The Efficiency of Indigenous and Designed Consortia in Bioleaching Stirred Tank Reactors

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Abstract

Fundamental to the efficiency of bioleaching is the establishment of an active microbial population. There is some debate as to whether an indigenous microbial population is necessarily superior to one composed of microbial strains selected for specific physiological traits. To this end, the bioleaching efficiency of three microbial populations was studied: The indigenous population of a commercial bioleaching system (KCCL), a reconstituted consortium of the three major organisms which comprise KCCL that had been 'un-adapted' through a period of continuous maintenance in synthetic media (KCCR) and a specifically designed consortium of bioleaching organisms (KCCD). *Acidithiobacillus caldus* was unable to re-establish itself in the reconstituted, un-adapted consortium. However, the bioleaching rate of this consortium improved over time, and its overall performance was ultimately very similar to that of the indigenous population. This is despite the absence of an obligate sulfur-oxidising species, which resulted in the generation of substantially less acid. The performance of the designed consortium was

poor, and the results imply that bioleaching consortia cannot be assembled ‘off-the-shelf’, at least not without a substantial period of adaptation.

Introduction

Biomining is the exploitation of biohydrometallurgy to process metal ores. These processes can be divided into two main types: irrigation-types and stirred tank-types. Irrigation-type processes involve the irrigation of crushed rock with a leaching solution, followed by the collection and processing of the leachate or pregnant liquor solution (PLS) to recover the target metals. Stirred tank-type operations involve the processing of mineral concentrates in a continuous series of large bioreactors (stirred tank reactors; STRs), and offer much more control than irrigation-based operations.

Most stirred tank operations are employed for the biooxidation of refractory gold ores; the value of the gold produced displacing the higher capital and running costs required for the implementation of these processes. However, a commercial stirred tank operation at the Kasese Cobalt Kilembe Mine in Uganda is used to recover cobalt from a 900 Kt dump of cobaltiferous pyritic tailings stockpiled on the site during the mine's operation between 1956 and 1982. The plant processes some 245 tonnes of tailings per day, recovering approximately 92% of the cobalt (Briggs and Millard, 1997; Morin and d'Hugues, 2007).

The earliest commercial biomining systems were not designed to specifically promote microbial activity. This has since changed, and greater effort is made to provide optimal leaching conditions for the bioleaching population, and to understand the biological processes therein. Due to the constant process monitoring and control, stirred tank biomining operations offer relatively constant and homogeneous conditions for the growth of acidophiles. As a result, populations tend to be a stable mixture of a limited number of physiologically- and phylogenetically-distinct prokaryotes, which generally includes a sulfur-oxidiser, an iron-oxidiser and a mixotrophic or heterotrophic acidophile (Norris, 2007; Okibe *et al*., 2003).

All current commercial stirred tank plants operate using mesophilic or moderately thermophilic microbial cultures. Most use the BIOX process, which operates at 40-45℃. These operations tend to be do minated by the iron-oxidising *L. ferriphilum* and the sulfur-oxidising *At. caldus* (Rawlings, 2007). Analysis of a pilot-scale polymetallic sulfide stirred tank operation running at ~45℃ found that the primary tank was dominated by *L. ferriphilum*, *At. caldus* and *Sulfobacillus* spp. However, in the secondary and tertiary tanks, the iron-oxidising heterotroph *Ferroplasma acidiphilum* became increasingly dominant, replacing *L. ferriphilum* completely in the final tank. Such 'heterotrophically-inclined' iron-oxidisers may tend to become increasingly dominant in secondary and tertiary reactors due to the increased availability organic carbon as a result of primary production in the initial tanks (Norris, 2007).

Foucher *et al*. (2001) reported that the culture used to inoculate the Kasese stirred tank system in Uganda mainly comprised *L. ferrooxidans*, *At. caldus* and a *Sulfobacillus* sp.. However, the microbial population of the commercially operating system is known to be dominated by *L. ferriphilum*, *At. caldus* and a distinct *Sulfobacillus* sp., with low levels of a *Ferroplasma* sp. (Johnson, unpublished).

Rawlings (2005) presents an in depth review of the microbial populations of biomining systems. The most important trait of an effective mineral oxidising population is the ability to generate the ferric iron and sulfuric acid lixiviant. However, each organism must be adaptable and able to compete in the non-sterile biomining environment. Early STRs treating gold ores were slow at first, as the organisms were not adapted to the reactor conditions (Rawlings and Silver, 1995). Over the next few years of pilot and commercial scale-up, performance increased, with the retention time required decreasing from 12 days to 3.5 days and solids loading increased from 10 % to 18 % (Rawlings, 2005). However, as the original culture was not retained, it is not possible to determine whether this increase in performance was due to adaptation of the existing population or through the acquisition of ‘better’ ones.

In a more recent review, Rawlings and Johnson (2007) raise many of the theoretical issues associated with the microbial consortia of biomining STRs.

Unlike most other industrial bioprocessing operations, selection, control and routine monitoring of the microbial population is minimal or non-existent. Therefore, it is not clear that the microbial population of a commercial system is necessarily the most effective.

In this review, two potential approaches are suggested for the design of bioleaching consortia. The ‘top-down’ approach envisages inoculating the STR with a wide range of organisms of which only the fittest will survive. The aim is to have a sufficiently high biodiversity at the start to provide a robust system. In contrast, the ‘bottom-up’ approach would employ a very specific, logically designed consortium with a small number of organisms selected on the basis of physiological traits needed to treat the target ore. Such traits may include high levels of resistance to certain metals. In both approaches, organisms would either have been isolated from existing biomining plants, or more likely from environmental samples.

