Using Isotopic Composition of Reduced Sulfur Species in Suflidic Mine Tailings as Signatures of Bacterial Sulfate Reduction

T. Praharaj and D. Fortin
Dept. Earth Sciences, University of Ottawa, Ottawa, ON, Canada, K1N 6N5

Abstract

Unlike marine and freshwater sediments, information about sulfur isotopic compositions of sulfides in Cu-Zn mine tailings and their application to understand the process of sulfate reduction in mine tailings by sulfate-reducing bacteria (SRB) is scanty. Although SRB are known to be present in those mine tailings and contributing to the formation of Fe-sulfides, sulfur isotopes have never been used to conclusively establish the formation of biogenic sulfides. The present study is the first attempt to separate the Cu-Zn mine tailings into reduced sulfur species (acid volatile sulfides, AVS and chromium reducible sulfides, CRS) and determine their isotopic compositions to find signatures of bacterial sulfate reduction in those environments. In most of the CRS fractions of tailings from Cu-Zn mines, $\delta^{34}S$ values were within a relatively wide range; being mostly low and varying from positive to negative values, which is characteristic of bacterial sulfate reduction. $\delta^{34}S$ values of AVS fractions from Cu-Zn mines were found to be positive and higher. The isotopic composition of the AVS fraction might have been biased by the presence of pyrrhotite, as most of the values were comparable to that of pure pyrrhotite extracted by the same method. In general, $\delta^{34}S$ values of reduced inorganic sulfides (RIS) were found to be very low in deeper tailings compared to the shallow ones. This depletion of ^{34}S possibly indicates the role of SRB in the formation of Fe-sulfides in mine tailings. From the present study, it is believed that low and negative $\delta^{34}S$ values of reduced sulfide species in mine tailings are probable signatures of biogenic sulfides formed by SRB. Results from this study also corroborate previous geochemical and microbiological evidence of sulfate reduction obtained for the same tailings.

Introduction

The widespread occurrence of sulfate reducing bacteria (SRB) in various types of environments, including extreme environments like acidic mine tailings, are well documented in research publications. SRB are known to reduce the oxidized forms of sulfur to produce hydrogen sulfide (H_2S), which immediately reacts with iron to form Fe-sulfides. Under such conditions, the first biogenic sulfide to form in iron-rich mine tailing is usually the amorphous, poorly crystalline mineral iron-monosulfide, which is diagenetically converted to the more stable mineral pyrite (FeS_2) by addition of sulfur. Bacterial sulfate reduction (BSR) is an important geomicrobiological process where the SRB bring about fractionation of sulfur isotopes (Kendall and McDonnell, 1998) by preferentially utilizing the lighter sulfur isotope ^{32}S, leaving pore fluids progressively enriched in heavier sulfur isotope, ^{34}S (Rudnicki et al., 2001). H_2S formed during the BSR becomes depleted in ^{34}S and the remaining sulfate left behind is enriched in ^{34}S (Habicht et al., 1998). This fractionation process brought about exclusively by SRB results in a unique $^{34}S/^{32}S$ isotopic composition in waters and sediments and is, therefore, widely used as a signature to differentiate biogenic sulfides from abiogenic sulfides. As a result, the isotopic composition of metal sulfides in sediments provides an indication of the activity of sulfate reducing bacteria in sulfide formation (Canfield, 2001). Many researchers have separated and determined $\delta^{34}S$ of reduced inorganic sulfides in anoxic sediments, as they are critical to biogeochemical studies of sulfur cycles. The most common reduced inorganic sulfide (RIS) compounds extracted from the sediments are AVS (= H_2S + FeS) and CRS (= S^0 + FeS_2). Mineralogically, AVS is known to be composed Fe-monosulfides while CRS is known to be composed of pyrite and elemental sulfur (Huerta-Diaz et al., 1993; Bruchert et al., 2001; Canfield, 2001; Detmers et al, 2001; Bolliger et al, 2001). While AVS is commonly extracted with concentrated HCl, the CRS is extracted with reduced chromium chloride solution. Most of the of AVS and CRS extractions are reported from marine sediments and some from freshwater/lacustrine sediments, but information

