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I have over thirty eight years of experience as an Environmental Chemist working on surface water and ground water treatment. I am the owner and founder of RNAS Remediation Products a Minnesota Corporation that provides bioremediation products for groundwater remediation. As part of our groundwater remediation strategies we often work to stimulate both biological and abiotic dechlorination of chlorinated solvents using sulfate/sulfide chemistry.

The complex, site-specific sulfate standard that was previously proposed by MPCA and ultimately rejected by the courts was to some degree based in science but not practical. It would be a huge task to properly evaluate every potential lake and stream environment to study and determine a site specific tolerance for sulfate inputs.

More importantly it ignores some common sense requirements for reactive iron to protect sediments from high sulfate inputs. The form of iron is very important. Zero valent iron with a high specific surface area will rapidly capture sulfide, while larger particles (with a low specific surface area) of oxidized iron are not effective in capturing sulfide. In review of "Sulphide Scavengers" in the attached article they discuss how treating sulfide contaminated drilling mud with magnetite is not effective at neutral or alkaline pH because reaction rates are very slow. Ferric iron has minimal water solubility and neutral pH water with more than 50 ppb of dissolve oxygen has little or no ferrous iron - there is little or no natural iron flux to replenish iron in sediments from water while soluble sulfate can be delivered at high concentrations. If the flux of reactive iron is not sufficient to react with the flux of unnaturally high sulfate inputs the end result is that what little reactive iron is present in the sediments will be depleted and toxic sulfide will poison wild rice.

Unnaturally high sulfate inputs are well known to cause other negative impacts to our streams, lakes and wetlands. The attached AGU Publication describes how not only do sulfate inputs create toxic sulfides but this alternative electron acceptor is used by microbes as an oxygen alternative, rapidly consuming natural organic matter in sediments which releases mercury, phosphates and TOC into our waters. The combination of sulfate reduction and releasing sequestered ionic mercury in sediments leads to greater production of methylated mercury which is of course the main toxic insult to humans and wildlife that consume fish.

The bottom line is that we must remove sulfate pollution at the source, before it reaches our natural waters and sediments. There are not that many high sulfate sources with the potential to impact wild rice waters. We now have low cost biological treatment methods that can take the sulfur out of the system in the mine pit lakes and at the power plant discharge locations before they harm our environment.



RESEARCH ARTICLE

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This article is a companion to Myrbo et al. (2017), <https://doi.org/10.1002/2017JG003787> and Pollman et al. (2017), <https://doi.org/10.1002/2017JG003785>.

Key Points:

- Sulfate addition increased organic matter mineralization in wetland sediment, releasing C, N, P, and Hg to the water column
- Sulfate reduction caused not only higher methylmercury concentrations but higher total mercury concentrations in the surface water
- Increased sulfate loading to freshwaters can cause deleterious effects separate from direct sulfide toxicity to organisms

Supporting Information:

- Supporting Information S1
- Figure S1
- Data Set S1

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Increase in Nutrients, Mercury, and Methylmercury as a Consequence of Elevated Sulfate Reduction to Sulfide in Experimental Wetland Mesocosms

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Abstract Microbial sulfate reduction (MSR) in both freshwater and marine ecosystems is a pathway for the decomposition of sedimentary organic matter (OM) after oxygen has been consumed. In experimental freshwater wetland mesocosms, sulfate additions allowed MSR to mineralize OM that would not otherwise have been decomposed. The mineralization of OM by MSR increased surface water concentrations of ecologically important constituents of OM: dissolved inorganic carbon, dissolved organic carbon, phosphorus, nitrogen, total mercury, and methylmercury. Increases in surface water concentrations, except for methylmercury, were in proportion to cumulative sulfate reduction, which was estimated by sulfate loss from the surface water into the sediments. Stoichiometric analysis shows that the increases were less than would be predicted from ratios with carbon in sediment, indicating that there are processes that limit P, N, and Hg mobilization to, or retention in, surface water. The highest sulfate treatment produced high levels of sulfide that retarded the methylation of mercury but simultaneously mobilized sedimentary inorganic mercury into surface water. As a result, the proportion of mercury in the surface water as methylmercury peaked at intermediate pore water sulfide concentrations. The mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, perhaps enhancing the removal of nutrients and mercury to periphyton. The presence of wild rice decreased sediment sulfide concentrations by 30%, which was most likely a result of oxygen release from the wild rice roots. An additional consequence of the enhanced MSR was that sulfate additions produced phytotoxic levels of sulfide in sediment pore water.

Plain Language Summary In the water-saturated soils of wetlands, which are usually anoxic, decomposition of dead plants and other organic matter is greatly retarded by the absence of oxygen. However, the addition of sulfate can allow bacteria that respire sulfate, instead of oxygen, to decompose organic matter that would not otherwise decay. The accelerated decay has multiple consequences that are concerning. The bacteria that respire sulfate “breathe out” hydrogen sulfide (also called sulfide), analogous to the conversion or respiration of oxygen to CO₂. Sulfide is very reactive with metals, which makes it toxic at higher concentrations. In addition to the release of sulfide, the sulfate-accelerated decomposition of plants releases phosphorus and nitrogen, fertilizing the waterbody. Decomposition also mobilizes mercury (which is everywhere, thanks to atmospheric transport) into the surface water. The microbes that convert sulfate to sulfide also methylate mercury, producing methylmercury, the only form of mercury that contaminates fish. This study demonstrates that adding sulfate to a wetland can not only produce toxic levels of sulfide but also increase the surface water concentrations of nitrogen, phosphorus, mercury, and methylmercury.

1. Introduction

Organic matter (OM) accumulates in the sediments of aquatic systems when sediment concentrations of terminal electron acceptors (TEAs) are too low for microbes to completely decompose OM, especially when the supply of the most energy-efficient TEA, oxygen, is low. In water-saturated, organic-rich sediment, microbial sulfate reduction (MSR) can be a dominant pathway for the respiration of OM because oxygen is depleted in the uppermost sediment (Boye et al., 2017). Dissolved sulfate (SO₄) concentrations in continental surface

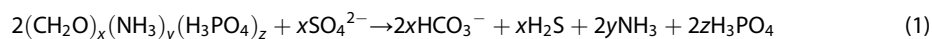
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waters are often low (less than 50 mg L^{-1} or 0.5 mmol L^{-1}) (e.g., Gorham et al., 1983) compared to ocean concentrations ($2,800 \text{ mg L}^{-1}$ or 29 mmol L^{-1}). Because of lower SO_4 concentrations, and because MSR rates can be limited by SO_4 concentrations (Holmer & Storkholm, 2001), the biogeochemical significance of MSR is often considered minimal in freshwater and low-salinity systems (e.g., Capone & Kiene, 1988; Nielsen et al., 2003; Stagg et al., 2017). However, absolute rates of MSR are not clearly lower in freshwater systems than in marine systems (Pallud & Van Cappellen, 2006), and in some cases, rapid cycling between oxidized and reduced forms of S can occur (Hansel et al., 2015).

In this study, we investigated the cascade of biogeochemical effects associated with increased MSR that result from increased surface water SO_4 . We simultaneously quantified three different categories of biogeochemical responses related to MSR: (1) mineralization of organic matter and associated release of dissolved C, N, P, and Hg; (2) methylation of Hg; and (3) production of sulfide.

The stoichiometric release of the constituents of OM during MSR, notably C, N, and P, is a phenomenon long recognized by marine scientists. For instance, Boudreau and Westrich (1984) constructed a model of the MSR-mediated decomposition of marine sediment. They showed that SO_4 is reduced to sulfide (H_2S) in stoichiometric proportion to the mineralization of C, N, and P according to the reaction



C is released as both dissolved inorganic carbon (DIC, from complete oxidation, produced as bicarbonate alkalinity in stoichiometric proportion to sulfide (reaction (1); Boudreau & Westrich, 1984)) and dissolved organic carbon (DOC, from partial oxidation). The nutrients N and P are released in forms that are readily taken up by plants; N is released as ammonia, and P as phosphate. The mineralization of sediment organic matter associated with MSR releases sulfide (S^{2-}) into sediment pore water, which speciates, depending on the pH, into hydrogen sulfide (H_2S) and bisulfide (HS^-), henceforth collectively termed sulfide. If reduced S compounds accumulate in the sediment, there may be additional consequences to an aquatic system, such as toxic concentrations of sulfide in pore water (Lamers et al., 2013; Pastor et al., 2017; Myrbo et al., 2017) or conversion of sediment Fe(III) to FeS compounds, which enhances the mobilization of P (Curtis, 1989; Maynard et al., 2011).

The multiple biogeochemical consequences of MSR in freshwater systems have been investigated and documented in more than two dozen publications (Table S1 in the supporting information), which typically address a single issue, such as the production of alkalinity that neutralizes atmospherically deposited H_2SO_4 (Baker et al., 1986; Cook et al., 1986; and others) or the methylation of Hg (Gilmour et al., 1992; Branfireun et al., 1999, 2001; and others). Experimental studies addressing SO_4 reduction, sulfide production, associated OM mineralization, and release of nutrients have been broader (Lamers et al., 2001, 2002; Weston et al., 2006, 2011; and others), but aside from the results reported in this paper, only the experiments of Gilmour, Krabbenhoft, et al. (2007) and Gilmour, Orem, et al. (2007) have investigated all three categories of biogeochemical consequences of SO_4 reduction: OM mineralization, Hg methylation, and sulfide accumulation (Table S1). We also investigated the potential for Hg to be released by mineralization, a phenomenon proposed by Regnell and Hammar (2004).

Sulfate-driven enhanced mineralization of sediment OM and release of dissolved sulfide, N, P, DOC, DIC, and associated increases in alkalinity and pH have the potential to change the nature of an aquatic ecosystem. The immediate release is to the sediment pore water, but these dissolved materials can diffuse into the surface water. Increased internal loading of N and P can drive a system toward eutrophy, which can increase carbon fixation and amplify the cascade of biogeochemical effects associated with increased MSR. Increases in DOC also have the potential to fundamentally change the nature of a waterbody. DOC influences many processes in freshwater ecosystems, including light availability for macrophyte growth, thermal stratification, and bioavailability of metals, P, and C. In addition, DOC interferes with drinking water purification (Williamson et al., 1999). Increases in DIC, alkalinity, and pH can also change the nature of a system. Aquatic macrophyte and algal species often have different optimal alkalinity concentrations (e.g., Moyle, 1945; Vestergaard & Sand-Jensen, 2000), so increases in alkalinity may change aquatic community composition. Because pH is a master variable in aquatic systems (Stumm & Morgan, 2012), increases in pH can cause changes in both aquatic chemistry and the biota that dominate a system, as best documented by changes in diatom assemblages (Patrick et al., 1968).

The release of sulfide into sediment pore water has multiple biological and geochemical consequences, several of which are related to the reactivity of sulfide with metals. If dissolved sulfide accumulates in pore water, it can negatively affect multicellular organisms inhabiting the sediment because sulfide can denature a range of metal-containing biomolecules, including cytochrome C oxidase, which is essential for respiration by both animals and plants (Bagarinao, 1992). Because aquatic sediment is a primary site of sulfide production, plants that root in sediment are vulnerable to toxic sulfide concentrations (Lamers et al., 2013; Pastor et al., 2017). However, if the watershed supplies sufficiently high loading of reactive Fe or other metals to the sediment, pore water sulfide concentrations may stay below toxic levels even while MSR proceeds as an important mineralization process (Pollman et al., 2017). The formation of FeS compounds effectively detoxifies sulfide (e.g., Marbà et al., 2007; Van der Welle et al., 2007). When Fe availability exceeds the production of sulfide, the accumulation of FeS is a measure of cumulative SO_4 reduction, which can be quantified as acid-volatile sulfide (AVS) (Heijs & van Gemerden, 2000). In addition, phosphorus is mobilized when oxidized Fe compounds with significant capacity to bind phosphate are converted to FeS compounds, which are incapable of binding phosphate (Lamers et al., 1998; Maynard et al., 2011). Thus, MSR mobilizes P both by mineralization of P-containing OM and by changing the form of Fe in sediment.

In addition to releasing C, N, and P, producing potentially toxic concentrations of sulfide, and reducing the solubility of metals, MSR is a primary process leading to the formation of MeHg, the bioaccumulative form of Hg (Gilmour et al., 1992; Hsu-Kim et al., 2013), although other microbial groups can also methylate Hg (Podar et al., 2015). In some cases, MSR can lead to toxic levels of MeHg higher in the food chain. The relationship between SO_4 concentrations and MeHg production is complex, however, and both field and laboratory studies in freshwater and saline ecosystems suggest that there is a dual effect of S on Hg methylation. At low SO_4 concentrations, the addition of SO_4 can stimulate MSR and Hg methylation (Jeremiason et al., 2006). At higher SO_4 concentrations, a greater abundance of inorganic sulfide appears to decrease the availability of inorganic Hg for Hg methylation (Hsu-Kim et al., 2013; Johnson et al., 2016). Because it has been observed that low SO_4 additions often increase Hg methylation and higher SO_4 concentrations decrease methylation, it has been proposed that there is a range of SO_4 and sulfide concentrations are optimal for Hg methylation, above which methylation is inhibited (Hsu-Kim et al., 2013). There is some debate regarding the underlying mechanism, but there is substantial evidence suggesting that dissolved inorganic sulfide above concentrations of 300–3,000 $\mu\text{g L}^{-1}$ has an inhibitory effect on Hg methylation (Bailey et al., 2017).

