Microbial activity of iron-reducing bacteria and sulfate reducing bacteria isolated from mine tailings in the presence of various electron donors

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Abstract
The microbial activity of anaerobic bacteria, especially iron-reducing bacteria (IRB) and sulfate-reducing bacteria (SRB), in sulfidic mine tailings is still poorly understood. Both IRB and SRB require strict anaerobic conditions and simple organic electron donors, such as acetate, lactate, formate, etc. In addition, both processes generate alkalinity. IRB can reduce various forms of Fe(III)-oxides and generate large concentrations of soluble Fe(II) in the porewaters, whereas SRB reduce sulfate to sulfide, which in return can react with Fe(II) to form fresh Fe-monosulfides. Both SRB and IRB have then the ability to affect the chemical and mineralogical evolution of the tailings . It is also known that IRB and SRB can compete for the same electron donors under specific physico-chemical conditions and that the predominance of one group over the other can drastically impact the geochemistry of the tailings. The present study was designed to investigate if SRB and IRB could out compete each other in the presence of various electron donors and at various pH conditions. Sediments from acidic Cu-Zn tailings and from neutral Pb-Zn tailings were used for batch experiments. Individual electron donors (either lactate, acetate or formate) were added to the systems and the pH was adjusted to the *in situ* pH of the tailings. The activity of SRB was also inhibited in some systems in order to isolate the activity of the IRB alone. Our results first indicate that the activity of both SRB and IRB had a greater buffering effect than the IRB alone. The production of sulfide was also greater in the IRB and SRB systems, whereas the release of Fe(II) was not necessary linked to the activity of the IRB, since large concentrations were observed in the abiotic control systems. The largest Fe(II) concentrations were indeed observed in the systems containing large quantities of acidic abiotic tailings. The exact mechanism for abiotic Fe(III) reduction in those systems is still under investigation. SRB growth did not appear to be a function of the type of electron donors in the systems, but the presence of both SRB and IRB limited the growth of IRB in the acetate systems. This suggests that IRB cannot compete with SRB when acetate is the only electron donor present.

Introduction

The microbial activity of anaerobic bacteria in mine tailings can affect the chemical conditions within the mining residues. Sulfate-reducing bacteria (SRB) have been identified in various Cu-Zn and Au tailings, but the presence and activity of IRB remains uncertain (Fortin et al., 2000; 2002). Recent studies have shown that there is a competitive behaviour between IRB and SRB, which depends on the availability of electron donors and on the *in situ* chemical conditions. For instance, a recent study by Roden et al., (2000) has shown that SRB are able to inhibit the presence of IRB in regions where sulfate reduction is high. Recent studies (Blodau et al., 1998; Cummings et al., 2000 and Wielinga et al., 1999) have however indicated that both IRB and SRB are active in sediments and are able to coexist under anaerobic conditions. Further more, it is suggested that the selectivity of both types of bacteria for the electron donors is responsible for their co-existence. A study by Peine et al. (2000) indicated that lactate, as an electron donor, favored SRB activity, but it also supported IRB activity, but at a smaller rate. The purpose of the present study was to examine the co-existence of SRB and IRB in the presence of different electron donors (lactate, formate and acetate) in two different mining environments. Acidic mine tailings were sampled at a Cu-Zn mine (Potter) near Timmins, Ontario, Canada, whereas alkaline tailings were sampled at a Pb-Zn mine (Calumet) near Ottawa, Ontario, Canada.

Methods

Tailings were sampled at the two abandoned mines mentioned above. For the alkaline mine tailings, samples were collected in July 2002 within the reducing zone located just below the

surface. At a depth of 5 cm, a black layer composed of plant roots and black iron sulfides was present. In the acidic mine tailings, the samples were collected in July 2002 just below the oxic-anoxic interface at a depth of 15-20 cm. The growth medium used in all batch experiments was a modified version of Postgate's Growth Medium G (Postgate, 1984). The pH of the medium was adjusted to *in situ* pH conditions, i.e., 7.38 and 5.22 for Calumet and Potter respectively. All systems were setup in duplicate. The medium was dispensed in 500 ml serum bottles under CO_2:N_2 (80%:20%) atmosphere and autoclaved (121°C) for 15 minutes. Lactate, formate and acetate were added as separate electron donors and the final concentration was adjusted to 5 mM. The activity of SRB was inhibited in some systems by adding 20 mM (final concentration) of sodium molybdate. In order to inoculate each system, a sediment suspension was prepared with 10 g of tailings dispersed in 50 ml of sterile deionized water. From this suspension, 10 ml of the slurry was added to the bottles using a sterile syringe. The control systems were inoculated with dried autoclaved sediments. All systems were sampled bi-weekly and analyzed for dissolved Fe(II) and HS^- using the ferrozine and Cline methods, respectively. pH and Eh measurements were also performed over time. The remaining samples were acidified with omnitrace HNO_3 acid (0.1% final concentration) and analyzed for $SO4^{-2}$, total dissolved iron, dissolved organic (DOC) and inorganic carbon (DIC). SRB and IRB enumerations were performed with the Most Probable Number (MPN) technique according to the procedure outlined in Fortin et al. (2002) and Cummings et al. (2000).

