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BIOCHEMICAL INHIBITION OF SULFATE REDUCTION IN BATCH AND CONTINUOUS ANAEROBIC DIGESTERS

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ABSTRACT

Two classes of biochemical inhibitors of sulfate reduction were tested in batch and continuous anaerobic digesters. At high phosphate concentrations, molybdate was an effective and selective inhibitor of sulfate reduction in fed-batch systems, although non-specific effects were observed at lower phosphate concentrations. Transition metal divalent cations were effective and selective inhibitors of sulfate reduction at all conditions tested in fed-batch digesters. In continuous digesters, molybdate inhibited sulfate reduction but also resulted in non-specific inhibition independent of phosphate concentration; transition metals proved to be ineffective in curbing sulfate reduction. Experiments were conducted to explain the non-success of inhibitors in continuous digesters.

KEYWORDS

Sulfate reduction; methanogenesis; inhibition; molybdate; transition metals.

INTRODUCTION

Sulfate is present in many wastewaters due to the pervasive use of sulfuric acid in chemical processing. Sulfate may also be present in certain food and beverage processing wastewaters, such as in those which use or produce molasses. Anaerobic biological treatment of such wastewaters results in the production of hydrogen sulfide via sulfate reduction. Gaseous and dissolved sulfides give rise to a wide range of physically-based problems, including corrosion, odor, and augmenting the chemical oxygen demand (COD) of the liquid effluent (Hamilton, 1985; Sarner et al., 1988).

Sulfate reduction may also cause problems at the microbial level. Dissolved, unionized H_2S has been shown to inhibit methanogenic bacteria (Karhadkar et al., 1987; Isa et al., 1986; Koster et al., 1986; Khan and Trottier, 1978; Hilton and Oleszkiewicz, 1989) as well as sulfate-reducing bacteria (SRB; Hilton and Oleszkiewicz, 1989). In addition, SRB compete with methanogens for available electrons, resulting in a decrease in the methane yield.

These problems associated with sulfate reduction are significant to the extent that many industries are unwilling to consider anaerobic digestion as an option for the treatment of high sulfate-containing wastes. Methods of dealing with sulfate reduction may be classified as either corrective or preventative. Corrective methods allow sulfate reduction to occur and deal with its effects thereafter. Thus the problems of methane yield loss and (in many cases) disposal of sulfur-containing compounds are not addressed.

Prevention of sulfate reduction would alleviate the problem of methane yield loss caused by SRB competition. Sulfate removal via precipitation with barium has been investigated but does not appear practical (King et al., 1975; Lo et al., 1990b). Selective inhibition of SRB would potentially prevent sulfate reduction without affecting digester performance. Two promising classes of potentially SRB-specific biochemical inhibitors have been identified: 1) sulfate analogs such as molybdate, and 2) transition metal divalent cations.

Molybdate is thought to act by depleting the ATP pool in SRB (Taylor and Oremland, 1979). Molybdate in concentrations ranging from 0.2 to 200 mM has been used extensively as a selective inhibitor of SRB to study competition and metabolism of anaerobic consortia found in lake and marine sediments. (See for example Westermann and Ahring, 1987; Dicker and Smith, 1985; Phelps et al., 1985.) Only one group of investigators reported a detrimental effect of molybdate on methanogenic bacteria in sediments (Jones et al., 1982). In wastewater digesters, molybdate was shown to be an effective SRB inhibitor, but non-specific inhibitory effects were reported (Lo et al., 1990a; Gao, 1989; Hilton and Archer, 1988; Quatibi and Boiries, 1988; McKinney et al., 1988; Koepp et al., 1985; Puhakka et al., 1985) in all but one study (Ueki et al., 1988). Though many of the investigators concluded that molybdate was inhibitory to methanogenesis, it is generally unclear whether the observed behavior was the result of a direct inhibitory effect or of a dynamic imbalance resulting from the inhibition and/or organic overloading of higher trophic groups (such as acetogens).

Studies demonstrating the inhibitory effects of transition metals on sulfate reduction are more limited. Koyoma and Sugarawa (1953), Hata (1960) and Capone et al. (1983) showed that transition metals could inhibit sulfate reduction in batch tests, and (with the exception of the Capone et al. study) that the metal-to-iron molar ratio was an important determinant governing the extent of this inhibition. Mountfort and Asher (1979) and Hersch (1989) presented ecological evidence that transition metals resulted in a decline in sulfate reduction. Metals are also inhibitory to methanogens (Muller and Steiner, 1988), but at higher concentrations than required for inhibition of SRB. Nandan and coworkers (1990) showed that inhibition of methanogens by transition metals could be lessened by addition of iron.