However, as stated above, the population may require a significant period of time to adapt to the STR conditions. Therefore, it is probable that any unadapted organisms (particularly those from environmental samples) may lose out to adapted ones, even if they may eventually be superior given sufficient time. The situation is clearly complex and is compounded by a lack of microbial succession studies from lab to pilot to commercial scale. Before the use of designed consortia (top-down or bottom-up) can be pursued, several key questions must be addressed. These include:

- How long does it take for environmental organisms to become competitive in a biomining STR?
- Is there only one ideal combination of microorganisms for a given process or substrate?
- Are indigenous organisms inherently more efficient than selected strains?

The non-sterile nature of biomining systems makes the question very difficult to address. While there has been much work done using defined populations

on sterilised ore (for example Dopson and Lindström, 1999; McGuire *et al.*, 2001; Edwards *et al*., 2000a) such studies are usually performed using simple shake flask experiments and present much less challenging environments than that found in a typical biomining STR (both chemical and sheer stresses).

This study presents an effort to begin the process of providing empirical evidence for the theoretical issues raised. Three different consortia were tested on a cobaltiferous pyrite originating from Kasese (Uganda). These consortia were: the indigenous bioleaching population taken from the commercial plant in Uganda; a defined population of the four principal microorganisms that comprise this population which had been isolated and cultured as pure strains; and a defined consortium consisting of organisms selected to fulfil the collective roles of the indigenous population. Comparison with the designed consortium would show whether certain organisms are simply better evolved to STR conditions, or whether foreign organisms, given time, could become as effective.

Materials & Methods

Bacterial Inocula

The indigenous consortium (KCCL) was taken from the Kasese Cobalt Company Ltd. bioleaching plant in Uganda. The reconstituted consortium (KCCR) comprised the four microbial strains which had been shown to be present in, and were subsequently isolated from, the indigenous Kasese bioleaching population (d'Hugues *et al*., 2008; Johnson *et al*., 2008). These were: *Leptospirillum ferriphilum* BRGM-1, *Sulfobacillus benefaciens* BRGM-2, *Acidithiobacillus caldus* BRGM-3 and *Ferroplasma acidiphilum* BRGM-4. The prerequisites for the designed consortium (*KCCD*) were that it contain an iron-oxidising and a sulfur-oxidising microorganism, that the capacity for both autotrophic and heterotrophic growth were present and that all the strains could grow at 40℃. The following strains were chosen: G1, an iron- and sulfur-oxidising Gram-positive mixotrophic firmicute (Johnson *et al*., 2005); *Acidimicrobium ferrooxidans* TH3, an iron-oxidising mixotrophic actinobacterium (Clark and Norris, 1996); *At. thiooxidans* SDE4, a sulfur-

oxidising autotroph; SDE2, an iron- and sulfur-oxidising firmicute (both Bryan *et al*., 2006) and Y008c, an iron-oxidising, mixotrophic Gram-positive organism contaminating a culture of strain Y008 (Johnson *et al*., 2003).

Pure cultures were maintained in appropriate liquid media (Bryan *et al*., 2006). In all cases, a preculture was prepared in a shake flask containing 200 mL 0km medium (d'Hugues *et al*., 2008) with 5 % w/v sterilised KCC pyrite (10% v/v total inoculum) which was used to inoculate the initial reactor. The cultures were incubated at 38℃ in a reciprocal shaker. Samp les were taken at regular intervals and pH, redox, and soluble sulfate, cobalt and iron concentrations determined as described below.

Sulfide Mineral and Bioleaching Experiments

The sulfide material used in the bioleaching experiments was a cobalt-rich pyrite flotation concentrate that had been stockpiled at Kasese (Uganda) downhill from the Kilembe Mine. It had been stored on the BRGM premises for two years prior to its use in the current study. The material contained on average 80% pyrite and 1.3% cobalt mainly disseminated within the pyrite matrix and had a P80 of 43 µm. The lot was quartered into aliquots of approximately 200 g which were sterilised by gamma irradiation (40-70 KGy external exposure).

To test the mineral sterility and amenability to bioleaching, triplicate flasks of 100 mL sterile 0Km medium were inoculated with either 5% w/v mineral, 5% w/v mineral + 5% v/v KCCL bioleaching consortium or 5% w/v non-sterilised mineral. Flasks were incubated as above. At regular intervals flasks were weighed and water lost through evaporation replaced from a sterile stock. 2 mL samples were taken and pH and redox and soluble sulfate concentrations determined turbidimetrically as described by Kolmert *et al*. (2000). Soluble [Co] and [Fe] were determined by AAS.

Bioleaching experiments were performed in batch mode in 2 L (working volume) laboratory-scale glass bench-top reactors. To minimise the risk of contamination of the unit the air line included a 0.22 µm (pore size) in line filter

and a cotton-wool bung was used in the exit to the condenser. The entire unit was autoclaved and strict aseptic technique was used during sampling.

The reactors were operated at 40℃, and CO_2-enriched air (1%) was injected at a rate of 100 L h^{-1}. The reactors contained 10% w/v sterile KCC pyrite, and a 10% v/v inoculum was used. The impeller speed was 700 rpm and pH was maintained at pH 1.0-1.2 through the addition of a sterile calcite suspension (8:10 w/v $CaCO_3$:H_2O). Sampling was performed twice-daily, and pH and Redox measured; 2 mL samples were stored at -20℃ for eventual DNA extraction, and, if desired, 1 mL of sample was fixed for analysis by fluorescent microscopy. Approximately 2 mL sample was filtered through a 0.45 µm pore-sized filter and stored at 4℃ for soluble Co and Fe analysis by AAS.

At the end of the KCCL and KCCR reactor series, one reactor from each series was selected for residue analyses as described by d'Hugues *et al*. (2008). Complete material balances including cobalt, copper, iron, nickel and total sulfur were then established using data obtained both from this analysis and from daily running results with the BILCO software. Dissolution rates and final recoveries were then estimated.

