

# Accumulation and consumption of odorous compounds in feedlot soils under aerobic, fermentative, and anaerobic respiratory conditions<sup>1,2</sup>

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**ABSTRACT:** Agricultural odors present an increasingly difficult challenge to livestock producers, yet very little information is available on the microbiology of odor production or microbial factors that regulate the emission of odors. This study examined the microbial potential for odor production and odor consumption in two soils from a cattle production facility in central Nebraska. The two soils tested were collected from a feedlot pen and a runoff ditch below the pen and contained high- and low-fecal matter content, respectively. These soils were tested for their ability to produce and consume a mixture of VFA and aromatic compounds (phenols and indoles) under aerobic, fermentative, and anaerobic respiratory conditions, with  $\text{NO}_3^-$ , Fe(III), Mn(IV), and  $\text{SO}_4^{2-}$  serving as anaerobic terminal electron acceptors, over a 6-wk incubation. The pen soil had greater ( $P < 0.05$ ) initial total VFA content ( $40 \mu\text{mol/g}$  soil) and produced more VFA during incubation than the feedlot ditch soil, whereas total aromatic compound concentrations were not significantly different between soils. The general pattern of odor compound

accumulation and consumption did not differ between soils. Oxygen and nitrate treatments produced very little VFA and consumed acetate more rapidly than the other treatments, which produced large quantities of short-chain VFA and consumed acetate only after all other VFA were consumed. When VFA and aromatic compound consumption was compared across all the treatments, aerobic incubation proved most effective, and all compounds were rapidly consumed by the second day of incubation. Of the anaerobic treatments examined, nitrate proved most effective, followed by Fe, with VFA consumed by d 5 and 21, respectively. Anaerobic incubation with sulfate produced more VFA than the fermentative incubation, and anaerobic incubation with oxidized Mn produced the largest quantities of VFA, which remained high throughout the six-wk incubation. Aromatic compounds were more easily consumed aerobically and were only slowly consumed in the anaerobic treatments. We conclude from this study that cattle feedlot soils possessed a varying, potentially exploitable capacity for odor consumption when alternate electron acceptors were available.

Key Words: Bacteria, Cattle, Feedlot, Odors, Volatile Fatty Acids

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## Introduction

Livestock odors are produced primarily during the incomplete fermentation of livestock waste by bacteria (Mackie et al., 1998). Fermentation products include VFA, alcohols, aromatic compounds, amides (including  $\text{NH}_3$ ), and sulfides (O'Neill and Phillips, 1992; Hartung and Phillips, 1994). Aromatic compounds and VFA most

closely correlate to odor intensity (Zahn et al., 1997; Zhu et al., 1997; Powers et al., 1999); however, alcohols, amides, and sulfides affect odor character (Barth et al., 1974; Yasuhara, 1980; Yasuhara et al., 1984).

Aerobic bacteria can utilize odorous compounds in livestock waste for energy and carbon. Many odor control technologies (biofilters, bioscrubbers, and biocovers) depend upon their odor-consuming activities, but only a few swine waste studies have examined the microbiology of aerobic odor consumption (Ishaque et al., 1985; Bourque et al., 1987; Chen et al., 1994). These studies demonstrate that indigenous microorganisms, belonging to a wide variety of bacterial groups, can aerobically degrade odorous compounds. Respiratory anaerobic bacteria are capable of utilizing a wide variety of oxidized compounds, including nitrate, oxidized metals, and sulfate. To date, no studies have examined the potential of respiratory anaerobic bacteria to control odorous compounds in agricultural

<sup>1</sup>Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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environments. I hypothesize that the concentration and duration of cattle feedlot odorous compounds can be decreased when alternate electron acceptors are available to feedlot microorganisms.

The objective of this study was to determine the potential of indigenous cattle feedlot microorganisms to produce and consume odorous compounds under a variety of environmental conditions. Feedlot soils were spiked with a mixture of odorous compounds including straight and branched-chain VFA, phenols, and indoles and were incubated under aerobic, anaerobic fermentative, and anaerobic respiratory conditions with  $\text{NO}_3^-$ , Fe(III), Mn(IV), or  $\text{SO}_4^{2-}$ .

