

MICROBIAL SULFATE REDUCTION FOR THE TREATMENT OF ACID MINE DRAINAGE: A LABORATORY STUDY

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ABSTRACT

A laboratory study was conducted to determine if sulfate-reducing ecosystems can effectively treat acid mine drainage in the presence and absence of calcareous substrates. Four anaerobic mesocosms (20 Q) were created in the laboratory by inundating carboys containing spent mushroom compost and allowing native species of fermentative organisms and sulfate-reducing bacteria to become established. The mushroom compost in two carboys was underlain by calcareous gravel; the other two carboys contained a noncalcareous gravel substrate. Simulated acid mine drainage was passed through each carboy and samples of the inflow and outflow were analyzed to determine pH, acidity, alkalinity, and concentrations of iron, manganese, sulfate, and calcium. At influent pH 4-5, about 90 pct of the iron and 80 pct of the manganese were removed with a single pass through the mesocosms. At influent pH 2.5-3.5, about 80 pct of the iron and 50 pct of the manganese were removed. About 55 pct of the sulfate was removed irrespective of influent pH. All effluents from the mesocosms exhibited a circumneutral pH and had slight net alkalinity. The presence or absence of a calcareous substrate did not significantly affect the pH and alkalinity of the effluents or the quantity of iron and sulfate removed. However, mesocosms with calcareous substrates were more effective in removing manganese. Results of this study indicate that existing anaerobic organisms will remain active in the presence of acid mine drainage and significantly improve water quality.

INTRODUCTION

Drainage from high-sulfur coal mines in the Appalachian Coal Basin is commonly acidic and contains elevated concentrations of sulfate, iron, aluminum, and manganese. Prior to release into receiving streams, contaminated mine effluents must be neutralized and the concentration of iron and manganese reduced to meet statutory limits. This treatment is usually accomplished with aeration and chemical neutralization at great expense to the mine

operator. Recently biological treatment has become popular because it offers long-term, low-maintenance treatment at low-cost.

In 1979, a group of Japanese researchers (6) found that acid mine drainage (AMD) could be successfully treated using dissimilatory sulfate-reducing bacteria. However, their laboratory results were based on nutrient addition to wood dust in an anaerobic reactor and were never applied to the field. In 1988, the Bureau of Mines found that microbial sulfate reduction was occurring in the thick organic substrates in some wetlands constructed to treat AMD (2). In fact, sulfate reduction was found to be an important process contributing to the successful treatment of AMD in these wetlands. When inundated with AMD, spent mushroom compost, a byproduct of mushroom cultivation consisting of composted manures, straw, corncocks, and an occasional horseshoe or syringe, was found to support the growth of sulfate-reducing and fermentative bacteria. However, all constructed wetlands found to be actively reducing sulfate had limestone gravel underlying mushroom compost (1). This made it impossible to determine if the circumneutral pH in the wetland substrate only a few centimeters below pH 2.5 surface waters was due to: (1) alkalinity naturally in the compost, (2) alkalinity generated by sulfate reduction, or (3) alkalinity resulting from carbonate dissolution. This paper contains a brief introduction to dissimilatory sulfate-reducing bacteria and results of laboratory AMD treatment using sulfate-reducing bacteria grown on mushroom compost.

Dissimilatory Sulfate Reduction

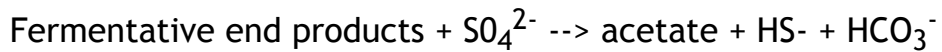
Dissimilatory sulfate-reducing bacteria reduce inorganic sulfate or other oxidized sulfur forms to sulfide. This sulfide is not incorporated into the organism but is released as "free" H_2S . Desulfovibrio and Desulfotomaculum are the two best known genera of dissimilatory sulfate-reducing bacteria. Others include Desulfobulbus, Desulfococcus, Desulfosarcina, Desulfobacter, and Desulfonema, although the latter three genera are restricted to marine environments (4). Dissimilatory sulfate-reducing bacteria are strict anaerobes that are severely inhibited by even small amounts of oxygen. They will, however, survive long periods of oxygen exposure and become active when anaerobic conditions are restored.

Dissimilatory sulfate-reducing bacteria are heterotrophs and therefore require an organic carbon source. In the case of Desulfovibrio spp., this carbon source can be supplied by simple organic molecules such as lactate, pyruvate, and malate. These are subsequently oxidized to acetate and CO_2 with the concurrent reduction of sulfate to sulfide (2, p. 263). Like Desulfovibrio spp., Desulfotomaculum Up. prefer to oxidize lactate and pyruvate to acetate and CO_2 , although one species, Desulfotomaculum ruminis, can also oxidize formate to CO_2 . Several species, including Desulfovibrio baarsii, Desulfococcus multivorans, and Desulfotomaculum acetoxidans are capable of oxidizing acetate (4, pp. 12-13) to CO_2 with the concurrent reduction of oxidized sulfur species. Because sulfate-reducing bacteria can oxidize simple organic compounds and will only oxidize carbohydrates under rare circumstances (Z, pp. 263-264), they generally rely on fermentative bacteria and fungi to break complex organic compounds into simple molecules prior to utilization.

In the natural environment, dissimilatory sulfate-reducing bacteria can be found growing at temperatures between 0 and 70 $^{\circ}C$. Desulfovibrio spp. grow between 0 and 44 $^{\circ}C$ with optimum growth occurring between 25 and 30 $^{\circ}C$. Desulfotomaculum Up. prefer higher 0

temperatures (30-70 °C) with optimum growth occurring between 35 and 55 °C (Z, p. 264). Sulfate reduction is thought to cease when the pH drops below 4 (5). This is supported by the observation that mixed cultures of sulfate-reducing bacteria and heterotrophic bacteria can become established in solutions with an initial pH as low as 2.7 (6), but sulfate reduction does not commence until the pH rises above 4.

