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## **Bioprospecting and the Microbial Ecology of a Coal Production Waste Dump.**

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**Abstract:** In order to develop an optimised coal production waste bioleaching unit, a sampling campaign was carried out on the existing waste dump. Eleven samples from various surface locations of the heap were used for enrichment cultures at three temperatures (30°C, 42°C and 48°C). Interestingly, none of the 11 cultures at 42°C grew well (low cell numbers and poor pyrite dissolution). Bioreactors inoculated with pooled enrichments showed the same results, indicating that the waste heap harboured organisms capable of growth and mineral oxidation at 30°C and 48°C but not 42°C. Analysis of the 48°C culture used for subsequent reactor tests using a variety of molecular microbiology methods (CE-SSCP, T-RFLP and high-throughput sequencing) revealed the culture was dominated by *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus* and an unknown member of the Acidimicrobiaceae. Further tests at 42°C using a well-characterised bioleaching consortium, BRGM-KCC, performed well in initial reactors. However, during each batch the relative abundance of *Leptospirillum ferriphilum* decreased, and its numbers declined gradually with each subsequent subculture indicating some form of accrued inhibition. Coupled with the absence of a 42°C culture from the dump it was theorised that the waste was inhibitory to *Leptospirillum* spp. In order to further explore the possible reasons for the absence of *Leptospirillum* in the enrichments and bioreactors, a detailed analysis of the waste heap was undertaken using a range of geochemical and molecular biology tools. Results suggest the role of an as-yet unidentified organic carbon compound from the coal itself.

**Keywords:** Coal Waste, Microbial Ecology, *Leptospirillum*, Inhibition

## 1. Introduction

Acid mine drainage from sulfidic coal production wastes is a significant challenge to the European Coal industry. This drainage can be treated, but most wastes will continue to produce AMD for decades. At the same time, the generation of electronic waste is growing year by year, nonetheless, recovering valuable raw materials from this waste by existing processes it is not always possible. The EU RFCS-funded CEReS project aims at developing a co-processing strategy where i) the acid-generating potential of coal mine waste is removed through accelerated bioleaching and (ii) base metals from electronic waste are recovered, while concentrating precious and critical elements into enriched substrates. The selection and adaption of an effective and efficient bioleaching consortium is key stage for achieving these two goals (Bryan et al., 2015).

It was hypothesised that microbes isolated from coal itself may be more efficient than allochthonous ones but the literature reports conflicting results (Ye et al., 2018). Cultures enriched from a hard coal mine in Poland and a well-characterised consortium, KCC, were tested for their ability in suiting both goals of the CEReS project. In order to design an optimal consortium, the microbial ecology of the sampling site and of experimental reactors was investigated in relation to their geochemistry.

## 2. Materials and methods

### 2-1. Bioleaching experiments

A sampling campaign was carried out on a waste dump in Janina mine (Libiąż, Poland). Samples, collected from various surface locations, were used for enriching bioleaching cultures at three temperatures, here named TW-30, TW-42 and TW-48, according to their enrichment temperature (30°C, 42°C and 48°C). Additional samples have been collected to characterise the microbial community of the heap.

### 2-2. Bioleaching experiments and process scale-up

Bioleaching experiments were performed in batch reactors using unweathered tailings from a spiral separator in Janina mine ((i.e. JST, Janina spiral tails)). Reactors were purged with CO<sub>2</sub>-enriched air (2% v/v) operating at 0.5 L/min. Experiments were carried out with increasing solid load, (5%, 10% and 15%) and at increasing working volumes (0.5L, 2L). Bioreactors were inoculated at 10% (v/v) with consortia described above, each at its respective temperature. Further experiments were performed inoculating with the allochthonous, moderately thermophilic consortium KCC. This consortium is well characterized (the main strains are *Leptospirillum ferriphilum*, *Sulfobacillus benefaciens*, *Acidithiobacillus caldus* and *Ferroplasma acidiphilum*) and has been already applied with high performances in several bioleaching applications (Bryan et al., 2011; Hubau et al., 2018). Consortia performances were assessed monitoring the following variables: redox potential, pH, planktonic cell count, Fe concentration and speciation. At the end of each bioleaching run, samples of the slurry were centrifuged to remove the supernatant and stored at -80°C until subsequent extraction of genomic DNA (Power Soil DNA Isolation kit, Qiagen).

### 2-3. Molecular biology

Samples collected in Janina mine were used for characterization of the microbial community by sequencing V4-V5 region of 16S rDNA (Illumina MiSeq, universal primer set 515WF/928WR). Obtained sequences were analysed using FROGS bioinformatics pipeline in the Galaxy platform (Escudí et al., 2017). Variations in the community structure during bioreactor experiments and in enrichment cultures were monitored by CE-SSCP (Bryan et al., 2011). In addition, TW-48 consortium was sequenced by Oxford Nanopore MinIONS (universal primer set 515F/1492R), which allows to resolve lower taxonomic levels than Illumina MiSeq.