Results

The results presented in this paper are preliminary because the study is still underway. The pH of the systems containing the alkaline tailings remained relatively stable for both the control and inhibited SRB systems in the presence of the various electron donors (Fig. 1). A small pH increase was however observed in the systems containing both active IRB and SRB populations (Fig.1). Fe(II) concentrations increased over time in all systems containing active IRB (i.e., in the inhibited SRB systems), but also in the control system containing lactate (Fig. 1). Low levels of Fe (II) were also observed in some systems containing both IRB and SRB (Fig. 1). Sulfide concentrations increased with time in all non-inhibited SRB systems, whereas concentrations remained very low in the other systems (Fig. 1). SRB grew in the presence of all electron donors, but the fastest growth was observed in the lactate systems (Fig. 3) IRB populations were generally lower than SRB populations, with the exception of the inhibited SRB system with acetate where IRB populations exceeded the SRB (Fig. 3)

The systems containing the acidic tailings showed a fairly constant pH over time, but the systems containing both IRB and SRB slightly increased in pH during the course of the experiment (Fig. 2). Large concentrations of Fe(II) were observed in all control abiotic systems, but they remained very low in the systems containing the bacteria (Fig. 2). Sulfide concentrations increased over time in the non-inhibited SRB systems in the presence of all electron donors (Fig. 2). SRB populations grew in all systems, but they appeared to grow better in the presence of formate (Fig. 3). IRB populations are not reported here because they require a long time to grow (i.e., 3 months) and the results are still not available.

Discussion

From our preliminary results, it is clear that both microbial sulfate and iron reduction generated alkalinity and buffered the systems, especially in the alkaline tailings (Fig. 1). In the acidic systems, the activity of both IRB and SRB slightly increased the pH of the systems, but the overall effect was less pronounced than in the alkaline tailings, with the exception of the formate system (Fig. 2). Such trends are consistent with the fact that both iron and sulfate reduction produce alkalinity (Fortin et al., 2002; Wendt-Potthoff et al. 2002) and that the buffering capacity of the acidic tailings is very low because the tailings have undergone acidic oxidation, resulting in carbonate dissolution (Fortin et al., 2002). Our preliminary data do not allow us to assess the effect of electron donors on the buffering capacity of the systems.

The activity of the iron reducers is very difficult to assess because our MPN numbers are incomplete (Fig. 3). It appears however that the presence and growth of IRB are not always linked to the release of Fe(II) in the systems, because Fe(II) was present in the abiotic control systems, especially in the acidic tailings (Fig. 2). In the alkaline tailings, Fe(II) increased over time in the presence of inhibited SRB. This indicates that IRB alone were active and capable of reducing Fe(III), as shown by Wendt-Potthoff et al. (2002). It is also clear that abiotic Fe(III) reduction also occurred in some systems because the lactate control system contained large concentrations of Fe(II) in solution (Fig. 1). The same trends were also observed in the acidic tailings in the presence of all electron donors (Fig. 2). It is known that sulfides can abiotically reduce Fe(III) in aquatic environments, but this mechanism is unlikely because the abiotic systems contained no dissolved sulfides (Fig. 2). It is possible that the electron donors themselves acted as reducing agents in the abiotic systems, but we do not have supportive data to corroborate our findings. It is also possible that autoclaving the tailings promoted the dissolution of Fe(III)-rich minerals over time. Further investigations will be necessary. It is also clear from our results that Fe(II) concentrations in the active IRB and SRB systems were controlled by the precipitation of Fe-monosulfides, following the reduction of sulfate to sulfides (Figures 1 and 2), because in the systems containing inhibited SRB, Fe(II) concentrations increased over time. Fe-monosulfides were indeed visible in some systems containing both SRB and IRB.