The overall sulfate reduction process can be represented by the following generalized equation:



The end products of biomass fermentation and sulfate are converted by this reaction to acetate, bisulfide or hydrogen sulfide, and bicarbonate. Application of this process to acidic mine effluents may improve water quality in five respects:

1. The process consumes sulfate which, although unregulated at present, is undesirable in high concentrations,
2. sulfide generated either in the form of bisulfide or hydrogen sulfide will react quickly with many metals to form insoluble precipitates and decrease dissolved metal concentrations,
3. alkalinity generated in the form of bicarbonate and acetate will help neutralize acidity and raise pH,
4. aluminum will precipitate as a hydroxide in response to the pH increase, and
5. manganese may precipitate as a carbonate in the presence of high pCO_2 .

In fresh waters, sulfate reduction is commonly limited by the availability of sulfate. However, because high sulfate levels are characteristic of AMD, sulfate reduction in the presence of AMD would not be sulfate limited and would likely occur at a faster rate than in fresh waters.

An organic-rich, anaerobic environment must be engineered and an active population of sulfate-reducing bacteria and fermentative organisms established before the treatment of mine effluents can begin. These engineered environments can take the form of tanks such as the anaerobic digesters used for the treatment of municipal wastewaters or the constructed wetlands with thick organic substrates used for AMD treatment. It is desirable that AMD be introduced with minimal dissolved oxygen because sulfate-reducing bacteria are inactive in the presence of oxygen. Aerated mine waters will require systems of higher capacity or longer flowpaths to allow aerobic organisms time to remove dissolved oxygen.

In order to evaluate sulfate reduction as a possible AMD treatment method, experiments were carried out in the laboratory where reactions could be carefully monitored. Specific objectives of this research were:

1. To determine if fermentative and sulfate-reducing bacteria indigenous to mushroom compost remain active in the presence of AMD,
2. To determine if water quality is improved by passage through a sulfate-reducing ecosystem, and
3. To determine if a calcareous substrate is beneficial to the performance of an AMD treatment system using sulfate reduction.

EXPERIMENTAL

Four mesocosms were constructed from 21.5 X 31 X 34.5 cm plastic carboys (20 L capacity). Each carboy was filled with 4 kg (6 cm) of either calcareous or noncalcareous gravel (minus 5 cm) overlain with 8 kg (25 cm) of spent mushroom compost. Initially, all mesocosms were leached with deoxygenated, deionized water until the effluents contained less than 5 mg/L sulfate and 1 mg/L iron. Twenty 50-L batches of simulated AMD were made by dissolving reagent-grade ferrous sulfate heptahydrate, manganese sulfate monohydrate and sodium sulfate in 16 megohm deionized water. The pH was adjusted by adding 0.5 M sulfuric acid. Each batch was analyzed to determine pH and concentrations of iron, sulfate, and manganese. The batches were divided into three groups based on composition of the simulated AMD (table 1).

Table 1. Composition of simulated AMD.

Batch nos.	Average pH	Average batch composition (mg/L)		
		iron	sulfate	manganese
1-7	4.8	89	310	-
8-12	4.3	91	328	44
13-20	3.2	100	394	49

An argon atmosphere was maintained over the solution to prevent ferrous iron oxidation. Simulated AMD from each batch was split into four equivalent flows that were introduced into the base of each carboy (fig. 1). Upon entering the carboy, the solution contacted either a calcareous or a noncalcareous substrate and then flowed upward through the mushroom compost. An outlet on the side of the carboy was positioned to maintain a water level about 5 cm above the top of the mushroom compost. Because the system was gravity fed, flow rates through each carboy varied between 90 and 150 mL/hr depending on the hydraulic head. Effluents from the four carboys were collected in 30 L glass bottles. At the end of each batch application, the volume of effluent from each carboy was measured and a sample analyzed for pH, alkalinity, iron, manganese, sulfate, and calcium. Acidity and alkalinity values were converted to CaCO_3 equivalent and reported as net alkalinity. Metal and sulfate loads were calculated for influent and effluent streams. Because the mesocosms were always kept inundated, the effluent for a particular batch contained, in part, effluent from the previous batch which had remained in the mesocosms between batch applications. Because of the carryover of effluents between batches, the response of the system to a change in influent water quality could not be fully evaluated until two batches had flowed through the system.

RESULTS AND DISCUSSION

Upon the introduction of ferrous iron solutions, a pervasive, black, iron-monosulfide precipitate was observed to form throughout the mesocosms and in effluent lines. The distinctive odor of hydrogen sulfide was detected when the mesocosms were disturbed, although no hydrogen sulfide odor was noted under normal operation. The formation of iron sulfides and hydrogen sulfide with the concurrent removal of sulfate was taken as substantive proof that active populations of sulfate-reducing bacteria were established in each mesocosm.

pH and Alkalinity

Although the influent pH ranged from 4.8 to 2.5, the outflow from the mesocosms was consistently circumneutral (6.4 - 7.2), irrespective of the presence or absence of a calcareous substrate (fig. 2). The influent acidity (negative alkalinity), effluent alkalinity, and effluent calcium content were measured for batches 11 through 20. Figure 3 shows the average alkalinity of effluents from two mesocosms with calcareous material and two mesocosms containing no calcareous material. There is no difference in alkalinity between mesocosms with calcareous substrates and those without.

Calcium in the effluents was monitored (fig. 4) to determine if dissolution of calcium carbonate was responsible for the increase in pH and alkalinity. Calcium concentrations in the effluents of both calcareous and noncalcareous mesocosms were about equal, suggesting that the calcareous substrate was not the source of alkalinity. Therefore, the source of alkalinity must be either alkalinity naturally in the compost or alkalinity generated by sulfate reduction.