## Materials and Methods

### *Feedlot Description, Soil Sampling, and Soil Characterization*

Soil samples were collected at the 6,000-animal-capacity cattle feedlot at the USDA's, U.S. Meat Animal Research Center located in south-central Nebraska. Soil samples were collected in June 1999 from the open-air feedlot at both a feedlot pen (15 × 63 m) that held 26 heifers (high fecal content) and at the drainage ditch immediately down-slope from the pen (low fecal content). These sites were selected to represent areas having either a high input of fresh feces and urine or a low input of more-degraded material with differing microbial activities. Cattle in the pen were fed 203 kg DM/d of a diet that contained 30% alfalfa haylage and 70% corn silage on a DM basis. One month after cattle were removed from the pen, nine soil core samples spanning a depth from 0 to 10 cm below the surface were collected from each site using open-ended 60-mL syringes. The pen sample sites were collected immediately behind the concrete apron, whereas the drainage ditch samples were collected from the bottom of the drainage ditch. Soil cores were immediately capped with rubber stoppers and transported to the laboratory. All subsequent soil manipulations were performed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, MI). To reduce sample-to-sample variability at each site, the nine cores were distributed randomly into three groups of three cores and mixed to form three composites that were subsequently characterized. Dry matter content and OM content of the composites were measured by weight differences upon drying at 100°C overnight and after combusting the sample overnight at 560°C, respectively. Total C and N were measured on the dried composites using a LECO CN-2000 carbon/nitrogen analyzer (LECO Corp., St. Joseph, MI). Soil pH was determined in distilled  $\text{H}_2\text{O}$  slurries (33% wt/vol) using a combination pH electrode and PHM 80 Portable pH meter (Radiometer Analytical, Westlake, OH).

### *Soil Slurry Incubations*

Each composite was blended by a Waring blender (New Hartford, CT) in sterile, anaerobic 1 mM  $\text{KH}_2\text{PO}_4$

buffer (pH 7) to make a 20% (wt/vol) inoculation slurry. Inoculation slurry (5 mL) was allocated to a series of 100-mL serum bottles (500-mL brown serum bottle for  $\text{O}_2$  treatment) containing 45 mL of AT minimal salts medium (Imhoff, 1988) supplemented with trace elements, vitamins, and yeast extract. The AT medium contained (g/L)  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (0.5),  $\text{NH}_4\text{Cl}$  (1.0),  $\text{NaHCO}_3$  (3.0),  $\text{Na}_2\text{SO}_4$  (0.7),  $\text{NaCl}$  (1.0),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.1),  $\text{KH}_2\text{PO}_4$  (1), phenol red (0.001), yeast extract (0.5), trace elements (1 mL/L), and vitamin solution (1 mL/L) at pH 7. The trace element solution contained (mg/L)  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (1,800),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (250),  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  (10),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (10),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (70),  $\text{ZnCl}_2$  (100),  $\text{H}_3\text{BO}_3$  (500),  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (30), and  $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$  (10). The vitamin solution contained (g/L) biotin (0.1), nicotinic acid (0.35), thiamine dichloride (0.3), *p*-aminobenzoic acid (0.2), pyridoxine ( $\text{B}_6$ ) (0.1), Ca-pantothenic acid (0.1), and vitamin  $\text{B}_{12}$  (0.05). Depending upon the treatment, the following alternate electron acceptors were amended to the AT medium before sterilization: nitrate (4.24 g  $\text{NaNO}_3/\text{L}$ ), iron (3.1 g Fe/L as amorphous iron oxides), manganese (0.8 g Mn/L as amorphous manganese oxides), or sulfate (7.1 g  $\text{Na}_2\text{SO}_4/\text{L}$ ). These alternate electron acceptors were selected because microorganisms in a variety of anaerobic terrestrial and aquatic environments utilize these compounds during decomposition (Tiedje, 1988; Widdel, 1988; Lovley, 1991).

