AN EVALUATION OF A PEAT-WOOD CHIP-MICROFLORA ADMIXTURE TO ACT AS AN AMELIORANT FOR ACID MINE DRAINAGE[1]

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Abstract: Peat was investigated as a potential source of indigenous microflora capable of: (1) providing a suitable carbon source for sulfate-reducing bacteria (SRB) through the anaerobic degradation of black spruce (*Picea mariana*) and trembling aspen (*Populus tremuloides*) wood chips, (2) complexing metals to form relatively insoluble sulphide minerals through the reduction of sulfate to sulphide, and (3) surviving exposures to extremely acidic conditions. Enumeration studies, combined with measurements of dissolved organic carbon, indicated sufficient available carbon was generated by anaerobic degradation of cellulose to support a viable population of SRB. The bacterial systems showed signs of recovery within 2 to 3 weeks following acidification to pH 3.0. The reestablishment of active microbial systems was indicated by the formation of a black iron sulphide precipitate.

Additional Key Words: acid mine drainage, sulphate reducing bacteria, cellulolytic bacteria, peat, wood waste.

Introduction

Acid mine drainage (AMD), associated with the weathering of sulfide-rich tailings and waste rock, is the single greatest environmental problem facing the Canadian mining industry. The acidic waters, containing high levels of potentially toxic trace elements, pose a substantial threat to aquatic ecosystems located within the immediate vicinity of sulphur-rich coal, base metal (Cu, Ni, Pb, Zn) and precious metal (Ag, Au) mining activities.

One of the most promising areas of AMD amelioration research is the study of organic-biological-inorganic interactions. In laboratory studies, Tuttle et al. (1969a) demonstrated that significant decreases in the concentrations of sulfate and iron in AMD could be achieved by treatment with wood dust inoculated with both cellulose-degrading bacteria and SRB. These results were visually manifested by the formation of a black iron sulfide precipitate through the reduction of sulfate to sulfide. In addition, during a 10-day culture period the pH increased from 3.6 to 7.0. The ability of the SRB to significantly reduce concentrations of sulfate and subsequently form complexes with iron and other heavy metals depends upon the availability of a soluble organic carbon source, and a suitable pH-Eh environment. According to Postgate (1984), the activity of SRB is inhibited at a pH<4.5 and at redox potentials exceeding -100 mV (SHE).

Field examination of an acid leachate stream seeping through a wood dust dam (Tuttle et al., 1969b) showed significant increases both in pH, from 2.84 to 3.38, and in the activity of heterotrophic bacteria. These increases were associated with significant decreases in both sulfate, from 8.765 to 6.1 µmol/mL, and iron, from 1.067 to 0.313 µmol/mL, in water downstream from the dam relative to that in the upstream pond. These changes were attributed to an active population of heterotrophic microflora, including dissimilatory SRB, occurring within and below the sawdust dam. Furthermore, with sawdust as the sole nutrient source, Tuttle (1969b) noted that mixed cultures containing cellulose-degrading bacteria and SRB were capable of reducing sulfate at pH 3.0, whereas pure cultures of SRB did not reduce sulfate below pH 5.5. Such studies demonstrate the potential benefits of biotreatment and suggest that indigenous microflora could be utilized when developing biologically mediated amelioration techniques.

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In northern Canada, where metal mining is prevalent, the success of biological approaches may largely depend on the ability of the microorganisms to remain metabolically active under near-freezing conditions. Organic deposits native to the northern regions, such as peat, may provide a potential source of indigenous microflora capable of degrading cellulosic material, reducing sulfate to sulfide, and also surviving unfavorable thermal conditions. Locally-available wood products, such as chips and process waste, may provide a suitable appropriate carbon source capable of supporting a viable microbial culture.

Peat is composed of organic materials such as fulvic acids, humic acids, cellulose, hemicellulose, lignin, and bitumen as well as mineral material, water, and dissolved gases such as carbon dioxide, oxygen, and methane. In addition to peat providing a consortium of microorganisms, the reactive organic component is capable of adsorbing and complexing cationic forms of metals (Stevenson 1982; Henrot and Weider 1990) and reducing anionic forms of certain metals to cationic forms (Szalay and Szilagyi 1967, Goodman and Cheshire 1982) which may then be adsorbed. Furthermore, Gambrell et al. (1977) found that the stability of the metal complexes, especially of copper, lead, and cadmium, decreased as reduced materials were subjected to oxidizing conditions. Consequently, anaerobic conditions are required to fully utilize the adsorption capacity of peat materials as an important sink for metals. This adsorption capacity for metals may be especially critical during periods of low pH and relatively low bacterial activity encountered during spring thaw.