The neutralization potential (5) of fresh mushroom compost is about 35 tons CaCO_3 equivalents/1000 tons. Therefore, the alkalinity in each mesocosm, neglecting any contribution from sulfate reduction would be 140 g CaCO_3 equivalent. Because less than 100 g CaCO_3 equivalent of acidity were added to each mesocosm during the experiment, the initial alkalinity of the mushroom compost was not exhausted. Therefore, no estimate of alkalinity generation by sulfate-reducing bacteria can be made.

Iron

About 90 pct of the iron load was consistently removed upon passage through the mesocosms when the influent pH ranged between 4 and 5 (fig. 5). When the pH decreased to the 2.5-3.5 range, iron removal decreased to 78 pct and became more erratic. Overall, 86 pct of the iron was removed. The presence or absence of a calcareous substrate had no significant effect on iron removal efficiency.

Manganese

Starting with batch 8 (fig. 6), manganese was added to the simulated AMD. At pH 4.0-4.5, 80 pct of the manganese was removed by the mesocosms with calcareous substrates. For the same pH range, mesocosms with noncalcareous substrates removed only 64 pct of the manganese. At pH 2.5-3.5, manganese removal dropped to 52 pct in all mesocosms. Overall, throughout the course of the experiment, 57 pct of the influent manganese was removed. Manganese removal declined with each successive batch (fig. 6). When the experiment was terminated with batch 20, there was no clear indication whether manganese removal efficiency would continue to decrease or become constant (fig. 6). The overall effect of the calcareous substrate is also unclear. At pH 4.0-4.5, mesocosms with calcareous substrates exhibited higher manganese removal rates whereas at pH 2.5-3.5, calcareous and noncalcareous substrates were equally effective. More detailed work is necessary to determine the ability of anaerobic mesocosms to remove manganese.

Sulfate

Although sulfate removal was variable (fig. 7), the mesocosms averaged about 55 pct removal of influent sulfate. Slightly more sulfate was removed by mesocosms containing calcareous material, although the difference appears insignificant. Sulfate removal percentage was independent of influent pH between pH 2.5 and 5.0. The fact that sulfate removal continued even at low influent pH, suggests that sulfate-reducing bacteria may be active at a pH less than 4. This also suggests that the lower metal removal efficiencies observed at pH 2.5-3.5 may result from the increased solubility of metal sulfides rather than decreased rates of sulfate reduction.

Relationship Between Metal Removal and Sulfate Removal

In figure 8, the millimoles of iron removed upon passage through the mesocosms is plotted versus the millimoles of sulfate removed. These points represent batches 1 through 7, before manganese was added to the simulated AMD and include data from calcareous and noncalcareous mesocosms. The least-squares regression line through these data has a slope of 0.67, and indicates that about 3 millimoles of sulfate are removed for every 2 millimoles of iron removed. Iron sulfide precipitates initially formed in sulfate-reducing systems exhibit a 1:1 stoichiometry of iron to sulfur (1). With time, iron monosulfides react with elemental sulfur to form pyrite, which has a 1:2 stoichiometry of iron to sulfur. There are no known iron sulfides with the 1:1.5 iron to sulfur stoichiometry that is suggested by this plot. The two sulfides closest in stoichiometry are smythite and greigite, both with Fe:S ratios of 1:1.33. Obviously, not all sulfide is precipitated as iron monosulfide, even though iron is present throughout the mesocosms and in the effluents. Sulfide that is formed but not precipitated as FeS may: (1) escape from the mesocosm as H_2S ; (2) be partially oxidized to form elemental sulfur; (3) be assimilated by bacteria or fungi; (4) form a disulfide mineral such as pyrite or; (5) be unable to compete with strong organic complexes for iron. The location of the y-intercept indicates that about 9 millimoles of iron are removed by reactions not related to sulfate removal. These reactions may include iron and substrate interactions such as adsorption, complexation, and ion exchange.

Figure 9 shows the millimoles of manganese removed with respect to the millimoles of sulfate removed. These points represent data from both calcareous and noncalcareous mesocosms for batches 8 through 20. Interpretation of this plot is complicated because the effect of iron on manganese removal is unknown. However, under these conditions one millimole of manganese is removed for every five millimoles of sulfate removed. It is clear that manganese removal is related to sulfate removal, although it is unknown whether the manganese is absorbed onto iron monosulfides or is precipitated as manganese carbonate or manganese sulfide. Sulfide and bicarbonate produced by sulfate reduction would promote the formation of manganese sulfide and manganese carbonate, respectively. Based solely on solubility considerations, the less soluble manganese sulfide ($pK_{sp} = 13.27$) would probably predominate over manganese carbonate ($pK_{sp} = 10.4$). The species kinetically favored is not known at present. The removal of about 3 millimoles of manganese (y-intercept) is unrelated to sulfate reduction.

CONCLUSIONS

Results of this experiment indicate that ecosystems of sulfate-reducing bacteria growing in spent mushroom compost remain active when simulated AMD with a pH as low as 2.5 is

introduced into the system. Because of the natural alkalinity of mushroom compost and the alkalinity generated by sulfate reduction, it is not known if sulfate-reducing bacteria were actually contacted by low-pH waters. Water quality was significantly improved upon passage through the mesocosms. About 78-90 pct of the iron and 64-80 pct of the manganese were removed from simulated AMD at influent pH 2.5-5.0. Iron and manganese removal efficiency decreased at lower pH probably reflecting the increased solubility of metal sulfides. Manganese removal efficiency decreased with time even at a constant influent pH. The system lowered influent sulfate concentrations by 50-60 pct and resulted in an effluent of circumneutral pH. The effluents from these ecosystems were alkaline despite an influent acidity between 220 and 430 mg/L CaCO_3 equivalent. The presence or absence of a calcareous substrate did not make any significant difference in the amount of iron or sulfate removed nor did it affect the pH or alkalinity of the effluents. At influent pH 4-5, manganese removal was higher in mesocosms with calcareous substrates. However, at pH 2.5-3.5, the manganese removal in mesocosms with calcareous substrates did not differ from that of mesocosms with noncalcareous substrates. Results of these experiments indicate that the calcareous substrate conventionally used in constructed wetlands is unnecessary for sulfate reduction unless manganese removal is a major concern.