about AVS and CRS extractions in extreme low-pH environments, like sulfidic mine tailings, is scanty, making it difficult to predict the response of tailings to the above extraction procedures. The only clue to this regard has been the published work of Friese et al. (1998) who have used $\delta^{34}S$ composition of RIS to study the activity of SRB in the sediments of an acidic mining lake in Germany. Moreover, the $\delta^{34}S$ compositions of the RIS fractions in published literatures have been reported to be falling in a wide range of values. This wide divergence of isotopic data in the published literature makes further research more difficult.

Fortin (1999); Fortin et al. (2000) and Fortin et al. (2002) have successfully studied the role of SRB in the formation of sulfides in Cu-Zn mine tailings from Timmins, Northern Ontario, Canada with the help of geochemical studies and microbiological enumerations. They have shown increasing populations of SRB with depth in the above mine tailings. So the present work was undertaken to support the above findings with the aid of sulfur isotopic compositions. The present study looks at SR from sulfur isotopic composition of different RIS fractions of Cu-Zn mine tailings to see if they can be used as signatures of BSR.

Methodology

Tailings samples were collected from a Cu-Zn mine (Potter) located in the Timmins area, in northern Ontario, Canada, during the summer of 1999. The tailing samples were collected from depths of 2.5 cm to 37.5 or 42.5 cm at regular intervals of 5 cm at two sites; one with vegetation (PO-01) and the other without vegetation (PO-02). They were immediately sealed in acid-washed vials after collection and freeze-dried for later use. The tailings samples were separated into AVS and CRS fractions using the procedures of Huerta-Diaz (1993) and Friese et al. (1998). As elemental sulfur is known to be soluble in CRS method (Hsieh and Shieh, 1997), it was first extracted from the tailings. For this, 2 g of tailings was extracted three times, each time ultrasonified for 15 minutes with 5 ml of dichloromethane, followed by centrifugation at 3000 rpm for 10 minutes, and the residue after extraction was air-dried. AVS and CRS extractions were carried out by acid distillation using N_2 as the carrier gas. The whole setup was first flushed with N_2 before starting the extraction. AVS was first extracted in cold (room temperature) for 1 hour with 40-80 ml of 6 M HCl under continuous flow of N_2 and the evolving H_2S was trapped in 10 ml. of a 3% ZnAc solution. If the sample did not produce any ZnS precipitate at room temperature, it was then heated (temperature kept at about 80 ^{0}C) for 1 hour and the evolving H_2S was trapped in the same manner as described above. The ZnS precipitates were converted to Ag_2S with 20 ml 0.1 M $AgNO_3$ solution. The Ag_2S precipitates were filtered using vacuum ultrafiltration and air-dried. The CRS fractions were extracted from tailings by boiling (temperature kept between 150-175 ^{0}C) them in 40 ml of freshly prepared acidified 1 M $CrCl_2$ solution and 20 ml of 6 M HCl for 1 hour and the H_2S was trapped in the same manner as in case of AVS. The Ag_2S obtained from both the sulfidic fractions were air dried and combusted at high temperature (1050 ^{0}C) with V_2O_5 and the SO_2 gas produced was subsequently purified on a high vacuum extraction line and analyzed by Continuous flow isotopic ratio mass spectrometer (CFIR-MS) for determining the sulfur isotopic composition. The sulfur isotopic composition was expressed in the standard δ notation given by: $\delta^{34}S = (R_{sample}/R_{standard}-1) \times 1000$, where $R = {}^{34}S/{}^{32}S$. The isotopic values were expressed on a per mil ($^0/_{00}$) basis relative to the Canyon Diablo Troilite (CDT) standard.