### 3. Results and Discussion

#### 3-1. Microbial ecology of the site and enrichments of Fe/S oxidizing consortia

Despite a certain variability among sampling points, the microbial community of the waste dump was largely dominated by strains typically found in metal rich, low pH environments (e.g. Acidithiobacillaceae). Several strains commonly found in saline environments were also present in large amount and ubiquitous in the dump (e.g. *Acidihalobacter* spp., *Maritimimonas* spp.).

Of the three microbial cultures enriched from this initial community, TW-42 showed poor pyrite dissolution and very low cell numbers (data not shown). On the contrary, TW-48 showed the best performances. Results obtained by combining CE-SSCP profiles and sequencing by MinIONS revealed that TW-48 was largely dominated by an unidentified strain affiliated to Acidimicrobiaceae family, followed by *Sulfobacillus thermosulfidooxidans* and *At. caldus* in lower relative abundances. Due to its instability, it was not possible to obtain enough DNA for characterising TW-42.

#### 3-2. Microbial communities in batch reactor experiments

Best pyrite dissolution performances were obtained in bioreactors inoculated with the consortia TW-48 and KCC (data not shown). The mesophilic consortium TW-30 showed significantly lower

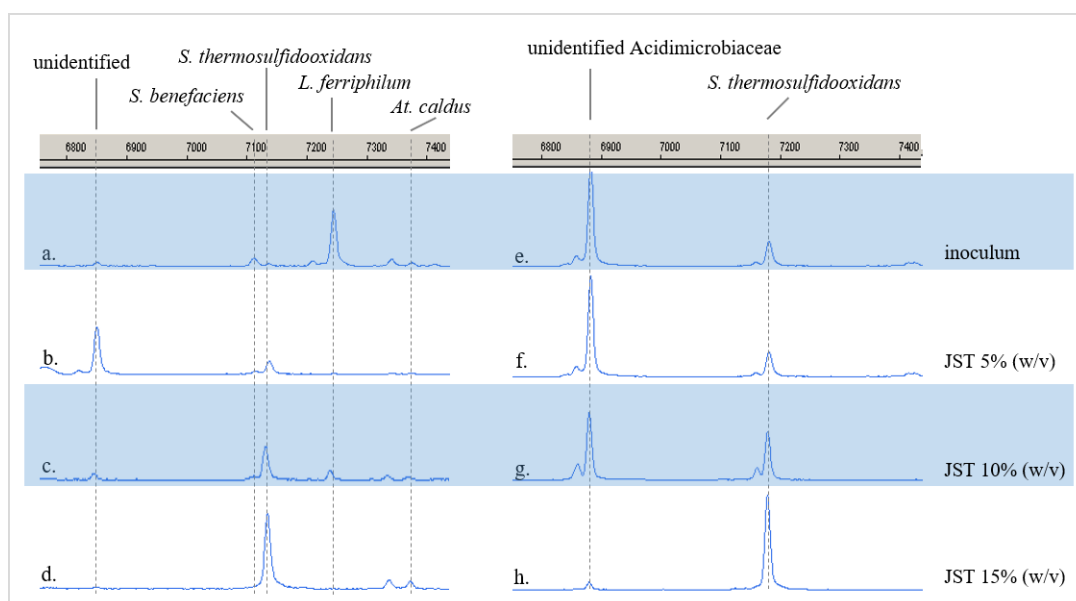


Fig.1 CE-SSCP profiles of BRGM-KCC (panels “a”, “b”, “c” and “d”) and TW48 (panels “e”, “f”, “g”, “h”) in 2L reactor experiments, increasing solid load.

pyrite dissolution yields and slower kinetics. As observed in shake flasks, bioreactors inoculated with TW-42 showed poor results, indicating that the waste heap harboured organisms capable of growth and mineral biooxidation at 30°C and 48°C but not 42°C. For these reasons, TW-48 and KCC were selected for deeper investigations. During scale-up both consortia became dominated by *S. thermosulfidooxidans* (Fig.1). In particular, the relative abundance of *L. ferriphilum* in KCC inoculating reactors declined gradually. Coupled with the absence of a 42°C culture from the dump, it was theorised that the waste was inhibitory to *Leptospirillum* spp. Due to the presence of Cl (3% w/w) in JST we hypothesized an inhibition due to salt dissolution from coal. However, the highest concentration of chloride observed in the reactors (at 10% w/v solids) was found to be less than 15 mM, well below the range normally tolerated by this organism.

#### 4. Conclusions

The waste dump sampled in this study harboured several strains known for their bioleaching potential, including *Leptospirillum* spp.. However, the scale-up of the bioleaching process seemed to drive consortia to a convergent dominance by *Sulfobacillus* spp.. In order to explore the possible reasons for the absence of *Leptospirillum* in the enrichment cultures and in reactors, a detailed analysis of the waste heap was undertaken using a range of geochemical and molecular biology tools. The effect of the solid load, as well as the salt content of the waste, were excluded as possible causes of such alterations. Results seems to suggest the role of an as-yet unidentified organic carbon compound liberated from the coal itself in bioreactor conditions.

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