CHAPTER III

INFLUENCE OF SULFUR AND LACTATE ON IN VITRO SULFIDE ACCUMULATION AND REDOX POTENTIAL IN FLOODED SAND

Abstract. Qualitative research into turf black layer suggested it was an accumulation of S^{-2}. Other researchers reported that S^{-2} production in rice paddy soil was dependent on the concentration of organic C present, and the redox potential of the surroundings. The purpose of this study was to determine how S^{-2} production and pE + pH in flooded sand of low organic matter content was affected by adding different levels of S and $C_3H_5O_3Na$. Sulfide production in response to S was dependent on concentration of lactate and vice versa. Results indicated that S was limiting for S^{-2} accumulation, not organic C. Results also indicated that only when S or S + lactate were added did pE + pH become significantly depressed. It was concluded that soil S, organic matter status, and pE + pH dictated $[S^{-2}]$ hence black layer potential.

Black layer appearing in turfgrass soils was suggested to result from accumulation of metal sulfides (2, 3, 9, 10). Rankin (9) reported incidence of FeS and MnS in the analysis of a New Zealand black layer deposit. He concluded that the black deposits were the end result of reactions between S, organic matter and waterlogging. Smith (10) reported the occurrence of a sulfidic precipitate resulting from anaerobic bacterial activity in Saskatchewan turfgrass soils after waterlogging. He concluded that S seemed to be the key element in this black layer formation

and suggested that other factors leading to the development of the black layer were heavy rainfall, waterlogging and perching of water tables. Hodges (5, 6, 7) hypothesized that algae may induce a perched water table in high sand content soils which receive frequent, close mowing. He also reported a relationship between Desulfovibrio spp., and Oscilatoria or Nostoc spp. in forming a black precipitate in high sand content cultures in vitro. Only when both algae and sulfate-reducers were present did the black precipitate quickly form. He reported that organic substances produced by the algae probably helped to form the perched water table, generate anaerobic microsites, and supply growth substrate conducive to activity of sulfate-reducers.

The objective of this research was to test the hypothesis that addition of S and $C_3H_5O_3Na$ to low organic matter content flooded sand would increase the $[S^{-2}]$, and decrease the redox potential, thereby enhancing the black layer formation potential.

MATERIALS AND METHODS

Experiments with Sulfur and Lactate

Two hundred grams washed Lake Michigan dune sand (99% > 0.1 mm and 84.5% > 0.25 mm, 0.0125% om) placed in 125 ml glass serum bottles (Wheaton) was treated with flowable 52% S at rates of 0.0, 73.2 and 146.4 kg ha^{-1}. Experimental units were then flooded so that no headspace existed with either tap water, or $C_3H_5O_3Na$ solution at either 112 or 1,120 mg kg^{-1}. Sulfate content of the tap water was 30 ug ml^{-1}. Units were then sealed with butyl rubber stoppers, crimped with aluminum, and then

incubated for 21 da in the dark at 30 C. Experimental design was completely randomized design and the treatments were arranged factorially with three replications.

At the end of 21 days solution from each unit was sampled first for free H_2S and SO_4^{-2}. Two mls of sample solution were obtained by displacing headspace solution with O_2 free N_2 using 5cc syringes. The syringe to receive the solution was equipped with a 0.22 um syringe filter to exclude particulates. In this way headspace solution from each experimental unit was sampled from completely filled vessels without contact from atmospheric O_2 or interference from sand debris.

To measure H_2S, 0.4 ml aliquots of sample drawn from the receptor syringe with a 1 cc tuburculin syringe were injected into 4 mls moving $HCl:CuSO_4$ reagent (4) in a 10 cc cuvette. Absorbance was then determined at 480 nm with a Spectronic 20 (Milton-Roy). Reagent without sample served as a blank. The A between blanks and water at 480 nm was zero. A calibration curve made with dilutions of Na_2S served as a S^{-2} concentration reference. Sulfate analyses were performed with remaining sample using a low pressure liquid anion chromatograph (Dionex).

Next, the crimp and butyl stoppers were removed under a constant stream of N_2 and the pE + pH of headspace solution was determined. Hydrogen ion concentration was measured with a Ag/AgCl combination electrode (Fischer Scientific) and pE determined using a Pt redox electrode with a calomel reference (8). A pH/mV microprocessor (Orion model 811) was used in conjunction with both electrodes. Lastly, 2 ml de-aired water was added to each bottle, the butyl stoppers were replaced and

re-crimped, and 2 ml 37% HCl was injected into each unit using
another syringe as a vent. This step solublized any acid
volatile S^{-2}, which was subsequently measured colorimetrically in
HCl:$CuSO_4$ reagent in the same fashion as H_2S.

RESULTS AND DISCUSSION

Experiments with S and Organic Matter

After 21 days the soil in experimental units was observed to
be in a darkened state. Visually, it appeared that the soil was
darker where S and lactate were added.