Microbiological Analyses

DNA was extracted from bioreactor and shake flask samples immediately or after short-term storage at -20℃. Between 0.5 and 2.0 g of each sample were pre-treated by repeated washing with 100 mM TRIS, pH. 7.5 until the supernatant pH was greater than pH 7.0, prior to DNA extraction using the FastPrep Kit for Soil (Bio101), with the following modifications to the manufacturer's protocol: Lysis using the FastPrep Instrument was performed at speed 5.0 rather than 5.5; If, as was frequently the case, whole cells could still be seen in the sample following lysis, this step was repeated once more; Following lysis, the tubes were centrifuged at 14 x 10^3 *g* for 30 min, rather than 30 s; Following the addition of the PPS reagent, tubes were centrifuged at 14 x 10^3 *g* for 20 min, rather than 5 min; Finally DNA was eluted in 100 µL DES solution, rather than 50 µL.

In the case of pure isolates on gelled overlay media (Johnson *et al.*, 2001), a small amount of biomass from several colonies was suspended in 20 µL cell lysis solution (0.05 M NaOH, 0.25% sodium dodecyl sulfate) and heated to 95℃ for 15 min in a PCR thermocycler. The crude cell lysates were allowed to cool and 180 µL MilliQ:Tris buffer (0.01 mM Tris, pH 7.5) added. Biomass from liquid cultures was harvested by centrifugation (1-3 mL, 16.1 x 10^3 *g*, 15 min) and processed as above.

Extracted DNA was analysed using capillary electrophoresis single strand conformation polymorphism (CE-SSCP) and in some cases using 16S rRNA gene clone libraries, as described by d'Hugues *et al*. (2008). Counts of metabolically active microbial cells were determined using fluorescent *in situ* hybridisation (FISH). Samples were harvested and fixed with 4 % (w/v) formaldehyde (Bryan, 2006) and deposited by filtration onto a black filter (0.22 µm, Millipore black GS). The filter was coated by dipping it into a 46℃ agarose (0.2% w/v) solution for 5 seconds and then air-dried, and then processed as described in Bryan (2006). Four FISH probes were used: LEP636 (González-Toril *et al*., 2003) specifically targets *L. ferriphilum* species, SUL141 (Amils, personal communication) was designed for the detection of *Sb. thermosulfidooxidans* but also targets *Sb. benefaciens*, THC642 is specific to *At. caldus* species and FER656 allows the detection of Archaea of the genus *Ferroplasma* (both Edwards *et al*., 2000b).

Results & Discussion

KCC mineral shake flasks

In uninoculated flasks, pH and soluble sulfate concentrations (fig. 1) remained stable, even after 69 days incubation, indicating that the gamma-irradiated mineral was indeed sterile. With flasks inoculated with an active KCC bioleaching population, the pH decreased and there was a concurrent increase in soluble sulfate concentrations within the first 9 days. This confirms that gamma-irradiation is a suitable method of mineral sterilisation and has no effect on its amenability to bioleaching. In uninoculated flasks containing untreated KCC mineral, the pH began to decrease after 16 days, with a

concurrent increase in soluble sulfate concentrations. By the end of the experiment, soluble sulfate concentrations were comparable to those of the inoculated cultures. This shows that even after two years of dry storage, a viable population of bioleaching organisms remains. This highlights the necessity for sterilisation of the mineral substrate in bioleaching experiments with defined populations.