After inoculation, the serum bottles of the anaerobic treatments (fermentative, nitrate, iron, manganese, and sulfate) were stoppered, capped, transferred from the anaerobic glove box, and flushed for 60 s with an  $\text{O}_2$ -free mixture of  $\text{N}_2/\text{CO}_2$  (4:1). Aerobic treatments (oxygen and autoclaved), which had air headspaces, were flushed for 60 s with filtered, compressed air and capped. The autoclaved treatment was sterilized for 1 h at 121°C and 1.05 kg/cm<sup>2</sup> in an autoclave before flushing with air and capping. A sterile, anaerobic mixture containing straight-chain VFA (acetate, propionate, butyrate, valerate, hexanoate, heptanoate, and octanoate), branched-chain VFA (isobutyrate, isovalerate, and isohexanoate), phenols (phenol and cresol), and indoles (indole and skatole) was aseptically added to each serum bottle by syringe. The final concentration of odor constituents ranged from 0.1 to 0.5 mM. Anaerobic treatments were incubated and sampled at room temperature in the anaerobic glove box. The oxygen treatment was shaken gently at room temperature to facilitate  $\text{O}_2$  transfer into the slurries. The autoclaved treatment was incubated statically at room temperature in the dark.

At periods ranging from several hours to several days, samples were withdrawn by syringe (18-gauge needle) from well-mixed serum bottles. A portion of the slurry was combined with an equal volume of ethanol to help dissolve precipitated aromatic compounds and was frozen at -20°C for subsequent analysis of VFA and aromatic compounds. Control samples preserved in this fashion could be stored for at least 6 mo and analyzed

with <5% loss of the VFA and aromatic constituents. Depending upon the treatment, a portion of the sample was preserved or analyzed immediately to determine the concentration of oxidized and reduced terminal electron acceptors. Finally, pH was determined in a clarified (centrifugation at  $10,000 \times g$  for 5 min) portion of the sample by ColorpHast pH paper (EM Science, Gibbstown, NJ). Before every other sampling period, an excess volume of the appropriate headspace gas mixture was added to ensure that the headspace pressure never dropped below ambient pressure.

### *Analytical Techniques and Statistical Analyses*

On the day of analysis, ethanol-preserved, frozen samples for VFA and aromatic compound analysis were centrifuged at  $10,000 \times g$  for 5 min. A 0.5-mL portion of the supernatant was combined with an internal standard (ethyl butyrate; 0.25 mM final conc.) and acidified by the addition of 40  $\mu\text{L}$  of 3 M HCl. Two preparations, acidified for VFA analysis and unacidified for aromatic compound analysis, were necessary for the analysis of odorous compounds in the nitrate treatments because aromatic compounds rapidly reacted with nitrate/nitrite under the acidified conditions. Odorous compounds were analyzed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector and a Hewlett Packard 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). Compounds were separated on a 30-m  $\times$  0.32-mm diameter (0.5- $\mu\text{m}$  film thickness) HP-INNOWax (cross-linked polyethylene glycol) column (Agilent Technologies) using the following program parameters: flow rate = 1.9 mL/min, initial temperature = 140°C, initial time = 3 min, temperature ramp = 7.5°C/min, final temperature = 230°C, final time = 4 min. Injector and detector temperature were 250°C. Headspace gases ( $\text{O}_2$ ,  $\text{H}_2$ , and  $\text{CH}_4$ ) were monitored during the incubation using a Varian 3300 gas chromatograph (Walnut Creek, CA) equipped with a thermal conductivity detector (200°C). Typically, 0.5 mL of headspace gas was injected directly onto a Mol Sieve 5A, 80/100 mesh column (3.05-m  $\times$  3-mm, oven temp = 70°C, flow rate = 20 mL  $\text{N}_2$ /min). The identities of sample compounds were confirmed by electron ionization mass spectrometry (EM voltage = 1700; MS Quad = 150°C; MS Source = 230°C; NIST 1998 reference library). The concentrations of odorous compounds and headspace gases was determined relative to the responses of known concentrations of standards.