This paper presents results from a series of experiments conducted to examine the following: the ability of peat material to provide a suitable consortium of microflora; the ability of indigenous cellulolytic microflora to degrade black spruce (*Picea mariana*) and trembling aspen (*Populus tremuloides*) wood chips under optimum ionic and ambient thermal conditions and, subsequently, provide an adequate supply of available carbon to support a viable population of SRB; and the ability of the microflora to withstand chronic and acute exposures to acidity levels characteristic of AMD.

Materials and Methods

Spruce and poplar wood chips were shredded and sieved to less than 4-mm. The peat, collected from a minerotrophic organic deposit, is characterized by a pH of 5.66 and a high base saturation (10,000 to 15,000 ppm Ca, 1,500-6,500 ppm Mg).

The degradation experiments were conducted at 25 ± 2 °C. For each experiment 20 g each of wood chips and peat were placed in nine 500-mL Erlenmeyer flasks with 200 mL of a balanced salt solution (pH 7) and stoppered with aluminum foil. The balanced salt solution consisted of 1.0 g $(NH_4)_2SO_4$, 1.0 g K_2HPO_4, 0.2 g $MgSO_4$, 0.1 g $CaCl_2$, and 10.0 g $FeCl_3$ made up to 1L with deionized water adjusted to pH 7.0 with 5M H_2SO_4, and then sterilized at 121°C for 15 min.

The pH and Eh of the supernatant were recorded weekly. The redox potentials presented in this paper were standardized to the hydrogen scale. Five-milliliter solution samples were pipetted, filtered through Whatman No. 44 filter paper, and analyzed colorimetrically for dissolved organic carbon. Following each extraction, 5 mL of the balanced salt solution were added to maintain the initial volume.

The first stage of each experiment was allowed to proceed for 11 weeks to allow development of viable microfloral populations. The degradation of a piece of filter paper (Whatman No. 44) placed in each flask provided visible evidence of an active population of cellulose-degrading microflora. The second stage involved exposing the mixed bacterial cultures to acid, followed by an evaluation of their ability to survive and rebound to the activity levels that would be required to ameliorate AMD. Of the nine replicates, three were given an acute exposure to acid, and three were given a chronic exposure; the remaining three were not exposed to acid.

Acute exposure consisted of acidification by the addition of 5M H_2SO_4 until the system stabilized at pH 3.0 twice within a 4-day period. This treatment was considered a worst-case scenario, with recovery of the system demonstrating

the ability of indigenous mixed cultures to survive harsh conditions not normally associated with laboratory cultures. However, for both wood species, difficulty was experienced during the attempt to acidify the systems to pH 3. Although the samples were vigorously mixed during the acidification process, the pH rebounded to values exceeding 3.0 within 3 to 7 days suggesting that some pockets of alkalinity may have survived the mixing process. Chronic exposure involved the lowering of the pH using 5M H_2SO_4 by one pH unit per week until pH 3 was reached. Following acidification, the same monitoring procedures were used with no additional nutrient solution being added to the flask after the solution phase was sampled.

For microbiological analyses, the bacteria were enumerated 1 day prior to the initial acidification at week 11 and 12 for poplar and spruce, respectively, and 1 and 3 weeks after acidification. Flasks were agitated weekly and aliquots of the fluid phase were removed from within the peat-wood chip material. A modified Hungate technique (Hungate, 1969) was used to enumerate the cellulolytic bacteria, whereas the SRB were enumerated with the standard five-tube most probable number (MPN) method (Mara and Williams, 1970). All cultures were incubated at 25° C. An additional control, consisting of peat and the balanced salt solution, was set up to determine whether a viable population of SRB could be established with peat as the sole carbon source.

Results and Discussion

The effects of anaerobic degradation of spruce and poplar wood chips are shown on figures 1 and 2. The initial pH of the solution produced from poplar wood chips (pH 6.0) is typically higher than that for spruce (pH 5.0), and represents the equilibrium pH for the simple hydrolysis of different wood species. Physicochemical data obtained from the solution phase are presented below and discussed by wood species.