The purpose of this study was to determine if sulfate reduction could be specifically inhibited in fed-batch and continuous anaerobic digesters by molybdate or transition metals. It was also intended to understand the process factors affecting inhibition, and the causes and mechanisms of any non-specific inhibitory effects.

MATERIALS AND METHODS

Fed-batch tests were conducted in 73 ml glass serum bottles containing 15 ml of basal reaction mixture. The basal reaction mixture was comprised of the following: 18.5 g/L NaH_2PO_4; 47.8 g/L $Na_2HPO_4.12H_2O$; 667 ml/L granular sludge from upflow anaerobic sludge bed-filter (UBF) digesters described below; inhibitor in concentrations as noted on plots. 0.15 ml of a propionate (the energy substrate) and sulfate stock solution were injected into the test bottles on a daily basis, such that their time-zero concentrations were as follows: 0.37 g/L sodium propionate; 0.37 g/L propionic acid; sulfate (as Na_2SO_4) in concentrations as noted on plots. The daily addition of the propionate/sulfate stock solution ensured that their concentrations were significantly greater than their respective K_S values resulting in zeroth order consumption kinetics. The tests were carried out at a temperature 36 +/- 1°C under N_2/CO_2 (80%/20%). In experiments where phosphate concentrations were lowered, a 131 mM MOPS buffering system was substituted.

1.4 litre (76.2 mm diameter) upflow anaerobic sludge bed-filter (UBF) digesters, operated at 36 +/- 1°C with a minimum recycle ratio of 61.3, were used as the test vehicles for continuous digester experiments. The carbon/energy source used was sucrose (5.00 g/l). Feed sulfate levels used were 0.35, 14.4 and 21.5 mM, and were accomplished by supplementing the basal nutrient system with 0, 2.0, or 3.0 g/L Na_2SO_4 respectively. Low sulfate (0.35 mM) reactors were buffered with 5.0 g/L $NaHCO_3$, and high sulfate (14.4 or 21.5 mM) reactors were buffered with 2.5 g/L $NaHCO_3$. The basal nutrient system used was a completely defined mixture based on the formulations of Parkin et al. (1983), Guiot et al. (1988) and Nel et al. (1985) and contained the following (in mg/L): NH_4Cl, 400; KCl, 400; $Na_2S.9H_2O$, 308; $MgCl_2$, 133; $MgSO_4.7H_2O$, 86.3; $CaCl_2.2H_2O$, 48.6; $(NH_4)_2HPO_4$, 80.0; L-cysteine, 40.0; $FeCl_2.4H_2O$, 33.9; KI, 10.0; $(NaPO_3)_6$, 10.0; $CoCl_2.6H_2O$, 7.33; $Na_2SiO_3.9H_2O$;

1.16; $NiCl_2.6H_2O$, 0.920; $AlCl_3.6H_2O$, 0.910; $MnCl_2.4H_2O$, 0.790; $CuCl_2.2H_2O$, 0.630; NH_4VO_3, 0.500; Na_2SeO_3, 0.500; $Na_2MoO_4.2H_2O$, 0.500; H_3BO, 0.500; $ZnCl_2$, 0.500; $Na_2WO_4.2H_2O$, 0.500.

Pure culture experiments were carried out in 163 ml serum bottles. *Methanobacterium formicicum*, obtained from Michigan Biotechnology Institute, East Lansing, Michigan, was used for these experiments. Cultures were grown on H_2/CO_2 (80%/20%) in a carbonate buffered medium.

Liquid phase samples were analyzed for sugars and organic acids via high performance liquid chromatography (HPLC), using a HPX-87H jacketed column (Biorad Laboratories, Richmond, California) operated at 60°C, and refractive index detection (Knauer-Sonntech, Hillsdale, New Jersey). Gas phase samples were analyzed for methane and hydrogen sulfide with a Perkin Elmer (Pamona, California) Model 900 gas chromatograph, equipped with a HayeSep Q 80/100 mesh column operated at 100°C (Alltech Associates Inc., Deerfield, Illinois). Thermal conductivity detection (150°C) was employed. Iron analysis of liquid and solid samples (centrifuged sludge pellet) was conducted by Galbraith Laboratories (Knoxville, Tennessee).

