Formation of hydrocarbon gaseous compounds during bioleaching of copper sulphide minerals with mesophilic microorganisms

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ABSTRACT

An experimental study was conducted for assessing the formation of hydrocarbon gaseous compounds during bioleaching of copper sulphide ores with mesophilic microorganisms. Three different mineral samples were used: a pyrite concentrate, a chalcopyrite concentrate and a copper sulphide ore rock from El Teniente mine, Chile, containing 1.2% copper. Mineral samples were bioleached in 250 ml shake flasks containing 100 ml of basal medium inoculated either with a pure strain of *Acidithiobacillus ferrooxidans* or a natural bacteria consortium obtained from the acid leaching of the El Teniente copper ore in columns. Each sealed shake flask system was fed with a flow of synthetic air and the exit stream was passed through a column containing an adsorbent material and next through a water trap to avoid back-contamination with air from the environment. Compounds present in the adsorbent material after 90 days of bioleaching were analysed using a gas chromatography mass spectrometry technique with a procedure that detects 162 different hydrocarbon gaseous compounds. Experimental results permitted to identify the formation of significant amounts of five different types of hydrocarbon gaseous compounds: di-,tri- and penta-methyl benzene, 11th-ethyl alkane and branched alkanes. The number of formed gaseous hydrocarbon compounds increased in experiments conducted with the natural bacterial consortium, where their formation appears to be enhanced by the presence of heterotrophic microorganisms. This work provides the first experimental evidence of the formation of hydrocarbon gaseous compounds in bioleaching.

1. Introduction

Bacterial metabolism of both aerobes and anaerobes, involving redox reactions with carbon, sulphur and metals, can have profound effects on the geochemistry of dissolved metals and metal-bearing minerals and can contribute to the supergene enrichment of sulphide ores (Southam and Saunders, 2005; Enders et al., 2006). The metabolic processes associated to microbial populations present in ore deposits can result in the production of various gases, e.g.: CO_2, CH_4, H_2S (Ehrlich, 1996). Major mineral oxidation/reduction boundaries in sulphide deposits are very often heavily colonized by bacteria which can be a significant source of organic carbon, subject to volatilization as hydrocarbons. Light and heavy hydrocarbons which move upward through the overburden of a deposit can then be postulated to be somehow linked to bacterial activity related to mineral sulphide oxidation processes (Highsmith, 2004).

In bioleaching processes the dissolution of minerals occurs under the combined action of autotrophic and heterotrophic microorganisms and the production of important concentrations of organic carbon has been observed (Johnson et al., 1995). The production of hydrocarbon gaseous compounds (HGC) is very likely to occur under these conditions, but there are no reports or studies of their detection and occurrence. In some respect, however, the identification of some characteristic smells which have been reported to appear during the operation of bioleaching heaps and reactors can be considered perhaps as the first indication of the formation of some type of organic gaseous compounds in these processes (Brierley and Brierley, 2007).

Identification and characterization of HGC compounds in bioleaching operations could provide valuable complementary information for assessing the efficiency of bacterial activity during the process. The present work represents an exploratory study in the assessment of the formation of HGC in the bioleaching of copper sulphide minerals.

2. Materials and methods

2.1. Minerals

Three different mineral samples were used: a) a copper concentrate from Codelco-Andina containing 90% chalcopyrite, 8% pyrite and quartz; b) a -150#+200# pyrite sample prepared from a pure massive crystal of pyrite; c) a 3/4” copper ore sample of Codelco-El Teniente with 1.2% copper mainly present as chalcopyrite, containing 2% pyrite

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Table 1
Experimental data on pH and bacterial population just before sealing the flasks (first two columns) and metal recoveries and bacterial population at the end of the three month period (last two columns), in each of the experiments

Experiment	pH	Bact/ml	Bioleached metal ions	Bact/ml
1	1.6	2.7×10^8	85% Fe	1.5×10^8
2	2.0	2.5×10^8	43% Cu	1.9×10^8
3 (blank)	–	–	–	–
4	2.0	2×10^8	43% Cu	3.0×10^6
5	3.2	1.5×10^8	50% Cu	1.97×10^6

and smaller amounts of bornite (0.7%), digenite (0.1%) and molybdenite (0.3%).

