Effect of Lactate/Acetate and Glucose Amendments on Low Temperature Performance of Anaerobic Bioreactors Treating Simulated Mine Drainage

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Abstract

This paper presents the results of a study evaluating the effect of two different carbon additions on the performance of a series of six anaerobic bioreactors operating at low temperature. The feed contained 100 ppm Zn and 600 ppm SO_4 at pH 6.5. The columns contained a mixture of pulp mill biosolids, sand and limestone. During initial operation at 20°C the columns removed approximately 99% of the zinc. The temperature was lowered to 4°C after which zinc removal fell to approximately 60%. Subsequently, carbon sources were added to the feed entering four of the columns and two of the columns were unamended controls and received only the zinc sulphate feed. Two columns received glucose and two of the columns received a mixture of acetate and lactate. At 4°C the two unamended columns continued to remove only 60% of the zinc while the lactate/acetate and glucose amended columns achieved >95% zinc removal within 156 and 170 days respectively. The experiments confirm previous results that show that at low temperatures, labile metabolizable carbon is the main limiting factor in anaerobic bioreactors and that the addition of a labile carbon source can improve low temperature performance.

Key words: mine drainage, low temperature, treatment, carbon amendment, sulphate reduction

Introduction

The use of passive systems to treat mine drainage was first suggested and studied in bench scale applications as early as 1978. Passive systems can be based on chemical or biological processes or a combination of both and include anoxic limestone drains, limestone channels, constructed wetlands and anaerobic bioreactors (Johnson and Hallberg 2005; Neculita *et al*. 2007). More than 300 artificial wetlands to treat mine drainage were constructed in the eastern United States in the period between 1984 and 1990 but the results were varied and unpredictable. After 1990 it was suggested (Dvorak *et al*. 1991; Drury 1999) that the metabolism of sulphate reducing bacteria (SRB) was the most important process occurring in wetlands treating metal contaminated water. Anaerobic bioreactors (ABR) for the treatment of acid mine drainage were designed to enhance sulphate reduction. ABR consist of organic matter to provide substrates for microbial activity, sand and gravel for permeability, and limestone for alkalinity generation (Johnson and Hallberg 2005; Kaksonen and Puhakka 2007; Mattes *et al*. 2011). Initially these systems produced inconsistent results but additional research has provided information on how to design and build better bioreactors for the treatment of metal contaminated effluents. SRB are obligate anaerobes that use either sulphate or thiosulphate as terminal electron acceptors in place of oxygen. SRB are generally heterotrophic bacteria requiring an organic carbon source but some can grow autotrophically using hydrogen as an electron donor, although growth rates are slow. SRB ameliorate acid mine drainage by generating alkalinity as bicarbonate and producing bisulphide, which precipitates metals as their sulphides.

SRB generally metabolize low molecular weight compounds such as organic acids, alcohols and amino acids but some species of SRB can also metabolize low molecular weight aromatic compounds such as benzene and toluene. The most critical limiting factor for the microbial activity is the amount of labile metabolizable carbon from an added organic carbon source (Gilbert *et al*. 2004; Neculita *et al*. 2007; Zagury *et al*. 2006). Waste biomass is usually used as both electron donor and carbon source in ABR. The main components of the wastes used are lignin and cellulose, both of which must be broken down by other bacterial groups before they can be used by the SRB. Two key groups of bacteria are the cellulose

degraders and the fermentative bacteria (acid producing bacteria or APB). Some of the fermentative bacteria can also degrade cellulose. Microbiological studies of laboratory ABRs indicate that anaerobic cellulose degrading bacteria are the key organisms in the successful operation of ABRs and that cellulose hydrolysis is the rate limiting step (Logan *et al*. 2005; Pruden *et al*. 2007).