The concentrations of oxidized and reduced nitrogen-, iron-, manganese-, and sulfur-containing electron acceptors were monitored throughout the course of incubation using standard methods. Nitrite, nitrate, and sulfate were measured using a DX600 ion chromatograph (Dionex Corp., Westmont, IL) equipped with Ion Pac AG14 guard and IonPac AS14 analytical columns and an ED50 electrochemical detector (Dionex). A 3.5 mM  $\text{Na}_2\text{CO}_3$ /1 mM  $\text{NaHCO}_3$  buffer was used as eluent

(1.2 mL/min; 1,100 psi). Reduced iron, Fe(II), was extracted from 1-mL samples of the iron enrichments during a 15-min incubation in 0.5 M HCl as previously described (Lovley and Phillips, 1988). Total iron was reduced to Fe(II) by the addition of 1 mL of conc. HCl, 4 mL of  $\text{H}_2\text{O}$ , and 0.5 mL of 10% (wt/vol) hydroxylamine solution. The iron in both fractions was then quantified using the phenanthroline colorimetric method (APHA, 1980). Reduced manganese [Mn(II)] and oxidized manganese [Mn(IV)] were determined in the manganese enrichments. A reduced manganese fraction was prepared by combining the sample supernatant [soluble Mn(II)] with a 10 mM  $\text{CuSO}_4$  (pH 4) wash of the particulates as described by Kostka and Nealson (1998), which liberated any sorbed or insoluble Mn(II). Manganese(IV) remaining in the pellet after the  $\text{CuSO}_4$  wash was resuspended in 0.5 M NaCl, and a portion was transferred to a fresh tube and quantified. Manganese in each fraction was quantified using the formaldoxime colorimetric method (Kostka and Nealson, 1998). Samples for sulfide and sulfate were collected anaerobically from the sulfate enrichments. Sulfide samples were immediately added to a zinc acetate solution, which precipitated and preserved the sulfide as insoluble zinc sulfide, and stored until analysis. Sulfide content was then determined using the methylene blue method (Kelly and Wood, 1998).

Data were analyzed as a split plot in time. The model was treatment, soil, day, treatment  $\times$  day, treatment  $\times$  soil, soil  $\times$  day, and treatment  $\times$  soil  $\times$  day with treatment, soil, and day as main effects. Treatment and soil were tested with treatment  $\times$  soil as the source of error. The other factors were tested with the residual error. Differences between least-square means were tested with a protected *t*-test. For discussion, responses with probabilities <0.05 were considered to be different. Statistical analyses were tested with the GLM procedure of SAS v. 7.0 (SAS Inst. Inc., Cary, NC).

## Results and Discussion

### *Differences Between Soil Types*

Initial soil composition was very different between the two soils (Table 1). The feedlot pen samples were significantly wetter, and had greater OM content, with correspondingly greater C and N, presumably due to greater fecal content. The pH was also different between soils and elevated (>7.5) for highly organic soils. It is likely that the underlying calcareous subsoil mixed with the feces and urine and helped to buffer any initial acid fermentation at the feedlot pen before sampling. Similarly, the elevated pH drainage ditch samples were composed of the same subsoil washed from the feedlot pen surface. Although not measured initially, the VFA concentrations immediately after adding the stock odorous compounds were greater in the pen samples, whereas no differences ( $P = 0.60$ ) were detected for initial aromatic compound concentration. The initially

**Table 1.** Initial feedlot pen and drainage ditch soil characteristics<sup>a</sup>

Constituent	Feedlot pen	Drainage ditch <sup>b</sup>
Dry matter, %	50.5 ± 1.2	69.5 ± 1.4***
Organic matter, % <sup>c</sup>	32.4 ± 0.3	14.3 ± 0.5***
Total N, % <sup>c</sup>	1.82 ± 0.01	0.83 ± 0.03***
Total C, % <sup>c</sup>	17.2 ± 0.3	7.8 ± 0.3***
pH	8.56 ± 0.03	7.92 ± 0.06***
VFA, μmol/g soil <sup>d</sup>	176 ± 9	135 ± 11*
Aromatics, μmol/g soil <sup>d</sup>	75 ± 6	77 ± 6

<sup>a</sup>Mean and standard error, n = 3.