RESULTS AND DISCUSSION

Fed-Batch Testing

Molybdate added to fed-batch reactors completely and rapidly inhibited sulfate reduction with no adverse effects to methanogenesis over a five day period. (See Figure 1.) Based on the MoO_4-SO_4 competitive inhibition mechanism determined by Taylor and Oremland (1979), it would be expected that the MoO_4/SO_4 ratio (rather than the absolute MoO_4 concentration) would be the primary determinant affecting inhibition of sulfate reduction. This is indeed the case, as shown in Figure 2 for 5-day fed-batch assays. The threshold MoO_4/SO_4 ratio to achieve inhibition was between 1/5 and 1/1. No concentration of molybdate used resulted in any inhibition of methanogenesis at 288 mM phosphate. Subsequent testing demonstrated that molybdate did inhibit methanogenesis when phosphate concentrations were reduced. (See Figure 3.)

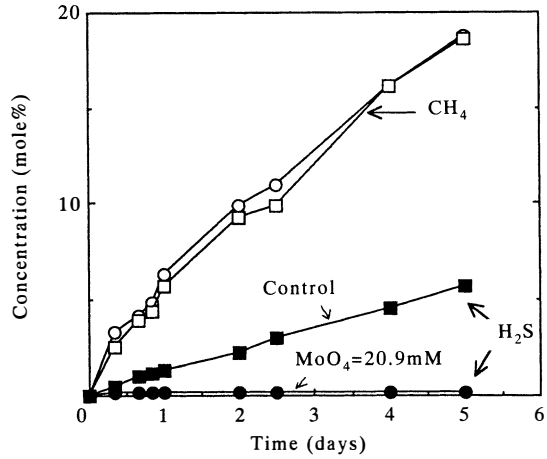

Figure 1. Effect of MoO_4 on SO_4 reduction and methanogenesis. $[SO_4]_{t=0}$ = 4.18 mM

Copper, zinc, manganese, cobalt, nickel and cadmium were also effective inhibitors of sulfate reduction in 5-day fed-batch experiments. Inhibition of methanogenesis was observed, but at higher metal concentrations than required to inhibit sulfate reduction. (See Figure 4 for copper.) In accordance with the findings of Koyoma and Sugarawa (1953) and Hata (1960), the inhibition of sulfate reduction was a function of the metal-to-iron for all metals tested. The metal-to-iron ratio also governed the inhibition of methanogenesis, as observed by Nandan et al. (1990). However, a slight effect of the absolute metal concentration could be observed for very low iron concentrations (0.05 mM). (See Figure 5.)

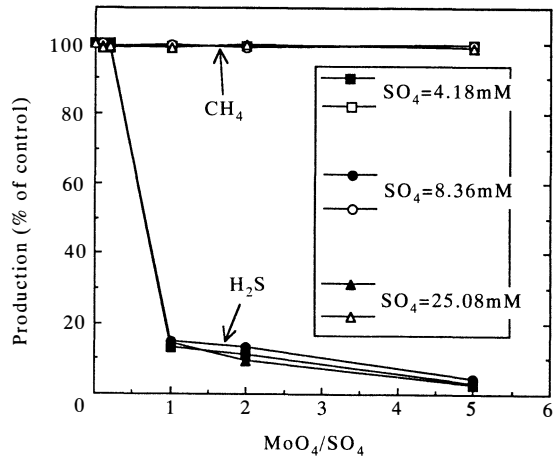

Figure 2. Effect of MoO_4/SO_4 ratio on methane and sulfide production in fed batch digesters.

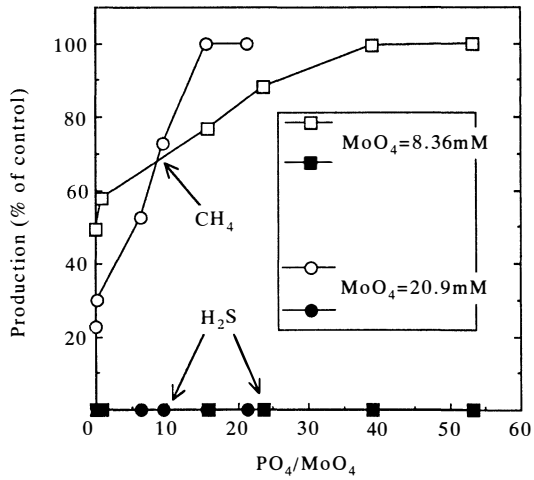

Figure 3. Effect of PO_4 on inhibition by MoO_4 in fed-batch digesters. $[SO_4]_{t=0}$ = 4.18 mM.