Enrichment cultures for the SRB were done for the tailings from PO-01. The growth medium was prepared according to the method described in Fortin et al. (2000). The cultures were incubated in the dark at room temperature for two weeks. The SRB cultures were then fixed with 10 ml of 20% Zn-acetate to terminate the bacterial activity. The ZnS was collected after centrifuging and filtration and distilled in cold with 6 M HCl. The liberated H_2S was trapped in $AgNO_3$ as Ag_2S. $BaCl_2$ was added to the supernatent after filtration to precipitate the dissolved sulfate as $BaSO_4$. The Ag_2S and $BaSO_4$ were air dried and measured for sulfur isotopic composition in the same way as mentioned above.

The mineralogy of the finely powdered mine tailings was determined with a Philips X-ray Diffractometer on a zero-background sample holder at 45 kV and 40 mA, using a Cu-source. Quantitative determination of the relative proportions of pyrite and pyrrhotite was done

from a two-phase calibration curve using pure pyrite and pyrrhotite mineral phases.

Results

The isotopic compositions of the RIS (AVS and CRS) fractions of mine tailings from PO-01 and PO-02 as a function of depth are shown in Figures 1 and 2. In both sites, the AVS phase could not be extracted from the tailings during the cold extraction step and, therefore, the extraction temperature was increased and kept around 80 ^{0}C. The δ^{34}S values in the AVS extracted fractions in PO-01 were found to be positive and ranging from 0.04 to 4.16 ‰, whereas those of the CRS fractions ranged from –0.38 to 1.42‰. The δ^{34}S values of the AVS fractions generally decreased with depth, showing a high δ^{34}S at 17.5 cm. XRD analysis showed that pyrite and pyrrhotite were the major sulfide mineral phases in the tailings. Quantitative mineral analysis showed that the pyrrhotite content increased with depth in the tailings (Figure 3). At this stage, it was suspected that pyrrhotite could have contributed to the AVS fraction. To examine this aspect further, pure pyrrhotite minerals were submitted to the same AVS extraction procedure. It was observed that pyrrhotite was almost entirely extracted during the AVS step, and that the δ^{34}S values ranged from 1.3-1.8‰, being distinctly positive and higher. So it is possible that the presence of pyrrhotite in the tailings could have overlapped and biased the δ^{34}S isotopic composition of AVS fractions of the mine tailings. To study the possibility of any contribution to the isotopic composition of AVS fractions in these tailings by the other sulfide minerals viz. sphalerite and chalcopyrite, these minerals were extracted using the same procedure as outlined above. Unlike pyrrhotite, their δ^{34}S values were observed to be typically low and negative.

In the case of PO-02, AVS and CRS could not be extracted from the top few centimeters of the tailings (2.5 cm to 17.5 cm for AVS and 2.5 cm to 12.5 cm for CRS). Quantitative mineral analysis showed that the pyrrhotite and pyrite contents of these tailings were extremely low (with the exception of the samples at 17.5 cm, where the pyrrhotite content was slightly higher). The δ^{34}S values of the AVS fractions of the rest of the tailings were found to be ranging from 0.53 to 3.28 ‰, whereas those of the CRS fractions were found to be ranging from –1.04 to 0.74 ‰. In general, the δ^{34}S values in the AVS fractions were all positive and higher, while those of CRS were found to be lower and varying from positive to negative values, as also observed for PO-01. Mineralogically, the pyrrhotite content in these tailings was found to be increasing with depth up to 22.5 cm and then decreasing afterwards (Figure 4).

The isotopic composition of the sulfides and sulfates obtained from enrichment cultures of SRB from PO-01 and PO-02 are shown in Figure 5. It can be seen that the δ^{34}S values for the sulfides for both PO-01 and PO-02 are low and negative (-1.6 to –1.8 ‰), whereas those for sulfates are distinctly higher and positive (2.8 to 6.1 $^{0}/_{00}$).

