

The Microbial Immobilization of Zinc Sulfate

by

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE
in
Microbiology

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March, 1983
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Acknowledgements

I would like to express my appreciation to my committee members, Dr. R.E. Benoit, Dr. D.S. Cherry, and especially my major advisor, Dr. S.G. Hornor in pursuit of this degree. I thank Dr. J.D. Rimstidt for his geochemical advice and Judy Alls for her technical assistance. I also express my gratitude to Dr. G.W. Claus for his emotional support and encouragement. I finally give my greatest thanks to my brother, Dr. R.H. Yoon and my sister in law, M.S. Yoon. This work was supported by a grant from American Electric Power Company (230-11-110-312-801122-1).

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Introduction

The immobilization of heavy metals has recently drawn attention because of the potential hazardous effects of water soluble heavy metals in the environment. Incorporation of soluble metals into microorganisms and primary producers, such as algae, can result in the upward movement of metals to higher trophic levels, causing physiological effects to aquatic organisms in various levels of a food chain (Jones and Jarvis, 1981). It has been suggested that precipitation of water soluble metal complexes as insoluble metallic sulfides may function as an immobilization process within aquatic systems where metal-rich industrial effluents are introduced (Olson et al, 1979).

Huang et al. (1977) classified heavy metals in the aquatic environment into two categories; 1) in true solution as free or complexed ions, or 2) in particulates adsorbed onto other particles or incorporated into the biomass of living organisms and inorganic precipitates such as hydroxides, carbonates, sulfates, and sulfides. Free and complexed metal ions may be removed from the water column onto sediment surface by adsorption and precipitation mechanisms. Once deposited, the fate of a given metal is determined by biogeochemical factors such as pH, redox

potential (Eh), O_2 , carbonate activity, organic matter and sulfide activity (Khalid et al, 1978). In oxidized sediments with high Eh, coprecipitation of metals with iron and manganese hydroxides is prevalent, while metal complexation with insoluble humic substances and metallic sulfides play important roles in reducing environments (Khalid et al, 1976). Under reduced conditions, metals may become more solubilized due to the reduction of iron and manganese oxy-hydroxides, especially manganese hydroxides in the case of zinc (Suarez and Langmuir, 1976). In the presence of sulfide, however, Zn released from iron and manganese hydroxide precipitates would react with sulfide to form insoluble ZnS.

Bacterial sulfate reduction has been recognized as an important source of metallic sulfide precipitation, resulting in important sulfide ore deposits in some cases (Barnes, 1975). Due to the high degree of stability of SO_4^{2-} , SO_4^{2-} reduction does not occur under the earth's surface temperature and pressure, unless microbiologically mediated (Malinin and Khitarov, 1969).

The objective of this study was to investigate the feasibility of bacterial SO_4^{2-} reduction as an immobilization mechanism of $ZnSO_4$. Considerable research has been performed on metal behaviour and possible

immobilization processes of Zn with iron and manganese hydroxides, organic materials, and sulfides in nature (Hem, 1972; Berner, 1964; Pauli, 1975). No work has been done, however, to stimulate ZnS precipitation by directly enhancing metabolic activity of SO_4^{2-} reducing bacteria in the laboratory (S.G. Hornor and S.O. Yoon, abstr., Comparative rates of microbial community respiration in zinc-amended freshwater microcosms, Ann. Meet. Am. Soc. Limnol. Oceanogr., Raleigh, N. C., 1982). My hypothesis was that enhanced microbial SO_4^{2-} reduction would scavenge ZnSO_4 as an insoluble ZnS under reducing conditions. The experimental approach was to establish sediment-water microcosms in the laboratory to simulate certain characteristics of natural systems.

Sulfate reduction is known to be limited by low availability of degradable organic matter (Jackson, 1978), thus, cellulose was added as an organic carbon source to enhance anaerobic processes and subsequent activation of microbial SO_4^{2-} reduction. Zinc as ZnSO_4 was selected as the model metal for my microcosm experiments because it is an essential element for living organisms, relatively easy to determine, and abundant in coal ash effluent receiving stream where samples were collected for this study. The immobilization of added ZnSO_4 to microcosms was investigated by 1) precipitating the soluble form of ZnSO_4 in the water column, and 2) determining the fate of the precipitated Zn in the sediments.

Materials and Methods

Sample Collection

Sediment and surface water samples were collected from Adair Run, a second order mountain stream in Glen Lyn, southwestern Virginia. Adair Run receives the effluent from a fly ash settling basin of Glen Lyn coal-burning power plant. At the time of this study, the settling basin had not been in operation for more than a year. All glassware was acid-washed according to standard methods (U.S.EPA, 1979). Water and sediment samples were brought to the laboratory on ice and stored at 5°C until they were used for microcosm construction. The organic content of the sediments ranged from 2-3% of dry weight, which was of 75-80% wet weight. Sulfate concentration in water ranged from 11.8 to 53.0 mg L⁻¹ with an average of 30.0 mg L⁻¹, while alkalinity (bicarbonate) and hardness were 108 and 120 mg L⁻¹, respectively. Average Zn and iron concentration were 0.026 and 0.053 mg L⁻¹, respectively (Cherry et al, 1982).

Microcosm Design

Microcosms consisted of 9-L gas-tight plexiglass

chambers which were 40, 15, 15 cm in length, width and height. Each chamber was divided into four identical compartments and was fitted with a gas-tight lid with a port for gas sampling which was closed with a rubber stopper. Each compartment received approximately 400 g of wet sediment and 500 ml of water.

Each of the 4 microcosm compartments was amended with 1% reagent grade cellulose (Baker TLC) g^{-1} dry weight of sediment and 100 mg L^{-1} of Zn as reagent grade ZnSO_4 . Controls consisted of non-cellulose amended microcosms with or without 100 mg L^{-1} Zn. Sulfate was added to each microcosm at a concentration of 200 ppm. All microcosms were incubated at approximately 22°C for 3 weeks.

Gas Analyses

Oxygen, CO_2 , and CH_4 in the headspace were measured weekly by withdrawing a 250 μl sample with a syringe. Analyses were performed with a Varian 920 gas chromatograph equipped with a thermal conductivity detector (TCD). Temperatures for detector, injector and oven were 130, 95 and 40°C , respectively. Helium was used as the carrier gas (30 ml min^{-1}). Gases were separated by two stainless steel columns (183.0 x 0.32 cm) in parallel containing Porapak Q 80/100 and Molecular Sieve 5A 45/60 (Supelco, Inc.),

respectively. Known gas standards were used daily for apparatus calibration.

Sulfide (H_2S) was measured with a Varian 3700 gas chromatograph equipped with a flame photometric detector (FPD) sensitive to ng quantities of S compounds. The detector was fitted with a sulfur filter (365 nm) and operated at 220°C with an H_2 flow rate of 140ml min^{-1} and air flows of 80 and 170ml min^{-1} for the dual flames. Helium carrier gas flow rate was 30ml min^{-1} through a Teflon column (183.0×0.32 cm) packed with chromosorb 105(80/100). Temperatures for column and injector were 140 and 170°C , respectively.

pH and Eh measurement

The pH and redox potential (Eh) in the water column were measured weekly with an Orion 601A Ionalyzer. Eh measurements were performed with a platinum electrode which was calibrated with Zobell standard solution (Zobell, 1946).