When treatment effects were analyzed it was found that there
were significant statistical interactions for the S^{-2}
measurements. Thus, response to S was dependent on level of
lactate and vice versa. The effect of the S was to provide an
abundance of electron acceptors to be reduced while the lactate
provided necessary reducing equivalents.

Adding S at 73.2 kg ha^{-1} increased the [H_2S] to a detectable
level, and dramatically increased the acid volatile S^{-2} (AVS)
concentration compared to a no lactate, no S treatment (see
Table 1.). Increasing the level of S to 146.4 kg ha^{-1} did not
influence [H_2S] but did cause [AVS] to increase slightly,
although not significantly. Adding lactate at any level without
S did not result in significant H_2S or AVS accumulation compared
to the no lactate, no S treatment.

Adding S at 73.2 kg ha^{-1} with lactate at 112 or 1,120 mg kg^{-1}
caused no increase in [H_2S] but did increase [AVS] compared to
adding only S at 73.2 kg ha^{-1}. Increasing S to 146.4 kg ha^{-1}

Table 1. Influence of S and lactate on S^{-2} production and redox parameters in flooded Lake Michigan dune sand. Lactate was added at 0, 112 or 1,112 mg $C_3H_5O_3Na$ kg^{-1} solution. Tap water was used to flood the sand. Sulfur was added as a 52% flowable formulation at 0.0, 73.2 or 146.4 kg ha^{-1}. Experimental units were incubated in the dark for 21 da at 30 C. Means were the averages of 3 reps.

Treatment		Sulfur Compounds[z]			Redox Parameters[y]		
Lactate	S	H_2S	AVS	SO_4^{-2}	pE	pE+pH[x]	pH
mg/kg	kg/ha		mg/kg				
0.0	0.0	0.0 c*	5.1 e	19.1 e	-0.5 a	6.9 a	7.4 a
	73.2	4.2 c	46.7 d	27.4 cd	-2.2 bc	4.8 b	7.0 cd
	146.4	3.8 c	60.2 cd	33.6 ab	-2.1 bc	4.8 b	6.9 d
112	0.0	0.0 c	5.1 e	3.7 f	-0.8 a	6.5 a	7.3 b
	73.2	5.2 c	74.8 bc	28.8 bc	-2.2 bc	4.7 b	6.9 e
	146.4	18.3 b	65.5 c	34.4 a	-2.3 bc	4.4 bc	6.7 f
1,120	0.0	0.7 c	6.1 e	1.1 f	-0.8 a	6.2 a	7.0 cd
	73.2	2.7 c	86.7 ab	22.5 de	-1.8 b	5.0 b	6.8 f
	146.4	31.2 a	99.7 a	34.6 a	-2.6 c	4.0 c	6.6 g
$LSD_{.05}$ =		9.1	17.7	7.5	0.6	0.6	0.05

[z] mg S kg^{-1} soil solution as free H_2S, acid volatile S^{-2} (AVS), or SO_4^{-2}.

[y] pH measured with a Ag/AgCl combination electrode.

[y] pE = Eh/59.2 measured with Pt electrode and calomel reference.

[x] Denotes addition of pE and pH values.

* Means with similar letters within columns are not different by LSD @ P = 0.05.

with 112 mg kg^{-1} of lactate resulted in significant jump in [H$_2$S] but increased the [AVS] only slightly. Increasing S to 146.4 kg ha^{-1} with 1,120 mg kg^{-1} lactate increased both [H$_2$S] and [AVS] significantly.

Significant linear trends (see Table 2.) across S levels were detected for H$_2$S appearance where lactate was added. Increasing lactate to 1,120 mg kg^{-1} resulted in the detection of a significant quadratic response. Significant linear responses were detected for all 3 lactate levels regarding AVS. Where lactate was added at 112 mg kg^{-1}, and when it increased to 1,120 mg kg^{-1} significant quadratic responses were detected.

It appeared that free H$_2$S generation in response to S was limited by availability of S, and somewhere between the 73.2 and 146.4 kg ha^{-1} S levels lay a critical S application value above which free H$_2$S was readily generated provided organic matter was abundent. If that threshold S application level was reached, increasing organic matter increased the [H$_2$S]. If the threshold was not reached additional organic matter did not cause an increase in [H$_2$S].

For AVS formation in response to S application, it again appeared that S was limiting. For a given level of organic matter, if sufficient S was present AVS readily accumulated. Without S application little AVS accumulated even though organic matter was abundant. However, increasing the level of applied S from 73.2 to 146.4 failed to produce a significant increase in [AVS] concentration. Thus, once S was added the organic matter content dictated the S^{-2} intensity. If S was not added then organic matter content did not matter.

Table 2. Trend analysis summary for lactate treatments across S levels. Lactate was added as $C_3H_5O_3Na$ at 0, 112 or 1,112 mg kg^{-1} solution. Sulfur was added as a 52% flowable at 0.0, 73.2 or 146.4 kg ha^{-1}. Values for SSQ were calculated with treatment means. Means were the values of three replications.