Discussion

One important observation from the extractions was that the AVS phase could not be extracted from the mine tailings at room temperature. On the other hand, when the extraction temperature was increased, AVS could be extracted from these tailings. This is possibly due to the fact that pyrrhotite, which is a constituent of the AVS, is a relatively stable phase. It is well known that the dissolution of minerals depends on their crystallinity. More robust and crystalline sulfides could only be extracted on heating. The absence of AVS at room temperature indicates that amorphous FeS and more crystalline monosulfides, like mackinawite/greigite, were absent from the tailings. It is possible that they formed rapidly but they most likely transformed into more stable phases like pyrrhotite and pyrite overtime. This can be explained by the slow or rapid oxidation of the monosulfides formed by BSR to pyrrhotite or pyrite by Fe^{3+} (Seal et al., 2000). The transformation of diagenetic Fe-monosulfides to pyrite in the tailings has also been documented by Fortin et al. (2000). The higher δ^{34}S values in the AVS extracted fractions could have been influenced by the mineral pyrrhotite, which is simultaneously extracted with Fe-monosulfides during the AVS extraction (Morse et al., 1987). It is difficult to decipher whether the other sulfide minerals viz. sphalerite and chalcopyrite were contributing to the isotopic composition of AVS fractions of mine tailings,

based on their isotopic compositions. However, owing to the fact that the proportions of these minerals are very low in the mine tailings, and that their $\delta^{34}S$ values are low and negative, it is possible that they are not contributors to the isotopic composition of the AVS phase. Interestingly, the isotopic composition of the CRS fractions were found to be low, variable and fluctuating between positive and negative values which is known to be characteristic of biogenic pyrite (Ehrlich, 1996). The most characteristic feature of isotopic composition of the reduced sulfides in these tailings is the decreasing $\delta^{34}S$ values with depth. Such a drop in the isotopic composition is expected if there is bacterial sulfate reduction wherein the SRB bring about isotopic fractionation between ^{34}S and ^{32}S, resulting in a decrease in $\delta^{34}S$ values with depth. Fortin et al. (2000) showed decreasing sulfate concentrations and increasing populations of SRB with depth in the PO-01 tailings. Such trends in the isotopic compositions of AVS and CRS with depth have also been previously observed by Habicht and Canfield (1997). Bruchert et al. (2001) and Wortmann et al. (2001) have also attributed the decline of isotopic compositions of AVS and CRS with depth to bacterial sulfate reduction in marine sediments. Friese et al. (1998), from their work on mining impacted acidic lake sediments, have stated that the decrease in $\delta^{34}S$ values with depth is indicative of dissimilatory sulfate reduction. Although the $\delta^{34}S$ values of the AVS phase were higher than the CRS ones in the PO-01 tailings, the relatively lower values possibly originated from biogenic SR. For example, the $\delta^{34}S$ composition of mine tailings at 37.5 cm depth in PO-01 was 0.04 ‰, which corresponds to relatively lower pyrrhotite content than the tailings at 27.5 and 32.5 cm depths. The isotopic composition of the former is possibly affected by SRB activity as their $^{34}S/^{32}S$ signals are biased by pyrrhotite, which has a higher $\delta^{34}S$ composition.

In the case of the PO-02 tailings, AVS was found to be very low at shallow depths. This is possibly caused by sulfide-depleted layer in the tailings (Figure 4), which has also been reported by Fortin et al. (2000). While the isotopic composition of the AVS fractions of the rest of the tailings were all positive, those of CRS fractions were found to be much lower; varying from positive to negative values. Such low (<5 ‰) and variable $\delta^{34}S$ values of iron sulfides are believed to be a consequence of the activity of SRB and believed to be of biogenic origin, whereas high values indicate a abiogenic origin for the pyrite (Kohn et al., 1998; Thode and Goodwin, 1983). The $\delta^{34}S$ values reported for abiogenic sulfides from massive and ore sulfide deposits (Seal et al., 2000) are mostly higher than the values obtained in the present study. All these evidences possibly point to the fact that the lower $\delta^{34}S$ values mostly obtained from the CRS extracted fractions are representative of biogenic pyritic sulfides formed by SRB in mine tailings.