This study presents results from 30 wetland mesocosms in which the surface waters were treated to maintain a wide range of SO_4 concentrations over the course of 5 years (2011–2015) to assess the impact on wild rice, *Zizania palustris* (Pastor et al., 2017). We took advantage of this experiment to analyze the geochemical conditions in surface and pore water in the mesocosms during late summer 2013, 3 years into the experiment. Pastor et al. (2017) specifically examined the effect of increased SO_4 loading on wild rice, whereas this paper examines the broader biogeochemical impact of augmenting SO_4 to a low- SO_4 system.

2. Materials and Methods

2.1. Experimental Design

The experimental setup (Figure S1 in the supporting information), described in detail by Pastor et al. (2017), consisted of thirty 375 L polyethylene stock tanks containing sediment from a wild rice lake (Rice Portage Lake; +46.6987°, –92.6886°) in which wild rice was grown in self-perpetuating populations at five SO_4 treatment levels (control, 50, 100, 150, and 300 mg L^{-1}). SO_4 concentrations in six replicate mesocosms were routinely monitored, and amendments of SO_4 were added as Na_2SO_4 during the growing season as SO_4 was removed by MSR (Figure 1). Due to MSR, the mesocosm surface waters actually had time-weighted average concentrations of 7, 27, 59, 93, and 207 mg L^{-1} , respectively. Local well water containing an average of 10.6 mg L^{-1} SO_4 was added as needed to compensate for evapotranspiration. Precipitation in the region contains an average of 2.1 mg L^{-1} SO_4 , and Rice Portage Lake has an average SO_4 concentration of 2.2 mg L^{-1} (Fond du Lac Band, 2016), so the control was slightly elevated above the ambient SO_4 concentration of the sediment source for the experiment. During the ice-free period (generally May through October), the surface water temperature (T) measured in the morning was correlated with the previous day's mean air temperature (mesocosm $T = 0.72$ air $T + 4.4$ °C; $R^2 = 0.65$). Peak air temperature is reached in July, when the average

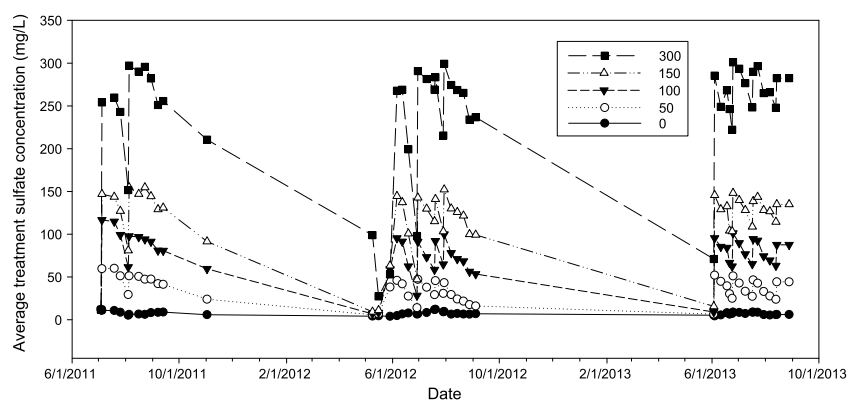


Figure 1. SO_4 concentrations in surface waters of each treatment, showing repetitive depletion and periodic amendment with Na_2SO_4 (average of six mesocosms per treatment on each sampling date).

temperature is 18.8°C (based on 1981–2010 air temperatures measured at the Duluth, Minnesota, airport, 10 km from the experimental site).

The experiments had been in progress for three growing seasons at the time of the sampling for this study, 27 and 28 August 2013, and for five growing seasons at the time of the second, less intensive, sampling (August 2015). The sediment of each mesocosm was divided into two parts for the 2013 growing season by a clear acrylic plate and all wild rice plants removed from one side in order to evaluate the effects of plant root presence on the geochemistry of the sediments. The plate was situated near one end of each mesocosm, such that about 10% of the surface area of 0.6 m^2 was plant-free (Figure S1). The plate was positioned to segregate the sediment without impeding the circulation of the surface water above all of the sediment. Sediment chemistry results presented here are from the side with wild rice plants present, except when analyzing the difference in AVS between the two sides.

2.2. Methods

2.2.1. Sample Collection

Rhizon™ samplers with a 10 cm long, 2.5 mm diameter, cylindrical porous tip (hydrophilic membrane pore size $0.12\text{--}0.18\ \mu\text{m}$ (Rhizosphere.com, Netherlands; Shotbolt, 2010)), were connected by Teflon-taped Luer-Lok connectors and silicone tubing to a syringe needle. The sampler was inserted into the sediment, and the needle was then inserted through the 20 mm thick butyl rubber septum of an evacuated serum bottle (Bellco Glass) to initiate pore water draw through the tubing and displace air. After water was observed entering the serum bottle, the needle was removed from the first sacrificial bottle and inserted through the septum of a second evacuated serum bottle to collect the sample. One Rhizon and bottle were used to collect a sample for dissolved iron, preserved with 20% nitric acid. A second Rhizon and evacuated, N_2 gas-flushed sealed bottle, preloaded with 0.2 mL 2 N zinc acetate, 0.5 mL 15 M NaOH, and a stir bar, was used to collect a sample for dissolved sulfide analysis. Each Rhizon was positioned to sample pore water from the top 10 cm of sediment and to avoid collecting water from above the sediment surface. However, it is conceivable that some surface water was able to follow the path of the Rhizon into the sediment and dilute or partially oxidize the pore water sample.

Surface water in each mesocosm was collected for analysis of nitrate + nitrite, TP, TN, DOC, pH, temperature, and alkalinity from 5 cm below the surface of the water. Surface water samples for analysis of total Hg (THg) and MeHg were collected using clean hands/dirty hands protocols in September 2013, filtered through $0.45\ \mu\text{m}$ glass fiber filters, and immediately acidified with 0.5% (by volume) trace metal hydrochloric acid. Samples were stored on ice during transport and at 4°C until analysis.

Pore water P availability was measured with three mixed bed ion exchange bags (Fisher Rexyn 300 resin) placed in the sediment of each tank in spring and harvested at the end of the growing season in 2013. A 3.8 cm diameter piston corer was used to obtain 10 cm long sediment samples for various analyses. Sediment samples for the analysis of AVS were taken monthly from June to October 2013 from replicate mesocosms of four SO_4 treatments (control, 50, 150, and $300\ \text{mg L}^{-1}$; no mesocosm was sampled more

than once). Sediment samples were also taken on 8 October 2013 for the analysis of THg in bulk sediment and on 6 October 2015 for the analysis of total organic carbon (TOC).

2.2.2. Laboratory Analyses

Surface water and pore water analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL). Total P was measured by in-line ultraviolet/persulfate digestion and flow injection (APHA, 2005, 4500 P-I), DOC by persulfate-ultraviolet oxidation and IR CO₂ detection (APHA, 2005, 5310-C), and alkalinity by automated titration (APHA, 2005, 2320-B). Pore water sulfide samples were prepared for inline distillation and flow injection colorimetric analysis using procedures that avoided exposure to oxygen. The sulfide serum bottle was weighed to determine the amount of sample collected and to adjust for the slight dilution factor of an alkaline antioxidant that was added by injection through the stoppers. The sealed samples were then placed on a stir plate for at least 1 h and subsamples withdrawn for analysis through a needle. Reanalysis of sealed, processed samples 12 months later shows no significant difference in sulfide concentrations, indicating that the sulfide samples were stable prior to analysis (data not shown). SO₄ concentration was measured using a Lachat QuikChem 8000 Autoanalyzer (Lachat Method 10-116-10-1-A). The resin was eluted using a KCl solution and analyzed for PO₄ using a Lachat Autoanalyzer, following the methods of Walker et al. (2006).

An aliquot of the nitrate + nitrite/TP/TN/DOC serum bottle was filtered in the lab within 10 days of sampling using a 0.45 μm filter, preserved to a pH < 2 with 10% sulfuric acid, and transferred to a 250 mL polyethylene bottle for DOC analysis. The remaining sample was preserved to a pH < 2, with 10% sulfuric acid and transferred to 250 mL polyethylene bottle for nitrate + nitrite/TP/TN analysis. The contents of the metal serum bottle were transferred to a 250 mL polyethylene bottle and preserved to a pH < 2 with 10% nitric acid. Analyses were conducted within 30 days of sampling.

THg in surface water and bulk sediment were analyzed with EPA method 1631 by MDHEL, and surface water MeHg was analyzed with EPA method 1630 by Frontier Global Sciences (Bothell, Washington). Inorganic Hg (iHg) was calculated as the difference between THg and MeHg. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid distillation and in-line alkaline trapping (APHA, 2005; SM 4500-S2). Sediment TOC was analyzed following SM5310C (APHA, 2005), using an OI Analytical Aurora 1030 at Pace Analytical Services, Virginia, Minnesota.

3. Data Analysis

3.1. Sulfate Depletion as the Independent Variable

Because SO₄ is relatively unreactive under oxidized conditions, its loss is attributable to diffusion or transpiration-driven advection (Bachand et al., 2014) into sediment and conversion to sulfide by bacteria. Surface water SO₄ concentrations decreased partly due to dilution by precipitation but largely from loss after movement into the sediment and reduction to sulfide. Sulfide would largely be retained in the sediment as FeS compounds, although some could be lost to the atmosphere as H₂S gas (Bagarinao, 1992) or as volatile organic sulfur compounds (Lomans et al., 2002). The cumulative SO₄ lost from surface water was calculated from a mass balance for each mesocosm from the inception of the experiment in spring 2011 through fall 2013; this quantity, termed here SO₄ depletion, (SO₄)_{Depl}, is used as a proxy for net MSR, following Weston et al. (2006). The surface water remained frozen from approximately 1 December to 1 April each winter, and the mesocosms were covered with plastic from November to late April each year and not amended with SO₄. SO₄ reduction was the major biogeochemical process altered by the experimental treatments, and therefore, (SO₄)_{Depl} is the independent variable used in subsequent data analyses. It was only possible to perform a complete mass balance for SO₄, the only parameter consistently quantified in source water, precipitation, and overflow water.

3.2. Calculation of DIC From Measured Alkalinity

Dissolved inorganic carbon (DIC ≡ [CO₃²⁻] + [HCO₃⁻] + [CO₂*], where [CO₂*] = [CO_{2(g)}] + [H₂CO₃]) was calculated from measured alkalinity and speciated using pH, temperature, and specific conductance of the surface water. At the pH range of the mesocosms (7.60–8.84), 95–98% of DIC is in the form of HCO₃⁻, so DIC concentration on a molar basis is nearly the same as alkalinity (ALK) on an equivalent basis (DIC = 0.988 ALK + 0.077, R² = 0.995). In studies of freshwater, most inorganic carbon data are presented in terms of alkalinity because

alkalinity is a familiar metric; however, in comparisons with DOC, inorganic carbon data are presented as DIC so that the units are directly comparable. PHREEQC version 3 geochemical modeling software (Parkhurst & Appelo, 2013) was used to calculate saturation indices for carbonate minerals.

3.3. Statistical Analysis

Statistical analysis was conducted with R version 3.2.3 and STATA (StataCorp, 2015). The effect of increased sulfate availability was assessed through both categorical analysis of the sulfate treatments (Kruskal-Wallis ANOVA test, followed by Dunn's test for multiple comparisons with Holm-Sidak corrections) and through linear regression and nonparametric Spearman rank correlations. We rely primarily on regressions against SO_4 depletion to detect the effects of enhanced sulfate-reduction driven mineralization, rather than categorical analysis of the sulfate treatment results, because (a) biogeochemical changes are not driven directly by SO_4 concentration, but rather by MSR, quantified as SO_4 depletion; (b) although SO_4 depletion may be highly correlated to SO_4 concentration, deviations between experimental mesocosms develop over time, so cumulative SO_4 depletion values eventually no longer align exactly with treatment categories, but rather become continuous variables; and (c) regression provides more statistical power than ANOVA and builds models that allowed us to describe the relationships between SO_4 depletion and response variables (Cottingham et al., 2005). However, when the relationship is not linear, ANOVA and comparison of treatments through Dunn's analysis can help describe the nature of a relationship.