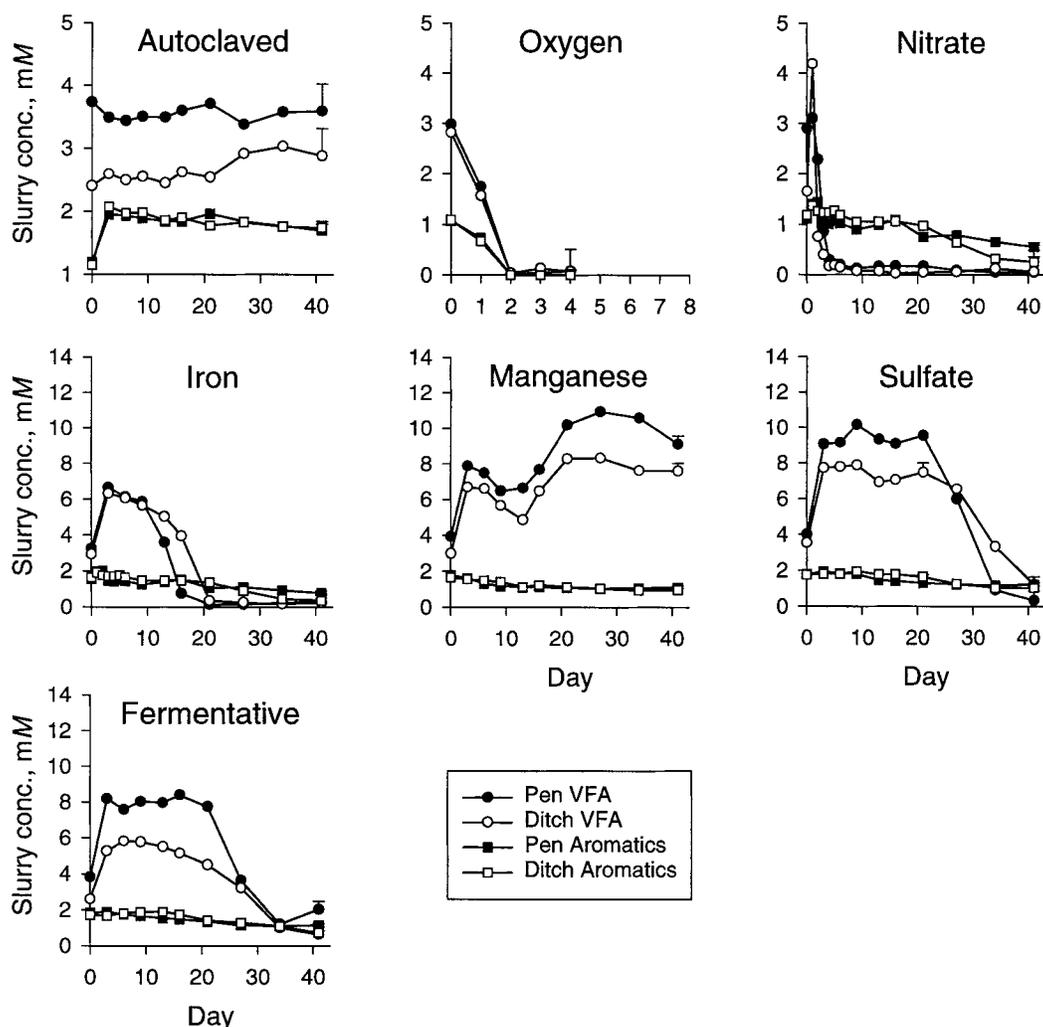
<sup>b</sup>Significant differences between soils are denoted by asterisk (\*  $P < 0.05$ ; \*\*\* $P < 0.001$ ).

<sup>c</sup>Expressed on a dry matter basis.

<sup>d</sup>Initial concentration after odor compound inoculation at time zero. Mean and standard error reported for the treatments, n = 7.

greater VFA concentrations compared with ditch soils are likely attributable to fermentation of fresh feces and urine before the time of collection. Whereas pen soils received animal manure continuously, ditch soils received more mineralized animal manures only rarely, during rainfall run-off events. I presume that, by the time of sampling, aerobic decomposition in the ditch had reduced the concentration of VFA fermentation products in the samples.

The patterns of odorous compound accumulation and consumption were very similar between feedlot pen and ditch soils for each particular treatment (Figure 1). This was contrary to initial expectations that the microbial communities would have very different activities based upon differences in waste inputs (i.e., fresh vs highly mineralized fecal material). All treatments showed a significant increase over initially added VFA except for oxygen (pen and ditch), nitrate (pen only), and auto-claved (pen and ditch), whereas the concentration of



**Figure 1.** Mean concentrations of total VFA and total odorous aromatic compounds for pen and ditch soil samples for each treatment. Standard error of the LS mean (n = 3 for oxygen, pen nitrate, iron, manganese, sulfate [except d-21 ditch], fermentative, and autoclaved samples; n = 2 for ditch nitrate and sulfate d-21 ditch sample) for each compound is reported on the last data point for last day sampled.