The $\delta^{34}S$ values obtained from enrichment cultures of SRB from PO-01 and PO-02 indicate that they are similar to the low and negative $\delta^{34}S$ values observed for the CRS extracted fractions of the same mine tailings. The fractionation factor between the sulfides and sulfates from bacterial cultures are found to be fairly high (7.9 ‰ for PO-01 and 4.4 ‰ for PO-02). However, these values are much lower than those reported for marine and freshwater sediments where the rate of sulfate reduction is known to be quite high.

Conclusion

In the present study, it was observed that in most cases, the $\delta^{34}S$ values were found to be very low in deeper tailings compared to the shallower ones indicating greater sulfide formation leading to a decrease in $^{34}S/^{32}S$. This depletion of ^{34}S possibly indicates the role of SRB in the formation of sulfides in mine tailings. Moreover, the $\delta^{34}S$ values generally decreased with depth, indicating greater isotopic fractionation and increasing formation of sulfides from bacterial sulfate reduction. In PO-01 and PO-02, a gradual decrease in $\delta^{34}S$ values with depth was observed for both AVS and CRS fractions. This is probably indicative of a biogenic origin of the sulfides. It can be seen that the more negative and lower $\delta^{34}S$ values are better representative of biogenic sulfides. Hence they can be used as indicators of sulfides formed by SRB.

In case of mine tailings, although there is ample evidence of SRB activity, the $\delta^{34}S$ values are believed to be contributed and dominated by the primary sulfide minerals. In all the tailings from

Potter mines, the $\delta^{34}S$ values of AVS fractions were however heavier than those of CRS. Our results show that pyrrhotite contributed to the AVS fraction, and that some pyrrhotite could have formed by the transformation of the early-formed amorphous diagenetic Fe-monosulfides to more stable sulfide phase. However, more work needs to be done to find out the relative fractions of biogenic and abiogenic pyrrhotites and pyrites in mine tailings. The lower $\delta^{34}S$ values of the corresponding CRS fractions indicate that pyrite was newly formed, possibly by SRB, which are already known to be present in these tailings. From the results and trends observed in the present study, it can be inferred that sulfur isotopic composition notably that of the CRS phase, can be used as a signature to indicate the activity of SRB in mine tailings.

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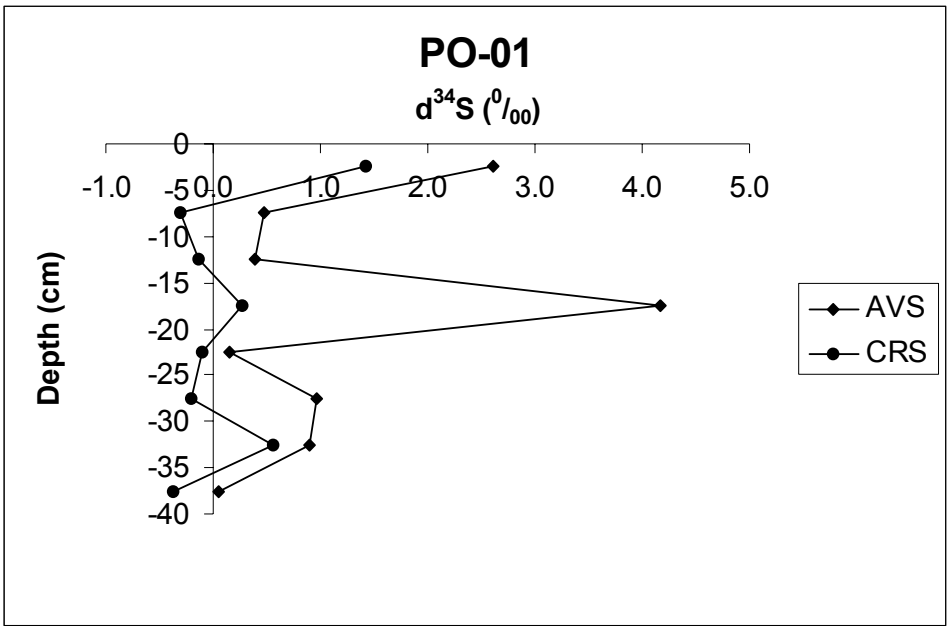

Figure 1. Variation of $\delta^{34}S$ values with depth of the AVS and CRS fractions of the PO-01 mine tailings.