4. Results and Discussion

4.1. The Impact of SO_4 Reduction on Mineralization of Sediment Organic Matter

Increased concentrations of surface water SO_4 resulted in increased sulfate reduction, which necessarily increased the mineralization of organic carbon, as described by reaction (1). Concentrations of surface water DOC and DIC increased in proportion to sulfate reduction, as measured by $(\text{SO}_4)_{\text{Depl}}$ (Table 1 and Figure 2). The marine literature generally assumes complete mineralization of particulate organic carbon (POC) to DIC in the water column (e.g., Boudreau & Westrich, 1984) (reaction 1), but in freshwater systems and especially wetlands, not all carbon is completely oxidized during decomposition, and a portion of POC may be mobilized as DOC (Howes et al., 1985; Selvendiran et al., 2008). In principle, the constituents of organic matter, such as the nutrients N and P, are mobilized in proportion to the mass of carbon mineralized as a result of MSR-driven decomposition. Surface water DOC and DIC, and the sum DOC + DIC, are therefore used as indicators of OM mineralization in interpreting the mobilization of N, P, and Hg to surface waters (Figure 2 and Tables 2 and 3).

In contrast to many marine systems, it is likely that SO_4 reduction in these sediments was limited more by SO_4 than by organic carbon, given that $(\text{SO}_4)_{\text{Depl}}$ was linearly proportional to the average SO_4 concentration (Figure S2a; $R^2 = 0.87$), without any obvious curvature to the relationship that would indicate saturation of MSR.

Regressions of surface water DOC and DIC against SO_4 depletion demonstrate that, on a net basis, about 60% more DIC than DOC was mobilized to the surface water as a result of MSR-driven mineralization (slope of 0.235 mM C per unit SO_4 depletion compared to 0.148; Table 2). The significantly positive slope of the DIC:DOC ratio against SO_4 depletion (Table 2) indicates that increasingly more DIC than DOC was observed in the surface water as sulfate depletion increased. Some mineralization of DOC to DIC likely occurs in the surface water as a result of exposure to oxygen, aerobic bacteria, and sunlight, processes that could have a larger effect as DOC increases.

Not only did surface water DIC and DOC increase in concert with sulfate reduction, but parallel increases occurred in surface water concentrations of constituents of organic matter: N, P, and Hg (Table 1 and Figure 2). DIC, DOC, total P, total N, ammonia, and total Hg in surface water all had increases from the control to the highest SO_4 addition of about twofold, (2.3, 1.7, 1.9, 1.8, 1.7, and 2.6-fold, respectively, Table 1). However, available phosphate in the sediment, an estimate of P availability in pore water, had a larger increase (7.5-fold). MSR consumes acidity as the DIC-based alkalinity is produced (Baker et al., 1986), which increased the average pH from 7.57 to 7.81, a 44% decrease in hydrogen ion concentration (Table 1). If the sulfide subsequently oxidizes (which could happen in a natural system during drought (Laudon et al., 2004) or intentional dewatering), a proportional quantity of alkalinity is consumed as acid is produced

Table 1

Summary of Effects of Experimentally Increased SO₄ Concentrations on SO₄ Reduction (Quantified as SO₄ Depletion), Organic Matter Mineralization, and Mercury Methylation

| Variable | Matrix | Average of each sulfate treatment (n = 6 for each treatment) | | | | | | Correlation with SO ₄ depletion (Spearman) | |
|--|--------------|--|----------------------|----------------------|---------------------|--------------------|---------|---|---------|
| | | Control | 50 | 100 | 150 | 300 | Max/Min | Rho | p value |
| <i>Variables mainly associated with SO₄ reduction</i> | | | | | | | | | |
| SO ₄ (T-W mean mg SO ₄ L ⁻¹) | sw | 6.7 ^a | 26.9 ^{ab} | 58.5 ^{abc} | 93.2 ^{bc} | 206.5 ^c | 31.0 | 0.93 | <0.0001 |
| SO ₄ depletion (mg S cm ⁻²) | sw | 0.14 ^a | 2.52 ^{ab} | 3.63 ^{abc} | 4.28 ^{bc} | 6.90 ^c | 48.5 | 1 | |
| Pore water sulfide (μg S L ⁻¹) | pw | 69 ^a | 184 ^a | 224 ^a | 393 ^b | 728 ^b | 10.5 | 0.81 | <0.0001 |
| Pore water iron (μg L ⁻¹) | pw | 12,883 ^a | 11,122 ^{ab} | 6,808 ^{abc} | 4,483 ^{bc} | 3,032 ^c | 4.25 | -0.82 | <0.0001 |
| AVS (mg S kg ⁻¹) | sed | 102 ^a | 483 ^{ab} | NA | 826 ^{ab} | 1,413 ^b | 13.8 | 0.77 | <0.0001 |
| pH | pw | 7.57 ^a | 7.52 ^a | 7.55 ^a | 7.75 ^a | 7.81 ^a | 1.03 | 0.39 | =0.03 |
| H ⁺ ion (μmol L ⁻¹) | pw | 0.027 | 0.030 | 0.028 | 0.018 | 0.015 | 1.72 | 0.39 | =0.03 |
| <i>Variables mainly associated with mineralization of organic matter</i> | | | | | | | | | |
| TOC (% dry mass) | sed | 9.26 ^a | 7.90 ^a | 8.18 ^a | 7.17 ^a | 8.22 ^a | 1.29 | -0.34 | =0.065 |
| DIC (mg C L ⁻¹) | sw | 28.9 ^a | 47.2 ^{ab} | 56.3 ^{bc} | 56.7 ^{bc} | 66.3 ^c | 2.30 | 0.94 | <0.0001 |
| DOC (mg C L ⁻¹) | sw | 16.3 ^a | 21.4 ^a | 26.8 ^{bc} | 24.0 ^{abc} | 28.3 ^{bc} | 1.74 | 0.79 | <0.0001 |
| Total N (mg N L ⁻¹) | sw | 1.42 ^a | 1.75 ^a | 2.35 ^{bc} | 2.03 ^{abc} | 2.57 ^{bc} | 1.81 | 0.77 | <0.0001 |
| Ammonia (mg N L ⁻¹) | sw | 0.09 ^a | 0.09 ^a | 0.10 ^a | 0.10 ^a | 0.16 ^a | 1.70 | 0.38 | =0.04 |
| Total P (μg P L ⁻¹) | sw | 13 ^a | 16 ^{ab} | 22 ^{ab} | 21 ^{ab} | 25 ^b | 1.92 | 0.73 | <0.0001 |
| Available P (μg P g ⁻¹ resin) | Resin in sed | 0.34 ^a | 0.40 ^a | 0.59 ^{ab} | 0.92 ^{ab} | 2.56 ^b | 7.45 | 0.86 | <0.0001 |
| Total Hg (ng L ⁻¹) | sw | 1.83 ^a | 2.09 ^a | 3.61 ^{ab} | 3.25 ^{ab} | 4.80 ^b | 2.63 | 0.82 | <0.0001 |
| <i>Variables mainly associated with Hg methylation</i> | | | | | | | | | |
| Methylmercury (ng Hg L ⁻¹) | sw | 0.20 ^a | 0.49 ^{ab} | 1.21 ^b | 1.08 ^b | 1.18 ^b | 5.91 | 0.66 | <0.0001 |
| Inorganic Hg (ng L ⁻¹) | sw | 1.63 ^a | 1.60 ^{ab} | 2.40 ^{abc} | 2.17 ^{bc} | 3.62 ^c | 2.22 | 0.80 | <0.0001 |
| Percent methylmercury | sw | 11% ^a | 23% ^{ab} | 30% ^b | 32% ^b | 23% ^{ab} | 2.90 | 0.45 | =0.02 |

Note. Matrix abbreviations: sw = surface water, pw = pore water, sed = bulk sediment. Averages with superscript letters in common are not significantly different at the 0.05 level.

(Hall et al., 2006). However, the sulfide reoxidation does not reverse the mobilization of the constituents of organic matter (C, N, P, and Hg) or the production of methylmercury (MeHg; see below). Rather, any production of SO₄ from sulfide oxidation creates the potential for additional MSR-driven OM mineralization and Hg methylation (Coleman Wasik et al., 2015; Hansel et al., 2015).

The slope of linear regressions of the C, N, and P in surface water against (SO₄)_{Depl} is an estimate of the increase of that variable in mesocosm surface waters per unit SO₄ reduction (Table 2). The regression slopes provide a basis for estimates of stoichiometric ratios of the constituents mobilized from the sediment solid phase, similar to the calculation that Weston et al. (2006) performed for pore water. The calculation of stoichiometric ratios from the slopes of regressions with (SO₄)_{Depl} is more accurate than calculating ratios from surface water concentrations alone, as the use of slopes accounts for the concentrations of the control (the intercept of the linear regression).

The regression slopes of surface water C versus surface water N, P, and Hg in mesocosms are estimates of the net release of each element relative to that of C (Table 3). These estimates can then be compared to the ratio of these constituents in the primary source material—the sediment—to determine the efficiency of mobilization of sediment N, P, and Hg to surface water, compared to C (Table 3). Although we present efficiency relative to only DOC and only DIC, calculating efficiency relative to the sum of mineralized OM (DOC + DIC) represents the overall net efficiency of mineralization, which ranges from 8% to 38% for the three constituents (Table 3). Although the increases in surface water N, P, and Hg are consistent with the hypothesis that those elements were released to the surface water through sulfate-enhanced mineralization of sediment OM, their lower mobilization efficiencies relative to carbon suggest that other processes were operating to either increase carbon, decrease N, P, and Hg mobilization relative to carbon, and/or increase N, P, and Hg losses. It is likely that some carbon was introduced to the surface waters from sources other than the sediment (e.g., photosynthetic fixation of atmospheric carbon) and that there were losses for N, P, and Hg from the surface water (though adsorption, settling, biological uptake, or atmospheric evasion of N and Hg).

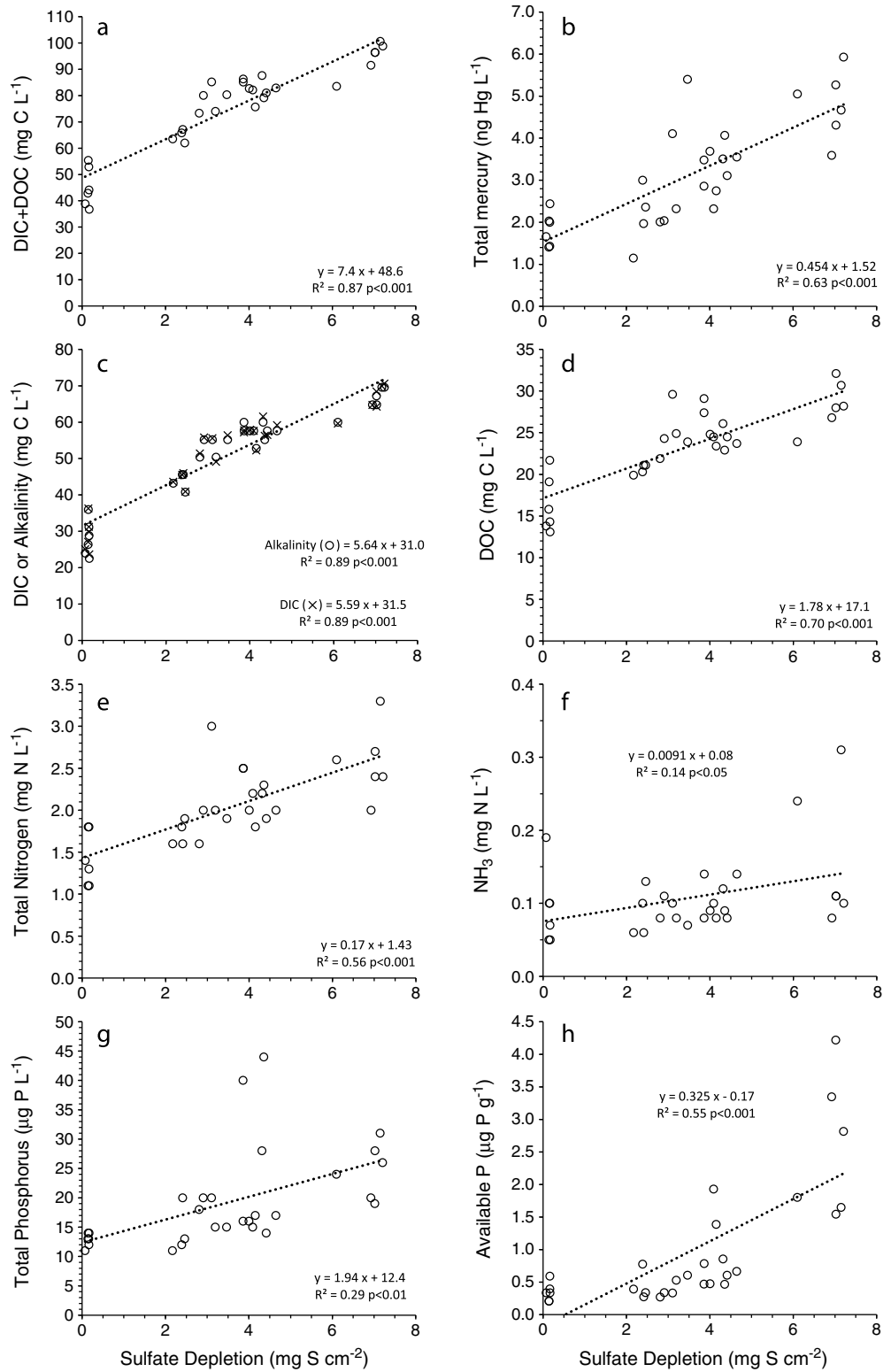


Figure 2. The release of constituents of sedimentary organic matter as a function of SO₄ depletion, showing linear regressions (dotted lines). (a) Sum of surface water DIC and DOC; (b) surface water total mercury; (c) surface water alkalinity and DIC (symbols ○ and ×, respectively; the two regressions are superimposed); (d) surface water DOC; (e) surface water total nitrogen; (f) surface water ammonia; (g) surface water total phosphorus; (h) available phosphate in the sediment, as quantified on ion-exchange resin.