In natural settings, or ones where a readily metabolizable carbon source is not available, sulphate reduction is temperature limited. The critical time for the performance of anaerobic bioreactors is during spring runoff when the flow is higher, the effluent is cold and the bioreactor is not yet acclimatized. Anaerobic bioreactors need a minimum of two weeks to acclimatize when they have been started after a dormant period. Other studies have shown that at higher temperatures, SRB require lower concentrations of volatile fatty acids to maintain activity than at lower temperatures. The rate limiting activity in these systems might be the microbial group responsible for generating the appropriate carbon substrates rather than the SRB. This project will address that hypothesis. The objectives of this project are to:

1. Establish that the rate limiting step of cellulose hydrolysis is kinetically hindered at low temperatures so that it is the rate limiting step in SRBs operating at low temperatures.
2. Determine if the addition of supplementary carbon sources will improve the performance of anaerobic bioreactors at low temperatures.

Materials and Methods

Six PVC columns with two sampling ports each were constructed for the study. The column dimensions are 15.2 cm diameter, and an overall height of 80 cm (Figure 1). The bottom layer consisted of 10 cm of 1.2 cm size limestone (3 kg limestone), and the second layer consisted of a 60 cm layer of a mixture of pulp mill biosolids + sand + limestone in the following proportions by weight: biosolids 72% (33.9% carbon), limestone 14%, and sand 14% (total amount 10 kg). The biosolids were covered by a 10 cm layer of water. The two sampling ports are spaced 20 (Port #1) and 40 cm (Port #2) respectively from the bottom of the biosolids layer. The feed contained zinc and sulphate at a pH value of 6.5 based on the criterion of 0.3 mmoles/L/day (Gusek and Wildeman 1997). The feed was similar to that being treated by the anaerobic bioreactor in Trail, B.C. and the feed contained approximately 100 ppm Zn and 600 ppm sulphate at pH 6.5. The flow rate in each column was 1 mL/min which was equivalent to 77 L/m^2/day as specified by Gusek and Wildeman (1997). The columns were operated in the upflow mode.

At each sampling interval a total of eighteen liquid samples were taken from six columns at each of the two ports and the final column effluent. The water samples from the columns were split into four subsamples. One set was filtered through a 0.45µm filter for the determination of soluble ions and half of the sample was acidified for the determination of zinc (subsample #1) and the other half was not acidified and was used for the determination of sulphate (subsample #2). The other two subsamples were not filtered but also split into acidified (subsample #3) and unacidified (subsample #4) subsamples and used for the determination of total zinc and sulphate concentrations. Zinc was determined by ICP-AES and sulphate by ion chromatography.

The columns were initially conditioned at 20^{o}C by filling the column with feed solution and allowing them to stand for one week. The operating conditions consisted of four phases: Phase I: All six columns were run for 65 days at 20^{o}C. Phase II: All six columns were run at 10^{o}C for 13 days. Phase III: All six columns were run at 4^{o}C for 87 days. Phase IV: The duration of this phase was 140 days. The temperature was maintained at 4^{o}C but two columns had glucose added to the feed (final concentration 6 mM glucose); two columns had a mixture of acetate and lactate added to the feed (final concentrations of 3 mM acetate and 3 mM lactate), and the remaining columns (controls) were operated in the same fashion as in Phase III. The feed solutions containing the added carbon sources were filter sterilized (0.45 µm filter) and stored in sterile bottles prior to being added to the columns to prevent microbial growth in the storage containers.