Water Chemistry

The following measurements were performed on initial and final samples collected from microcosms:

Sulfate concentration was measured using the BaCl₂ turbidometric method (APHA, 1971); alkalinity was measured using the standard titrametric method (APHA, 1971) and later converted to carbon dioxide dissolved in water (U.S. Geological Survey, 1979). Dissolved oxygen in water was detected using the Winkler method (APHA, 1971) and chemical oxygen demand (COD) in water was measured by standard methods (U.S.E.P.A., 1979). Sulfide dissolved in water was determined by placing a 5 ml water sample into Hungate tubes (Bellco, Vineland, NJ) which were sealed and purged with O₂-free N₂ gas to displace air. Then 1 ml of 6N HCl was added through the septa and tubes were shaken for 2 min. The gas was collected from the head space of the tubes by syringe and sulfide content of the gas was determined by gas chromatography as described above.

Sediment Analyses

Percent dry weight of sediments was determined by drying at 85-95°C for 24 hours and percent organic content was determined by loss of weight on combustion of dried sediment at 500°C for 3 hours. Sulfate was extracted from the sediments with acetic acid according to Pepper and Miller (1978). The supernatant was centrifuged for 25 min and filtered through 0.45 um Gelman membrane filters and analyzed for SO₄²⁻ with the same procedure used for water

samples. Acid volatile sulfide in sediments was released as H_2S by addition of 6N HCl at solid to acid ratio of 1:1 and treated as water samples were.

Enumeration of Sulfate Reducing Bacteria

The number of viable SO_4^{2-} reducing bacteria was enumerated from water and sediment samples using the 3-tube MPN technique and Postgate's medium B (Postgate, 1979). This is a mineral medium enriched with sodium lactate $3.5g L^{-1}$, and yeast extract $1g L^{-1}$, with pH adjusted to 7-7.5 with NaOH.

Metal Extraction Method

Zinc and iron concentrations were measured directly by atomic absorption spectroscopy (Perkin-Elmer 460). Determination of Zn species in sediments was performed by selective chemical leaching techniques according to Figure 1. Zinc content of each fraction was determined by atomic absorption spectroscopy.

Zn Speciation

1) Water soluble fraction: sediment was treated with deionized water at a solid to water ratio of 1:8 and

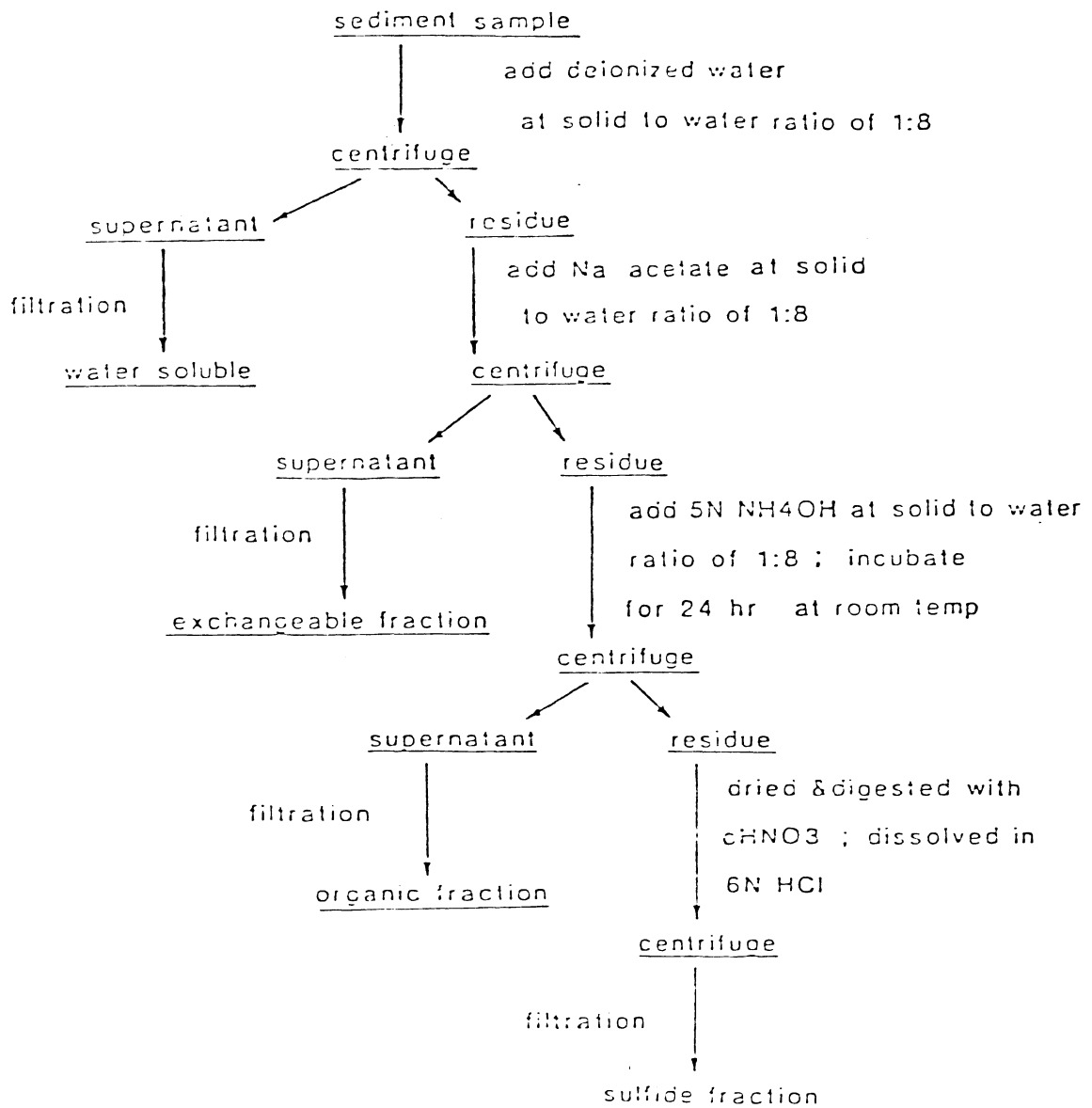


Figure 1. Flow Diagram of Metal Extracting Method. (See Materials and Methods for detailed description.)

centrifuged at 3170 x g for 25 min. The supernatant was filtered through 0.45 um Gelman membrane filter, and preserved with 3 ml of concentrated HNO_3 L^{-1} of sample.

2) Loosely-bound exchangeable fraction: 1N sodium acetate was added at a solid to water ratio of 1:8 to the residual solid from the water soluble fraction. The mixture was centrifuged and filtered as described for the water soluble fraction (Khalid et al, 1976).

3) Tightly-bound organic fraction: the residual solid material from the exchangeable fraction was extracted for 24 hours at 22°C with 5N NH_4OH at a solid to water ratio of 1:8. The supernatant was centrifuged and filtered as described in the water soluble fraction.