2.2. Microorganisms

a) A pure strain of *Acidithiobacillus ferrooxidans*, ATCC 19859, which was grown on ferrous iron in a basal medium with the following composition: $(NH_4)_2SO_4$ 0.4 g/l, $K_2HPO_4{\cdot}3H_2O$ 0.056 g/l, $MgSO_4{\cdot}7H_2O$ 0.4 g/l at pH = 1.6. b) A consortium of leaching microorganisms present in the leaching solution obtained after bioleaching the El Teniente copper ore in pilot columns for 2 years.

2.3. Experimental procedure

Leaching experiments were conducted in 250 ml Erlenmeyer flasks containing the respective mineral sample and 100 ml of basal medium. The flasks were incubated on an orbital shaker located in a thermostatic room which operated at 30 ° C. Bioleaching experiments were initially conducted with flasks open to the atmosphere. After 5 weeks each flask was sealed to the external atmosphere and synthetic air (21% v/v O_2, 79% v/v N_2, 0% CO_2) was fed through a glass tube. The exit gas stream was passed through a glass column containing 50 g of a proprietary in-house prepared adsorbent material and then passed through a water trap as to avoid any back air contamination from the environment. The air flow was very low, in the range 0.01–0.02 l/h, to optimize the efficiency in removing small concentration of HGC with the adsorbent. The flasks were operated in the sealed condition over a 90 day period. Leaching solutions were characterized prior to sealing the flasks and after the 90 day trial period: pH and Eh were determined, Fe^{+2} and total iron were determined by the *o*-phenantroline colorimetric method (Muir and Anderson, 1977), copper was determined by atomic absorption spectrophotometry and bacterial concentration by direct counting in a Petrof–Hausser chamber coupled to a Nikon Labophot microscope.

Table 2
Microorganisms identified with the T-RFLP technique in the El Teniente bacteria consortium and their relative abundance

Microorganisms	Relative abundance, %
Alpha Proteobacteria	2.6 %
Acidimicrobium ferrooxidans	2 %
Acidiphilium sp	
Acidocella sp	
Acidomonas methanolica	
Ferrimicrobium acidiphilium	
Acidithiobacillus ferrooxidans	1.4 %
Acidithiobacillus. thiooxidans	1.3 %
Leptospirillum ferrooxidans	1.9 %
Leptospirillum ferriphilium	1 %
Sulfobacillus acidophilus	1.2 %
Sulfobacillus thermosulfidooxidans	1.2 %
Shewanella sp	1.1 %
Arthrobacter sp	1 %
Others	80 %

"Others" corresponds to microorganisms with concentration below 1%, not identified yet.

At the end of the 90 days with synthetic air the columns with absorbent material were removed and sealed and then the compounds present in the adsorbent material were analysed with gas chromatography mass spectrometry using a proprietary technique (SGH). This technique enabled to simultaneously evaluate the presence of 162 different hydrocarbon gaseous compounds. The SGH analytical technique by Actlabs, Canada, reports detection limits and resolution at 1 ppt, with a calculated precision at 6.6% relative difference (http://www.actlabs.com/gg_soil_sgh.htm).

The following experiments were conducted: experiment (1): 1 g pyrite concentrate leached with 100 ml basal medium containing 2 g/l of ferrous iron and inoculated with *Acidithiobacillus ferrooxidans* ATCC 19859; experiment (2): 1 g chalcopyrite concentrate leached with 100 ml basal medium containing 2 g/l of ferrous iron and inoculated with *Acidithiobacillus ferrooxidans* ATCC 19859; experiment (3): a blank experiment in which the flask contained only distilled water; experiment (4): 1 g chalcopyrite concentrate leached with 100 ml basal medium containing 2 g/l of ferrous iron and inoculated with the El Teniente bacteria mesophiles community; experiment (5): approximately 50 g of El Teniente ore leached with 100 ml basal medium containing 2 g/l of ferrous iron and inoculated with the El Teniente mesophiles community.

Finally, microorganisms present in the El Teniente bacteria consortium were identified with the T-RLFP analysis (Bryan et al., 2005), using 341f and 1100r as universal primers (Liu et al., 1997; Osborn et al., 2000) and RsaI and MspI as the digestion enzymes. To obtain the amount of DNA required for conducting this determination successfully the bacteria consortium was further cultivated in 8 shake flasks over three weeks. Each flask contained 10 g of El Teniente copper ore and 100 ml of basal medium at pH 1.6 containing 3 g/l of ferrous iron.

3. Results and discussion

Table 1 shows the data of bacterial population and pH in each of the flasks after one month of starting the experiment, just before sealing the flasks and starting the flow of synthetic air (first two columns). Table 1 also includes metal recoveries and bacterial population obtained in each flask at the end of the 90 day trial period (last two columns). It can be seen that in every experiment there was a significant bacterial population, indicating the occurrence of an active bioleaching process.