Prior to Acidification

Immediately prior to acidification both spruce and poplar showed a final solution pH of approximately 7.0 (fig. 1 and 2). A similar trend was noted for the peat control, which also had an initial equilibrium pH of less than 6.0. In both the treatments and the peat control, the rise in pH was accompanied by a significant decrease in redox potential, from an initial value of ~+300 mV to -150 mV. Even after several weeks of purging with N_2, the Eh remained relatively constant. Such data suggest that active microbial cultures exist that are capable of altering their environment to establish favourable Eh/pH conditions.

The existence of active microbial populations is further supported by the presence of significant concentrations of relatively low molecular weight organic compounds in solution (DOC). In the case of spruce, the DOC content, which was ~300 µg/mL C after 1 week, levelled off at ~400 µg/mL C after 3 weeks. The poplar DOC level, on the other hand, remained relatively constant at 200 µg/mL C throughout the preacidification period. The observed trends may be the result of predominantly hydrolysis reactions and not due to bacterial degradation of cellulose.

Period Following Acidification

Distinctly different trends in pH, Eh, and DOC are evident for the treatments following acidification (fig. 1 and 2). For spruce, the unacidified samples showed no significant deviations from the apparent equilibrium values for all measured parameters. In contrast, acidification resulted in immediate decreases in DOC, with the rate of change being proportional to the rate of acidification. Once pH 3 conditions were established, the DOC content of the solution remained relatively constant at 200 µg/mL C for both treatments. The redox potentials for acidified samples increased significantly, from -100 to +100 mV, possibly due to the strong oxidizing effect of sulphuric acid at low redox potentials and, to a lesser degree, the decrease in bacterial activity. However, by the 19th week both acid treatments showed signs of recovery towards the earlier equilibrium conditions. Redox potentials decreased with a concomitant slow increase in pH. These results indicate that the spruce systems appear to recover, with the rate of recovery being independent of the method of acidification.

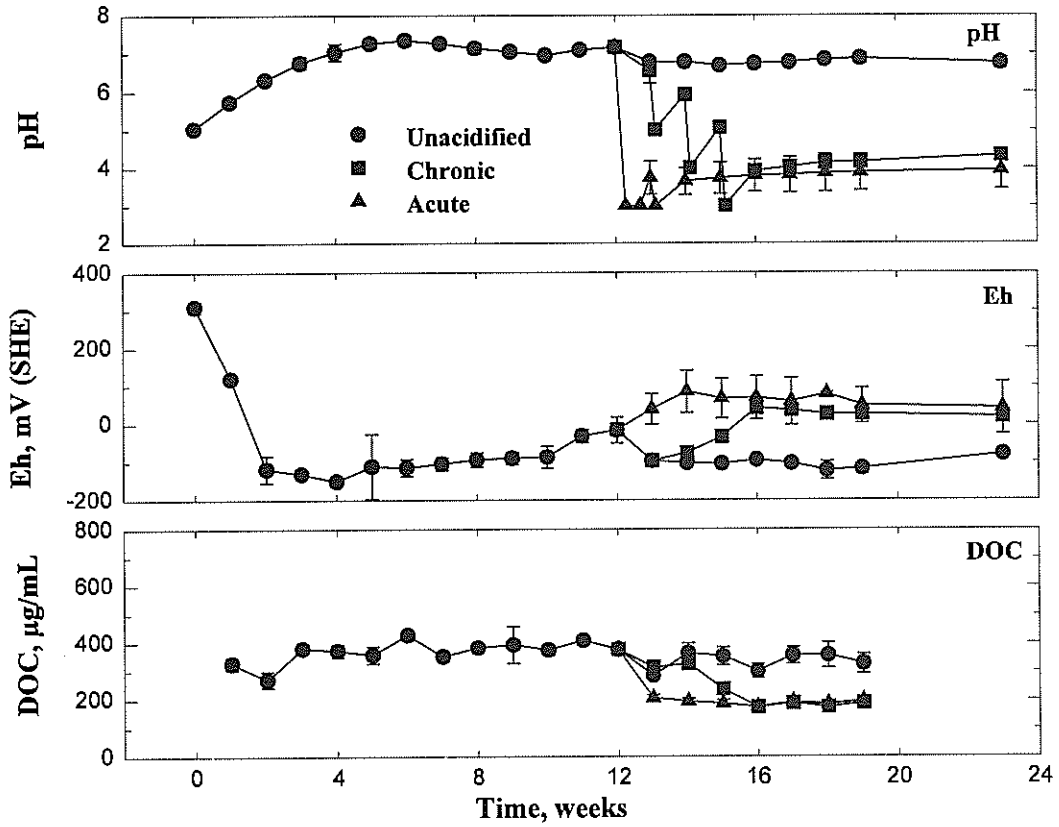

Figure 1. Temporal changes in pH, Eh, and DOC for the solution phase of spruce wood chips under anaerobic decomposition with various acidification treatments.