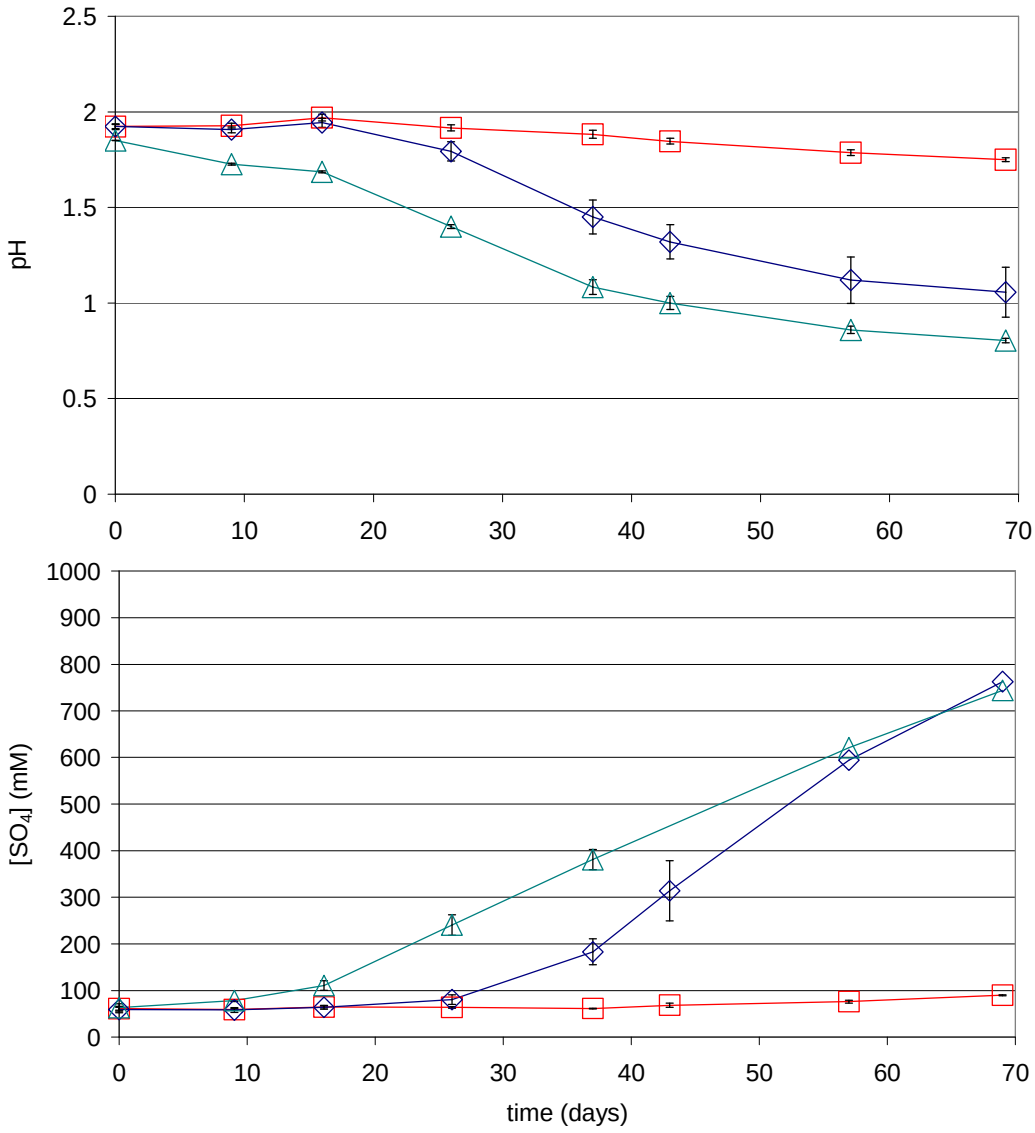

Figure 1 pH and soluble sulfate data for gamma-irradiated KCC ore (KCC-γ; □), non-irradiated KCC ore (KCC; ◇) and gamma-irradiated KCC ore, inoculated with an active bioleaching microbial culture (△). Error bars, where visible, show standard deviation; *n* = 3.

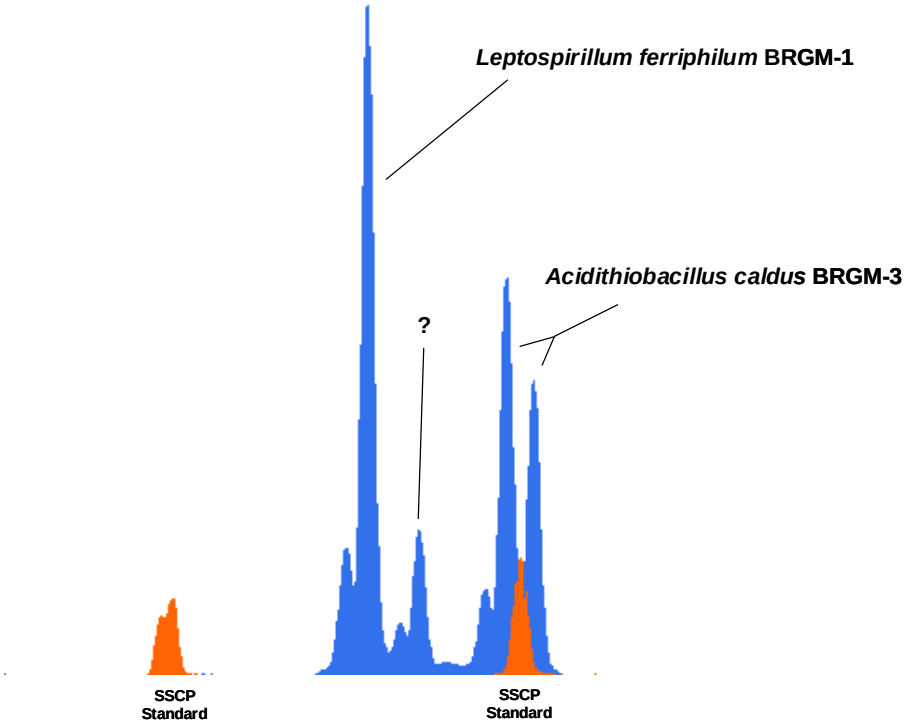

Figure 2 SSCP profile of bioleaching population originating from non-sterilised KCC pyrite (KCCM) highlighting the unknown OUT ('?').

SSCP analysis of the population from the untreated mineral, denoted KCCM, at the end of the experiment found it to be dominated by operational taxonomic units (OTU; SSCP peaks) corresponding to *Leptospirillum ferriphilum* BRGM-1 and *Acidithiobacillus caldus* BRGM-3, along with an unidentified OTU (fig. 2). *Sulfobacillus benefaciens* BRGM-2 was not detected, even though it is known to produce endospores. This should make it more resistant to desiccation than the other, Gram-negative, organisms and therefore a more likely candidate for long-term survival. Several organisms were isolated from this culture (table 1). Isolates KM2 and KM3 had identical SSCP profiles as the OTU hitherto ascribed to *At. caldus* BRGM-3. However, KM3 oxidised ferrous iron on FeSo gelled media. Subsequent 16S rRNA gene sequence analysis revealed that while KM2 was very closely related, if not identical, to *At. caldus* BRGM-3, isolate KM3 was closely related to the Gram-positive iron- and sulfur-oxidising acidophile SLC66. This implies that SSCP alone cannot be used to distinguish between these two very different

organisms. Isolate KM7 had the same SSCP profile as *L. ferriphilum* BRGM-1, and presented colony morphologies typical of *Leptospirillum* spp. grown on this type of medium.

Clone library analysis of the KCCM community was performed in order to identify the unidentified SSCP OTU. Of 20 clones selected, 3 had identical SSCP profiles to this OTU. Sequence analysis revealed this to be a novel organism most closely (though distantly) related to an uncultured clone taken from a copper mine (accession ID EF409850; approximately 92% sequence identity) and more distantly the Gram-positive iron- and sulfur-oxidising acidophile SLC66 (approximately 89% sequence identity). It is important to note that this does not correspond to isolate KM3. This implies that while *Sb. benefaciens* BRGM-2 may be absent from this population, another Gram-positive organism was present. It is thus probable that *Sb. benefaciens* BRGM-2 is not indigenous to the KCC mineral itself, but came from the inoculum used in the reactors which was developed by BRGM (Battaglia-Brunet *et al.*, 2002).

Table 1 Descriptions of isolates and their closest relatives, based on 16S rRNA gene identity.