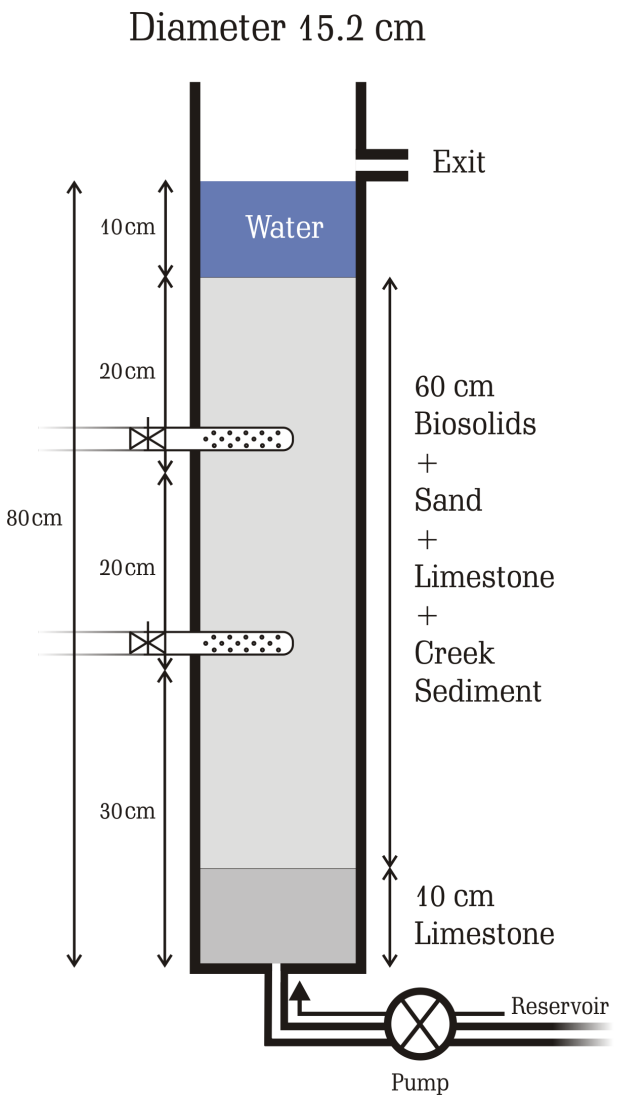

Figure 1. Column design.

Results and Discussion

The initial removal efficiency of the total zinc from the feed at 20°C (Phase I) was 92% and required 30 days to increase to greater than 99% removal (Figure 2). On day 15 a spike was added to the influent to increase the feed zinc concentration to 230 ppm. The total zinc concentration in the bottom port increased to 60 ppm but then declined rapidly after the spike. The final effluent concentration was unaffected by the spike. The low concentrations of zinc in the two ports and the response to the spike event indicate that the treatment system is robust and has the capacity to handle shock loadings. The removal efficiency of soluble zinc was 100% for the duration of Phase I (Figure 2). The discrepancy between total zinc and soluble zinc in the final effluent during the initial period indicates that some particulate zinc was passing through the column. However, after 30 days even the particulate material was also being removed. Studies of large scale wetlands and anaerobic bioreactors have also shown that filtration of particulate contaminants is one of the functions of these treatment systems (Batty *et al*. 2008; Johnson and Hallberg 2005; Mayes *et al*. 2009).