In contrast to spruce, the pH of the poplar unacidified samples decreased from 7.0 to stabilize at approximately 6.0. The redox potentials remained relatively constant at ~-100 mV. Following week 11, DOC increased linearly, with a twofold to threefold increase by week 17. On acidification, the redox potentials in both the acute and chronic systems increased initially, from -100 to approximately +150 mV. Again, these changes are a result of the strong oxidizing effect of H_2SO_4. The redox potentials slowly decreased to approximately +50 mV (acute) and 0 mV (chronic) by week 22. This decrease is attributed to the activity of the bacteria. The DOC content for the acute treatment remained relatively constant, whereas that for the chronic treatment initially increased and then declined to the pre-acidification level of ~200 ug/mL by week 17.

Thus, although differences apparently exist in the individual microcosm dynamics, the apparent pH and Eh trends for the acute and chronic acidification treatments of spruce and poplar suggest that both systems are capable of recovering from exposures to high levels of acidity.

Bacterial Studies

Cellulolytic Bacteria. The existence of active cellulolytic bacterial populations is indicated by complete breakdown of filter paper in all flasks by Week 22. Although the numbers are relatively low, the initial enumeration results show that cellulolytic bacteria appear to have been more successful at colonizing poplar than spruce (table 1). Lower DOC values were observed for poplar, at 200 µg/mL C, than for spruce, at 350 µg/mL C. Since dissolved organic carbon measurements represent the residual net soluble carbon resulting from both cellulose degradation and microbial

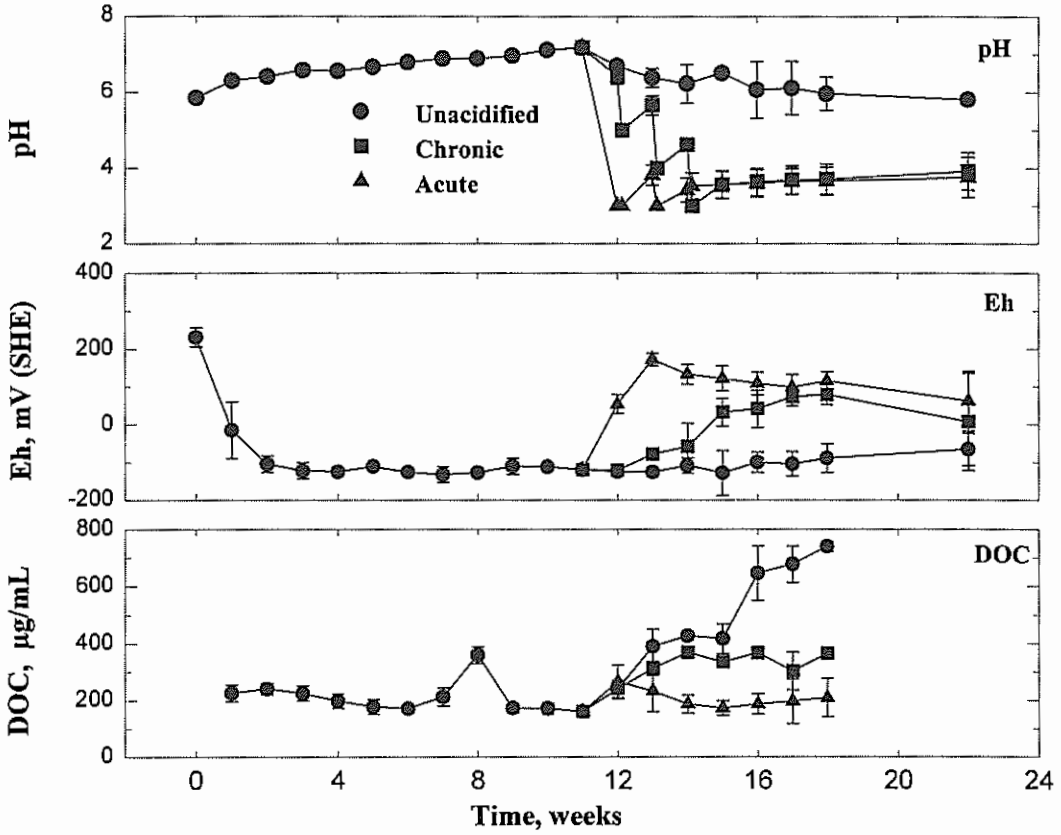

Figure 2. Temporal changes in pH, Eh, and DOC for the solution phase of poplar wood chips under anaerobic decomposition with various acidification treatments.