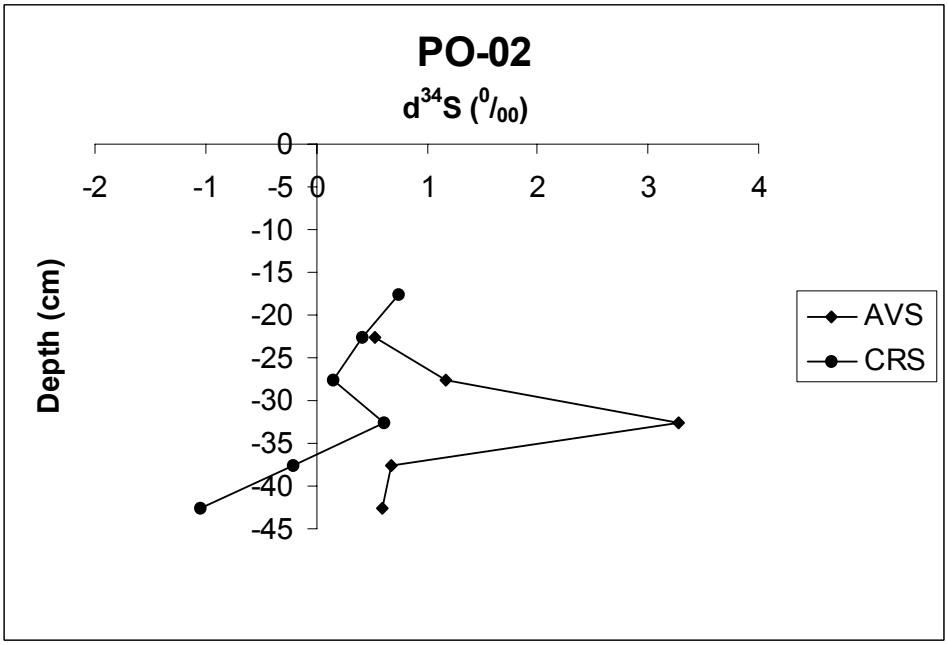

Figure 2. Variation of $\delta^{34}S$ values with depth of the AVS and CRS fractions of the PO-02 mine tailings.

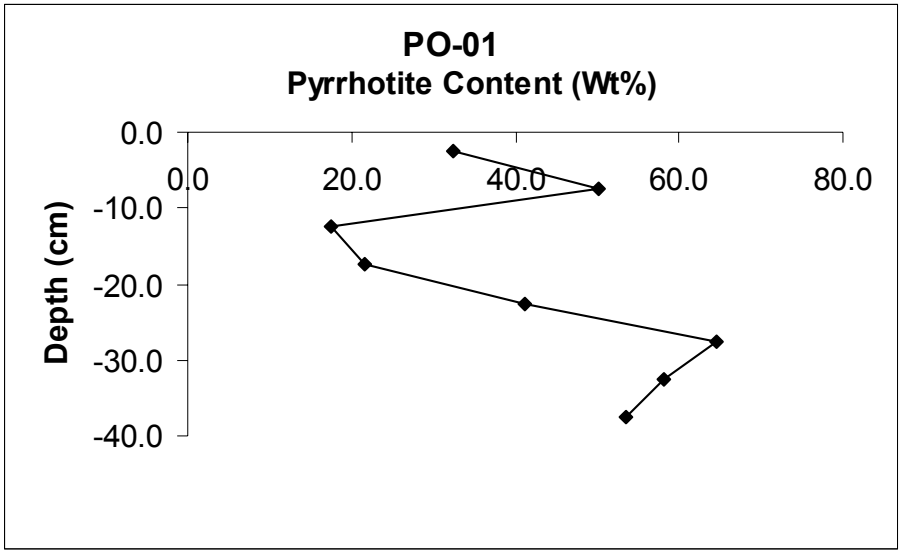

Figure 3. Pyrrhotite content of the PO-01 tailings as a function of depth.