Continuous Digester Experiments

Based on the success of the fed-batch experiments, the feasibility of using molybdate as an inhibitor of sulfate reduction in digesters was investigated. Figures 6a and 6b shows the responses of a high (21.5 mM) and a low (0.35 mM) sulfate digester to continuous 1 mM molybdate addition at a 1 day HRT. Sulfide production was nearly completely inhibited in the low sulfate digester, and initially inhibited by about 50% in the high sulfate digester, although some evidence of acclimation was observed. Shortly after the start of molybdate addition, fatty acid concentrations began to increase in both digesters. Propionic acid accumulated initially, followed by acetic and eventually butyric. Methane production was not affected until acids had accumulated to significant levels. These observations suggest that fermentative acetogenic bacteria are more susceptible to non-specific inhibition by molybdate than are methanogens.

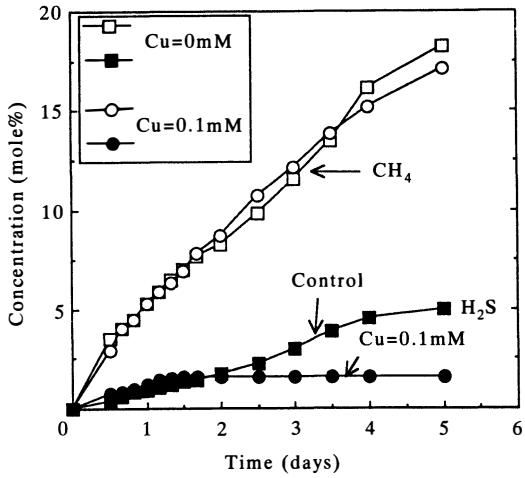

Figure 4. Effect of copper on SO_4 reduction and methanogenesis in fed-batch digesters.

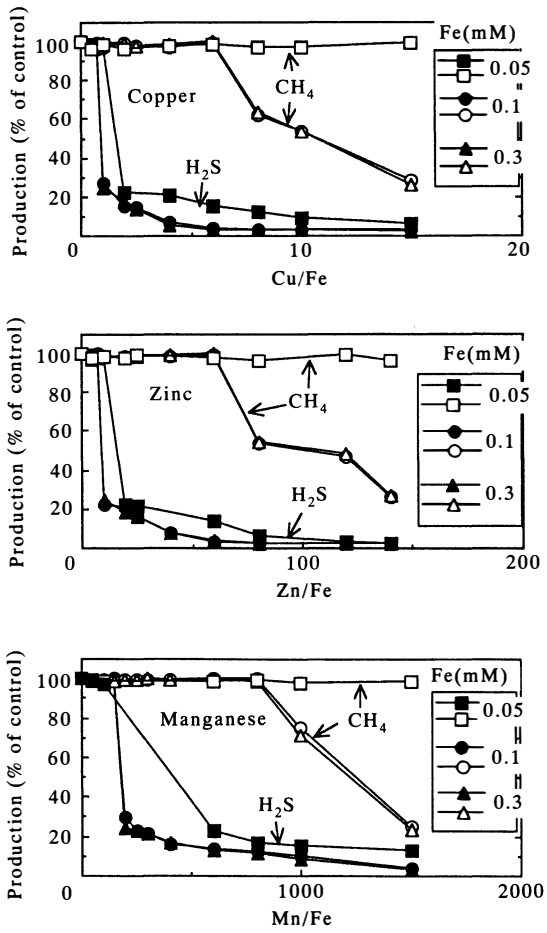

Figure 5. Effect of metal-to-iron ratio on methane and sulfide production for Zn, Cu and Mn.

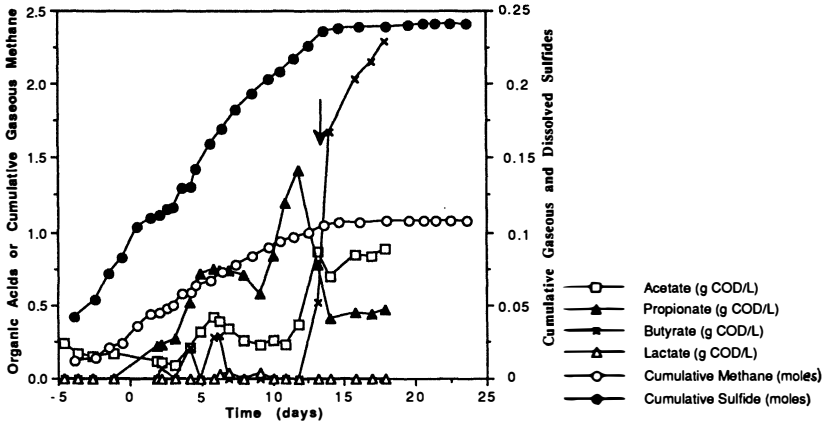

Figure 6a. Continuous MoO_4 addition to a high SO_4 digester. $[SO_4]$ = 21.5 mM; HRT = 1 day; $[MoO_4]$ = 1 mM for t > 0; MoO_4 removed at arrow