Isolate	Description	Nearest organism(s) (accession ID)	Identity
KM2	Pale, sulfur-oxidising colony from FeSo plate	*At. caldus* DSM 8584 (Z29975)	99.2%
		At. caldus BRGM-3	98.7%
KM3	Tiny, white sulfur-oxidising colony from FeSo plate	Gram-positive iron-oxidizing acidophile SLC66 (AY040739)	98.1%
KM7	Large, brown iron-oxidising colony from iFeo plate	*L. ferriphilum* BRGM-1	SSCP*
KL2	Tiny, white colony from Feo plate	*Sb. benefaciens* BRGM-2 (EF679212)	SSCP*
KL5	Dry, powdery iron-oxidising colony from iFeo plate	*Sb. benefaciens* BRGM-2 (EF679212)	99.6%
KL6	Large, black-centred iron-oxidising colony from FeSo plate	Gram-positive iron-oxidizing acidophile SLC66 (AY040739)	SSCP*

*Identity inferred due to identical SSCP profile and colony morphology. (FeSo, ferrous iron, tetrathionate and TSB overlay; Feo, ferrous iron, TSB overlay; iFeo, ferrous iron overlay; Johnson *et al*., 2001.)

Classical microbiological analysis using gelled media revealed further diversity than that determined using molecular methods alone. Moreover, SSCP was shown to be inefficient when used in isolation as two phylogenetically and physiologically distinct organisms were shown to give identical SSCP profiles.

KCCL bioreactors

The SSCP profile of the indigenous (KCCL) inoculum was typical of the KCC bioleaching population of the commercial reactors, with OTUs corresponding to *L. ferriphilum* BRGM-1, *Sb. benefaciens* BRGM-2 and *At. caldus* BRGM-3 (fig. 3a). Again, several organisms were isolated from this culture. Isolates KL2 and KL5 were iron-oxidising, spore-forming bacteria isolated on FeSo gelled media and had identical SSCP profiles to *Sb. benefaciens* BRGM-2. Sequence analysis of the 16S rRNA gene of isolate KL5 revealed it to be very closely related, if not identical, to *Sb. benefaciens* BRGM-2.

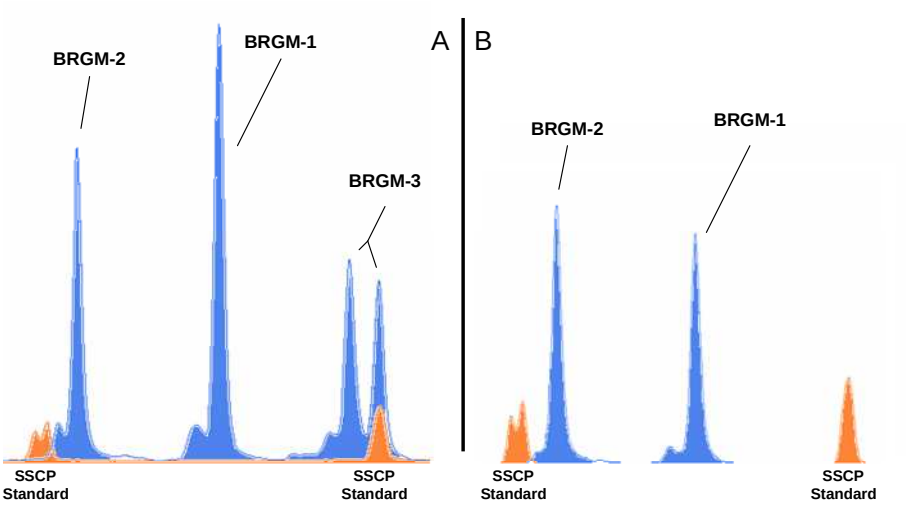

Figure 3 SSCP profiles of the KCCL (A) and KCCR (B) cultures used to inoculate the bioreactors.

Isolate KL6 had an identical SSCP profile to the SLC66-like isolate KM3, and appeared also to oxidise iron. This shows that this organism is present in both the KCCM and KCCL consortia. However, it never appears in the SSCP analysis of either population, suggesting that it exists below the limit for detection.

A suite of reactors run in triplicate (denoted KCCL-6, KCCL-7 and KCCL-8) were used to determine the bioleaching kinetics of the KCCL bioleaching population.

The three reactors had nearly identical cobalt dissolution kinetics (see Bryan *et al*., 2009). The mean maximum rate of cobalt dissolution during the exponential phase of reactors was 8.61 mg L^{-1} h^{-1} (± 0.610 SD). Residue and material balance analysis revealed an overall leaching efficiency of 96.4% total cobalt.

SSCP and FISH analysis showed that the population was dominated by *L. ferriphilum* with low levels of *Sb. benefaciens* BRGM-2 at the end of the batch run. *At. caldus* was not detected after 19 h by SSCP. However, its continued presence was confirmed by FISH analysis (see Bryan *et al*., 2009).

KCCR bioreactors

SSCP analysis of the reconstituted (KCCR) inoculum presented just two OTUs, corresponding to *L. ferriphilum* BRGM-1 and *Sb. benefaciens* BRGM-2 (fig. 3b). This shows that despite the preculture having been inoculated with numerically equal populations of each strain, *At. caldus* BRGM-3 rapidly dropped below the limits of detection of the methods used.

The reactor kinetics were more variable than those of the KCCL series (see Bryan *et al*., 2009). Over the course of the KCCR series, the mean maximal dissolution rate improved from 6.48 mg L^{-1} h^{-1} (± 0.411 SD) to 7.45 mg L^{-1} h^{-1} (± 1.128 SD). Table 2 shows a summary of the residue analysis conducted on reactors KCCL-8 and KCCR-L at the end of their respective batch runs. These analyses showed that the cobalt dissolution efficiency of the KCCR consortium was 95.6% total cobalt extracted; equal to that of KCCL. Indeed, analysis of the liquid fraction of the two residues found cobalt concentrations of 1240 mg L^{-1} and 1230 mg L^{-1}, respectively. While there was a greater abundance of cobalt in the KCCR solid residue, there was only half as much solid in the pulp (100 g, compared to 232 g in the KCCL pulp).