Results of the identification of the microorganisms present in El Teniente bacteria consortium using the T-RLFP technique are presented in Table 2. It can be observed that an important part of the microflora is formed by chemolithotrophic, autotrophic microorganisms (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, *Sulfobacillus*), which are expected to be found in bioleaching operations. However, there is also an important presence of heterotrophic microorganisms (*Acidocella* sp., *Arthrobacter*, *Ferrimicrobium acidiphilium*, *Shewanella*) and mixotrophic microorganisms (*Acidiphilium* sp., *Acidimicrobium ferrooxidans*, *Acidocella* sp.).

The analysis of the solid adsorbent at the end of the leaching experiments for 162 gaseous hydrocarbons compounds using the SGH technique showed significant concentrations of only 5 types of

Table 3
List of gaseous hydrocarbon compounds which showed significant concentration and the respective leaching experiments in which these compounds were detected

Hydrocarbon	Experiment
Di-methyl benzene	4
Tri-methyl benzene	4
Penta-methyl benzene	2, 5
Branched alkane	2, 4, 5
11th-ethyl alkane	4

hydrocarbon compounds, which are listed in Table 3, indicating the respective experiment where they were detected. Figs. 1, 2 and 3 show values of concentration measured for the cases of di-methyl benzene, branched alkanes and tri-methyl benzene, respectively, in each of the 4 bioleaching experiments and the blank experiment. Concentrations are in part per trillion and correspond to the concentration of the respective organic compound accumulated after 90 days in the 50 g adsorbent material bed. The continuous horizontal line in each of these figures represents the base value detected in the pure adsorbent material, while the dotted lines represent the range of uncertainty of the analytical method. To determine the base line concentration of any given HGC a total of 15 original samples were sent for analysis. The average value represents the concentration of HGC's in the original material and the analytical variance is represented within ±2 standard deviations. Variations of concentrations above or below upper and lower limits for *in-vitro* experimental results evidence those compounds which show significant variations with respect to initial conditions, in other words, the original material. The blank experiment is intended to evaluate variations with respect to the original material resulting from pure air circulation.

Results showed that experiment 4, where a chalcopyrite concentrate was leached with the bacteria consortium grown in the El Teniente ore, presented the largest production of HGC, 4 out of the 5 detected. Experiment 5, where the same El Teniente bacteria consortium was used to leach El Teniente ore, presented significant production of just two of the 5 detected HGC, penta-methyl benzene and branched alkanes. However, there was also significant production of the same two compounds in bioleaching of chalcopyrite concentrate with a single strain of *Acidithiobacillus ferrooxidans* (experiment 2). Therefore, a major number of hydrocarbon gaseous compounds were detected in experiments where both autotrophic and heterotrophic microorganisms were present. In addition, even in the presence of one single strain of an autotrophic microorganism, some of these HGC compounds were also detected (experiment 2).

Chemolithotrophic, autotrophic microorganisms grow out of the oxidation of inorganic substrates such as ferrous iron and reduced sulphur compounds, and utilize inorganic carbon. Metabolic processes associated to the activity of these microorganisms normally produce a wide variety of organic compounds, many of which are released into the environment (Madigan et al., 2000). In experiments related to bioleaching of minerals with *Acidithiobacillus ferrooxidans* and *L. ferrooxidans*, compounds such as rhamnose, fucose, arabinose, xylose, manose, glucose and glucuronic acid have been detected in low concentrations in the growth media. All these compounds are sugar-related and form part of the exopolymeric substance (EPS) (Gehrke et al., 1995). In addition, cellular lysis of autotrophic microorganisms produces additional organic compounds which also help to increase the concentration of organic carbon in solution (Johnson et al., 1995). Therefore, it is possible to conclude that in the presence of autotrophic microorganisms some gaseous compounds could be produced simply out of the chemical decomposition of some of the released organics, which could lead to formation of more volatile products. That could explain the formation of hydrocarbon gaseous compounds in leaching of chalcopyrite with a pure strain of *Acidithiobacillus ferrooxidans* (experiment 2).