FUTURE WORK

Future work will concentrate on determining if sulfate reduction will continue in the presence of AMD after the natural alkalinity of mushroom compost is exhausted. The ability of a sulfate-reducing ecosystem to generate alkalinity will determine the long-term feasibility of using a sulfate reduction strategy for treating AMD. Further work is necessary to determine:

1. the optimum system configuration,
2. the minimum pH and maximum metal concentrations tolerated by an active fermentative/sulfate-reducing ecosystem,
3. the organic substrate requirements,
4. the optimum flow rates and residence times, and
5. the metal loading capacity for each metal.

REFERENCES

1. Berner, R. A. Sedimentary Pyrite Formation: An Update. *Geochimica et Cosmochimica Acta*, Vol. 48, 1984, pp. 605-615
2. Ehrlich, H. L. *Geomicrobiology*. Marcel Dekker, Inc., New York, 1981, 393 pp.
3. Hedin, R. S., D. M. Hyman, and R. W. Hammack. Implications of Sulfate-Reduction and Pyrite Formation Processes for Water Quality in a Constructed Wetland: Preliminary Observation. *BuMines IC 9183*, 1988, pp. 382-389.
4. Postgate, J. R. *The Sulphate-Reducing Bacteria*. Cambridge University Press, New York, 2nd ed., 1984, 208 pp.
5. Sobek, A. A., W. A. Schuller, J. R. Freeman, and R. M. Smith. Field and Laboratory Methods Applicable to Overburdens and Minesoils. EPA 600/2-78054, 1978, 203 pp.
6. Wakao, N., T. Takahashi, Y. Sakurai, and H. Shiota. The Treatment of Acid Mine Water using Sulfate-reducing Bacteria. *J. Ferment. Technol.*, Vol. 57, No. 5, 1979, pp. 445-452.

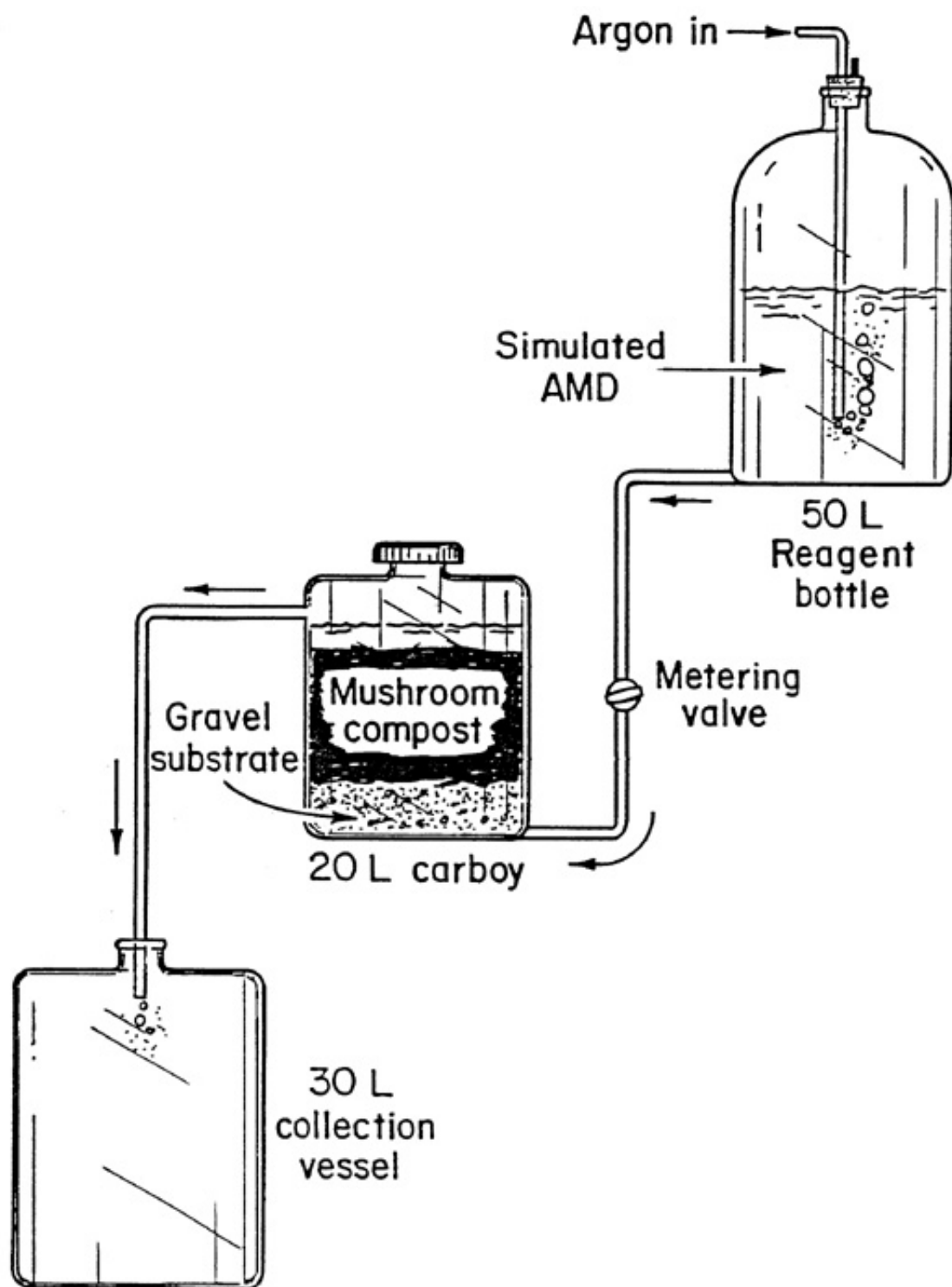


Figure 1.-Sketch of sulfate reduction mesocosm.