The slow growth of IRB in our systems could be related to the lack of nutrients available, especially in the systems where SRB were active. However, it has been argued that iron-reducing bacteria (IRB) are good scavengers in nutrient limited conditions (Straub et al. 2001). This can be seen in our results, especially in the alkaline systems containing lactate and formate and active SRB (Fig. 3). In these systems, IRB populations were greater that in the systems containing inhibited SRB, indicating that IRB can easily compete for available nutrients and electron donors. Our limited results also indicate that acetate appeared to be the only electron donor favoring IRB growth in the alkaline tailings, because IRB populations dominated the systems containing inhibited SRB (Fig. 3).

The activity of IRB in the acidic tailings is still under investigation, but we expect that the pH should favor the growth of IRB because SRB are usually more sensitive to acidity (Kusel and Dorsch, 2000).

Conclusion

Our preliminary results suggest that the microbial activity of IRB and SRB in tailings could buffer the tailings and prevent the development of acidic conditions. In addition, the activity of both IRB and SRB partially control the cycling of iron and sulfur and, the presence of specific electron donors, namely acetate, could favor the iron reducers over the sulfate reducers.

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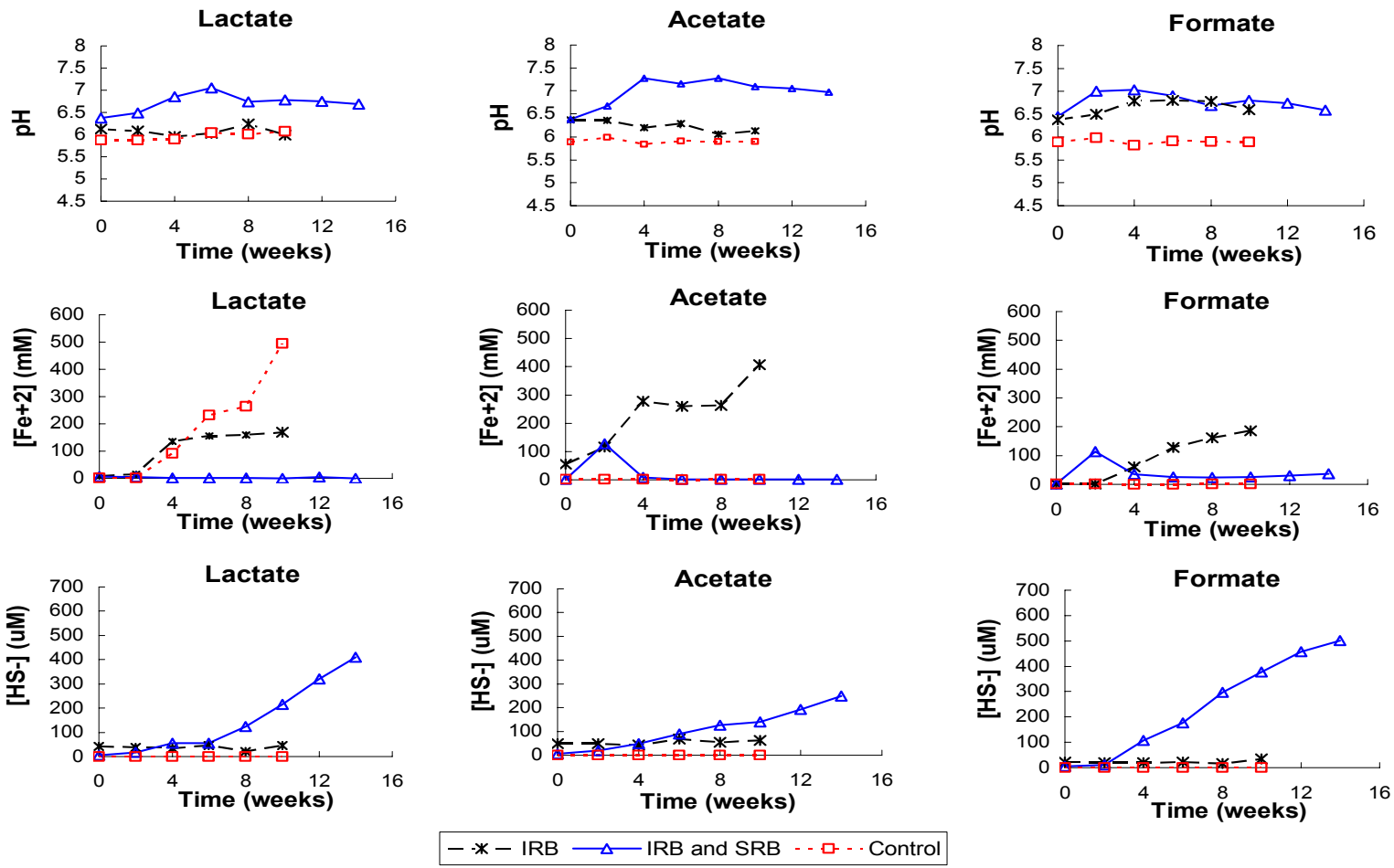