		Sulfur Compounds[z]			Redox Parameters[y]		
Source	df	H_2S	AVS	SO_4^{-2}	pE	pE+pH[x]	pH
Reps	2						
S Level	2						
0.0							
Linear	1	1 ns	43 **	38 **	31 **	52 **	375 **
Quadratic	1	1 ns	4 ns	1 ns	13 **	17 **	45 **
112							
Linear	1	18 **	52 **	168 **	27 **	52 **	540 **
Quadratic	1	1 ns	29 **	23 **	7 *	9 **	20 **
1,112							
Linear	1	50 **	124 **	200 **	40 **	57 **	240 **
Quadratic	1	13 **	22 **	5 *	1 ns	1 ns	1 ns
Error	18						

*, ** Denotes significance at P = 0.05 and P = 0.01 respectively.

[z] Sulfur as free H_2S, as acid volatile S^{-2} (AVS), or as sulfate-S (SO_4^{-2}).

[y] pH measured with a Ag/AgCl combination electrode.

[y] pE = Eh/59.2 measured with a Pt electrode and calomel reference.

[x] Denotes addition of pE and pH values.

No statistical interactions were detected regarding pE + pH. Adding lactate without S at 112 mg kg^{-1} tended to depress pE + pH, and increasing lactate concentration to 1,120 mg kg^{-1} tended to increase the depression (see Table 1.). However, the change in pE + pH between lactate levels without S was subtle and was not statistically significant. Adding S at 73.2 kg ha^{-1} without lactate depressed the pE + pH significantly compared to the no S control, and compared to the lactate only treatments. However, increasing the level of S without lactate did not result in a greater pE + pH reduction. The only additional reduction in pE + pH due to increasing level of S occurred at the 1,120 mg kg^{-1} lactate level. Organic matter at high concentrations might be enough to depress the pE + pH to such a level that intense S^{-2} production occurs but this was not accomplished in the present study. Addition of S at near reccommended rates did depress pE + pH to the point where reduction was iminent.

Reduction of pE + pH with lactate was assumed to involve increasing bacterial respiration rates so that O_2 was eventually consumed. This assumption ws not unreasonable due to the fact that our experimental units were sealed, and O_2 diffusion was stopped. Occurrence of lactate in situ is probably slight but occurrence of sloughed root mass in turfgrass soils should accomplish the same end, while also providing a source of S.

Reduction of pE + pH with S probably involved the oxidation of S to SO_4^{-2} consuming O_2 in the process:

$$S + 3/2\ O_2 + H_2O \longleftrightarrow H_2SO_4$$

This would also account for the observed pH reduction (see Table 1.). As S application to lower soil pH is a common cultural practice in turfgrass management, this too may impact the aeration status of treated soil, especially during rainy periods, to the point where SO_4^{-2} reduction occurs. Thus, addition of lactate and S led to conditions environmentally conducive to S^{-2} accumulation, low redox potential.

CONCLUSIONS

The results of this study suggested that S was limiting for S^{-2} production in our experimental soil. Addition of lactate increased the S^{-2} production only where [S] was sufficient; where interactive effects were operative more S^{-2} was produced. Compared to lactate, addition of S also appeared to reduce redox potential to a greater extent. However, the combined effects of S and lactate produced the lowest redox. Thus, where S and soluble organic debris are plentiful, and O_2 diffusion or availability is retarded for whatever reason, potential of black layer S^{-2} production is enhanced.

rated lower on 27%.

Rate of application induced differences in visual quality, when averaged over carrier types, were detected on 100% of ratings, with 92% of the differences highly significant. High rate treatments were significantly better than medium rate treatments 83% of the time. Medium rates rated higher than low rates 83% of the time.

Regarding 1985 ratings (N=17, see Table 8)), Mean treatment rating values ranged from 5.5 to 9.0.

Carrier type induced differences in visual quality, when averaged over rates of application, occurred on 75% of the ratings, with 75% of the differences highly significant. When differences occurred, compared to sulfur-coated urea- treated turf, Lawn Restore-treated turf never rated better and rated lower 66% of the time. In comparison to urea-treated turf, Lawn Restore treated turf always rated lower.

Differences in visual quality due to increasing rates of application, averaged over carrier types, occurred on 100% of the ratings, with 94% highly significant. High rate treatments consistently rated better than medium rate treatments which rated significantly better than the low rate treatments of application.

Treatment means for each collection or rating date were collapsed across dates within years and equivalent conditions correlated for degree of variable association (see Table 9). All correlation

9)   Rankin, P.C.   1988.   When the black layer hit the fan.   Turf
     Craft Aust. 6: 18-20.

10) Smith, J.D.   1988.   Black plug layer on Saskatchewan golf
     courses.   Greenmaster 8: 6-21.

11) Steel, R.G.D., and J.H. Torrie.   1980.   Principles and
     procedures of statistics.   McGraw-Hill, New York.