Higher dissolved iron and total sulfur concentrations were found in the KCCR liquid phase, although the ratio of iron to sulfur remained unchanged (approximately 1:1.7 Fe:S). The KCCR solid residue contained less iron and sulfide than KCCL. There was three times more sulfate in the KCCL solid residue, and twice as much total sulfur.

Table 2. Summary of KCCL-8 and KCCR-L final residue analysis.

			Pulp quantity (g)	Total	Co	Cu	Fe	Ni	S^{2-}	SO_4^{2+}	S^{total}
KCCL	Solid	(%)	2275	10.2	0.03	0.15	7.76	0.01	2.7	37	15
		(mg)		232 g	77	341	18009	21	6266	85870	34812
	Liquid	(mg L^{-1})		-	1230	-	24000	-	-*	-*	23700
		(mg)		2043 mL	-	-	49037	-	-*	-*	48424
KCCR	Solid	(%)	2220	4.5	0.08	0.35	8.94	0.03	3.62	29.2	18.6
		(mg)		100 g	80	350	8930	30	3616	29169	18580
	Liquid	(mg L^{-1})		-	1240	-	33600	-	-*	-*	32430
		(mg)		2119 mL	-	-	71231	-	-*	-*	68751

* Analysis of S in the liquid phase did not determine different sulfur species.

Just over half of the difference in solid mass between the KCCL and KCCR residue is accounted for by the fact that there was approximately 69 g less iron, sulfide and sulfate in the KCCR solid phase. Also, over the course of each series of reactors, on average only a third as much $CaCO_3$ was used in the KCCR reactors to maintain the pH at the desired level (34 mL and 115 mL, respectively), signifying that much less acid was produced by the KCCR consortium. 58.3 g more calcite was added to the KCCL reactor than the KCCR. This equates to 23.3 g calcium. Stoichiometrically if this amount of calcium were precipitated as $CaSO_4$, it would require approximately 56 g of sulfate; more or less identical to the difference in solid-phase sulfate between the two reactor residues.

The greater amount of neutralisation required with the KCCL reactors could also explain the difference in iron in the solid phase. Each time that the calcite suspension was added to the reactor, there was a localised formation of orange (presumably) ferric-hydroxy precipitates. While $Fe(OH)_3$ will form

rapidly at pH greater than around 3.0, it is very difficult to redissolve, even at the very low pH of the reactor.

It is often stated that effective sulfide and reduced inorganic sulfur compound (RISC) oxidation (and thus acid production) is beneficial to the bioleaching process as it removes sulfur compounds which may coat, and so occlude the mineral surface from attack (e.g. Dopson and Lindström, 1999). However, it is clear that the level of complete sulfide oxidation (to sulfate) was reduced (as evidenced by less acid production) yet the overall efficiency did not appear to be significantly affected. It is possible that the presence of the iron- and sulfur-oxidising bacterium *Sulfobacillus benefaciens* BRGM-2 prevented the build up of elemental sulfur on the mineral surface, but that oxidation was not complete, rather only as far as an intermediary sulfur species.

SSCP analysis of the KCCR populations found that *L. ferriphilum BRGM-1* and *Sb. benefaciens* BRGM-2 were co-dominant by the end of each batch run, in contrast to the KCCL reactors. Moreover, *At. caldus* BRGM-3 was never detected by SSCP and only transiently detected by FISH. When taken in the context of less acid production observed, this suggests that *At. caldus* BRGM-3 is responsible for a large amount of the acid produced in this bioleaching system. That *At. caldus* BRGM-3 is detected, albeit at low levels, sporadically throughout the KCCR series suggests that has not been able to re-adapt to the reactor conditions; it has in effect become ‘un-adapted’ since its isolation and maintenance in pure culture. However, given that the overall reactor efficiency was the same, and that it required less pH maintenance as a result, it seems that the presence of *At. caldus* BRGM-3 is not important, at least in this batch system. Rather, if there is a critical level of sulfur oxidation required for efficient performance, this is fulfilled by *Sb. benefaciens* BRGM-2.

KCCD

The KCCD population was incredibly slow to commence mineral oxidation in the 200 mL preculture. Indeed, despite the observation of a large microbial population by light microscopy the redox never reached greater than 500 mV (data not shown). SSCP analysis of this population revealed it to be

dominated by OTUs corresponding to *Am. ferrooxidans* TH3, *At. thiooxidans* SDE4 and strain SDE2 (fig. 4).

The pH, redox and dissolved cobalt and iron concentrations changed only very slightly over the course of the first reactor. After 1000 h, the redox and dissolved cobalt and iron concentrations began to increase (fig. 5). This was an exceptionally long time for a bioleaching population to commence biooxidation, and much longer than the KCCR and KCCL reactors.