Figure 1: Chemical evolution of the batch systems containing lactate, acetate or formate and the alkaline Pb-Zn mine tailings (CA-01).

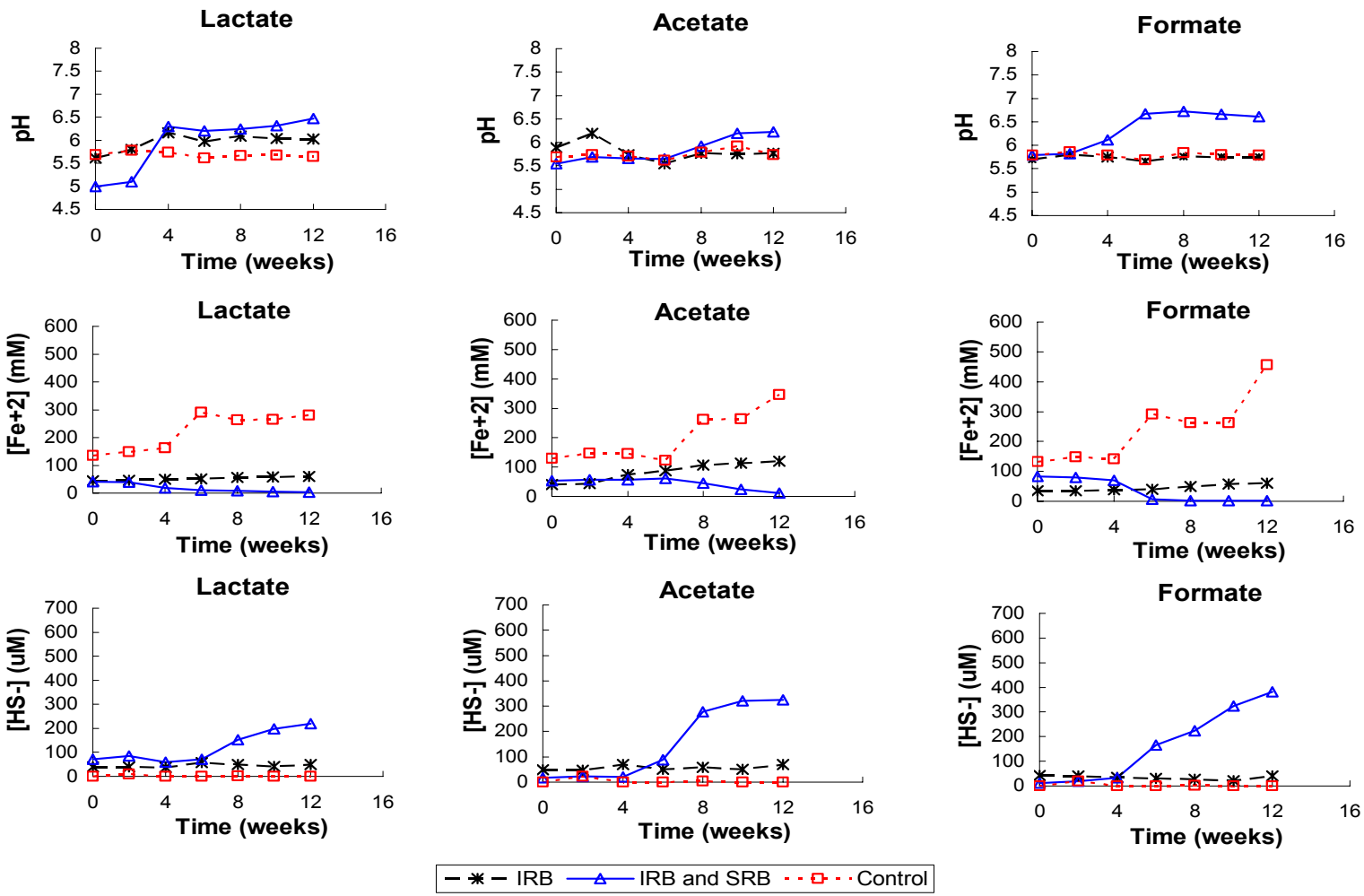

Figure 2: Chemical evolution of the batch systems containing lactate, acetate or formate and the acidic Cu-Zn mine tailings (PO-01).