Table 2
Slopes of Regressions of Surface Water Parameters (mM) Against SO_4 Depletion ($mg\ S\ cm^{-2}$)

| Surface water variable (molar basis) | Regression against $(SO_4)_{Depl}$ ($mg\ S\ cm^{-2}$) | | |
|--------------------------------------|---|-------|---------|
| | Slope | R^2 | p |
| DIC | 0.235 | 0.89 | <0.0001 |
| DOC | 0.148 | 0.70 | <0.0001 |
| DIC + DOC | 0.383 | 0.84 | <0.0001 |
| DIC: DOC | 0.044 | 0.56 | <0.0001 |
| TN | 0.0121 | 0.56 | <0.0001 |
| TN: DIC | -0.0028 | 0.25 | <0.01 |
| TN: DOC | 0.0004 | 0.01 | NS |
| TN: DIC + DOC | -0.0006 | 0.08 | NS |
| TP | 6.26E-05 | 0.29 | <0.002 |
| TP: DIC | -7.00E-06 | 0.03 | NS |
| TP: DOC | 7.00E-06 | 0.02 | NS |
| TP: DIC + DOC | -1.00E-07 | 0.00 | NS |
| THg | 2.26E-09 | 0.63 | <0.0001 |
| THg: DIC | 9.00E-06 | 0.46 | <0.0001 |
| THg: DOC | 6.00E-06 | 0.23 | <0.01 |
| THg: DIC + DOC | 2.00E-05 | 0.42 | <0.0001 |

Note. When a sediment constituent's ratio to DIC or DOC has a significant slope against sulfate depletion, it indicates that the constituent was mobilized to the surface water at a significantly different rate than the DIC or DOC.

In addition to increases of TP in the surface water, the sediment pore water in the highest SO_4 treatment contained 7.5-fold greater available phosphate than the controls, as quantified with ion-exchange resin (Table 1 and Figure 2h). In comparison, the increase in surface water TP was only 1.9-fold (Table 1 and Figure 2g). The difference between phosphorus response in the resin and the surface water may be partly due to (a) loss of TP from the surface water after mobilization or (b) irreversible trapping of mobilized P on the resin. If phosphorus is released from sediment en masse in response to an S-induced shift from iron oxides to iron sulfides, the sediment pore water would experience this release first, while release to surface waters would take longer due to diffusion-limited transport and potentially an iron-oxide barrier at the sediment-water (anoxic-oxic) interface.

DIC in surface water is not conservative, being subject to exchange across the air-water interface, carbonate mineral precipitation, and photosynthetic uptake. Surface water pCO_2 in all mesocosms was above saturation with respect to atmospheric equilibrium by a factor of 1.4–15.5 (based on the DIC speciation calculations discussed earlier; data not shown), so the mesocosms were losing, not gaining, C through gas exchange with the atmosphere. The pCO_2 values in the mesocosms are similar to those reported from epilimnia of small, organic-rich, temperate lakes of low to moderate salinity (Cole et al., 1994; Myrbo & Shapley, 2006). With respect to mineral precipitation, based on geochemical equilibrium calculations, surface waters were undersaturated with respect to all carbonate minerals. Thus, although DIC in surface water is subject to several transport and transformation processes, the sustained presence of CO_2 at quantities

significantly above saturation with respect to the atmosphere and the observation of increasing DIC and DOC with increasing $(SO_4)_{Depl}$ (Table 1) provide strong evidence of sulfate-induced increases in net carbon mineralization in the mesocosms.

In addition to the carbon originally present in the sediment, organic carbon was also photosynthetically fixed by wild rice and algae in the mesocosms and subsequently subjected to respiration and some decomposition, adding to the DIC and DOC in surface waters. DOC may also have been released into sediment pore water as an exudate from the wild rice roots (Rothenberg et al., 2014; Windham-Myers et al., 2009). Exudate DOC, however, does not account for the observed increase in DOC, since a negative relationship between the number of wild rice plants and DOC was observed (Spearman's rho = -0.63, $p < 0.001$, Table S2).

4.2. Effects of SO_4 Reduction on Mercury and Methylmercury in Surface Water

We interpret Hg mobilization to the surface water in an analogous manner to C, N, and P, as Hg tends to associate strongly with organic matter in sediment (Feyte et al., 2010). In the mesocosm surface waters,

Table 3
Elemental Ratios in Sediment and Surface Water Across the Range of SO_4 Depletion

| Molar ratio in sediment ^a | Molar ratio in surface water ^b | | | Efficiency of mobilization of sediment N, P, or Hg to surface water, relative to carbon | | |
|--------------------------------------|---|------------|------------|---|------|-----------|
| | DIC | DOC | DOC + DIC | DIC | DOC | DOC + DIC |
| C: N | 12 ^a | 19 | 32 | 63% | 100% | 38% |
| C: P | 463 ^a | 3,752 | 6,118 | 12% | 20% | 8% |
| C: Hg | 1.90E + 07 | 1.04E + 08 | 1.69E + 08 | 18% | 29% | 11% |

Note. Together, the ratios are used to calculate the efficiency of mobilization of the constituents of particulate organic matter into the surface water. ^aSediment data from Hildebrandt, Pastor, and Dewey (2012), a mesocosm study that obtained sediment from the same natural wild rice stand. ^bRegression slopes of C versus N, P, and Hg in mesocosm surface waters; calculations are made based on surface water DIC alone, surface water DOC alone, and the sum of surface water DOC + DIC.

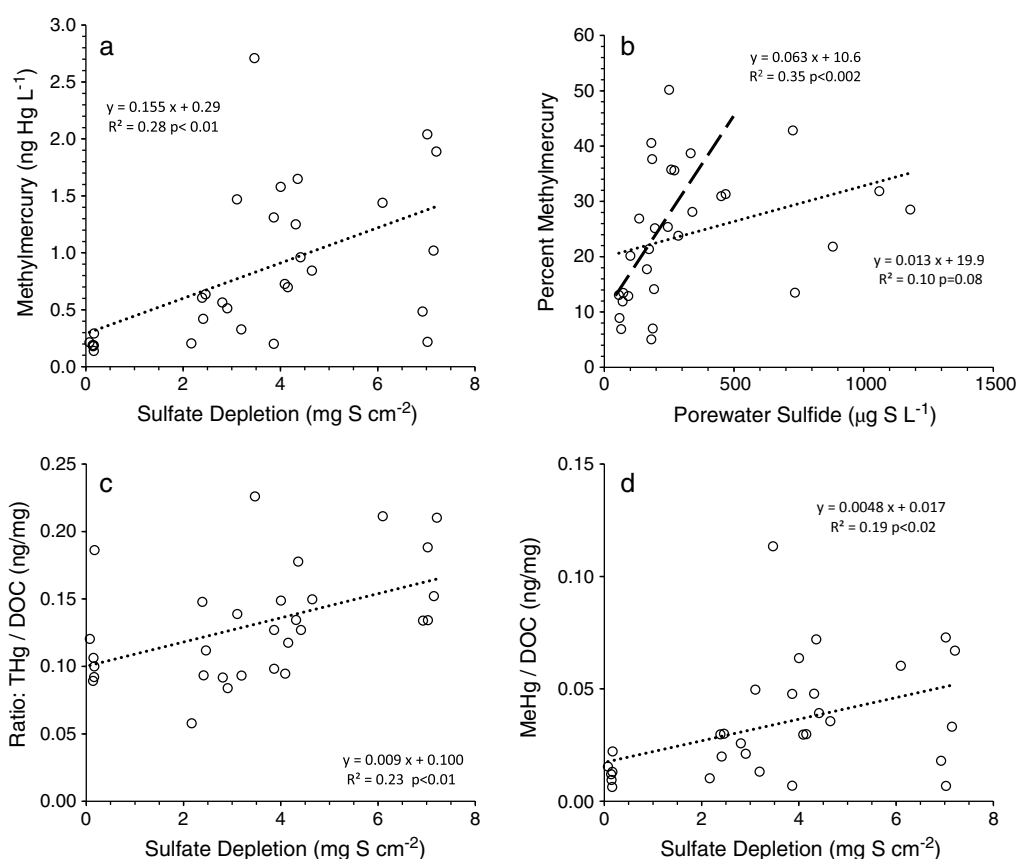


Figure 3. The response of surface water Hg variables to SO_4 depletion and the production of pore water sulfide, showing linear regressions. (a) MeHg as a function of SO_4 depletion; (b) percent MeHg as a function of pore water sulfide, showing regressions for all data (dotted line) and for the subset of data extending only to a pore water sulfide concentration of $468 \mu\text{g S L}^{-1}$ (dashed line); (c) ratio of THg to DOC as a function of SO_4 depletion; (d) ratio of MeHg to DOC as a function of SO_4 depletion.

THg, inorganic Hg (iHg), and MeHg all increased significantly with increased $(\text{SO}_4)_{\text{Depl}}$ (Table 1 and Figures 2b and 3a, $p < 0.0001$) and were greater in the highest sulfate amendment by factors of 2.6, 2.2, and 5.9, respectively (Table 1). The relative increase in THg (2.6-fold) is greater than that for DIC, DOC, TN, and TP, which range from 1.7 to 2.3-fold (Table 1). DOC enhances the solubility of both iHg and MeHg and can facilitate the movement of Hg from sediment into surface water (Ravichandran, 2004). The 5.9-fold increase in MeHg indicates that MeHg flux to surface waters was enhanced by sulfate loading disproportionately more than sedimentary release of THg (2.6-fold) and the increase in surface water DOC (1.7-fold).

The genes required to methylate Hg have been found in a wide variety of anaerobic bacteria, including SO_4 -reducing bacteria, iron-reducing bacteria, and methanogens (Podar et al., 2015). Though some pure culture and experimental evidence exist for mercury methylation by other bacteria, extensive pure culture, experimental, and landscape-scale observations suggest SO_4 -reducing bacteria dominate Hg methylation in many freshwater and marine environments. The relatively large increase in surface water MeHg in response to increased $(\text{SO}_4)_{\text{Depl}}$ in this experiment supports the assumption that MSR was responsible for most of the observed production of MeHg. It is likely that increased SO_4 loading to low- SO_4 aquatic systems with organic sediment will result in increased Hg methylation even though the relative importance of Hg methylation in the environment by different groups of bacteria is still a subject of debate (Paranjape & Hall, 2017).

If movement of DOC from sediment to surface water were the sole mechanism for the Hg increase in surface water, a constant Hg:DOC ratio would be expected on the $(\text{SO}_4)_{\text{Depl}}$ gradient. However, THg:DOC, iHg:DOC, and MeHg:DOC ratios in surface water are all significantly correlated with SO_4 depletion (Table S2 and Figures 3c and 3d). Therefore, all forms of Hg (THg, iHg, and MeHg) increase in surface waters more than

does DOC, indicating that a sulfate-induced enhancement of carbon mineralization may act in combination with either enhanced methylation or an enhanced capacity of DOC to carry Hg. Changes to the binding strength of the DOC in heavily S-impacted mesocosm sediment are possible, as thiol groups on DOC are dominant binding sites for Hg (Skylberg, 2008). The dual role of organic carbon and sulfur in driving both the production of MeHg and the transport of MeHg could be responsible for the substantially larger maximum increase in MeHg:DOC ratio relative to the increase in the THg:DOC ratio (an average 206% increase relative to a 63% increase, Figures 3c and 3d), as postulated by Bailey et al. (2017).