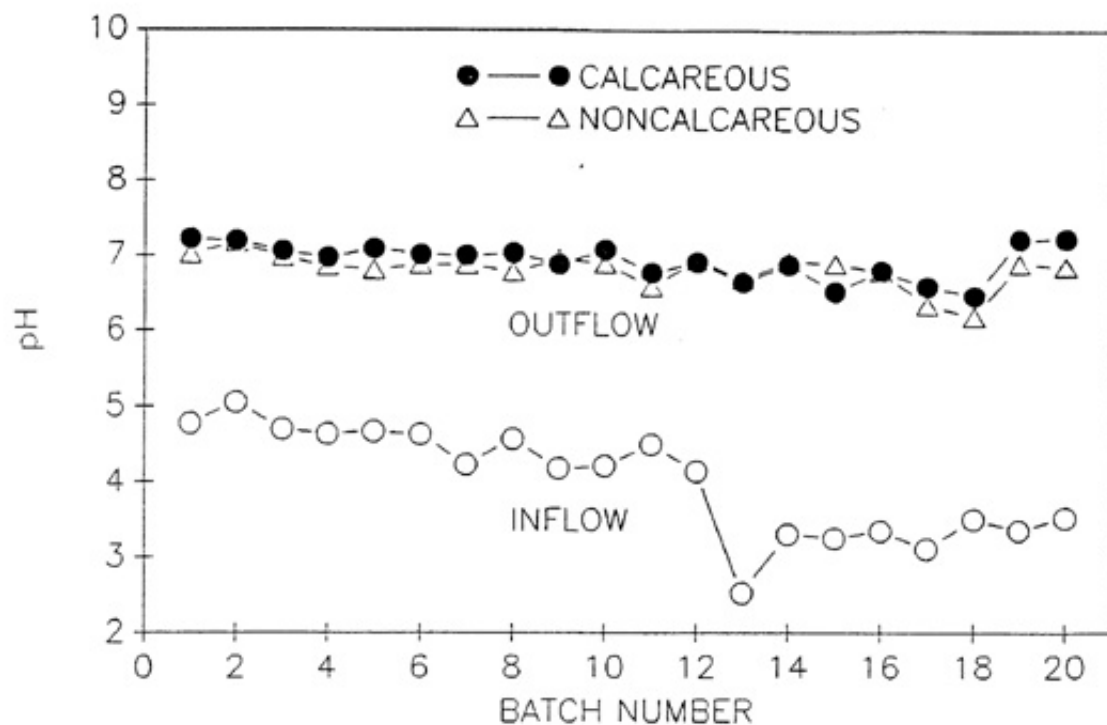


Figure 2.-Plot of influent and effluent pH for anaerobic mesocosms with calcareous or noncalcareous substrates.

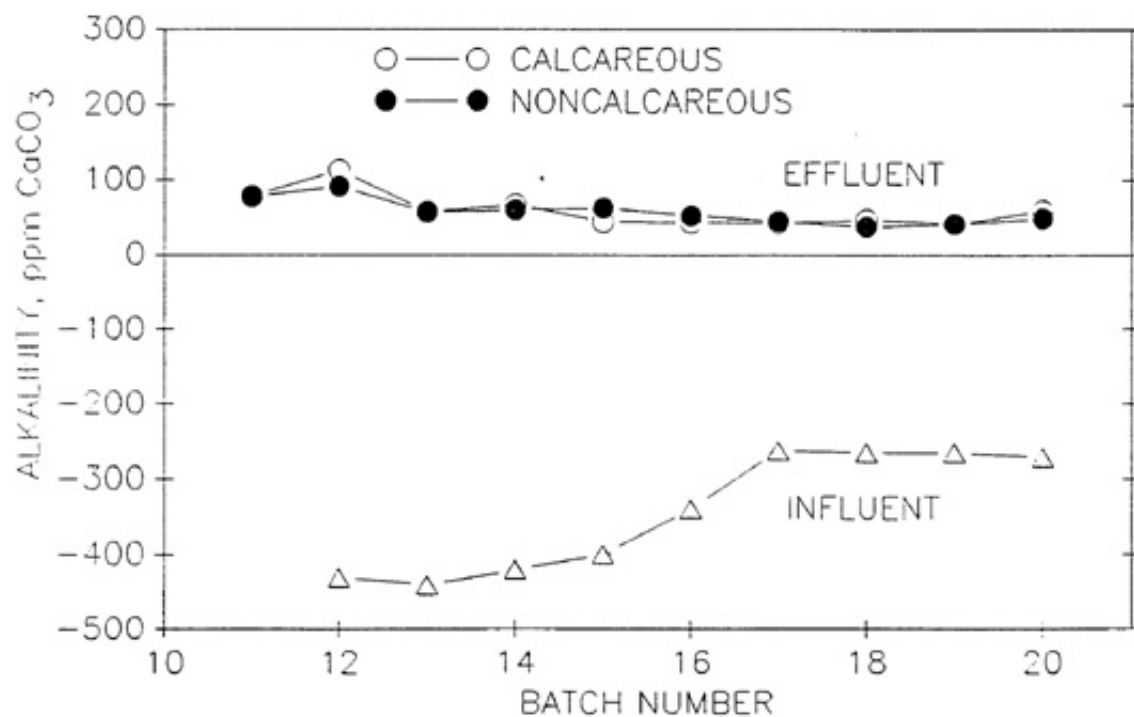


Figure 3.-Plot showing change in alkalinity upon passage through anaerobic mesocosms with calcareous or noncalcareous substrates.

