains and become less suitable for environmental applications over time.

A possible solution to overcoming environmental isolates losing their original traits is to study and work with microbial communities or mixed consortia rather than single isolates. Several studies have reported
improved leaching and robustness when mixed consortia were used in comparison to single strains [6,7]. However, in this study, the mixed Nifty consortium of isolates recovered at 45 °C did not perform better than the single isolates N22-45-01 and N39-45-02. This suggests that a greater knowledge of the synergistic and antagonistic interactions between different strains in the leaching microbial community is needed to identify and exploit an effective mixed consortium.

This study has demonstrated the successful recovery of Australian bioleaching microbes that can leach chalcopyrite significantly better than laboratory reference strains in the shake flask system. Further evaluation of their leaching capacity is required, such as stirred tank and column leaching tests. An advantage of indigenous microbes is that they are not restricted by the regulations governing genetically modified organisms or quarantine regulations, enabling them to be used for both biotech and environmental applications.

4. Conclusions

Phenotypic characterisation of the Nifty culture collection revealed isolates with very similar nutritional requirements, pH ranges and temperature profiles. However, water salinity and heavy metal characterisations indicate greater diversity, with potential to explore their importance and application.

Despite the limitation and possible bias associated with the enrichment and isolation methods, novel native bioleaching microbes have been obtained. The process of isolating, purifying and characterising native isolates and collecting leaching kinetic data through experiments took several months and was very labour-intensive. It is possible that the most active and important microbes perish before they can be cultured in the laboratory, leaving those microbes that are not as influential in the leaching process. Another issue concerns the cooperative nature amongst different microbial strains to provide nutrients to other strains.

These studies have demonstrated that novel Australian bioleaching microbes can be recovered and employed to leach chalcopyrite more effectively than an abiotic system and laboratory reference strains. By identifying an isolate that possesses traits necessary for highly efficient metal solubilisation, this system can be further optimised. This was demonstrated through the pre-conditioning of N39-45-02 that resulted in increased metabolic activity generating more oxidising agents and improving copper recovery.

Future work will involve evaluating isolates that show potential in stirred tank and column leaching systems. Isolates that indicate resistance to zinc, nickel and cobalt will be employed to leach other mineral sulphides such as sphalerite. Additionally, further exploration of the limits associated with sodium chloride tolerance and effect on bioleaching will be studied.

The increased sustainability and higher leaching capacity of the Nifty isolates indicates the benefits of using indigenous microbes that adapt readily to chalcopyrite and leaching conditions.

Acknowledgements

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