Regnell and Hammar (2004) identified three MSR-driven processes that might cause mobilization of Hg from sediment in a wetland, (1) mineralization of organic matter; (2) extraction of iHg by reduced S compounds, which could be associated with mobilized DOC; and (3) enhanced production of MeHg, which is more mobile than iHg. They argued that enhanced production of MeHg explained THg mobilization in the minerotrophic peat bog that they studied. However, in this study, increases in surface water MeHg concentrations (Figure 3a) are not sufficient to explain the linear increase in THg observed in this experiment (Figure 2b) because most (67%) of the increase is iHg (Table 1). Some of the increase in surface water iHg could be the result of increased production of MeHg that moved to surface water and was subsequently demethylated. Regardless of the underlying mechanism, our observations clearly show increases in surface water Hg that were greater than the increases in C, N, and P (Table 3); this corroborates other studies (Bouchet et al., 2013; Merritt & Amirbahman, 2007; Regnell & Hammar, 2004) that suggest sediment Hg may be synergistically mobilized to surface waters through mineralization, methylation, and enhanced mobility with DOC.

Recent research has shown that in many ecosystems, higher concentrations of pore water sulfide may inhibit MeHg production through either thermodynamically or kinetically controlled reactions with inorganic Hg (Benoit et al., 2003; Hsu-Kim et al., 2013). We plotted %MeHg, rather than the MeHg concentration, against pore water sulfide because we are interested in identifying the pore water sulfide zone of greatest efficiency for the methylation and mobilization of mercury. In this experiment the MSR-driven mineralization of OM released THg to surface water in addition to producing pore water sulfide. Accordingly, because THg is not constant, plotting %MeHg is the most accurate way to identify peak methylation efficiency. In principle, the restricted bioavailability of Hg to methylating bacteria results in a maximum in MeHg production at intermediate concentrations of pore water sulfide. Consistent with previous research in sulfate-impacted freshwater ecosystems (Gilmour et al., 1998; Gilmour, Krabbenhoft, et al., 2007; Gilmour, Orem, et al., 2007; Bailey et al., 2017), MeHg production was most efficient at intermediate sulfide concentrations. In the control, where average sulfide was $69 \mu\text{g S L}^{-1}$, MeHg averaged only 11% of THg in surface waters. In the intermediate SO_4 treatments, which had average sulfide concentrations of 224 and $393 \mu\text{g S L}^{-1}$, MeHg production efficiency peaked significantly higher, at averages of 30% and 32%, respectively (Table 1). %MeHg declined to an average of 23% in the highest SO_4 treatment, which had an average sulfide concentration of $728 \mu\text{g S L}^{-1}$. Given the relatively great scatter in the relationship between %MeHg and sulfide (Figure 3b), it would be most defensible to conclude that the decrease in %MeHg began to occur somewhere between 300 and $700 \mu\text{g S L}^{-1}$. There is a strong positive relationship ($p < 0.001$) between sulfide and %MeHg if the five sulfide concentrations greater than $727 \mu\text{g S L}^{-1}$ are excluded from the regression (which leaves only sulfide concentrations less than $468 \mu\text{g S L}^{-1}$, since there is a gap in sulfide concentrations; Figure 3b). Other studies have identified sulfide zones of peak methylation roughly comparable to that found here. In South Florida, Orem et al. (2011) found that sulfide ranging from 5 to $150 \mu\text{g S L}^{-1}$ did not inhibit methylation but that sulfide concentrations greater than $1,000 \mu\text{g S L}^{-1}$ did. In a subboreal Minnesota wetland enriched in SO_4 from mining discharge, Bailey et al. (2017) found that sulfide concentrations above $\sim 650 \mu\text{g S L}^{-1}$ inhibited methylation.

The relationship between surface water SO_4 and Hg methylation can be strongly affected by site-specific conditions. Because of the variable conversion of SO_4 in surface water to sulfide in pore water—primarily due to differences in OM and Fe availability (Pollman et al., 2017)—researchers have found a broad range in the SO_4 concentration associated with maximum efficiency of Hg methylation. For example, Orem et al. (2014) observed that two different areas in the Everglades Protection Area had peak surface water MeHg concentrations at SO_4 concentrations of 2 and 10–15 mg L^{-1} . In the mesocosms presented here peak surface water %MeHg was observed in the two sulfate treatments that averaged 59 and 93 mg L^{-1} (Table 1).

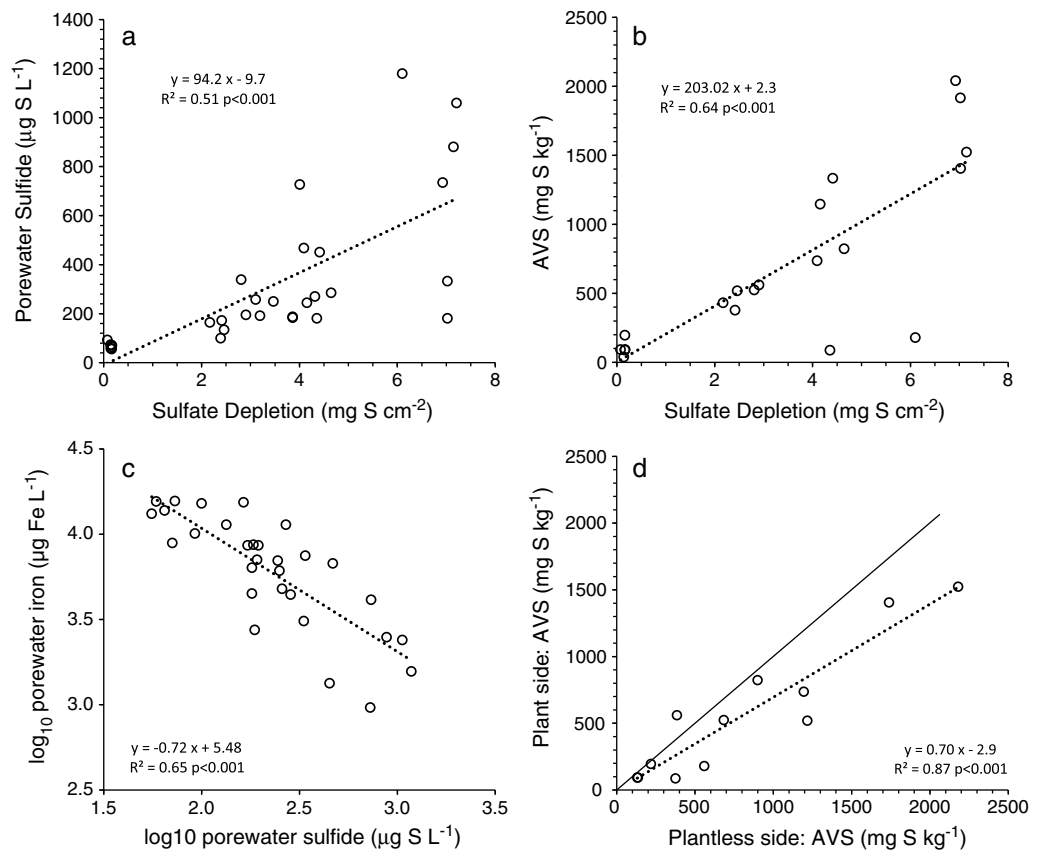


Figure 4. AVS and pore water sulfide, as related to SO_4 depletion, pore water iron, and presence of rooted plants. (a) Pore water sulfide as a function of SO_4 depletion; (b) AVS from the vegetated side of the mesocosms as a function of SO_4 depletion; (c) pore water iron as a function of pore water sulfide; (d) AVS compared between the vegetated side and nonvegetated side. The solid 1:1 line shows that in almost all mesocosms more AVS is found in the side without plants.

4.3. Effects of SO_4 Reduction on Pore Water and Sediment Sulfide

Pore water sulfide increased at higher $(\text{SO}_4)_{\text{Depl}}$, although with greater variance at higher $(\text{SO}_4)_{\text{Depl}}$ (Figure 4a), possibly as a result of variable oxidation of sulfide that may depend on the proximity of the Rhizon sampler to plant roots (Schmidt et al., 2011) or of variable bioturbation by invertebrates (Lawrence et al., 1982). When SO_4 is reduced through MSR, the sulfide produced has a number of nonexclusive potential fates: the sulfide could (1) be oxidized within the sediment; (2) remain in the sediment pore water as free sulfide; (3) diffuse into oxygenated surface water, to be oxidized; (4) react with metals in the sediment, forming insoluble precipitates (dominated by iron-sulfide compounds); or (5) be lost to the atmosphere as H_2S gas or as volatile organic sulfur compounds. Because precipitation reactions are fast relative to redox reactions and diffusion, most of the sulfide probably forms metal precipitates if metals are available. When precipitation dominates the fate of sulfide produced from MSR, the continuous reduction of SO_4 and precipitation of iron sulfides form quasi-steady states between surface water SO_4 and pore water sulfide (Figure S2b) and between pore water sulfide and pore water iron (Figures 3 and 4c). The overall mass of sulfide in the mesocosm sediment, quantified through analysis of AVS (from sediment in the vegetated area), is closely correlated with SO_4 depletion (Figure 4b) even though AVS may not include all the reduced sulfide in sediments. It is likely that most of the AVS in these sediments is present as an FeS precipitate because other metals are at low concentrations in these sediments, which came from a relatively pristine (unpolluted) lake (Fond du Lac Band, 2016; Pastor et al., 2017). Note that there are two mesocosms with especially low AVS concentrations (Figure 4b). It is possible that the AVS in the specific location in these mesocosms where sediment core samples were collected was influenced by

a spatially heterogeneous oxidization process (e.g., root oxygen or benthic invertebrates) that limited the accumulation of sulfide.

AVS was 30% lower in the vegetated side of the mesocosms, suggesting that wild rice released oxygen into the sediment, inhibiting the production of sulfide and/or decreasing sulfide concentrations through oxidation (Figure 4d; Wilcoxon paired test, $p = 0.007$). It is notable that this 30% difference developed in just one growing season, despite the previous 2 years of sulfate treatment. Pore water sulfide showed no statistically significant difference between the two sides owing to high variability within treatments. Numerous investigations have found that rooted aquatic plants release oxygen from their roots, a phenomenon that is usually interpreted as an adaptation to limit the toxicity of reduced chemical species in the pore water, especially sulfide (Lamers et al., 2013). Although oxygen release has been observed in white rice, *Oryza sativa* (Colmer, 2002), it has never been documented in wild rice, which is in the same tribe (Oryzaceae) of grasses as white rice, and also develops aerenchyma (Jorgenson et al., 2013), plant structures that provide a low-resistance internal pathway for movement of oxygen to the roots. Since the growth and reproduction of rooted plants can be inhibited by sulfide (Pastor et al., 2017), there may be a tipping point of exposure to sulfide above which oxygen release is insufficient to mitigate phytotoxic effects, and the plant population declines over time, possibly to extirpation. In this experiment, in the third treatment year, the increase in pore water sulfide was the apparent cause of a decrease in the average number of wild rice stems from 17 in the control mesocosms to 3 in the highest-sulfate treatment mesocosms (Pastor et al., 2017).