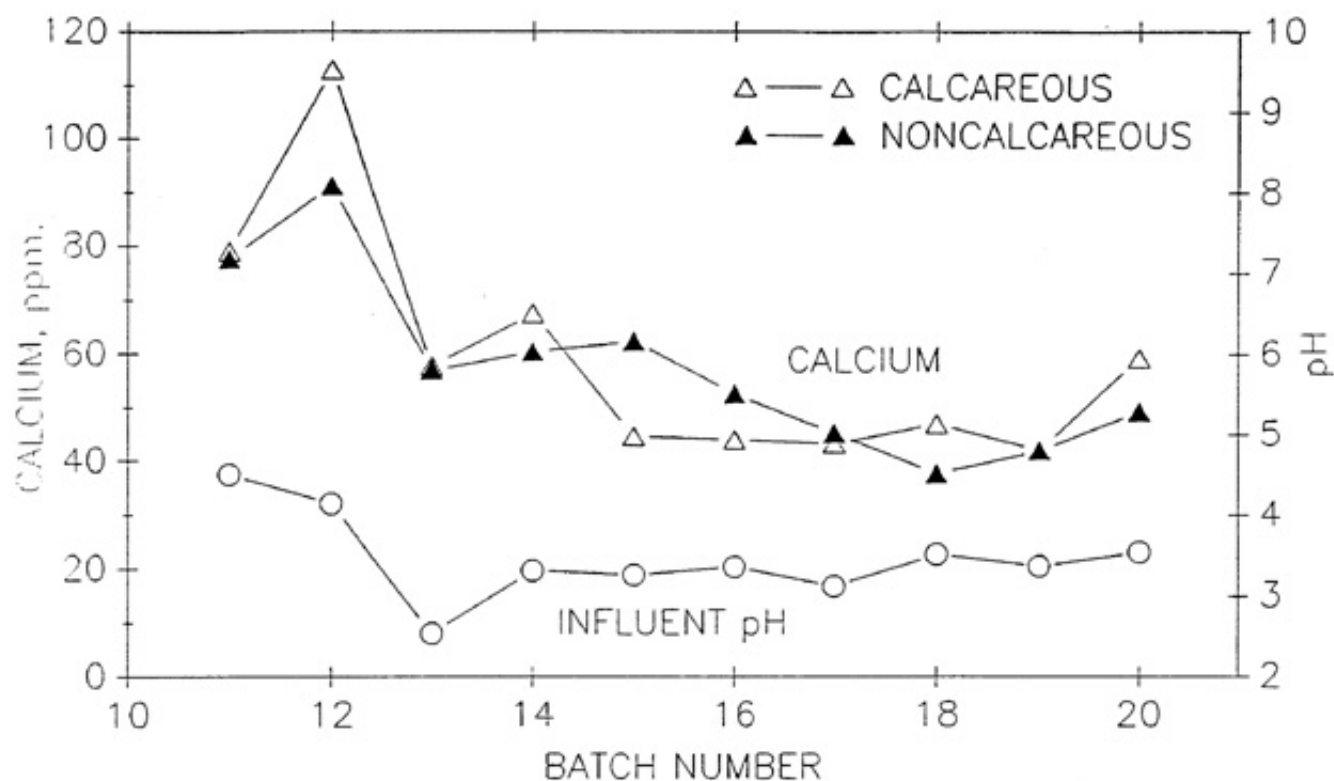


Figure 4.-Calcium concentrations in effluents from anaerobic mesocosms with respect to substrate mineralogy and influent pH.

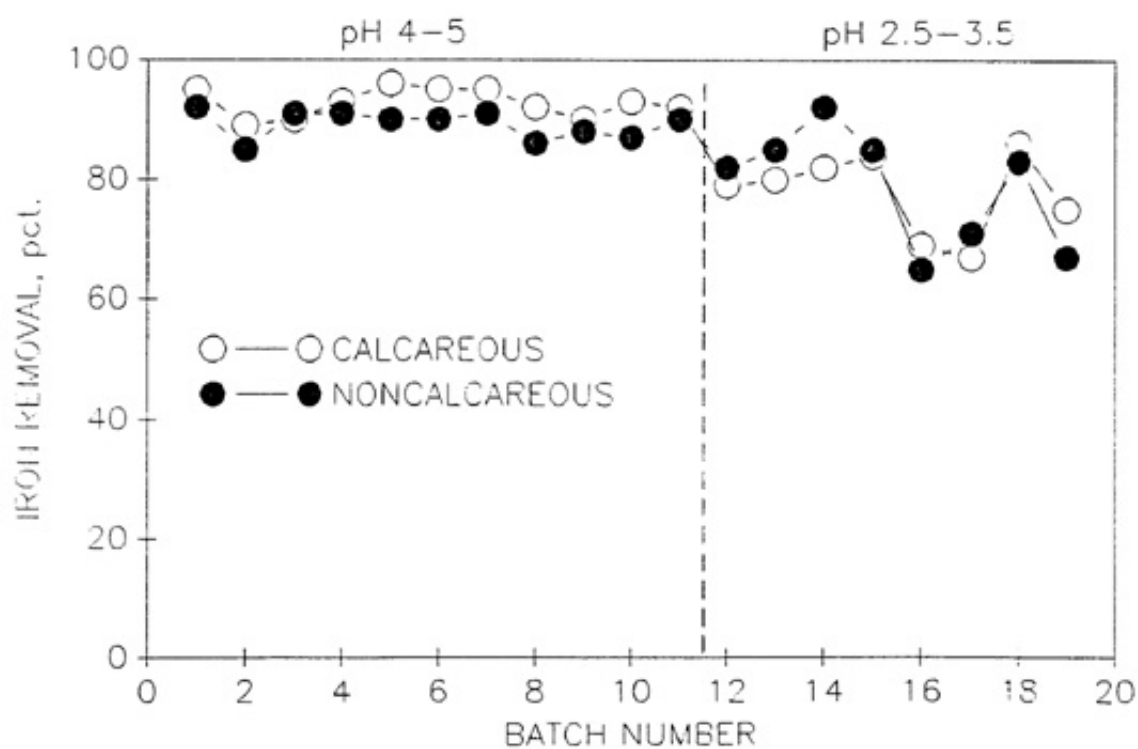


Figure 5.-Iron removal with respect to substrate mineralogy and influent pH.