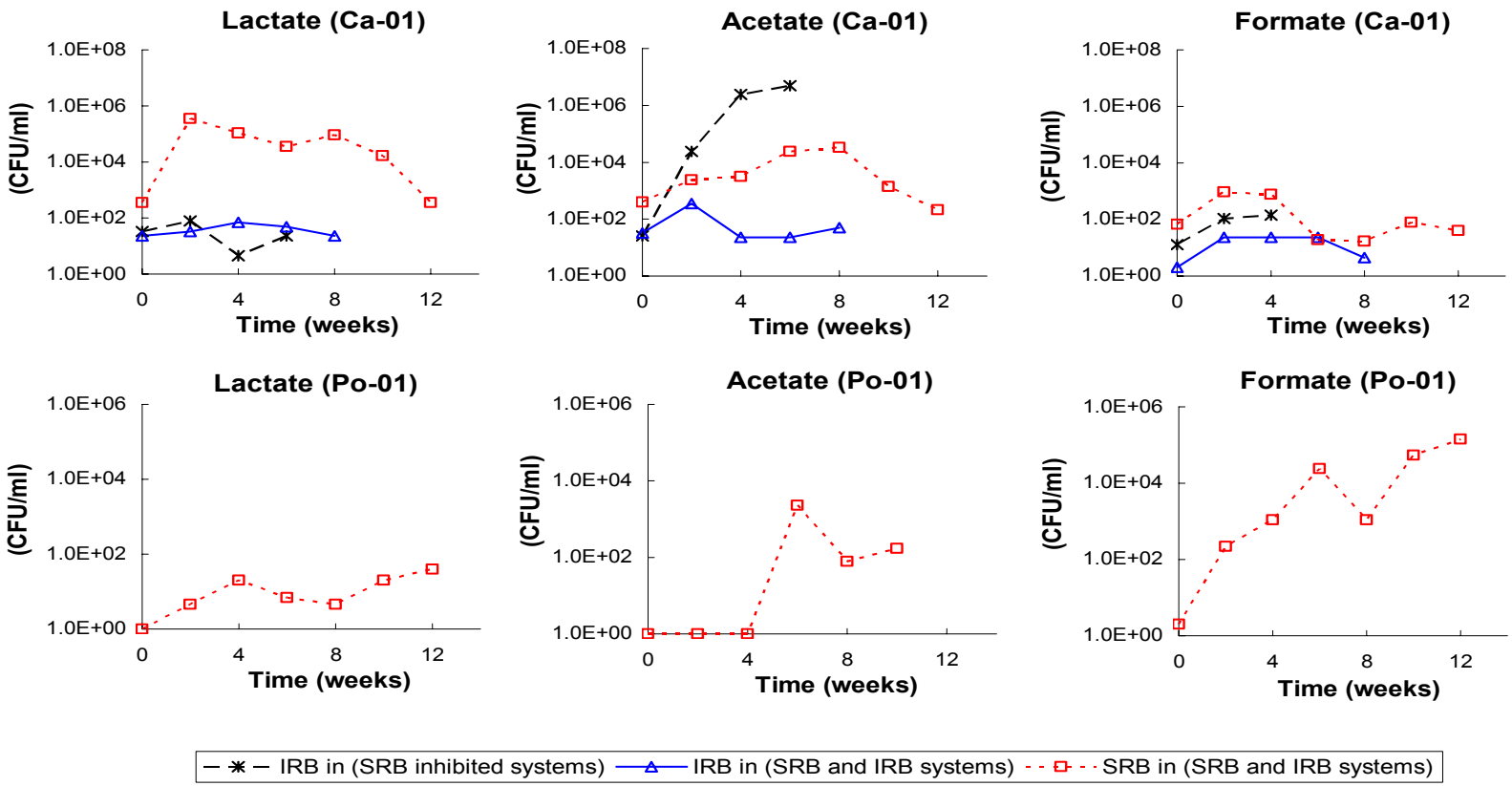

Figure 3: Bacterial populations of sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB) in the alkaline (CA-01) and acidic (PO-01) mine tailings.