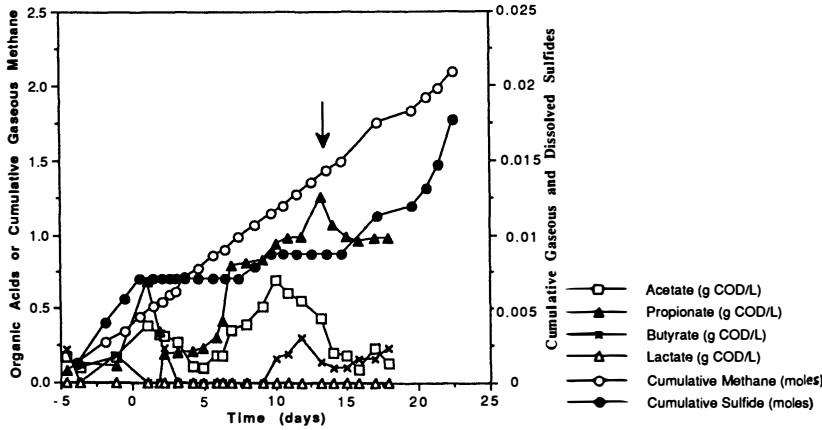

Figure 6b. Continuous MoO_4 addition to a low SO_4 digester. $[SO_4]$ = 0.35 mM; HRT = 1 day; $[MoO_4]$ = 1 mM for t > 0; MoO_4 removed at arrow

Other modes of inhibitor addition were tested: continuous addition at digester start-up (8 day HRT), pulse addition of molybdate, and pulse addition of molybdate coupled with phosphate limitation. In all cases, non-specific inhibitory effects were observed. In light of the fed-batch observation that increasing the phosphate concentration could temper or eliminate non-specific inhibition, experiments were conducted in which the feed phosphate concentration was increased from 0.704 mM to 50 and 350 mM. Whereas the high phosphate concentrations had no effect on digester steady state, molybdate addition resulted in non-specific inhibition for both elevated phosphate concentrations.

Three hypotheses to explain the non-specific inhibitory effect of molybdate were identified:

1) SRB are responsible for a substantial portion of higher fatty acid consumption due to respiratory (ie. sulfate-reducing) metabolism. Thus SRB have substantially displaced syntrophic hydrogen-producing (SHP) acetogens in the sulfate-rich digester environment. Inhibition of SRB by molybdate then results in a dynamic imbalance because the population of SHP acetogens is insufficient to consume acids as fast as they are being produced by the primary fermenters. The resulting acid accumulation then brings about digester failure.

2) In addition to sulfate-reducing acetogenesis, SRB are responsible for a substantial portion of higher fatty acid consumption due to fermentative metabolism (as has been reported by Thiele and Zeikus, 1988). Inhibition of both the fermentative and respiratory metabolisms of SRB by molybdate results in a dynamic imbalance and digester failure, as in Hypothesis 1.

3) Molybdate is generally inhibitory to acidogens, syntrophic hydrogen-producing acetogens and/or methanogens, which would also lead to digester failure.

The observation of higher fatty acid accumulation in the low sulfate digester, in which sulfate reduction was responsible for only a small portion of the total electron flux, suggests that Hypothesis 1 is invalid. To confirm that Hypothesis 1 is invalid, an experiment was designed to inhibit sulfate reduction in the absence of molybdate. The sulfate concentration in a high sulfate digester at steady state (0.75 day HRT) was reduced from 14.4 to 0.35 mM. (See Figure 7.) The sulfide rate production decreased immediately, methane production was slightly enhanced, but no decrease in waste treatment efficiency was observed. This suggests that non-specific inhibitory symptoms observed above are not a result of a dynamic imbalance caused by the shut down of sulfate-reducing acetogenesis (ie. Hypothesis 1 is eliminated). When the sulfate level was reinstated to 14.4 mM more than 40 days after it had been decreased, sulfide levels increased to their previous levels in about 5 to 7 residence times, suggesting that this digester had not lost any of its potential sulfate reducing activity. A high sulfate-containing feed (14.4 mM) was also introduced to a steady state (0.75 day HRT) digester which had been fed a low sulfate concentration (0.35 mM) since its seeding; onset of sulfate reduction was equally fast. These observations are consistent with a high population of SRB in the absense of sulfate, and are necessary but insufficient conditions for the demonstration of Hypothesis 2.