4) Sulfide fraction: the residue from organic fraction was dried and finely ground. One gram sediment was then digested with 25 ml of concentrated HNO_3 and extracted in 25 ml of 6N HCl (Holmes et al, 1974). The solution was centrifuged and filtered as described in the water soluble fraction.

5) Total zinc: One gram dry, ground sediment was separately prepared and digested with 25 ml of aqua regia ($\text{cHNO}_3:\text{cHCl} = 1:3$) twice until dryness (Agemian and Chau, 1976). The residue was then extracted in 25 ml of 6N HCl followed by centrifugation and filtration as described above.

The percent recovery of the loosely-bound exchangeable Zn was over 90%, when tested with ZnSO_4 , ZnCl_2 and Zn-carbonate. The percent recovery of tightly-bound organic Zn with Zn-acetate was over 95%, and the percent recovery for reagent grade ZnS was over 80%.

Statistical Analyses

The ANOVA and Duncan's multiple range tests were applied for statistical analyses to test the significant differences between treatments of cellulose and Zn amendments, using SAS (SAS, 1979).

Results

Enhancement of Anaerobiosis by Cellulose Amendment

Cellulose amendment was an effective stimulator of microbial community respiration as can be seen by the results shown in Table 1. Dissolved oxygen in the water column of cellulose amended microcosms was significantly ($p < 0.01$) lower than that of controls. Carbon dioxide production, COD and Eh also showed significant differences between cellulose and non-cellulose treatments ($p < 0.05$, $p < 0.01$, $p < 0.01$ respectively). Oxygen uptake and CO_2 production during incubation showed significant increases at $p < 0.05$ between cellulose and non-cellulose treatments (Figure 2 and 3). Methane production was only observed in cellulose amended microcosms near the end of incubation. RQ values (M CO_2 production/ M O_2 consumption) were > 0.85 in cellulose amended microcosms (Figure 3). Sterile controls in similar experiments did not show increases in O_2 uptake and CO_2 evolution and no CH_4 production was observed. The concentration of the soluble iron showed significant difference ($p < 0.01$) between cellulose and non-cellulose treatments (Table 2). The pH remained a neutral throughout the whole incubation period (Table 3).

Fractionation of Zn

Table 1. The Effect of Cellulose and Zn Amendment on Selected Water Quality Parameters in Microcosms.^a

Treatment		O ₂ Concentration	CO ₂ Concentration	COD	Measured Final Eh
cellulose	Zn ppm	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mV
-	-	1.6 ± 0.17 ^b	15.0 ± 0.40	306.1 ± 65.51 ^b	+558 ± 17.7
-	100	1.4 ± 0.04 ^b	9.2 ± 2.76	645.6 ± 35.67 ^b	+477 ± 5.5
+	-	0.2 ± 0.04 ^c	48.3 ± 0.69	1084.0 ± 176.57 ^c	+118 ± 10.2
+	100	0.2 ± 0.01 ^c	59.5 ± 0.55	1385.0 ± 7.57 ^c	+170 ± 10.4

^a Values are the mean of 4 replicates ± SD.

^b & ^c The same superscripts represent that the values are not significantly different at p<0.05 according to Duncan's Multiple Range tests.

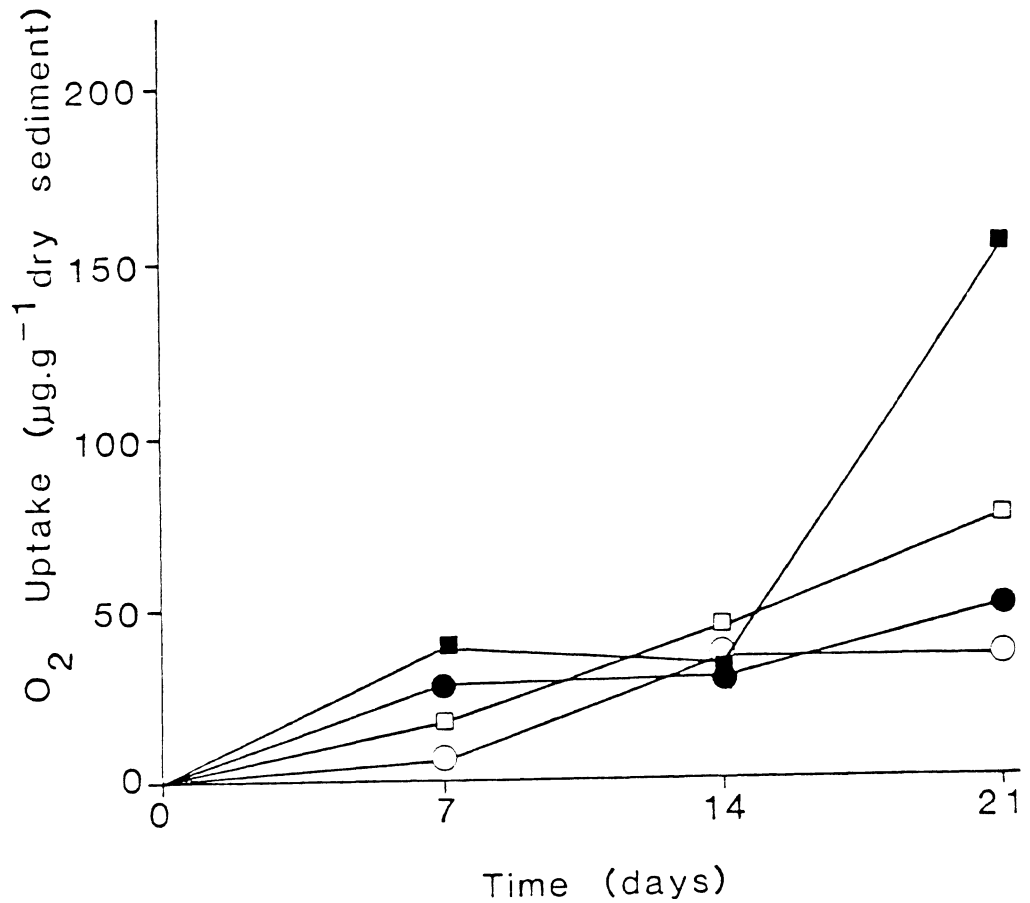


Figure 2. Oxygen Uptake Observed in the Headspace in Microcosm Experiments (symbols: □, cellulose amended microcosms with 100 ppm Zn as ZnSO₄, ■, cellulose amended microcosms without Zn, ○, non-cellulose amended microcosms with 100 ppm Zn as ZnSO₄, ●, non-cellulose amended microcosms without Zn). Values plotted represent the mean of 4 replicates.