4.4. Mesocosms as Models for Ecosystem-Scale Effects of SO_4 Reduction

Although mesocosms, as contained ecosystems, are useful because they mimic ecological and biogeochemical processes that occur in the field, extrapolating findings to nature is challenging when plastic walls have prevented exchange of water and materials (Petersen et al., 2009). These wall-based challenges are manifest in three phenomena in this experiment, (1) relatively long surface water residence times due to the lack of a constant throughflow; (2) the presence of the wall itself, which provides a surface for periphyton; and (3) lack of either overland or groundwater loading of external materials:

1. Relatively long surface water residence times: the increased loading of N, P, C, Hg, and MeHg to the surface water of the mesocosms was readily detected because the lack of hydraulic loading from a watershed minimized dilution and loss through the outflow. The impact of an increase in SO_4 loading on surface water concentrations of N, P, C, Hg, DIC, and DOC would be lower in waters with shorter residence times. For instance, Baker and Brezonik (1988), in modeling increases in alkalinity from atmospheric SO_4 loading, noted that net increases in alkalinity would be most important in waters with long residence times (>5 years) and that there would be little increase in alkalinity in waters with much shorter residence times (<1 year). However, the measured concentrations may not represent the maximum impact of MSR-driven mineralization because the mesocosm wall may enhance removal from the surface water (point number 2, below).
2. Presence of the mesocosm wall: the mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, enhancing the removal of surface water nutrients and Hg to periphyton or inorganic sinks such as iron oxyhydroxides. Natural aquatic systems have less proportional loss to surfaces. The quantitative estimates of internal loading of N, P, and Hg in response to MSR-induced carbon mineralization may have been underestimated by the measured surface water concentrations, given that significant loss of these constituents to periphyton may have occurred. In addition, THg was filtered prior to analysis, which would have removed any Hg associated with phytoplankton or other suspended particles.
3. Lack of either overland or groundwater loading of particulate and dissolved material, specifically iron: the availability of iron in sediment is a primary controller of the fate of MSR-produced sulfide (Pollman et al., 2017). In natural aquatic systems, iron would be supplied at a relatively constant rate from the system's watershed over the long term, although varying in magnitude from watershed to watershed (Maranger et al., 2006; Winter, 2001). This experiment was not an accurate long-term mimic of pore water sulfide concentrations because the external supply of iron was cut off at the inception of the experiment. With no loading of iron, but continued loading of SO_4 , the continued production of sulfide would be expected to eventually consume all available Fe, allowing pore water sulfide levels to exceed those expected in a natural system at equivalent surface water SO_4 concentrations. This mesocosm experiment provides

evidence for just such a result. The experiment continued for 2 years after the 2013 sampling presented here. In the fifth year (August 2015) pore water sulfide was much greater than had been observed in 2013, and disproportionately so in the highest SO_4 treatment, which was most likely to consume available Fe. Between the 2013 and 2015, pore water sulfide increased in the control SO_4 treatment (about $7 \text{ mg SO}_4 \text{ L}^{-1}$) from an average value of $69 \mu\text{g L}^{-1}$ in 2013 to $116 \mu\text{g L}^{-1}$ in 2015, a 68% increase. Pore water sulfide in the highest treatment (nominally $300 \text{ mg SO}_4 \text{ L}^{-1}$, Table 1) increased from an average value of $728 \mu\text{g L}^{-1}$ in 2013 to $9,350 \mu\text{g L}^{-1}$ in 2015, a 1,184% increase (Pastor et al., 2017). In a survey of 108 Minnesota waterbodies with a wide range of surface water sulfate, only two exceeded a pore water sulfide level of $3,200 \mu\text{g L}^{-1}$ (Myrbo et al., 2017).

5. Conclusions

This study demonstrates that increased SO_4 loading to inland waters with organic-rich sediments can significantly increase the decomposition of sedimentary organic matter, which increases internal loading to surface water of the chemical constituents of organic matter, including DIC, DOC, P, N, and Hg. Associated changes include increased production of sulfide and methylmercury and increased alkalinity and pH. Any one of these changes could alone cause significant secondary changes in the structure of an aquatic ecosystem but, taken together, could cause a cascade of primary and secondary environmental changes: increased availability of nutrients (N and P), which can alter dominant plant species, organic carbon production, oxygen consumption, and redox; increased pore water sulfide, which can be toxic to benthic animals and plants; increased MeHg production, which can affect fish and other consumers in the aquatic food web; increased DOC, which can alter light transmission, thermal stratification, and aquatic chemistry; and increased DIC production, which increases alkalinity and pH, affecting aquatic chemistry and biota. Each of these changes resulting from higher surface water SO_4 and consequent increases in MSR has been documented in the literature, but the entire suite of associated changes in aquatic chemistry has not heretofore been demonstrated in an integrated fashion. The degree to which an increase in SO_4 loading affects the ecological structure of the receiving water will depend on the relative increases in N, P, DIC, DOC, Hg, MeHg, pH, and sulfide, which will be a function of background geochemistry and hydrology of the specific system. In this experiment, the changes in these parameters were linearly proportional to SO_4 reduction, which, in turn, was linearly proportional to the time-weighted average SO_4 concentration. The linear responses of the parameters to SO_4 additions suggest that ecologically significant changes may occur even when SO_4 concentrations are elevated only modestly and that dramatic changes may occur with higher sulfate loading.

Acknowledgments

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Sulphide Scavengers in Oil and Gas Industry – A Review

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PROFESSIONAL PAPER

The application of sulphide scavengers is a widely adopted practice in production and processing operations in the Oil and Gas Industry. Particularly challenging is the search and development of new chemistries, which is aimed at safeguarding the health of personnel and maintaining good protection of materials under a variety of conditions whilst being environmentally acceptable. This paper includes an up to date history of the efforts which have been put forth so far in the industry to minimize or eliminate the various problems caused by hydrogen sulphide during Oil and Gas drilling operations by the use of some chemicals. The advantages and disadvantages of using the various chemicals for scavenging hydrogen sulphide drilling fluids and produced fluids are compared.

Key words: sulphide, sulphide scavenger, environmentally acceptable, drilling fluids, produced fluids

1. Introduction

We are fortunate to live in a world where awareness of the detrimental effects of pollution is on the increase. Such awareness will help ensure protection of the biosphere and safeguard the future generations of man and animals especially the aquatic creatures. One of the major industries responsible for all these pollutions is the Oil and Gas industry and also, among the major pollutants caused by this industry during oil and gas production and processing is hydrogen sulphide gas.

2. Description of Hydrogen Sulphide Gas

Hydrogen sulphide is a colourless gas, with an offensive odour and a sweetish taste. It is soluble in water, alcohol, oils, and many other solvents. It has a specific gravity of 1.1895 with reference to air. It is considered a weak acid, it is toxic to humans and corrosive to metals. Hydrogen sulphide can be dangerous to personnel on the surface as it is extremely toxic to human and even animal life, and is extremely corrosive to most metals as it can cause cracking of drill pipe and tubular goods, and destruction of testing tools and wire lines.

The hydrogen sulphide content of fluids in the permeable formations of oil wells has an important impact on the economic value of the produced hydrocarbons and production operations. Typically, the sulphur content of crude oils is in the range 0.3-0.8 weight percent and the hydrogen sulphide content of natural gas is in the range 0.01-0.4 weight percent, although concentrations of hydrogen sulphide in natural gas of up to 30 weight percent have been reported. Several recent reports have claimed a systematic increase in the sulphur content of crude oils over the past 10-20 years and anticipate further significant increases in the concentration of hydrogen sulphide in both oil and natural gas. The correlation between the hydrogen sulphide concentration of produced hydrocarbons from the Norwegian continental shelf and the reservoir temperature; above about 110 °C indicates that the

hydrogen sulphide content of produced hydrocarbons increases exponentially with temperature, while below this temperature the hydrogen sulphide concentration is negligible.¹⁴

When hydrogen sulphide enters the borehole during drilling, completion, or testing for hydrocarbons, it creates several very detrimental problems. These problems are encountered regardless of the source of the hydrogen sulphide. Hydrogen sulphide has created unsolved problems in the oil field.

3. Origin of Hydrogen Sulphide

During oil and gas operations, hydrogen sulphide can be found as a component of formation gases, dissolved in water, hydrocarbons, or even liquid sulphur.¹⁵ Thermal degradation of organic materials and sulphate reducing bacteria (SRB) can create hydrogen sulphide along with other gases.

4. Problems Caused By Hydrogen Sulphide Gas

4.1. Health and Safety of the Personnel

One of the main problems hydrogen sulphide causes is in respect to the health and safety and safety of the personnel.

A maximum of eight hours exposure to concentrations greater than 100 ppm H₂S will cause hemorrhage and death.¹³ Concentrations above 600 ppm can be fatal in three to five minutes. Highly stressed, high strength steel can fail in a matter of minutes in the presence of 50 ppm H₂S. At high pressure, as little as 0.1 ppm H₂S can greatly reduce the time to failure of highly stressed, high strength steel.¹³

Relying solely on its odour is not a good idea because at concentrations above 100 ppm it deadens a person's sense of smell within a few minutes. The pure gas is heavier than air and can collect in low areas such as pit rooms and accommodation.¹⁶ Table 1 shows the poten-

| Concentration (ppm) | Health Effect |
|---------------------|---|
| 0.01 – 0.3 | Odour threshold |
| 1 – 20 | Offensive odour, possible nausea, tearing of the eyes or headaches with prolonged exposure |
| 20 – 50 | Nose, throat and long irritation; digestive upset and loss of appetite; sense of smell starts to become fatigued; acute conjunctivitis may occur (pain, tearing and light sensitivity) |
| 100 – 200 | Severe nose, throat and long irritation; ability to smell odour completely disappears. |
| 250 – 500 | Pulmonary edema (build up of fluid in the lungs) |
| 500 | Severe lung irritation, excitement, headache, dizziness, staggering, sudden collapse (knockdown), unconsciousness and death within a few hours, loss of memory for the period of exposure (results in permanent brain damage if not rescued immediately). |
| 500 – 1000 | Respiratory paralysis, irregular heart beat, collapse and death without rescue. |
| > 1000 | Rapid collapse and death |

tial hazards hydrogen sulphide can cause to the personnel from short-term exposures.¹⁶

To buttressize this, there are many historical cases to document the ill effects of hydrogen sulphide exposure. The documented cases reported in Table 2 indicate that hydrogen sulphide is very toxic and it takes only a matter of minutes to affect unprotected workers. There is a report that some 26 persons died from exposure to hydrogen sulphide in the high-sulphur fields of Wyoming and West Texas between October 1, 1974 and April 28, 1976.⁶

4.2. Effects of hydrogen sulphide on Metals

Hydrogen sulphide mainly cause corrosion problems to drill strings, transport pipes, storage tanks etc. Hydrogen sulphide causes sulphide stress cracking, hydrogen embrittlement and pitting corrosion in oil and gas operations.

The corrosion of iron in the presence of hydrogen sulphide and water is dependent upon the dissociation of the hydrogen-sulphide molecule. Iron is oxidized to the

ferrous form at the anode and hydrogen sulphide undergoes a two-step -dissociation at the cathode. Tung et al²² propose the following equilibrium reactions below.

At the anode:

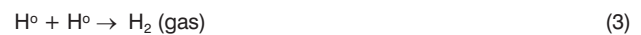


And at the cathode hydrogen is produced and either enters the steel or forms hydrogen gas and bubbles off:

If H₂S is present:



if H₂S is not Present:



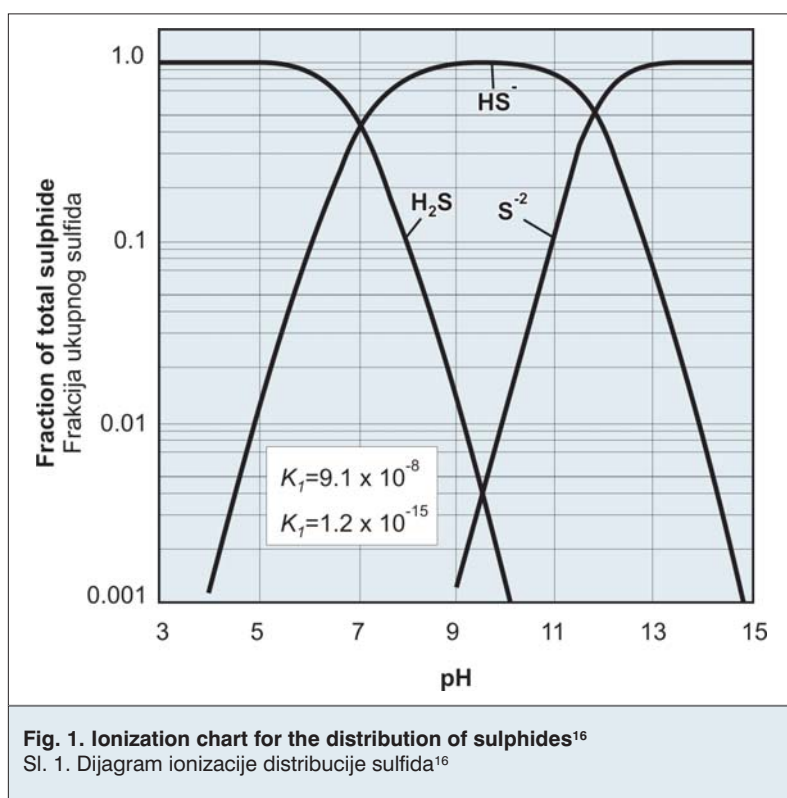
Hydrogen enters the steel first by adsorption onto the water to steel interface and then by being absorbed into the steel as hydrogen. The amount of hydrogen entry depends on the corrosion rate of the steel surface and the concentration of anions such as HS⁻ that reduce the tendency to produce hydrogen gas (as shown in second equation above) and promotes the hydrogen (H⁰) to enter the steel.²²

pH also has an influence on amount of corrosion damage. Basically, the term "sulphides" used in oil and gas operations includes all three water-soluble species, H₂S, HS⁻ and S²⁻, which coexist in a sulphide-water system. Shown in the ionization chart for hydrogen sulphide in figure 1, it can be observed that molecular H₂S predominates in the acidic range, where the pH is below 7. In the range of 7 to 12, monovalent HS⁻ predominates and above pH 12, divalent S²⁻ predominates.