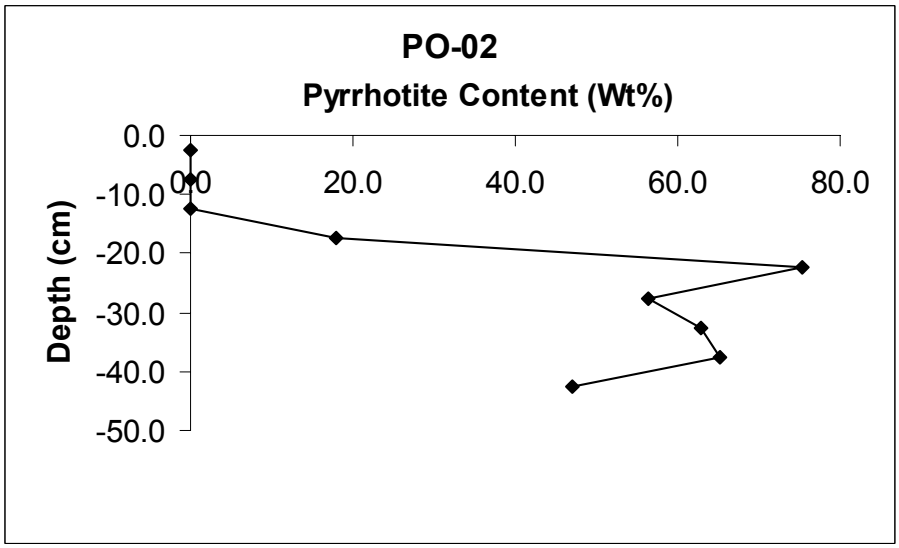

Figure 4. Pyrrhotite content of the PO-02 tailings as a function of depth.

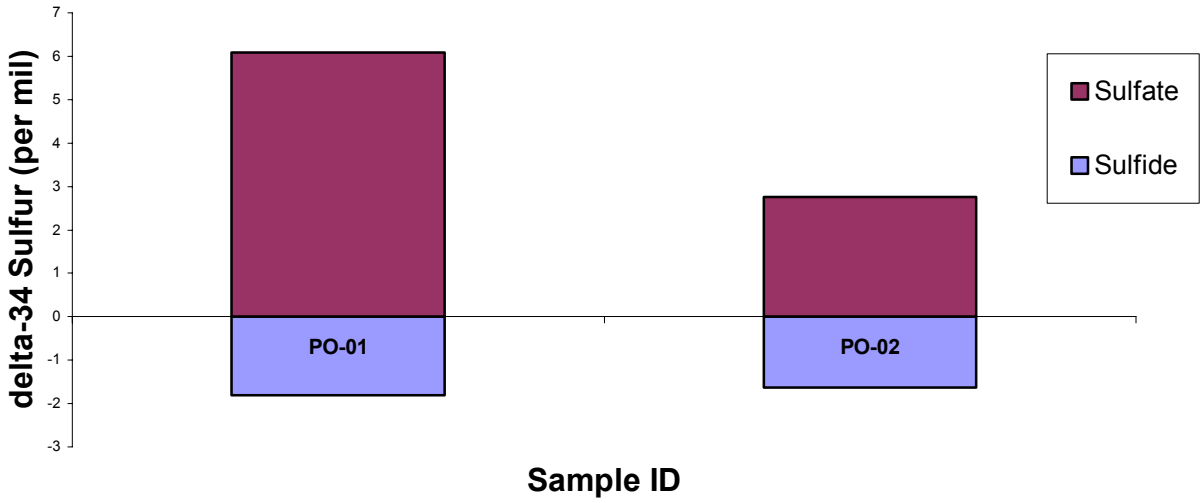

Figure 5. $\delta^{34}S$ isotopic composition of sulfides and sulfates formed in SRB cultures grown at room temperature in the presence of lactate as an electron donor.