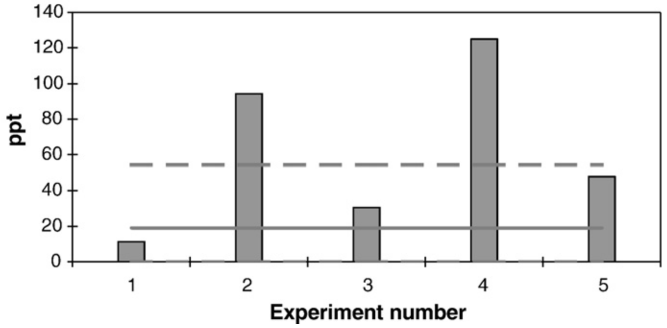

Fig. 2. Concentration of branched alkanes in absorbent material, in parts per trillion, determined with the SGH technique.

On the other hand, organic compounds released by autotrophic microorganisms can be used as an energy source for the growth of heterotrophic microorganisms, which result in the development of efficient mixotrophic bioleaching communities (Johnson et al., 1995). Heterotrophic microorganisms are able to oxidise a wide variety of organic compounds, including aromatic compounds and organic acids. Therefore, their presence should result in an acceleration of the rate of decomposition of the organics released from the activities of autotrophs and, very likely, in an increase of the variety of the resulting organic products. One can then postulate in principle that the increase in the number of gaseous hydrocarbon compounds observed in bioleaching experiments with the El Teniente bacteria consortium is mainly the result of the activity of heterotrophic microorganisms present in the system, which further metabolize the organic compounds produced by the autotrophic, chemolithotrophic microorganisms.

In the field, higher concentrations of HGC's in soils over ore deposits beneath cover are interpreted as the result of microbiological processes generating these compounds from biooxidation of sulphides, tested in many case studies around the world (Highsmith, 2004; Leng and Sutherland, 2004; Townley et al., 2007a,b). This has been emulated in analogue column experiments in which generation of HGC's from oxidising El Teniente sulphide mineralization was demonstrated, migrating through 1 m of overburden and captured within passive collector devices in a period of 90 days (Townley et al., 2007a,b). Direct linking of specific bacterial communities, sulphide biooxidation processes and generation of specific HGC's has so far not been published, then the *in-vitro* experiments here presented a first vital step in the understanding of these complex phenomena.

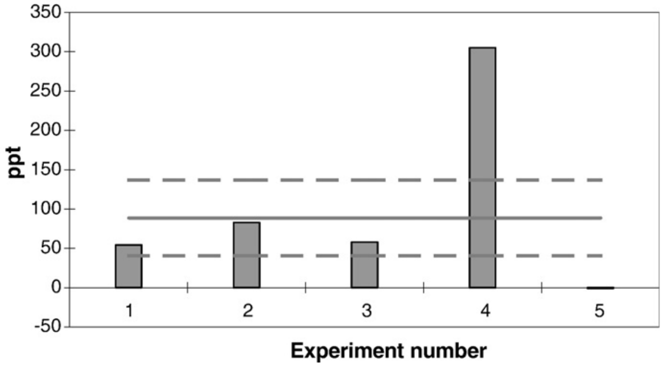

Fig. 1. Concentration of di-methyl benzene in absorbent material, in parts per trillion, determined with the SGH technique.

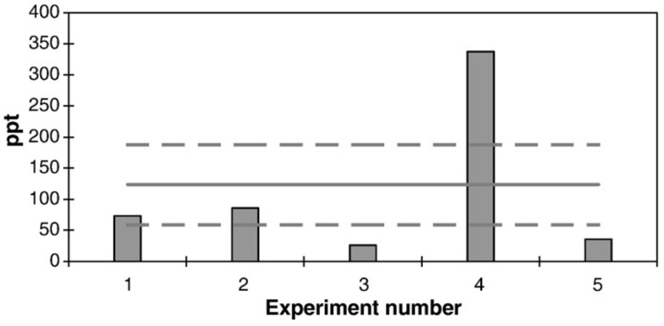

Fig. 3. Concentration of tri-methyl benzene in absorbent material, in parts per trillion, determined with the SGH technique.

4. Conclusions

Results showed that in bioleaching of copper sulphide ores and concentrates with mesophilic microorganisms there is formation of various hydrocarbon gaseous compounds produced as a result of bacterial activity. This is the first experimental evidence of the formation of these compounds during bioleaching.

The number of formed hydrocarbon gaseous compounds increased in experiments conducted with the natural bacterial consortium. Their formation in this case appeared to be enhanced by the presence of heterotrophic microorganisms.

Further work is now required to understand the chemistry of formation of these compounds and to find out how their formation is related to the oxidative activity of the leaching microorganisms.

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