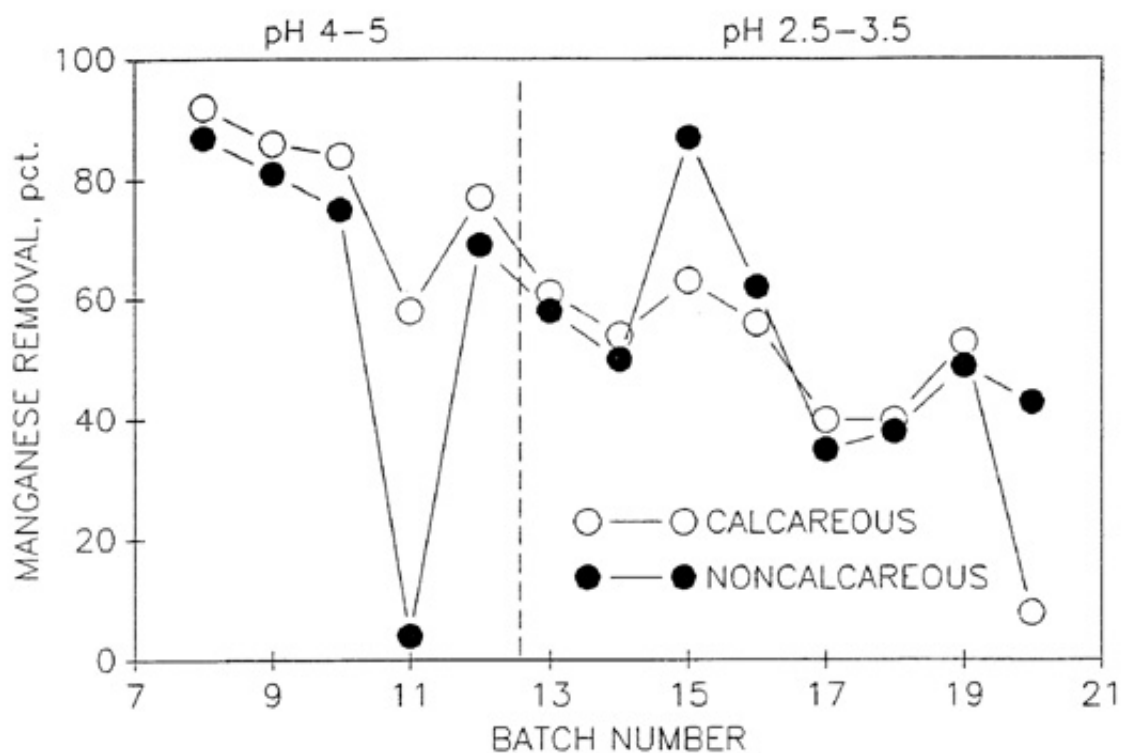


Figure 6.-Manganese removal with respect to substrate mineralogy and influent pH.

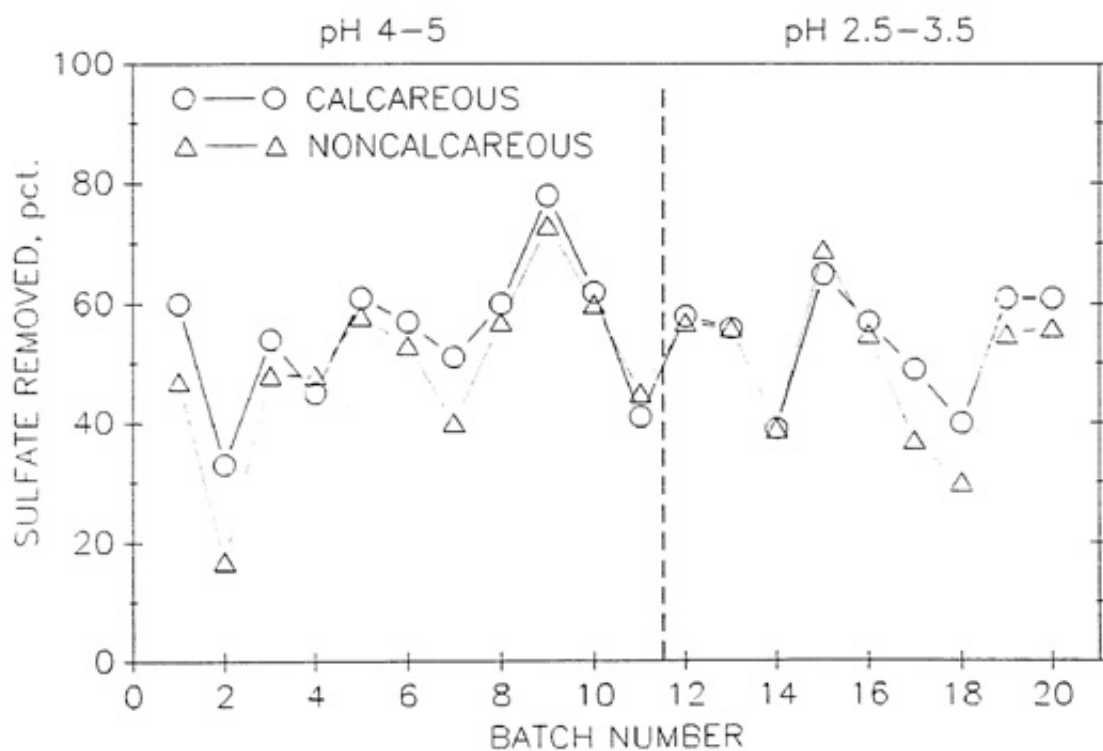


Figure 7.-Sulfate removal with respect to substrate mineralogy and influent pH.