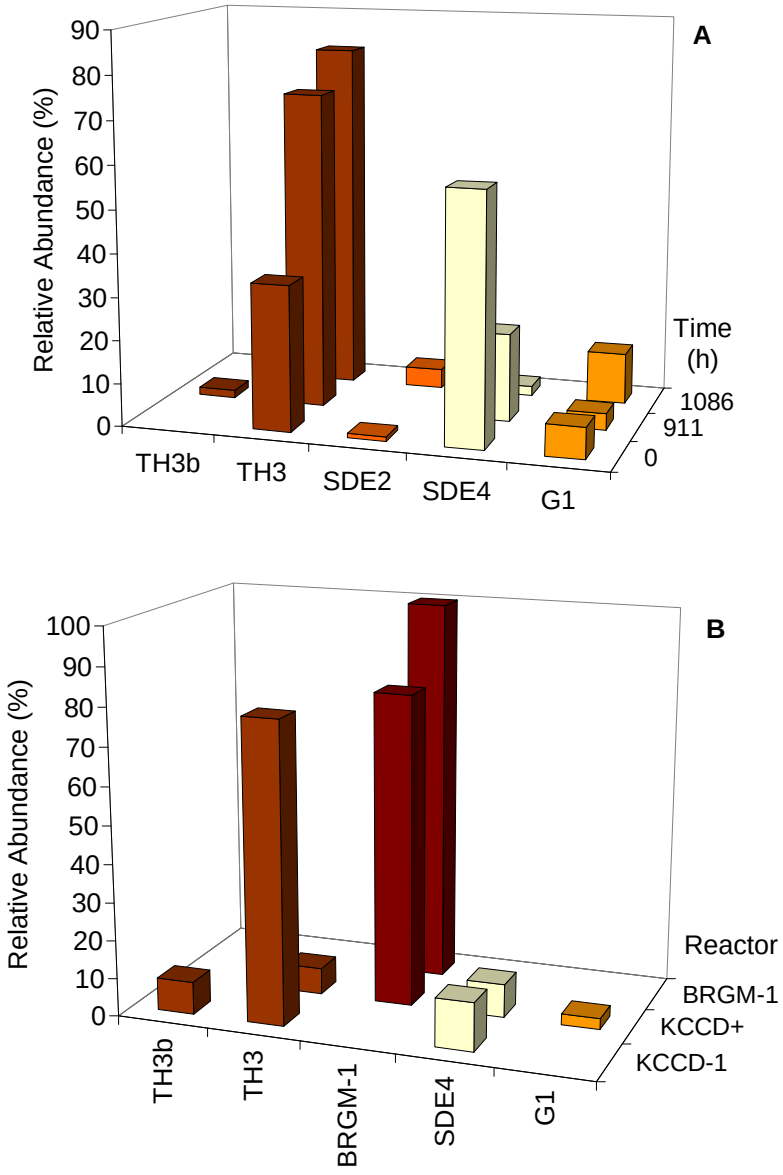

Figure 4. SSCP profiles of the initial KCC-D reactor (A) and the three subsequent reactors (B) after approximately 300 h. Despite being included in the initial preculture, strain Y008c was not detected at any point. The relative abundance of strain SDE2 also dropped below the limits of detection.

The SSCP profile (fig. 5) showed that with the exception of Y008c, all of the initial organisms added to the preculture were still present. This consortium still confers all of the metabolic traits originally sought on the system. The relative abundance of the iron-oxidising actinomycete *Am. ferrooxidans* TH3 increased, with a concurrent decrease in the relative abundance of SDE4. The Gram-positive organisms SDE2 and G1 maintained similar relative abundances, while G1 was the slightly more dominant. This probably implies that the numbers of *Am. ferrooxidans* TH3 were increasing with increasing dissolved iron concentrations.

It was reasoned that an autotrophic iron-oxidiser may be essential to the system, as even with very low numbers of an autotrophic sulfur-oxidiser (*At. caldus*), the KCCR population was substantially quicker. To investigate this, three further reactors were set up. The first, KCCD-1 was a sub-culture of the initial KCCD reactor. The second, KCCD+ was inoculated with the KCCD population and 100 mL of *L. ferriphilum* BRGM-1. The third reactor contained just *L. ferriphilum* BRGM-1 in order to be sure that any improvement in performance was not solely due to *L. ferriphilum* BRGM-1, but rather a synergy between all the organisms present.

The results (fig. 5) showed a great improvement in the performance of the KCCD culture following subculture, although still not comparable to either KCCL or KCCR. However, the rate of reaction was substantially higher in the KCCD+ culture, although again, the final cobalt and iron concentrations were much lower than for KCCL or KCCR. The pure *L. ferriphilum* BRGM-1 culture showed a similar rate of cobalt dissolution to the KCCD+ culture, implying that the presence of *L. ferriphilum* BRGM-1 was the main reason for the differences in performances observed.

The SSCP profile of the KCCD-1 reactor (fig. 5) showed that only the iron-oxidising actinomycete *Am. ferrooxidans* TH3 and the sulfur-oxidising obligate autotroph *At. thiooxidans* SDE4 are still present. That *At. thiooxidans* SDE4 persists is very interesting, as up until this point *At. thiooxidans* was assumed to be strictly mesophilic, with a T_{max} in the 35℃ range. *At. thiooxidans* SDE4 is an environmental isolate from southern Portugal (Bryan *et al*., 2006), where

summer temperatures may reach the high 30s and so is probably more thermotollerant than the type strain. In the KCCD+ reactor, it appeared that *L. ferriphilum* BRGM-1 became dominant at the expense of *Am. ferrooxidans* TH3, which is presumably unable to compete. The relative abundance of *At. thiooxidans* SDE4 remained similar, while G1 was once again apparent.