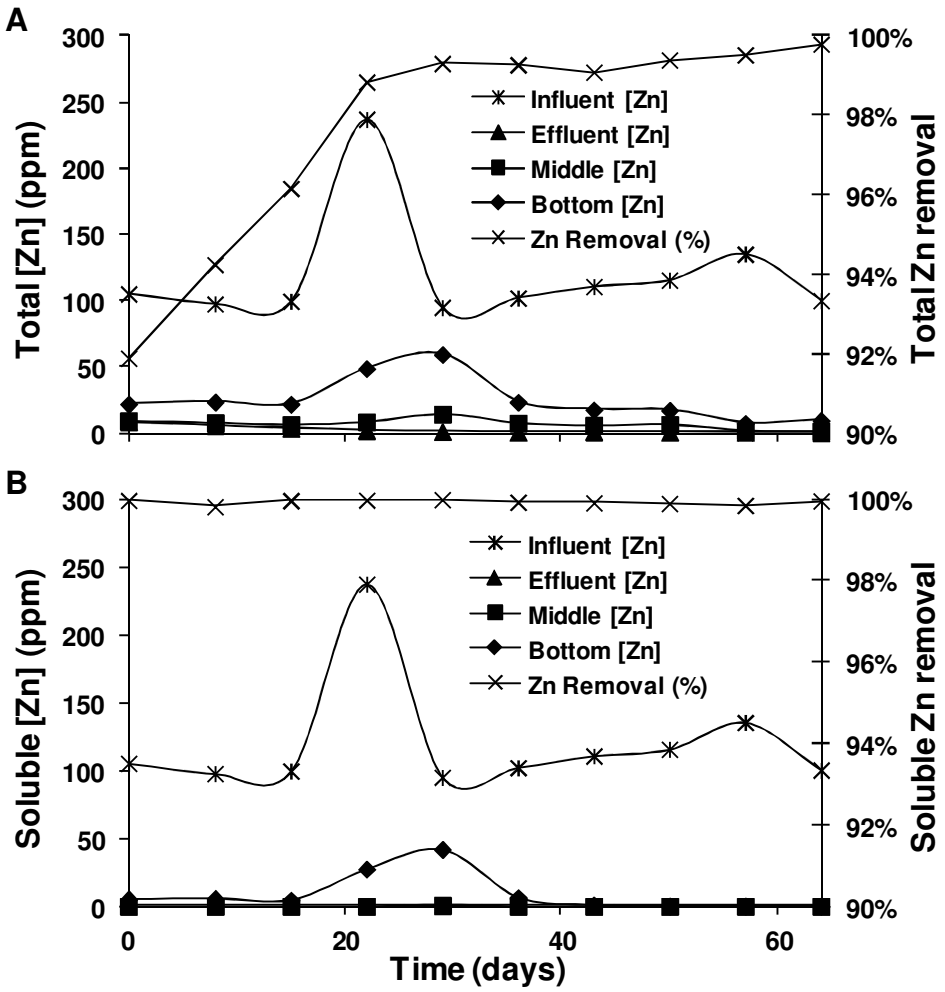

Figure 2. Phase I (20°C): A. Total Zn. B. Soluble Zn.

The temperature was decreased to 4°C in a stepwise fashion by reducing the temperature to 10°C for 13 days (Phase II) and then to 4°C for 87 days (Phase III). During Phase III, the zinc concentration in the final effluent from the columns was approximately 40 ppm which is equivalent to a treatment efficiency of 60% (Figures 3 and 4). The total and soluble zinc concentrations in all of the samples were equal which indicates that particulate contaminants are being filtered by the column matrix (Figures 3 and 4).