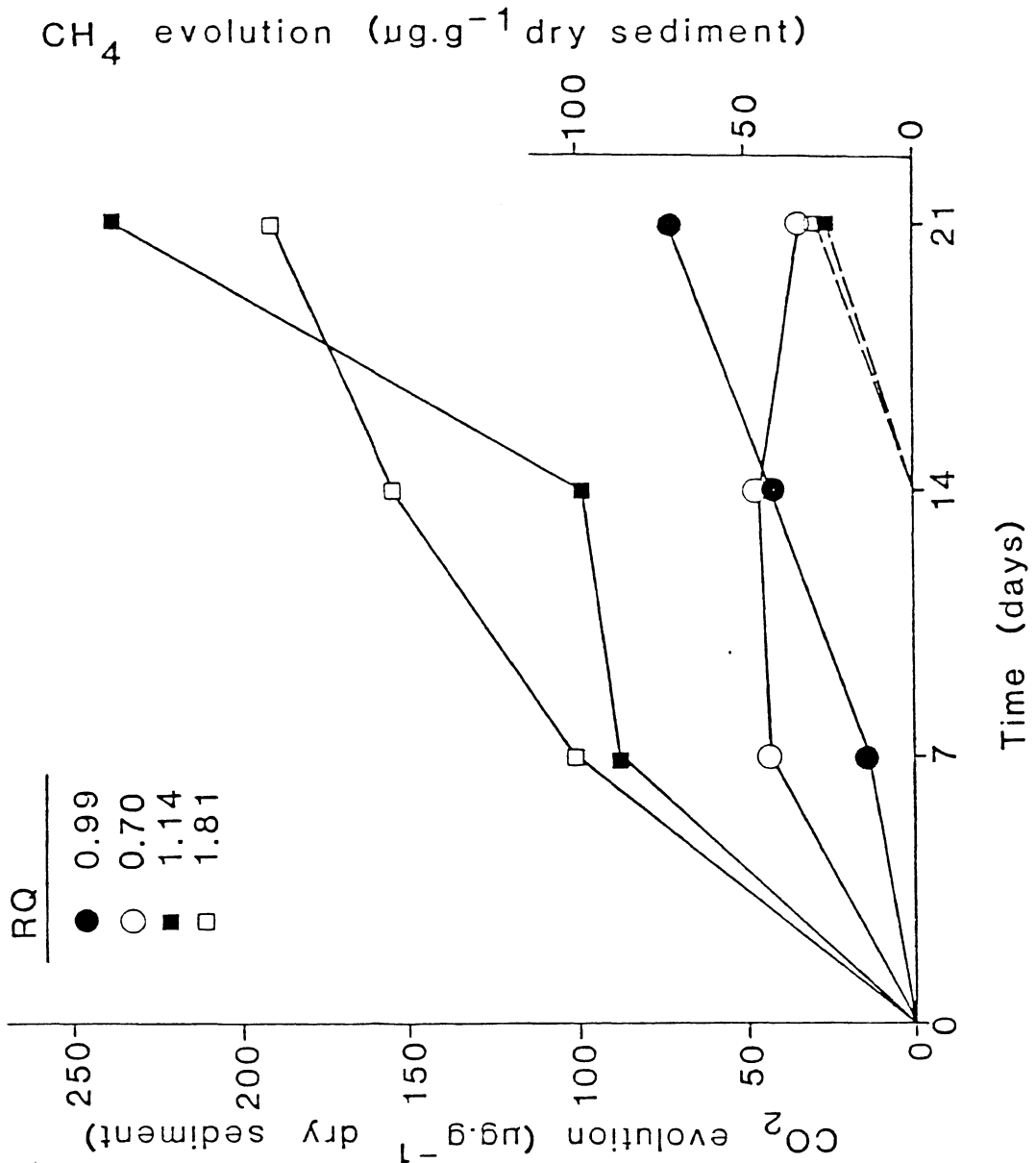


Figure 3. Carbon Dioxide and Methane Evolution Observed in the Headspace in Microcosm Experiments (symbols: □, cellulose amended microcosms with 100 ppm Zn as ZnSO₄, ■, cellulose amended microcosms without Zn, ○, non-cellulose amended microcosms with 100 ppm Zn as ZnSO₄, ●, non-cellulose amended microcosms without Zn). Methane evolution is shown by broken lines. RQ represents M CO₂ evolution/M O₂ consumption at 21 days, and values plotted are the mean of 4 replicates.

Table 2. Effect of Cellulose Amendment on Solubilization of Iron in the Water Column in Microcosm Experiments.

Treatment	Soluble Iron	% FeS in Sediment
cellulose	mg L ⁻¹	
-	nd ^a	94 ± 0.2
+	39.8 ± 7.28 ^b	97 ± 0.3

^a not detectable

^b Values are the mean of 4 replicates ± SD.

Table 3. The pH Change During the Whole Incubation Period in Microcosm Experiments.^a

Treatment	Zn	Initial	1st Week	2nd Week	3rd Week
cellulose	ppm				
-	-	7.16 ± .015	7.34 ± .053	7.73 ± .043	7.55 ± .022
-	100	6.72 ± .027	7.06 ± .020	7.67 ± .047	7.69 ± .067
+	-	7.30 ± .0]2	6.84 ± .029	7.01 ± .027	7.24 ± .074
+	100	6.80 ± .024	6.92 ± .021	6.86 ± .020	6.92 ± .026

^a Values are the mean of 4 replicates ± SD.

Greater sulfide production led to complete removal of soluble ZnSO_4 from the water column, and consequent Zn precipitation as ZnS (ZnS-Zn) (Table 4). After 3 weeks of incubation, soluble Zn levels in the water column dropped below detection limits both in cellulose and non-cellulose amended microcosms. In microcosms receiving 100 ppm Zn, the amount of volatile sulfide was less than the amount of ZnS-Zn. This discrepancy may be due to a limited supply of SO_4^{2-} . The SO_4^{2-} limitation was indicated by over 96% of SO_4^{2-} consumption and concomitant CH_4 production at the end of incubation (Table 5 and Figure 3). When the SO_4^{2-} supply was not limited, the percentage of ZnS-Zn was about 90% of total Zn detected in the sediments (Table 4). When the SO_4^{2-} supply was limited, however, so there was an insufficient amount of sulfide to bind with the added Zn, percent recovery of ZnS-Zn decreased to 60% out of the total. Zn concentration as ZnS, however, was ten times greater when 100 ppm Zn was added. The fate of Zn removed from the water column was then examined (Table 6). Approximately half of the Zn that disappeared from the water column was recovered as ZnS-Zn. When there was not enough sulfide produced to scavenge Zn as insoluble ZnS, the soluble Zn which was dissolved in water was recovered as a loosely-bound exchangeable Zn. The more tightly-bound organic Zn remained as a relatively small fraction.

Table 4. Acid-Volatile Sulfide and ZnS-Zn Values in Microcosm Sediments ($\mu\text{g g}^{-1}$ dry sediment).^a

Treatment	Zn ppm	Acid-Volatile Sulfide Fraction of Metallic Sulfide	ZnS-Zn	Final ZnS-Zn - Initial ZnS-Zn	% ZnS-Zn
-	-	71.5 \pm 6.19	61.4 \pm 3.23 ^b	9.7 \pm 2.94 ^b	91 \pm 0.5
-	100	142.4 \pm 4.92	174.2 \pm 8.60 ^c	122.2 \pm 8.29 ^c	55 \pm 3.8
+	-	148.5 \pm 14.67	65.4 \pm 1.78 ^b	17.7 \pm 1.59 ^b	88 \pm 2.4
+	100	168.4 \pm 3.37	219.1 \pm 9.30 ^d	160.7 \pm 7.06 ^d	61 \pm 1.6

^a Values are the mean of 4 replicates \pm SD.

b, c & d The same superscripts represent that the values are not significantly different at $p < 0.05$ according to Duncan's Multiple Range tests.