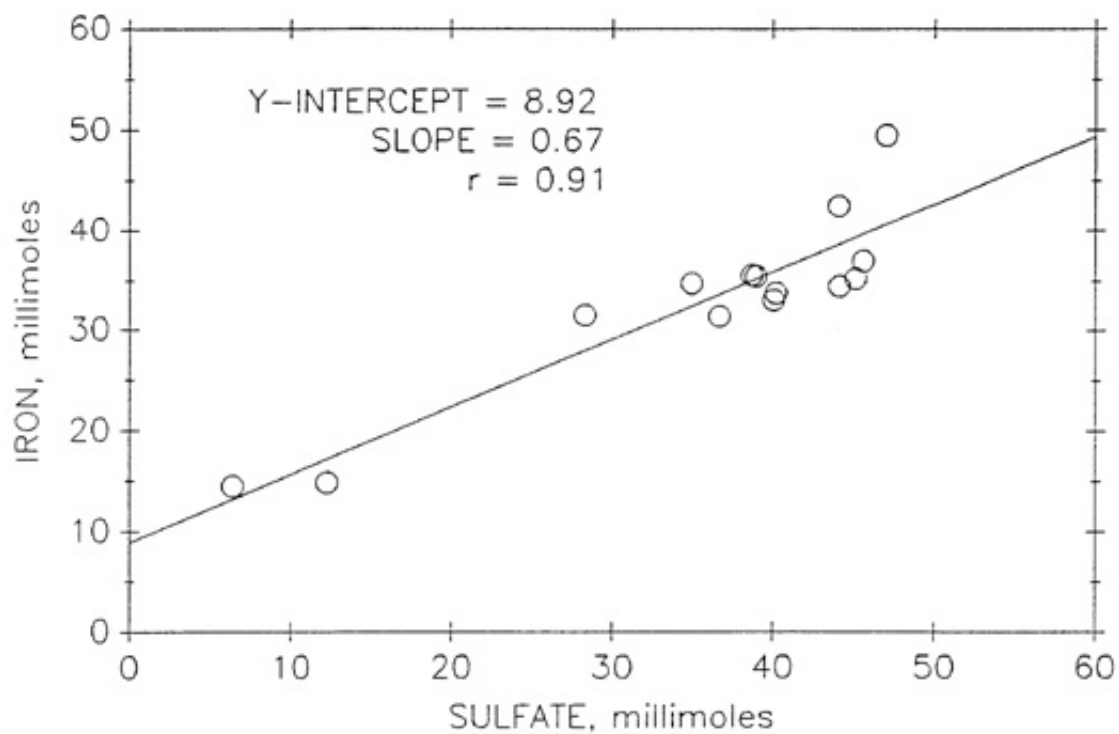


Figure 8.-The relationship between iron and sulfate removal.

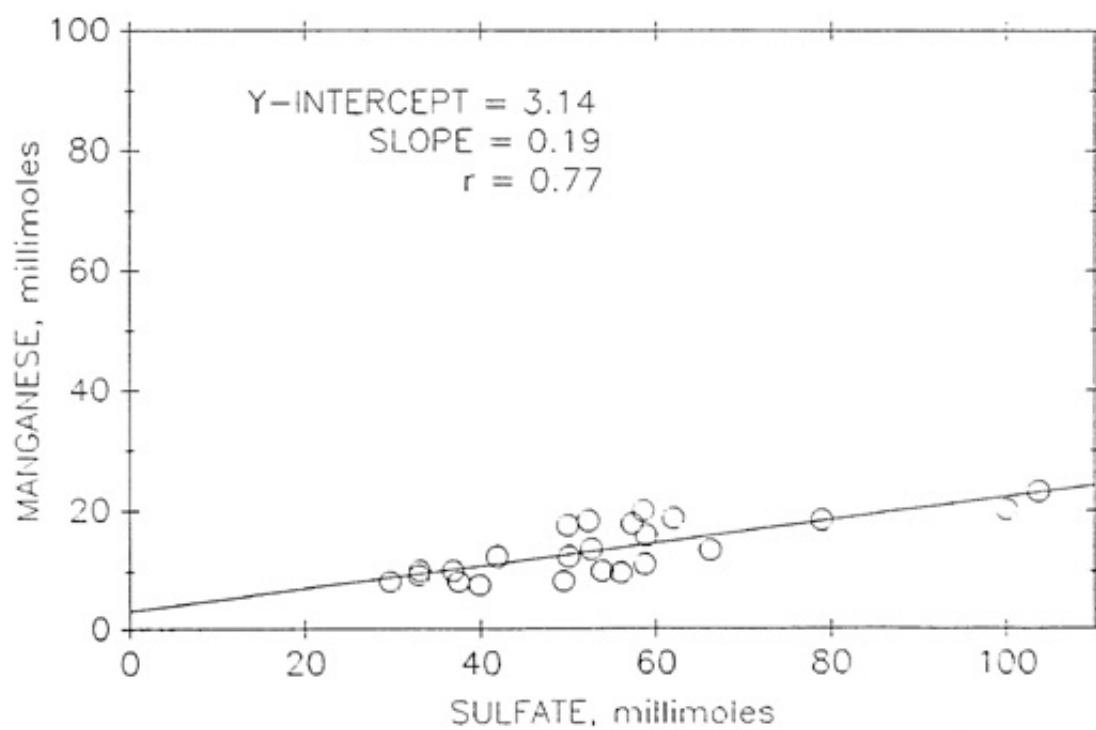


Figure 9.-The relationship between manganese and sulfate removal.