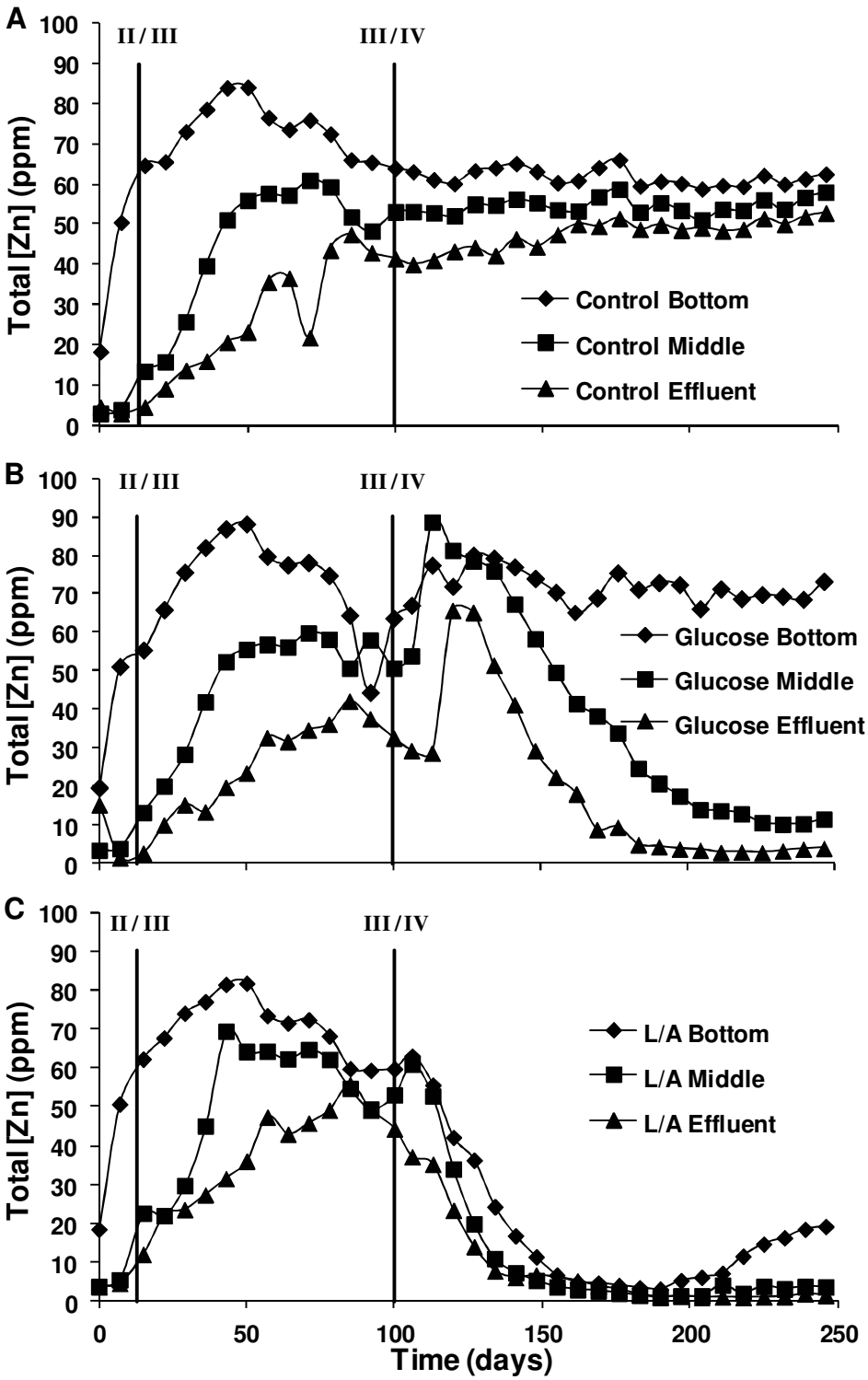

Figure 3. Phases II (10°C), III (4°C) and IV (4°C): Total Zn concentrations. A. Control columns B. Columns amended with glucose. C. Columns amended with acetate and lactate (L/A).