**Table 2.** Persistence (in days)<sup>a</sup> of VFA and aromatic compounds for each treatment and soil sample

Treatment and soil	Acetate	Propionate	Branched-chain VFA <sup>b</sup>	Medium-chain VFA <sup>c</sup>	Long-chain VFA <sup>d</sup>	Phenols	Indoles
Oxygen							
Pen	0	2	2	2	2	2	2
Ditch	0	2	2	2	2	2	2
Nitrate							
Pen	2	4	5	4	3	>41	>41
Ditch	2	2	4	3	3	34	>41
Iron							
Pen	21	9	16	13	13	>41	16
Ditch	21	9	16	13	13	>41	34
Manganese							
Pen	>41	9	>41	21	21	>41	>41
Ditch	>41	13	>41	21	21	>41	>41
Sulfate							
Pen	41	13	34	27	21	>41	34
Ditch	>41	21	41	27	21	>41	34
Fermentative							
Pen	>41	13	34	27	21	>41	>41
Ditch	41	21	41	27	21	>41	>41

<sup>a</sup>Compound persistence is the period of time required for concentrations to decrease to value not different from zero.

<sup>b</sup>Branched-chain VFA includes isobutyrate, isovalerate, and isohexanoate.

<sup>c</sup>Medium-chain VFA includes butyrate, valerate, and hexanoate.

<sup>d</sup>Long-chain VFA includes heptanoate and octanoate.

aromatic compounds in the treatments showed only a slow decrease from the initially low level. The maximum VFA concentration during incubation was also greater ( $P < 0.01$ ) in the pen samples than in the ditch samples for the fermentative, sulfate, and manganese treatments. The differences in the maximum VFA concentration indicate that the pen samples, which contained more organic matter, also had more easily degradable organic matter than the ditch samples. This organic matter was converted to VFA during the first few days of incubation, and then the VFA were subsequently consumed.

#### *Trends in Odor Consumption Within Treatments*

There was a wide range in the length of time needed to consume various odorous compounds between the different treatments (Table 2). In the oxygen and nitrate treatments, acetate was consumed first, followed by longer-chain VFA (heptanoate and octanoate), propionate and medium-chain VFA (butyrate, valerate, and hexanoate), then branched-chain VFA (isobutyrate, isovalerate, and isohexanoate), and finally phenols (phenol and cresol) and indoles (indole and skatole). In the iron, manganese, sulfate, and fermentative treatments, propionate was always consumed first, followed by the longer-chain VFA (long-chain VFA, medium-chain VFA, then branch-chain VFA) and indoles. In contrast with oxygen and nitrate, acetate was only consumed at the very end of incubation, and phenols were never totally consumed. Only the oxygen treatment resulted in complete odor compound removal. These results are consistent with earlier studies that investigated odor

consumption in swine wastes, in which researchers found that organisms indigenous to swine waste had the capacity to aerobically remove VFA and phenolic compounds (Ishaque et al., 1985; Bourque et al., 1987; Chen et al., 1994).

A general consistency between all treatments was that VFA was preferentially consumed before aromatic compounds. Based upon the physiology of microorganisms, this is not surprising. Volatile fatty acids are the dominant products of initial acid fermentations (Moat, 1979b), and many facultative or strictly anaerobic microorganisms possess the ability to utilize these substrates. Organisms capable of utilizing alternate anaerobic respiratory pathways (i.e., denitrifiers, metal-reducing organisms, and sulfate-reducing organisms) will compete for many of the short-chain VFA, as they are excellent energy and carbon sources. Anaerobic degradation of aromatic compounds requires specialized pathways necessary for opening up aromatic rings (Berry et al., 1987). Thus, if there are more readily utilizable substrates, such as VFA, microbes prefer them to aromatic compounds. Slow consumption of the indoles in all treatments can be attributed to their uptake for biosynthesis of aromatic amino acids, as these compounds are intermediates in aromatic amino acid biosynthesis (Moat, 1979a).