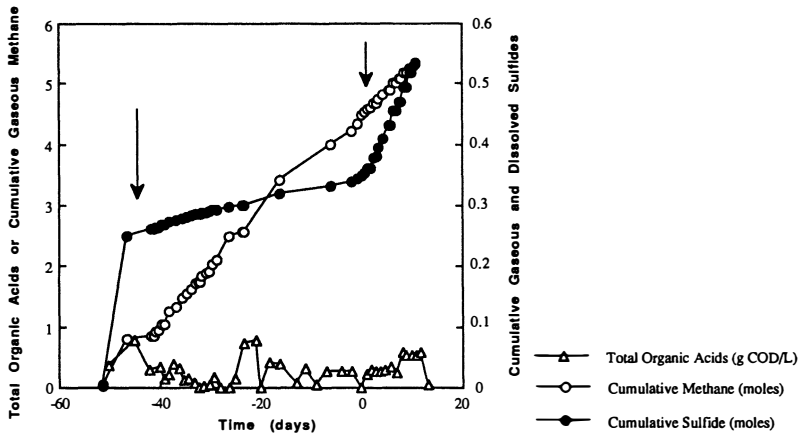

Figure 7. Effect of decreasing SO_4 in feed from 14.4 mM to 0.35 mM (1st arrow), and subsequent reinstatement to 14.4 mM (2nd arrow) in a continuous digester. HRT = 0.75 days.

To give an indication of whether molybdate is directly inhibitory to hydrogenotrophic methanogens, *Methanobacterium formicicum* was grown in fed-batch pure culture in the presence of high sulfate (21.1 mM) and molybdate (7.16 mM). Methane production in two cultures over a ten day incubation period was equivalent to that of two control cultures which contained only basal molybdate. Thus, molybdate is not inhibitory to hydrogenotrophic methanogenesis by (at least) this particular organism, which is consistent with the results of the molybdate addition experiments.

Continuous addition of copper or zinc to digesters had no effect on sulfate reduction, at metal-to-iron concentrations up to 10 times higher than required for inhibition in fed-batch tests. (See Table 1.) Several factors were investigated as potential reasons for the ineffectiveness of metal inhibitors in continuous digesters in light of their success in fed-batch testing. The primary cause for the ineffectiveness of transition metal inhibitors in continuous digesters was apparently iron stores in the sludge bed. The iron concentrations in the sludge beds of high and low sulfate digesters were 351 and 252 mg Fe/L reactor volume respectively. These were more than 100 times greater than the liquid phase iron concentrations (3.2 and 1.4 mg Fe/L reactor volume for high and low sulfate digesters respectively). The result is an overall digester metal-to-iron ratio which is 20 to 40 times lower than one would expect based on the feed composition.

Table 1: Summary of Experiments Evaluating Continuous Addition of Transition Metal Inhibitors to Digesters

Metal	Cu	Cu	Zn	Zn
$[SO4]_{in}$ (mM)	21.5	0.35	14.4	0.35
HRT (d)	1	1	0.75	0.75
Range MIR[1] Used	1-10	1-10	5-75	5-50
Effect on Sulfate Reduction	None	------	None	------
Effect on Treatment Efficiency	None	None	None[2]	Negative[3]

[1]MIR = Metal-to-iron ratio in feed.
[2]Sulfide levels in gas phase decreased, presumably due to precipitation; effluent sulfate levels indicated that 73 to 90% of feed sulfate was reduced.
[3]When Zn/Fe was stepped from 30 to 50, butyrate accumulated immediately, subsequently followed by an acetate build-up.

SUMMARY

Molybdate is effective and selective in fed-batch reactors under conditions of high phosphate, but results in non-specific inhibition in continuous digesters regardless of phosphate concentration. It appears that acetogenesis, rather than methanogenesis, was inhibited. Digester failure accompanying molybdate addition was not the result of a dynamic imbalance due to the absence of sulfate-reducing activity. Transition metals, though effective and selective inhibitors of sulfate reduction in fed-batch, were not effective in continuous digesters. The primary reason for this ineffectiveness appeared to be the presence of very high iron stores in the sludge bed.

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