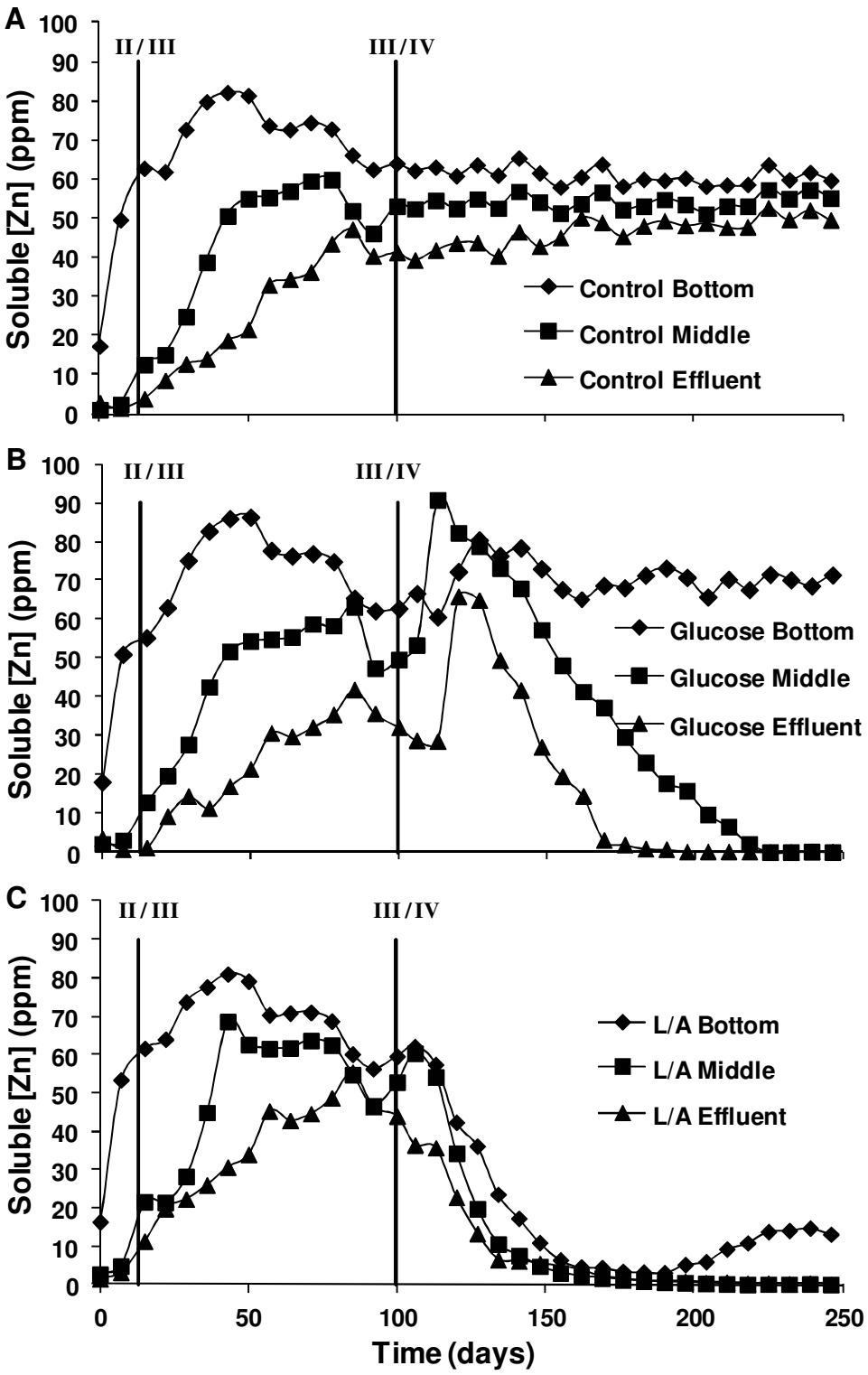

Figure 4. Phases II (10°C), III (4°C) and IV (4°C): Dissolved Zn concentrations. A. Control. B. Columns amended with glucose. C. Columns amended with acetate and lactate (L/A).

During Phase IV, the unamended columns continued to remove approximately 50 to 60% of the zinc. The zinc concentrations in the two lower ports were similar to the final effluent which indicates that most of the zinc is removed in the lower one third of the columns. The glucose amended columns required 170 days to achieve >95% removal. The bottom port of the glucose amended columns had similar (60%) treatment efficiency as the control columns (Figures 3 and 4). The lactate/acetate amended columns required 156 days to achieve >95% removal. All of the ports in the lactate/acetate amended columns had >95% treatment efficiency after the initial lag period (Figures 3 and 4). The glucose amended columns had a longer lag period and less effective treatment efficiency than the lactate/acetate amended columns because the glucose must be fermented to other products before it can be used by the SRB, whereas both lactate and acetate can be utilized directly by the SRB. These experiments show that cellulose degradation is the rate limiting step in the operation of SRB based anaerobic bioreactors treating mine drainage. These results are consistent with both microbiological and molecular studies of SRB anaerobic bioreactors (Logan *et al*. 2005; Pruden *et al*. 2007). In these systems the activities of the SRB are dependent on the amount of available carbon but are less dependent on temperature than the organic carbon degrading activities.