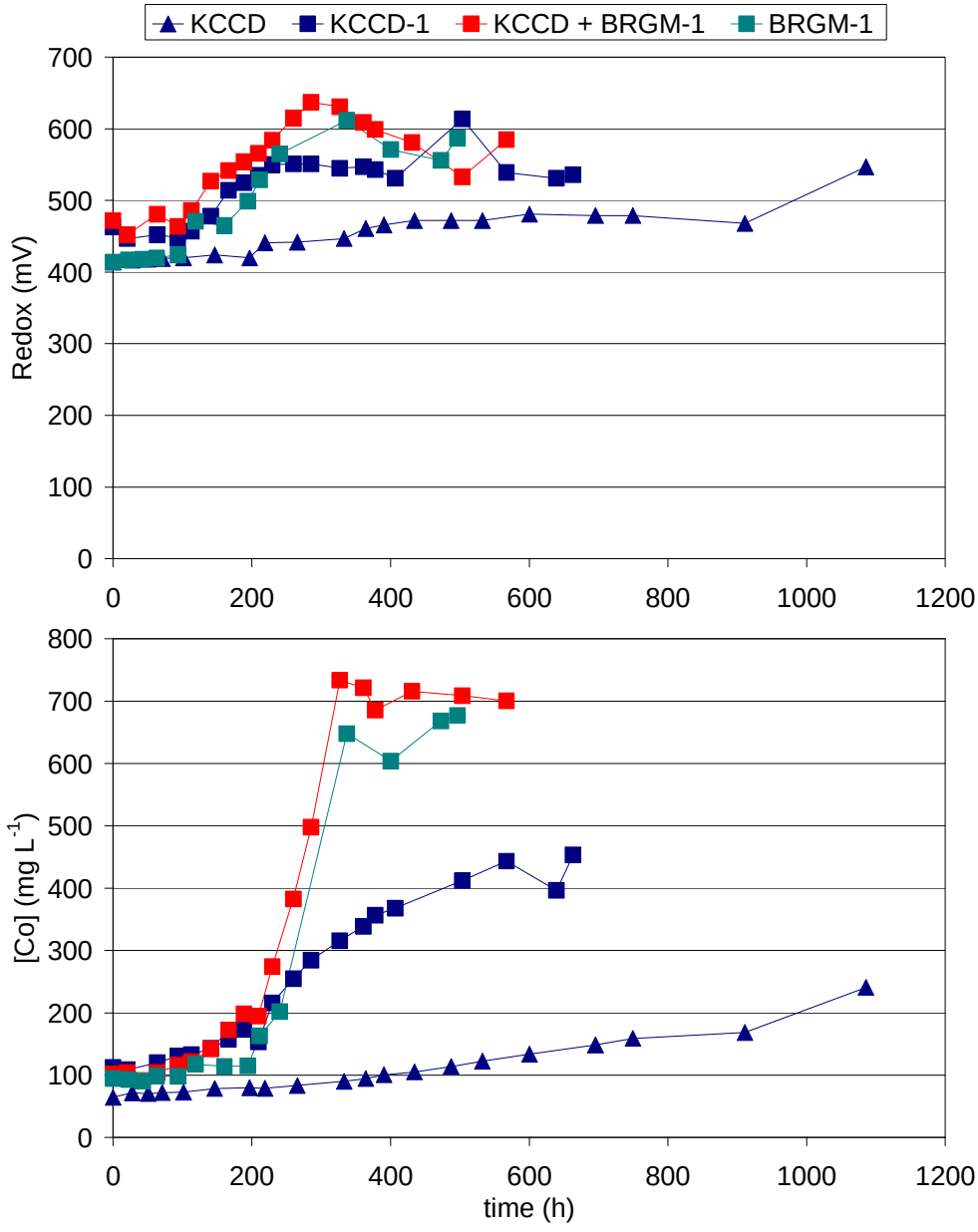

Figure 5 Redox and dissolved cobalt concentrations of the KCCD initial and subsequent reactors.

These results show that a designed consortium can establish itself in an STR containing the KCC mineral. They indicate that the bioleaching efficiency of

this consortium improves rapidly, but that it was unable to attain bioleaching efficiency anywhere close to that of the KCCL and KCCR consortia. The introduction of a culture of *L. ferriphilum* BRGM-1 greatly improved the bioleaching efficiency. However, this consortium was not substantially more effective than a pure *L. ferriphilum* BRGM-1 culture. Furthermore, the addition of *L. ferriphilum* BRGM-1 did not confer a dissolution rate comparable to the KCCL or KCCR cultures.

Conclusions

Gamma irradiation was an effective method for mineral sterilisation, whereas non-sterilised mineral rapidly gave rise to an effective mineral leaching community. Thus, any adaptation regime using a designed consortium must employ sterilised ore and robust aseptic technique. This is essential to prevent grow-through of a (presumably) better adapted population or contamination from elsewhere.

The bioleaching rate of the reconstituted population appeared to be slightly lower than that of the indigenous consortium. However, it improved within a relatively brief period of time and ultimately attained a very similar value. The indigenous and the reconstituted consortia were equally efficient in terms of total cobalt bioleached. While the majority of the bioleaching organisms readapted quickly to the bioleaching system following maintenance in synthetic media as pure cultures, *At. caldus* BRGM-3 did not. However, that it plays a beneficial role in bioleaching systems was unclear given the very similar leaching kinetics of the two consortia. Indeed, to an extent these results imply that a bioleaching system that lacks efficient sulfur-oxidising organisms such as *At. caldus* may be preferable, as it would require less pH regulation and produce less solid waste. However, how this would be achieved in practise at a commercial scale is not clear, given that biomining operations are inherently non-sterile environments.

Further work should focus on a consortium comprising just *L. ferriphilum* BRGM-1, *Sb. benefaciens* BRGM-2 and *Fp. acidiphilum* BRGM-4. This would confirm that the improvement seen in cobalt dissolution rate of the

reconstituted consortium was through the continued readaptation of these organisms, and not due to increasing, yet largely undetectable numbers of BRGM-3.

The results of the (logically) designed consortium show that adaptation of organisms to the bioleaching process (i.e. mineral type and general STR conditions) is likely to be a lengthy process. Before the examining whether a ‘Top-Down’ or ‘Bottom-Up’ approach is best, this adaptation process must be performed. Until this is done, comparison between strains taken from commercial reactors and environmental isolates is not possible on equal terms, regardless of whether in a strict physiological sense an environmental strain should be superior or not.

The poor bioleaching rates observed with the designed consortium, and the fact that *At. caldus* BRGM-3 was so slow to readapt to the KCC STR conditions indicate that the notion of being able to construct a logically designed consortium ‘off-the-shelf’ to treat this particular resource is not possible without a substantial period of preadaptation. While certain organisms may retain enhanced bioleaching capacity even after prolonged periods in ‘ideal’ synthetic liquid media, other organisms require continuous exposure to STR-conditions in order to maintain or obtain the adaptive mechanisms required.

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