The illustration in figure 1 indicates that the most important practice is that when H₂S enters in alkaline mud, it reacts to form an alkaline sulphide, most often sodium sulphide.¹⁷ While elevated pH is a good way to keep H₂S from causing problems under certain conditions, it does not remove it from the fluid and any drop in pH can create a significant hazard. This then brought about the advent of sulphide scavengers in the history of oil and gas operations.

| No of Subjects | Concentration (ppm) | Duration of Exposure | Effects |
|----------------|---------------------|----------------------|---|
| 1 | 12 000 | - | Death |
| 1 | 2 000 | Less than 20 minutes | Death |
| | 4 000 | - | - |
| 10 | 1 000 | Less than 1 minute | Death 1/10; unconsciousness, abnormal ECG. |
| 342 | 1 000 | Less than 20 minutes | Hospitalization of 320, death of 22 including 13 in hospital, residual nervous system damage in 4. |
| | 2 000 | | |
| 5 | 1 000 | Instant | Unconsciousness, death |
| 4 | 290-540 | - | Unconsciousness |
| 78 | 15-25 | - | Burning eyes in 25, headache in 32, loss of appetite in 31, weight loss in 20, - dizziness in more than 19. |
| 6 500 | 10-15 | 4 - 7 hours | Conjunctivitis |

Source: DHEN (NIOSH), 1977.



The only safe method for the total removal of hydrogen sulphide or soluble sulphides is with a sulphide scavenger.

5. Hydrogen Sulphide Scavenger Technology

According to Garrett *et al*¹¹, the term “sulphide scavenger” refers to any chemical (usually a commercial additive) that can react with one or more sulphide species and can convert them to a more inert form. Effective scavenging is based on attaining an *irreversible* and *complete* chemical reaction between the scavenger and one or more sulphide species. Because a mutual equilibrium exists between the three species in solution, irreversible end complete removal of one species serves to remove all three. Incomplete chemical reaction between a species and the scavenger cannot remove all soluble sulphides present.

Most H₂S scavengers function on a surface adsorption manner or through ionic precipitation. If the scavenger being used is based upon the surface adsorption technique, the mud must be in constant flow to assure that the additive and the sulphides actually collide with one another in order for the necessary reactions to take place. A turbulent flow type of situation would be ideal for this and would assure many random collisions of the two particles. When the scavenger is based on an ionic reaction, properties of the scavenger must be understood to assure that variables such as pH and salinity are conducive to the usage of the additive.

Before a particular scavenger may be selected, a determination of the form of sulphides that exist in a particular mud system must be made.

Under most conditions, sulphides will exist in one of three different forms, depending upon the pH factor of the mud. Refer back to Figure 1. It is interesting to note that the HS⁻ and the S²⁻ sulphide ions result from maintaining a high pH. Most government regulations require that a minimum pH level of 10 be maintained at all times in an H₂S environment.⁶

Popularly, only two types of scavengers are being marketed to the drilling industry currently: (1) zinc – containing chemicals; and (2) iron-oxide, Fe₃O₄. Other commercial chemicals are also available for the job but the aforementioned two dominates in the sulphide scavenging of the oil and gas industry.

In the first premise, it is pertinent to note that several hydrogen sulphide scavengers are being employed in the oil and gas industry. The type of the scavenger needed for a specific field application depends.¹ Some scavengers work best in drilling fluids while some render themselves more readily to sweetening processes in the produced oil and gas. Therefore, the reliability of a sulphide scavenger is best evaluated in a medium where it fits most. In fact, recent studies on sulphide scavenging from drilling fluids particularly are scarce in comparison with similar studies in produced fluids or gas.

For any of the types of scavenger to be employed in the production operations, it has to meet some stringent requirements because the safety of both personnel and equipment depends on it. According to Garrett *et al*¹¹, an ideal scavenger must have the following characteristics:

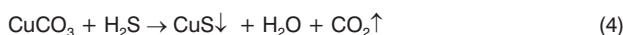
1. Its reaction with sulphide should be complete, rapid, and predictable. The reaction product(s) formed should remain inert under all mud conditions.
2. Scavenging should occur in a wide range of the system's chemical and physical environments. This includes a wide range of pH, temperature, pressure, competitive reactions, shear conditions — all in the presence of an array of active chemicals and solids found in muds.
3. General system performance, e.g. mud rheology, filtration and cake quality should not be impaired by the application of excess scavenging in the system, even at high temperatures.
4. The true amount of scavenger available for reacting in a mud should have the capacity to be measured quickly and easily at the rig-site.
5. The scavenger, as well as its reaction products, should be non-corrosive to metals and materials contacted by the mud.
6. Using a scavenger should not risk the safety and health of personnel or pollute the environment. On the contrary, the scavenger should make drilling in H₂S zones or sweetening processes safer.
7. The scavenger should be widely available and economical for industry acceptance by having a low unit

cost plus high sulphide reaction efficiency under all conditions of use.

6. Hydrogen Sulphide Scavengers Used So Far in the Industry

The first generation of hydrogen sulphide scavengers developed as could be observed from literatures are those to be discussed first.

Copper compounds, especially copper carbonate⁵ have been used in the drilling operations to remove H₂S. Actually if added to water-base muds, the H₂S will precipitated out as insoluble copper sulphide according the following equation:



Even though, tests have shown that the its reaction with sulphide is very fast and efficient, it is impractical to use it as a pre-treatment during drilling operations as copper will plate out on any ferretic material and set up a corrosion cell. There are reports that these scavengers have caused a lot of corrosion problems in production fields due to this fact.⁵

Moreover, it can be concluded that copper compounds can be used to remove hydrogen sulphide where there will be no contact with any ferretic material, for instance if hydrogen sulphide is brought to the surface due to a large influx of the gas.

Hydrogen peroxide has also been suggested as an additive to mud at the flowline to convert hydrogen sulphide to free sulphur according to the reaction:



The chemistry of the reaction is sound but the practical application is very limited as the hydrogen peroxide is too reactive with other components of the system.⁵ Due to this, it will be impossible to have a satisfactory removal of hydrogen sulphide.

Another approach is the addition of zinc-containing chemicals and zinc oxide (ZnO), zinc carbonate (ZnCO₃) and basic zinc carbonate (Zn₅(OH)₆(CO₃)₂) are still being used for the job in the industry.¹⁶ The solubility of ZnO and ZnCO₃ rapidly increase at either high pH or low pH because of the amphoteric nature of zinc compounds but basic zinc carbonate is soluble at both ends of the pH scale.¹¹ If mud pH is higher than about 11 (as is frequently the practice in drilling), zincate ions form, which greatly increase the solubility of the basic zinc carbonate. Zincate ions form because of the abundant OH⁻ combine with the zinc ions.¹¹



Due to this phenomenon, a zinc-base scavenger can dissolve completely in high pH muds giving high concentration of zinc or zincate ions for fast and complete precipitation of the sulphides. This makes the zinc-base scavengers efficient.

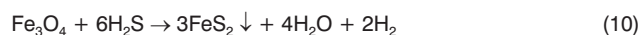
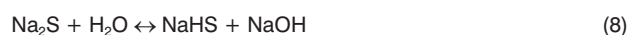
However, moderate to heavy additions of zinc-base chemicals causes detrimental effects to mud rheology, es-

pecially flocculation, and causes fluid loss. This is accentuated especially at high pH.¹¹

Iron compounds were also tested and put to practice up till the current time to remove sulphide during drilling or in the produced fluids. The only inorganic iron-base chemicals currently used as scavengers in drilling and produced fluids are iron oxides, which are insoluble in both water and muds. Reaction mechanisms between iron oxides and soluble sulphides that cause the formation of various iron-sulphur compounds may involve changes in oxidation state, precipitations, or combinations of both.

However, some drilling fluid specialists, especially Alvin³, Ray *et al*²⁰ and Garrett *et al*¹¹ have really researched into the scavenging technology of the iron oxides and they concluded that a synthetic magnetic iron oxide with the trade mark name *ironite sponge* (magnetite Fe₃O₄) is the best among the iron oxides for sulphide scavenging tasks. But, they observed in various tests that low pH speed the reaction of ironite sponge with hydrogen sulphide.

Laboratory tests of sulphide scavengers have used easily handled sodium sulphide crystals instead of highly toxic H₂S gas. In tests where basic sodium sulphide was added to the muds instead of H₂S gas and since iron oxide appears to react only with H₂S, the following reactions was proposed for a solution of sodium sulphide in water.



Since both solution reactions would tend to go to the left at high pH, the small partial concentration of H₂S would explain the observed very low reaction rates of iron oxide with sodium sulphide in muds with pH of more than 8.

The much higher reaction rates observed in iron oxide can be caused by higher pressure and temperature.³

But the fact is that the precipitation of sulphides by the iron oxide is essentially an oxidation-reduction reaction between the gas and the solid, which actually proceeds faster at low pH and slow at high pH.²⁰ This limitation does not allow the ironite sponge to be tagged ideal by the specialists because it is unusual for a mud to have acidic pH ranges.

The search for ideal scavengers continued up to the recent development sulphide scavengers. Some of the findings done in developing/sourcing new scavengers, especially organic compounds and chelates, after the first generation of H₂S scavengers are briefly reviewed below.

Charles and others⁷ observed that chemical techniques applied to oilfield waterflood operations (which are the main cause of biogenic sulphides in the industry) involve basically the use of neutralizers, oxidizers, and scavengers. Historically, neutralizers-such as sodium hydroxide, ammonia, and amines-were used first in an attempt to remove hydrogen sulphide from produced fluids. These materials are inexpedient because the hydrogen

sulphide can be easily regenerated within the oilfield waterflood systems when pH changes. Chemical oxidizers can remove hydrogen sulphide from oilfield waterflood operations but can produce undesirable side effects, such as corrosion and the formation of unwanted solids. Examples of oxidizers include chlorine, chlorine dioxide, hypochlorite, hydrogen peroxide, and thiosulphate.⁷

They also reported that some attempts to use metallic salts and oxides as scavengers in oilfield waterfloods have resulted in the formation of undesirable solid, metallic sulphides. Metallic salt coatings on zeolite filter agents have been suggested as an alternative.

Charles *et al*⁷ compared hydrogen peroxide and some chlorine-containing chemicals with the scavenging ability of acrolein and formaldehyde in the waterflood system. They reached a conclusion thus: "Hydrogen peroxide was able to provide some control, although some concern for concurrent accelerated oxygen corrosion in actual field systems is anticipated. The chlorine-containing chemicals (chlorine dioxide and sodium hypochlorite) appear to remove the hydrogen sulphide effectively in acidic and neutral waters but begin to decline in effectiveness under alkaline conditions. These chlorine-containing materials formed particulates (colloidal sulphur) during these experiments, which can be corrosive."

The final conclusion was that both formaldehyde and acrolein are aldehydes and the best performance of these sulphide scavengers can be seen in surface cleaning operations in the treatment of oilfield water flood systems. Good performance results depend on the nature of the system and the mechanism of the scavenging process. Once, these concepts are identified within a given system, economic judgments can be made.⁷

It can be observed from the above researches that formaldehyde, apart from being used as scavenger in muds and produced fluids and gases, can also be applied in the surface cleaning of waterflood systems.

However, in the case of possible processes based on organic reactants such as acrolein, formaldehydes and others, there were outstanding questions concerning health, safety, and environmental aspects of the reactants and reaction products. For instance, it is established fact that formaldehyde has a limited use in the oil industry because it is carcinogen.¹⁸

It is recommended that more of the aldehyde family should be put into test in order to decide whether some other aldehydes can be used as safe scavengers.

Sitz *et al*²¹ has once worked on amine-aldehyde condensates and they concluded that these condensates are suitably used for hydrogen sulphide removal from the produced fluids to meet the sales specification for the maximum allowable hydrogen sulphide concentration in the gas of 4 ppm. But one of the drawbacks associated with the products is their expensiveness considering the large amount of fluids to be treated.

They highlighted that nitrate solutions, acrolein, caustic scrubbing, formaldehyde and amine-aldehyde condensates are best applied as scavengers (for sweetening purpose) in produced oil, water and gas.²¹

Nasr-El-Din *et al*¹⁸ has also investigated that with pipelines or refinery operations, surface cleaning is the major goal of the operation. It was observed that several different suppression technologies have been developed for surface cleaning operations. Suppression chemicals that contain aldehydes were developed of which most efficient agent is formaldehyde, which reacts stoichiometrically with hydrogen sulphide to produce trithiane, a very insoluble material. But one of the biggest drawbacks associated with these organic chemicals, apart from the fact that they undergo very complex chemical reactions and expensiveness of some them, is their toxicity to humans, for instance the carcinogenic altitude of formaldehyde mentioned previously. Besides, they also observed that these chemicals are best applied in refinery operations such as gas sweetening or other surface cleaning operations.¹⁸

In a US Patent filed by Frenier¹⁰, he investigated that chelating agents such as ammonium salts of ethylenediaminetetraacetic acid (ammoniated-EDTA), hydroxyethylethylenediaminetriacetic acid (ammoniated-HEDTA), and ammoniated-DPTA are all useful as sulphide scavengers up to the temperature of 200 °C but their scavenging ability decrease at temperatures below 100 °C.¹⁰ Summarily, these chelating agents are not suitable as scavengers in drilling operating conditions where there can be temperature of up to 500 °C depending on the depth of the well.