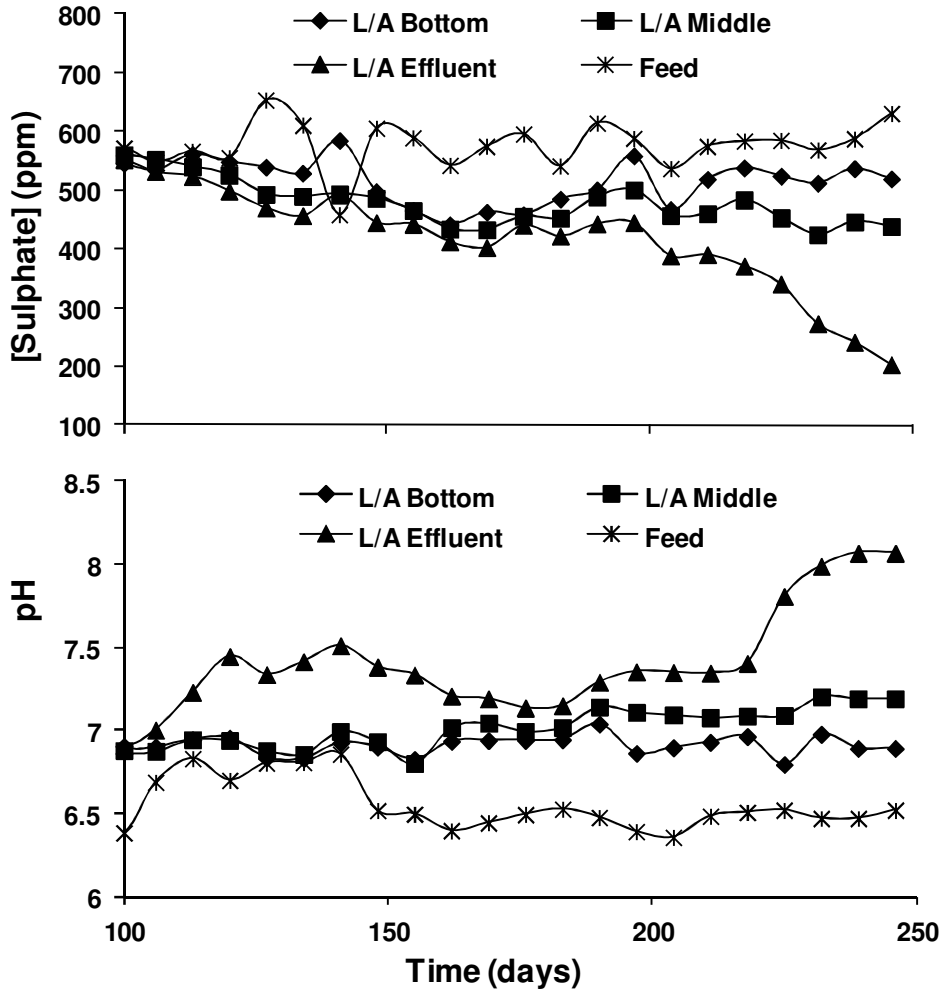

Figure 5. Concentration of sulphate and pH during Phase IV in columns fed the mixture of lactate and acetate.

The performance of the bottom port of the lactate/acetate amended columns decreased slightly at the end of the experiment (Figures 3 and 4). During that period the lower part of the columns had increased sulphate reduction as evidenced by the increase in pH and the decrease in sulphate concentrations (Figure 5). It is likely that sulphide toxicity may be responsible for the decrease in performance in the region of the bottom port. If sulphide is being produced more rapidly than it can be precipitated by the metals in the effluent, the accumulated sulphide could inhibit bacterial growth.

The results of this study also show that it is possible to enhance the performance of ABRs treating mine drainage during the critical start up period in the spring by supplementing the influent with a readily metabolizable carbon source.

Conclusions

The low temperature operation of an ABR treating mine drainage can be enhanced by the addition of a labile carbon source. The results of these are experiments are in agreement with other studies that show that cellulose degradation is the rate limiting process in an ABR based on sulphate reduction. If insufficient metals are present in the influent, the resulting accumulation of sulphide could inhibit bacterial growth.

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