Table 5. Effect of Cellulose Amendment on Sulfate Reduction in Microcosm Experiments.^a

Treatment		Sulfate Reduction ^b Rate (K) 10 ⁻² day ⁻¹	Total Sulfate Reduced %	MPN of Sulfate Reducers g ⁻¹
cellulose	Zn ppm			
-	-	1.25 ± 0.126 ^c	59 ± 3.0	2.30 x 10 ⁷
-	100	3.56 ± 0.328 ^d	96 ± 1.8	2.40 x 10 ⁸
+	-	3.41 ± 0.668 ^d	98 ± 0.03	1.5 x 10 ⁸ - 1.1 x 10 ⁹
+	100	4.60 ± 0.238 ^d	99 ± 0.01	7.5 x 10 ⁸ - 1.1 x 10 ⁹

^a Values are the mean of 4 replicates ± SD.

^b $\ln \frac{(SO_4^{2-})}{(SO_4^{2-}) - (S^{2-})} = Kt$ where (SO_4^{2-}) = initial concentration of sulfate, mg g⁻¹
 (S^{2-}) = amount of sulfide produced in time t, mg g⁻¹
t = time in days
K = rate constant

^c & ^d The same superscripts represent that the values are not significantly different at p<0.05 according to Duncan's Multiple Range tests.

Table 6. Fraction of Added Zn Total Accounted for as ZnS, a Loosely-Bound Exchangeable and a Tightly-Bound Organic Zn in the Sediments.^a

Treatment	cellulose Zn ppm	% Exchangeable	% Organic	% ZnS-Zn
-	100	49 ± 4.4	3 ± 0.2	47 ± 4.2
+	100	41 ± 3.7	3 ± 0.4	55 ± 3.6

^a Values are the mean of 4 replicates ± SD.

Discussion

Cellulose amendment was effective in stimulating greater anaerobiosis (The use of the word "anaerobiosis" in this discussion implies very low oxygen level within microcosm systems, for instance, 0.2 mg L^{-1} in water.) to enhance sulfide production (Table 1). Oxygen in the water column of cellulose-amended microcosms is significantly ($p < 0.01$) lower than that of non-cellulose amended controls. Oxygen consumption may include COD and the biological oxidation of some of the products of anaerobic processes, including sulfide from SO_4^{2-} reduction, and other organic acids as metabolic end products of fermentation. The uncoupling of CO_2 evolution from O_2 uptake resulted from vigorous anaerobic processes, which were indicated by RQ values > 0.85 (Figure 3). $\text{RQ} > 0.85$ indicates the extent of metabolism for which O_2 is not the terminal electron acceptor (Rich, 1979). The presence of alternate electron acceptors, such as ferric ion, nitrate and SO_4^{2-} , permits microorganisms to oxidize organic matter in the absence of O_2 . The concentration of soluble iron in the water column of cellulose amended microcosms were up to 40 mg L^{-1} at the end of incubation (Table 2). Under reducing conditions, large amounts of iron are released into the water column as Fe^{2+} , resulting from the dissolution of previously insoluble ferric hydrous oxides (Patrick et al, 1977). Nitrate respiration was not measured.

Another indication of anaerobiosis in cellulose amended microcosms were Eh values (Table 1). The measured Eh values in cellulose amended microcosms were significantly ($p < 0.01$) lower than those of the controls without cellulose. The calculated Eh values for cellulose amended microcosms according to sulfide activity (Berner, 1963) were -155 ± 1.6 mV and -170 ± 3.5 mV. Calculated Eh values may be closer to the actual Eh of the system than measured values, because Eh of the environment is difficult to assess when H_2S is present in significant concentration (Boulegne and Michard, 1979). The calculated Eh values of -150 mV in the water column were sufficiently low for the growth of SO_4^{2-} reducing bacteria (Connell and Patrick, 1968). Similar trends in microbial respiration were observed in replicate experiments, although results from only one experiment are presented.

The SO_4^{2-} reducing bacteria were found in higher numbers in the anaerobic systems (Table 5). Rates of SO_4^{2-} reduction calculated according to first-order reaction kinetics (Nakai and Jensen, 1964) were greater ($p < 0.01$) in the presence of cellulose than in its absence (Table 5). Sulfate reduction rates in this study were generally higher than those values reported by Nakai and Jensen (1964) for marine systems. This discrepancy may be due to the fact

that microcosms in this laboratory study were not aerated and thus oxidation of reduced sulfur compounds was O_2 limited. An alternate explanation for this discrepancy may be that heavy metals of potential toxicity became less available for microorganisms due to precipitation of metals as insoluble metallic sulfides. Thus, reduced availability of heavy metals may have enhanced microbial SO_4^{2-} reduction. The percentage of SO_4^{2-} reduced was greater with cellulose amendments than without cellulose. Zn amendment without cellulose also appeared to stimulate SO_4^{2-} reduction. This result was unexpected since Zn amendment did not stimulate microbial respiration (Table 1). One possible interpretation might be the metal resistance of SO_4^{2-} bacteria in the samples collected from Adair Run, since Adair Run was previously used as a receiving stream of a metal-rich coal ash effluent.

Methane production observed near the end of incubation indicated that SO_4^{2-} was limiting (Figure 3). When SO_4^{2-} was not limiting, SO_4^{2-} reducing bacteria may inhibit CH_4 production by lowering the H_2 partial pressure below a threshold level necessary for H_2 utilization by methanogens (Ingvorsen et al, 1981). When SO_4^{2-} level approximates that of marine systems (2000 ppm), SO_4^{2-} reduction is known to be limited by low availability of organic matter (Jackson, 1978). Goldhaber and Kaplan (1974) suggested that SO_4^{2-} reduction rates are independent of concentration above about

2-10 mM L⁻¹, and Ingvorsen et al (1981) suggested 0.1 mM L⁻¹ of SO₄²⁻ as a threshold level. The final level SO₄²⁻ in my system was 50 uM L⁻¹. At this lower concentration of SO₄²⁻ at the end of incubation, SO₄²⁻ reduction was limited by the availability of SO₄²⁻ in the presence of sufficient organic matter.

When SO₄²⁻ was not limiting, ZnS accounted for about 90% of total Zn in the sediments (Table 5). When SO₄²⁻ was limiting, however, the added ZnSO₄ to the water column was recovered as a loosely-bound exchangeable Zn (Table 6). The more tightly-bound organic Zn accounted for 3% of the total Zn removed from water. This may be due to lower affinity of Zn to organic compounds than that of other metals (Nordstrom, 1979), and a neutral pH of my system (Table 3). At a neutral pH with decreasing Eh, the metal humates release bound metal ions into solution, while these liberated cations will precipitate as metallic sulfides when sulfide is present in sufficient amounts (Pauli, 1975). Thus, with insufficient amount of sulfide resulted from the limited SO₄²⁻ supply, the added Zn was recovered as a loosely-bound exchangeable fraction rather than tightly-bound organic Zn which may include humic substances.