Alan *et al*² observed that triazine based scavenger is well established and effective when applied to oilfield process problems but the drawback is that at temperatures lower than 40 °C, triazine/H₂S scavenging rates become increasingly low. They further investigated that stabilized chlorine dioxide is an efficient scavenger at a wide range of temperatures in produced fluids. But these investigations were carried out in mitigating SRB-generated sulphides in long transport pipelines and storage tanks. They noted that chemical products such as aldehydes, nitrites, peroxides, chelated iron, amines, triazines and chlorine dioxide are efficiently applicable in sweetening processes, i.e., for scavenging sulphides in produced fluids and that the chemicals are not really applicable in drilling operations.

Norman *et al*¹⁹ also observed that although inorganic materials which have been tried as sulphide scavengers include copper compounds, sodium nitrite, hydrogen peroxide, sodium chlorite and chlorine dioxide. All have at least one or two disadvantages. Some compounds are harmful, corrosive or have corrosive by-products, may be unstable or potentially explosive and may require special handling technique. And that the organic scavengers which have been tried include formaldehyde, glutaraldehyde and particularly glyoxal as well as glyoxal/surfactant mixtures. They investigated that none of these materials is particularly satisfactory. They are very slow in reaction with hydrogen sulphide, are unstable at high temperature and pressure and solidify at low temperatures and on long term storage. Some are also expensive, bearing in mind the large volume of hydrocarbon-water mixture to be treated. If a slow reacting hydrogen sulphide scavenger is used, for example glyoxal, it may be necessary either to introduce it at a point which

would allow the maximum reaction time or to substantially increase the dosage.

They further searched for a suitable scavenger which will be stable at extreme conditions of well deepness of about and beyond 8 000 feet (2 438.4 m) and at elevated temperatures of 54 °C and above and elevated pressures of up to 5 200 psi (538 bar) and above. They finally selected ammonium bisulphite and they investigated that the material, but despite its high solubility in water, it works best as a scavenger when the weight ratio of scavenger to hydrogen sulphide is considered. The weight ratio of scavenger to hydrogen sulphide can be as low as 2:1 when long reaction times (e.g. 20 hours) are used, but for shorter time (e.g. minutes) a weight ratio of 7.4:1 or greater is required. Even in trying to achieve satisfactory results, weight ratio of scavenger to sulphide as high as 20:1 has been found.¹⁴ However, careful observations must be made as to whether the rheological properties of a mud will not be upset when larger concentrations of the scavenger are applied. Scavengers generally are required in small in small amounts. As majority of these scavengers are expensive, the proper method for adding them is important both practically and economically.

Also, an ideal scavenger must have a characteristic of instant and complete removal of sulphides in a system and this is contrary to what was observed in ammonium bisulphite tested.

Though in some areas where hydrogen sulphide may be encountered during drilling operations it has become a practice to use oil-base muds, but this seems to provide protection for the metal goods only. The solubility of hydrogen sulphide oil is greater than it is in water and more pressure dependent, therefore more hydrogen sulphide could be carried in an oil mud downhole and release all at once when pressure is removed causing a large volume of free hydrogen sulphide at the surface.⁵ This will potentially cause health hazards to the personnel working on the field. For this reason, application of hydrogen sulphide scavengers is pertinent even when using an oil-base mud for the safety of personnel to be ensured. Garrett *et al*¹² has tested some chemicals to be best applied in oil-base muds. The only concern raised concerning the application of scavengers in oil-base muds is in the aspect of their solubility in the muds.

Garrett *et al*¹² investigated two hydrogen sulphide scavengers (zinc oxide, an inorganic, insoluble, white powdered chemical containing 80 wt% Zn, and zinc naphthenate, an organic, oil-soluble, dark-brown liquid of mixed molecular composition containing approximately 12 wt% Zn) on an oil-based mud.

After the tests, they concluded that the inorganic compound is a good candidate for an H₂S scavenger for oil muds. It is readily available, low in cost, and high in the percentage of zinc. This compound scavenged all the H₂S in a quicker way than the zinc naphthenate by converting the active sulphides into a more inert form and the mud was tested "zero sulphide" after the analysis.

Nonetheless, a more efficient and environmentally benign sulphide scavenger was revealed as Eric⁹ disclosed in a US patent published in 2004 that ferrous gluconate could have an efficient sulphide removal from drilling

fluids. Though Eric performed some tests to investigate the desulphurization effects of the ferrous complex, major tests were performed on knowing the effects of the ferrous gluconate on the rheology of the drilling fluid and concluded that it has no adverse effect on it. The desulphurization showed that the complex has the ability to scavenge hydrogen sulphide efficiently.

Amosa⁴ later carried out more researches on the ferrous gluconate so as to determine the feasibility of sulphide uptake and also corrosion control by the addition of the ferrous gluconate to drilling fluids in hydrogen sulphide bearing wells. Since there has not been any work done yet to represent the realistic rig-site drilling conditions to test the stability of ferrous gluconate (in terms of its H₂S scavenging and corrosion inhibition effects) especially at HTHP conditions, it was felt that a research looking at temperatures and pressures of up to 350 °F (176 °C) and 6 000 psi (413 bar) would be worthwhile so as to examine if the iron complex can be stable, and still retain its expected scavenging and corrosion inhibition properties at such conditions.

The proposed/probable reaction of ferrous gluconate with sulphides is shown in equation 11⁴:



Ferrous gluconate + Sulphide → Ferrous sulphide + gluconate

Amosa⁴ performed tests on the desulphurization effect of the complex at various temperature conditions of 25 °C, 35 °C, 45 °C and 55 °C and agitated for a total time of 140 minutes. Same tests were also carried out on magnetite (*ironite sponge*) for comparison sake. The scavenger concentration to sulphide concentration was in the ratio of 1:1 for all the test conditions. The sulphide content was measured at 20 minutes interval of agitation and it was observed that temperature condition as low as of 25 °C can efficiently aid in the scavenging ability of the complex. It was also observed that the higher the temperature of the medium, the higher the rate of reaction between the scavenger and the sulphide. This was observed throughout the test conditions for both ferrous gluconate and magnetite. The mud was tested zero-sulphide content within 40 minutes of agitation when at 25 °C but the zero-sulphide content was achieved within the first 20 minutes of agitation at temperature of 55 °C (Table 3). The ferrous gluconate was able to scavenge the sulphide at 100% efficiency whereas magnetite's efficiency was only about 30%.⁴

It is an usual practice in drilling fluid technology that a scavenger's corrosion inhibition ability be evaluated so as to observe if the scavenger can assist in mitigating against corrosion of drilling tools. Knowing fully well that hydrogen sulphide is one of the causes of corrosion of drilling equipment, then the normal trend will be that the lower the sulphide content in the mud, the lower the corrosion rate of the drill tools. Various tests were carried out to evaluate the corrosion inhibition efficiency of the ferrous complex at various conditions of temperature and pressure of 150 °F/3000 psi (65.5 °C/206.8 bar), 275 °F/5 000 psi (135 °C/ 344.7 bar), and 350 °F/6 000 psi (176.6 °C/ 413.6 bar).⁴ Same tests were also carried out on magnetite (*ironite sponge*) for comparison sake.

| Time Taken (mins) | Remaining sulphide content with ferrous gluconate at 25 °C (mg/l) | Remaining sulphide content with magnetite at 25 °C (mg/l) | Remaining sulphide content with ferrous gluconate at 55 °C (mg/l) | Remaining sulphide content with magnetite at 55 °C (mg/l) |
|-------------------|---|---|---|---|
| 0 | 700 | 700 | 700 | 700 |
| 20 | 30 | 690 | 0 | 620 |
| 40 | 0 | 620 | 0 | 490 |
| 60 | 0 | 600 | 0 | 440 |
| 80 | 0 | 600 | 0 | 420 |
| 100 | 0 | 600 | 0 | 410 |
| 120 | 0 | 600 | 0 | 410 |
| 140 | 0 | 600 | 0 | 410 |

Source: Amosa (2009)

| Operating Conditions of Temperatures and Pressures | Corrosion Rate (mm/y) | | | |
|--|-----------------------|----------|----------|-----------|
| | pH = 5.5 | pH = 7.5 | pH = 9.5 | pH = 11.5 |
| 150 °F and 3 000 psi | 0.518 | 0.401 | 0.287 | 0.101 |
| 275 °F and 5 000 psi | 1.432 | 1.125 | 0.804 | 0.282 |
| 350 °F and 6 000 psi | 1.887 | 1.462 | 1.046 | 0.364 |

Source: Amosa (2009)

| pH | 5.5 | | | | 7.5 | | | | 9.5 | | | | 11.5 | | | |
|--------------------------|-------------------------|----------|-------------------------|----------|-------------------------|----------|-------------------------|----------|-------------------------|----------|-------------------------|----------|-------------------------|----------|-------------------------|----------|
| | Scav. G | | Scav.M | | Scav.G | | Scav.M | | Scav.G | | Scav.M | | Scav.G | | Scav.M | |
| Inhibitor Concen. (mg/l) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) | Corro-sion Rate (mm/yr) | I.E. (%) |
| 50 | 0.527 | 72.1 | 0.950 | 49.7 | 0.349 | 76.0 | 0.764 | 47.8 | 0.191 | 81.7 | 0.642 | 38.6 | 0.039 | 89.3 | 0.261 | 28.8 |
| 100 | 0.018 | 99.0 | 0.788 | 58.2 | 0.013 | 99.1 | 0.660 | 54.9 | 0.010 | 99.1 | 0.544 | 47.9 | 0.004 | 99.0 | 0.242 | 34.0 |
| 150 | 0.018 | 99.0 | 0.626 | 66.8 | 0.013 | 99.1 | 0.550 | 62.4 | 0.010 | 99.1 | 0.446 | 57.4 | 0.004 | 99.0 | 0.219 | 40.4 |
| 200 | 0.018 | 99.0 | 0.471 | 75.1 | 0.013 | 99.1 | 0.434 | 70.3 | 0.010 | 99.1 | 0.342 | 67.3 | 0.004 | 99.0 | 0.194 | 47.2 |

Higher condition of temperatures and pressures contributed to higher corrosion inhibition efficiencies as observed during the corrosion tests.⁴ Ferrous gluconate exhibited almost 100% corrosion inhibition efficiency as compared to the 47% efficiency exhibited by magnetite at alkaline pH which is best for drilling fluids. It was observed that the corrosion rates were very low and pitting corrosion on the coupons was markedly reduced in the results obtained at operating condition of 350 °F/6 000 psi (176.6 °C/ 413.6 bar) as shown in Table 5 compared to the corrosion rates obtained in the control tests in Table 4. The same trend of results was obtained in the case of other operating conditions of 150 °F/3 000 psi (65.5 °C/206.8 bar) and 275 °F/5 000 psi (135 °C/ 344.7 bar).⁴

The use of ferrous gluconate as scavenger in other media such as crude oil and produced fluid is being investigated.

From the foregoing, it seems the search still continues for an *ideal scavenger* for use in the Oil and Gas industry applications.

7. Conclusions and Recommendations

- Hydrogen sulphide scavengers are employed in more than one field applications like drilling operations and sweetening processes; and the type of scavenger needed for a particular application depends whether it can act better in that medium.
- Although there have been many findings on sulphide scavengers, each one of them has one or more limitations, ranging from attributed exorbitant prices to Health, Safety and Environmental (HSE) problems.
- Ferrous gluconate has been recently found to be efficient and environmentally benign but the laboratory tests still need to be translated into real rig-site operation. It should also be tested in other media other than drill-

ing fluid so as to evaluate its hydrogen sulphide scavenging ability in these media.

- Optimization studies on the reaction between ferrous gluconate and hydrogen sulphide need be done.
- There exist more research needs to develop or source for hydrogen sulphide scavengers, perhaps embodying complementary mixtures of chemicals or a compound specifically investigated to fit the needs and match more closely the qualities of an *ideal scavenger*.
- Thorough testing is necessary for any particular sulphide scavenger sourced or designed so as to know if it has satisfactory scavenging ability in every applications; and if it will not adversely affect the rheology (when used in drilling mud) at various conditions of temperatures and pressures. Otherwise, the researcher on the particular scavenger should state the medium where the scavenger is mostly fit for application.

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