Table 1. Plate counts in colony forming units per mL for cellulolytic bacteria

Wood species	Treatment	Enumeration date		
		1 day prior to acidification	Time following acidification	
			Week 1	Week 3
Spruce	Acute	10	7,800	1,300
	Chronic	120	1,650	900
	Unacidified	130	250	600
Poplar	Acute	1,200	3,300	160
	Chronic	2,550	5,000	15
	Unacidified	1,500	>31,000	700

metabolism, the DOC results support the preference for poplar. The apparent preference of the cellulolytic bacteria for poplar may be due to the lower lignin content relative to spruce.

Following acidification, the populations of cellulolytic bacteria for both spruce and poplar showed significant increases. Although solution samples were collected from within the wood chip-peat layer, the enumeration results are most likely representative of planktonic and not sessile forms of cellulolytic bacteria. Consequently, the higher counts likely resulted from the liberation of sessile forms, including spores, from cellulosic material on the addition of sulphuric acid. The general decrease in bacterial counts from week 1 to week 3 is due to a return to a predominantly sessile population.

Sulfate-Reducing Bacteria. The changes in the SRB populations for spruce and poplar are shown in table 2. Prior to acidification, the populations in the solution of all flasks with spruce were very low, 79 to 920 MPN/mL. The results are

Table 2. Most Probable Number per mL for sulfate reducing bacteria

Wood species	Treatment	Enumeration date		
		1 day prior to acidification	Time following acidification	
			Week 1	Week 3
Spruce	Acute	79	13000	1300
	Chronic	49	280	1700
	Unacidified	920	33	33
Poplar	Acute	11000	35000	1300
	Chronic	28000	160000	12000
	Unacidified	3300	23	180

similar to those for cellulolytic bacteria, in that significantly higher bacterial counts were obtained for the acute treatment, at 13,000 MPN/mL, immediately following acidification. Again, the higher counts may have resulted from the liberation of sessile forms from both suspended and accumulated precipitate material on the addition of sulphuric acid. After 3 weeks the bacterial counts for both treatments, acute (3,300 MPN/mL) and chronic (1,700 MPN/mL), suggest that mixed SRB populations are capable of surviving extremely acidic conditions. The unacidified spruce and poplar samples showed a decrease in the population of planktonic SRB during the same period. Sessile SRB were not enumerated because this would have disturbed pH-Eh microenvironments, and thus deleteriously affected the ability of the SRB's to withstand these acid conditions (Schindler and Turner, 1982).

The poplar treatments showed substantially higher counts (acute, 11,000 MPN/mL and chronic, 28,000 MPN/mL) than spruce prior to acidification, even though the pH and Eh data of the two systems were similar at approximately 7.0 and -100 mV. These data indicate that poplar may be a preferred cellulose substrate. The flasks were examined visually throughout the experiments. The formation of a black amorphous FeS precipitate was used as an indicator of an active SRB population (Postgate, 1984). All unacidified replicates for both spruce and poplar blackened to a point where the solution phase was opaque. For the poplar, one of three replicates for each treatment turned black. The remaining replicates, for both poplar and spruce, showed visual evidence suggesting active SRB populations. Furthermore, these observations suggest that anaerobic degradation of wood chips can provide sufficient available carbon to support an active microbial population capable of ameliorating AMD.

Since the concentration of iron in the balanced salt solution exceeded 34,000 mg/L, sulfide concentrations are assumed to have remained well below levels considered toxic to SRB. Consequently, sulphide toxicity is not considered responsible for the changes in the population size of SRB with time for the different treatments.

Conclusion

The results of this study clearly show that peat is an excellent source of indigenous anaerobic microflora capable of generating significant levels of dissolved organic carbon through biodegradation of wood chip cellulose. The DOC levels are sufficient to support a thriving population of SRB to reduce sulfate to sulfide, with the subsequent formation of relatively insoluble iron sulfide precipitates. The microfloral populations have demonstrated an ability to survive the very high levels of acidity characteristic of acid mine drainage.

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