Despite SO₄²⁻ limitation, the increase in ZnS in cellulose-amended microcosms at the end of incubation was

greater ($p < 0.01$) than that of the control without cellulose (Table 4). The lower than expected values of ZnS-Zn, based on the total sulfide production in non-Zn amended microcosms, may be due to the formation of other metallic sulfides, such as FeS. The proportion of sulfide bound to ZnS in these microcosms accounted for at least 45% (Table 4). This high portion of ZnS among total metallic sulfides may be due to the lower solubility product constant (K_{sp}) of ZnS, which is 1×10^{-23} compared to 2×10^{-17} of FeS (Masterton and Slowinski, 1973). Copper is known to show stronger affinity to sulfide than Zn (Stumm and Morgan, 1981), but the total concentration of Cu in my system did not exceed more than 0.02 mg g^{-1} dry sediment, which was less than 5% of total Zn concentration. This indicates that sulfide released from ZnS accounted for most of sulfide production.

The added soluble Zn in non-cellulose amended controls also disappeared completely from the water column by the end of the incubation period as it did in cellulose amended microcosms. It may have been adsorbed onto the wall of microcosm, as was observed by Santschi et al. (1980). The amount of added Zn unaccounted for in my experiments was approximately 10% greater in non-cellulose amended controls than in cellulose amended microcosms. Santschi et al. (1980) also found that Zn disappeared from water more rapidly when the microcosm had sediments than not. These results may indicate that adsorption is an important

mechanism in removing Zn from water, and sediments serve as a sink for added Zn. ZnS-Zn in cellulose amended microcosms, however, was greater ($p < 0.01$) than that of non-cellulose amended controls.

Summary

Enhanced SO_4^{2-} reduction was achieved from cellulose amendment, leading to greater sulfide production than in non-cellulose amended controls. The percent increase in ZnS at the end of incubation was twice as much in reduced microcosms with cellulose as in the controls without cellulose. The amount of ZnS-Zn accounted for about 90% of the total Zn in the sediments. When SO_4^{2-} limitation was observed, however, ZnS-Zn was over 50%, even if the absolute amount of ZnS-Zn was ten times greater in cellulose amended microcosms than non-cellulose amended controls.

These results indicate that if SO_4^{2-} is constantly supplied in the presence of sufficient amount of an organic carbon source, the soluble Zn in water would be immobilized as insoluble ZnS. In the field where SO_4^{2-} is constantly released into the coal ash effluent receiving stream at an average concentration of 75 mg L^{-1} , the addition of a sufficient amount of organic carbon source into an impoundment would precipitate the soluble Zn as ZnS to the sediments, thus reducing the potential hazardous effects of Zn. Chopped straw or hay could be a suitable organic matter which can be readily available and inexpensive.

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Appendix I. Chemical ZnS Formation with H₂S Purging in a Microcosm Experiment.^a

Treatment		Soluble Zn in the Water Column	ZnS
Zn	H ₂ S	mg L ⁻¹	µg g ⁻¹ dry sed.
+	-	13.27	131.2
-	+	nd ^b	147.6
+	+	0.24	869.4

^a Serum bottles (160 ml) contained 10 g of wet sediment and 5 mM of Zn as ZnSO₄. H₂S was purged for 5 min through a gas-tight rubber stopper which had a port for air displacement. White floc of ZnS was allowed to settle down the bottom of the bottles. Soluble Zn and ZnS were measured as described in Materials and Methods.

^b not detectable

Appendix I. (continued)

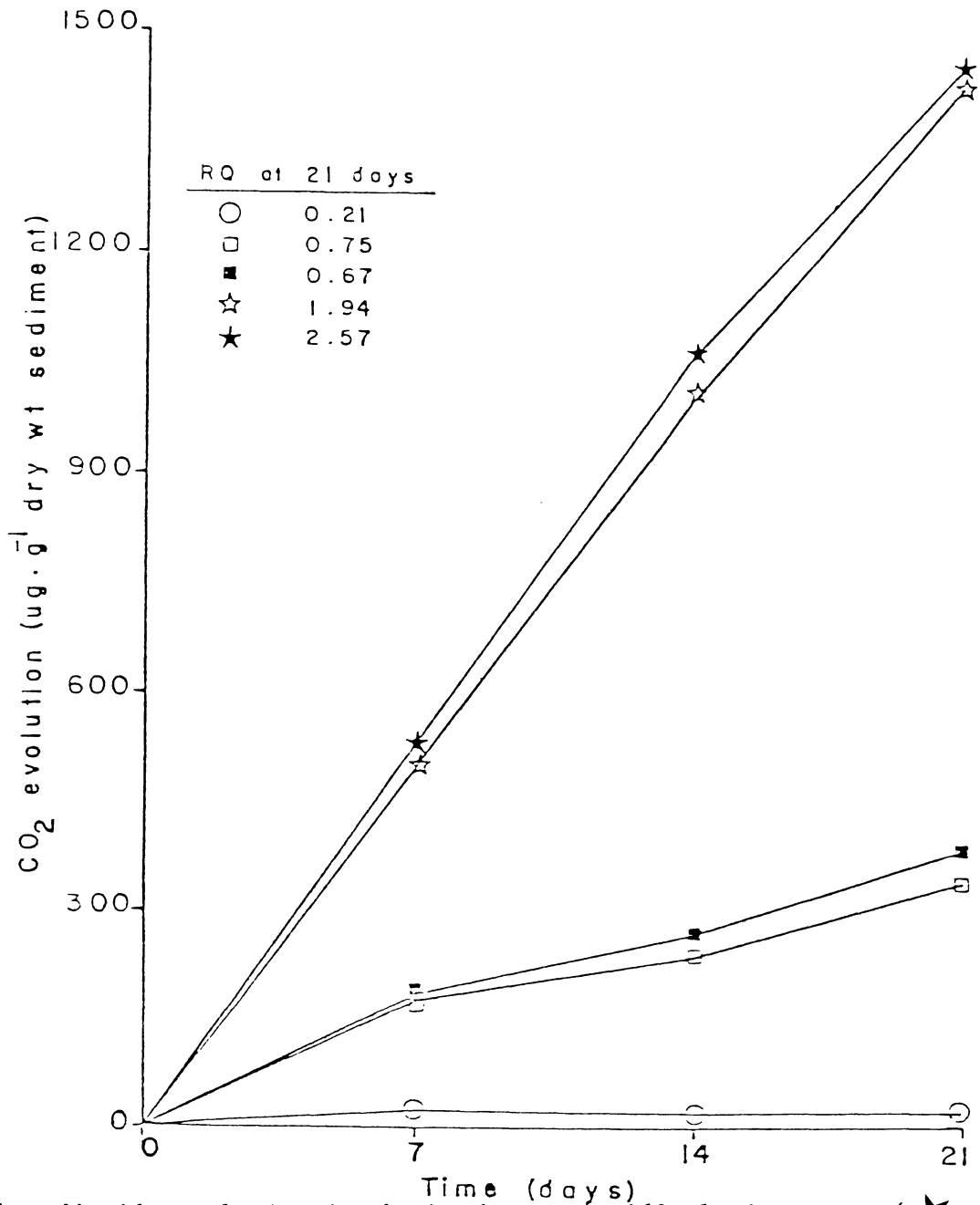
Results and Discussion

The scavenging activity of H_2S for $ZnSO_4$ was demonstrated. The concentration of soluble Zn in the water column was the highest when no H_2S was introduced. Zn as ZnS showed the highest level in the bottle amended with H_2S and Zn. Even when Zn was not added, but H_2S was purged, Zn as ZnS level was slightly higher than the control (Zn +, H_2S -). This may be because naturally present Zn in the sediments reacted with dissolved H_2S in water, while white floc of ZnS in the bottle (Zn +, H_2S +) was allowed to settle down the bottom. The increased portion of Zn as ZnS in the bottle (Zn -, H_2S +) may represent a loosely-bound exchangeable fraction.

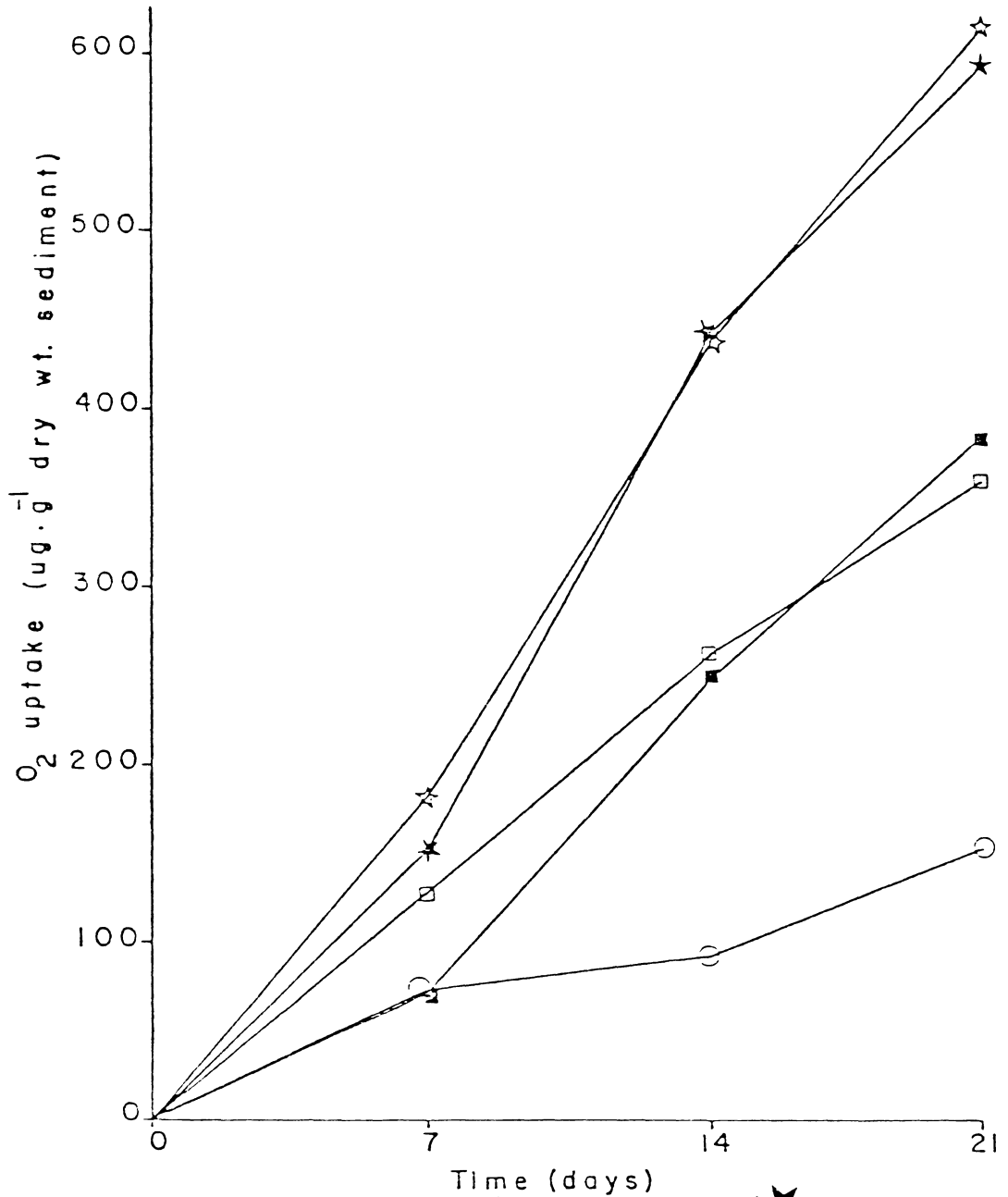
Appendix II.

Materials and Methods

Microcosms consisted of serum bottles (160 ml), containing 40 g of wet sediment and 40 ml of water sample taken from Adai Run (See Sample Collection.). Microcosms were sealed with gas-tight rubber caps. Gas, water and sediment samples were treated as described in Materials and Methods.



Carbon dioxide evolution in the headspace in 160 ml microcosms (★ represents cellulose amended microcosms with 1 ppm Zn as ZnSO₄, ☆ cellulose amended microcosms without Zn, ■, non-cellulose amended microcosms with Zn, □, non-cellulose amended microcosms without Zn, ○, sterile control with 1 ppm Zn and cellulose amendment). RQ represents M CO₂ evolution/M O₂ consumption.



Oxygen uptake in the headspace of 160 ml microcosms (★ represents cellulose amended microcosms with 1 ppm Zn as ZnSO₄, ☆, cellulose amended microcosms without Zn, ■, non-cellulose amended microcosms with Zn, □, non-cellulose amended microcosms without Zn, ○, sterile control with 1 ppm Zn and cellulose amendment).

Appendix II. (continued)

Enhancement of Anaerobiosis in the Presence of Cellulose in 160 ml Microcosm Experiments.

Treatment		Final Eh	H ₂ S in the Headspace	Total S ²⁻ Production
cellulose	Zn ppm	mV	µg g ⁻¹ dry sed.	µg g ⁻¹ dry sed.
-	-	+447	0.011	7.73
-	1	+434	0.012	13.42
+	-	+ 19	0.013	15.97
+	1	- 6	0.028	25.97

Final Eh for the sterile control was +346, H₂S was not detected, and total S²⁻ production was 0.46 µg g⁻¹ dry sediments.

Appendix II. (continued)

Effect of Cellulose Amendment on Sulfate Reduction in 160 ml Microcosm Experiments.

Treatment		Total Sulfide Production	Total Sulfate Reduced	MPN of Sulfate Reducers
cellulose	Zn ppm	$\mu\text{g g}^{-1}$ dry sed.	%	g^{-1}
-	-	7.73	10.60	1.42×10^8
-	1	13.42	19.65	1.41×10^8
+	-	15.97	29.16	4.35×10^8
+	1	25.97	47.24	2.42×10^9

The total sulfide production for sterile control was $0.46 \mu\text{g g}^{-1}$ dry sediments, total sulfate reduced, 57.26 %, and no viable cells were found in plate counts at the end of incubation.

Appendix II. (continued)

Fraction of Zn Total Accounted for as ZnS-Zn Compared to S^{2-} Production in 160 ml Microcosm Experiments.

Treatment		Total S^{2-} Production	ZnS-Zn	% Increase in ^c ZnS-Zn
cellulose	Zn ppm	$\mu\text{g g}^{-1}$ dry sed.	$\mu\text{g g}$ dry sed.	
-	-	7.7	64.1 ± 1.15^a	8.4
-	1	13.4	69.0 ± 3.79^a	8.7
+	-	16.0	46.0 ± 5.87^b	0
+	1	26.0	36.8 ± 2.17^b	10.3

^a Values are the mean of 4 replicates \pm SD.

^b Values are the mean of 6 replicates \pm SD.

^c % increase after 3 weeks of incubation

The level of ZnS-Zn in the sterile control was 57.6 ± 3.21 and % increase of ZnS-Zn was zero.

Appendix II. (continued)

Fraction of Fe Total Accounted for as a Loosely-Bound Exchangeable Fe and FeS in 160 ml Microcosm Experiments.

Treatment		Soluble Fe	% Exchangeable	% FeS
cellulose	Zn ppm	mg L ⁻¹		
-	-	nd ^a	0.01	99
-	1	nd	0.01	99
+	-	58.9 ± 2.75 ^b	0.01	99
+	1	55.4 ± 3.34	0.01	99

^anot detectable

^bValues are the mean of 6 replicates ± SD.

The level of soluble Fe in the sterile control was not detectable.

Appendix II. (continued)

Results and Discussion

Enhanced anaerobiosis and consequent sulfide production in greater amount were achieved by cellulose amendment. The soluble iron was detected only in cellulose amended microcosms, indicating greater extent of anaerobiosis. Hydrogen sulfide detected in the headspace of microcosms may have been due to the insufficient amount of Zn added. In other microcosm experiment with 100 ppm Zn amendment, no gaseous H_2S in the headspace was detected (See Results of main context.). Sulfate reduction and number of sulfate reducing bacteria showed similar results as discussed in main context. Even though ZnS-Zn in non-cellulose amended microcosms showed slightly greater values than cellulose amended microcosms, the percent increase of ZnS-Zn was higher in cellulose amended microcosms.

Appendix III.

Materials and Methods

Microcosms were constructed in the same way as described in Materials and Methods except that these microcosms did not have four compartments. Gas, water, and sediment samples were also treated in the same way.

Appendix III. (continued)

Effect of Cellulose Amendment on Microbial Community Respiration Measured in the Water Column in Microcosm Experiments.

Treatment		O ₂ Uptake ^a	CO ₂ Production ^b	COD ^c	Final Eh
Cellulose	Zn ppm	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mV
-	-	3.94 ± 0.00	1.19 ± 0.06	2.7 ± 0.23	+347
-	50	3.61 ± 0.00	1.71 ± 0.02	0	+371
+	10	6.14 ± 0.06	210.47 ± 0.03	1589.1 ± 195.23	- 16
+	50	6.08 ± 0.01	225.78 ± 0.75	1732.4 ± 7.60	- 39
+	-	6.22 ± 0.04	193.78 ± 0.13	1392.2 ± 3.74	- 32

^a Values are the mean of 2 replicates ± SD.

^b Values are the mean of 4 replicates ± SD.

^c COD = Chemical Oxygen Demand

Values for the sterile control for O₂ uptake was 2.09 ± 0.52, CO₂, 176.33, COD, 862.5, and Eh, +203, respectively.

Appendix III. (continued)

Effect of Cellulose Amendment on Sulfate Reduction in Microcosm Experiments.

Treatment		S ²⁻ Production in ^a Sediments	Total SO ₄ ²⁻ Reduced ⁴	MPN of Sulfate Reducers
cellulose	Zn ppm	µg g ⁻¹ dry sed.	%	g ⁻¹
-	-	61.7 ± 10.26	33.3	1.30 x 10 ⁸
-	50	68.2 ± 13.64	36.1	5.88 x 10 ⁷
+	10	105.8 ± 15.65	44.9	3.50 x 10 ⁹
+	50	179.2 ± 16.90	64.8	2.77 x 10 ⁹
+	-	118.7 ± 51.38	53.9	2.40 x 10 ⁹

^a Values are the mean of 4 replicates ± SD.

Values for the sterile control for each fraction were 12.9 ± 0.31 (S²⁻), 5.02% (total sulfate reduced), and no viable cells were found at the end of incubation.

Appendix III. (continued)

Fraction of Fe Total Accounted for as a Loosely-Bound Exchangeable and a Tightly-Bound Fe and FeS in Microcosm Experiments.

Treatment		Soluble Fe	% Exchangeable	% Organic	% FeS
cellulose	Zn ppm	mg L ⁻¹			
-	-	nd ^a	<0.01	1.2	99
-	50	nd	<0.01	1.5	98
+	10	33.5 ± 0.97 ^b	<0.01	2.8	97
+	50	27.3 ± 1.75	0.05	2.2	98
+	-	23.8 ± 0.37	<0.01	1.5	98

^a not detectable

^b Values are the mean of 4 replicates ± SD.

Values for the sterile control for each fraction 4.4 ± 0.06 (soluble Fe), <0.01% (exchangrable), 0.19% (organic), respectively.

Appendix III. (continued)

Results and Discussion

Enhanced anaerobiosis and consequent increase in sulfate reduction were observed as in the main context. The percent increase in ZnS-Zn in cellulose amended microcosms was almost twice greater than that of non-cellulose amended controls.

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THE MICROBIAL IMMOBILIZATION OF ZINC SULFATE

by

Sung OK Yoon

(ABSTRACT)

Microbial immobilization of soluble zinc (ZnSO_4) as ZnS was investigated to reduce the possible hazardous effects of soluble Zn in aquatic systems. Static three-phase microcosms were designed to simulate certain characteristics of natural systems in the laboratory. Microcosms (40, 15, 15 cm in length, width and height, respectively) contained water and sediments collected from a stream which had received metal-rich fly ash effluent from a coal burning power plant. To enhance sulfide production by sulfate reducing bacteria, an organic carbon source (1% cellulose g^{-1} dry sediment) and 200 ppm sulfate were added into the microcosms. The rates of microbial respiration were determined by the analyses of carbon dioxide, oxygen, methane and hydrogen sulfide in the head space and water using gas chromatography. Selective chemical leaching techniques were applied to determine the fate of the added Zn as ZnSO_4 in the sediments using atomic absorption spectroscopy. Cellulose amendment resulted in elevated RQ values ($\text{M CO}_2/\text{M O}_2$) and strongly reduced sediments. Greater volatile sulfide production was observed in these cellulose amended microcosms. The absolute amount of Zn as ZnS in

reduced microcosms was significantly greater ($p < 0.01$) than that of non-cellulose amended controls. The amount of Zn as ZnS accounted for over 90% when a sufficient amount of sulfate was present. When sulfate limitation was observed, however, the amount of Zn as ZnS accounted for approximately 50% of the total Zn in the sediments. The rest of the added Zn which could not bind with sulfide was mostly recovered as a loosely-bound exchangeable Zn. These results suggest that the addition of a suitable organic carbon source into the fly ash effluent receiving stream would remove soluble Zn from water and consequent precipitation of insoluble ZnS in the sediments.