#### *Differences Between Treatments*

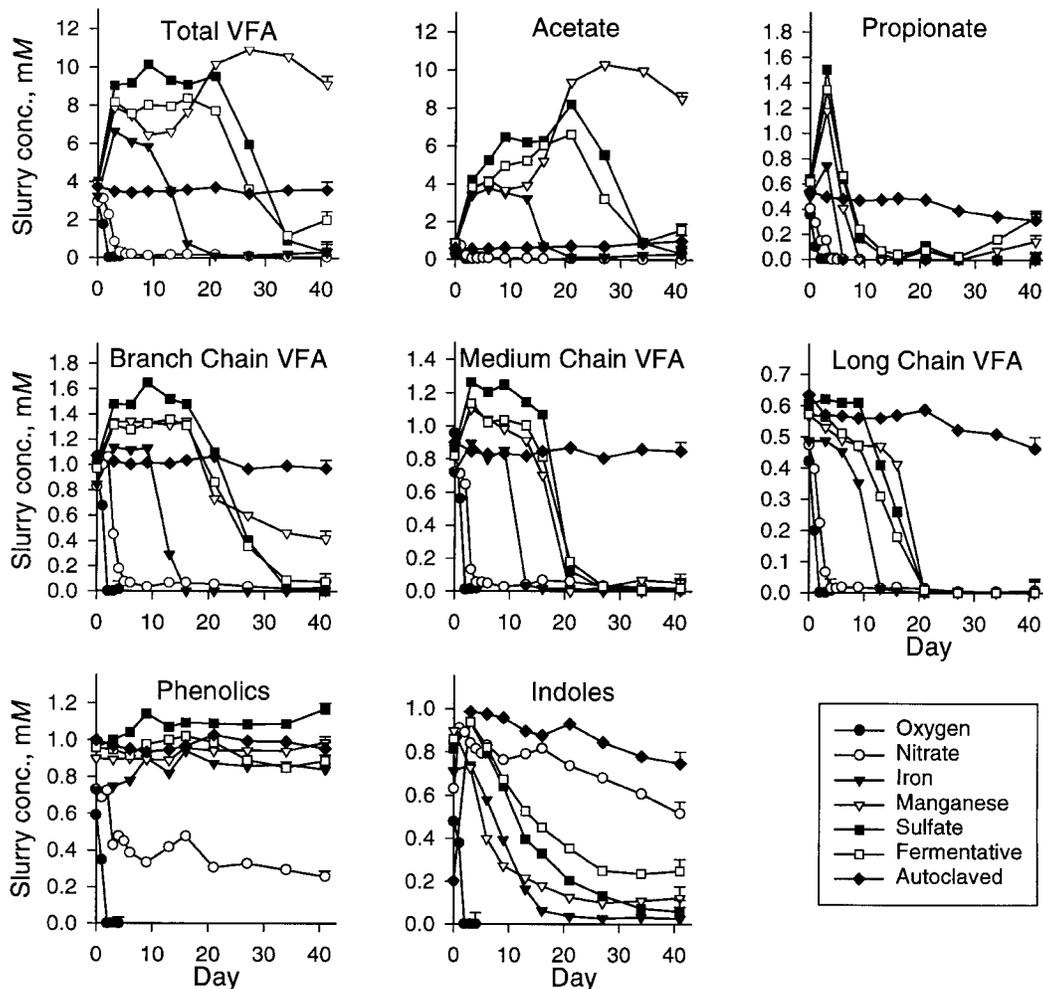
Treatments had a significant effect on both the accumulation of odorous compounds and their persistence in the slurries (Figures 1, 2, 3). In contrast to the oxygen and nitrate treatments, iron, manganese, sulfate, and

fermentative treatments had a large production of acetate, which comprised nearly all of the VFA late in the incubation. Similar results were observed for propionate, branched-chain VFA, and medium-chain VFA: there was no significant increase in these particular VFA for the oxygen and nitrate treatments, but there was a notable increase in these constituents in the iron, manganese, sulfate, and fermentative treatments. Oxygen and nitrate treatments also exhibited an ability to degrade phenolic compounds, whereas the other treatments did not. Long-chain VFA, phenols, or indoles did not accumulate in any of the treatments regardless of the soil sample.

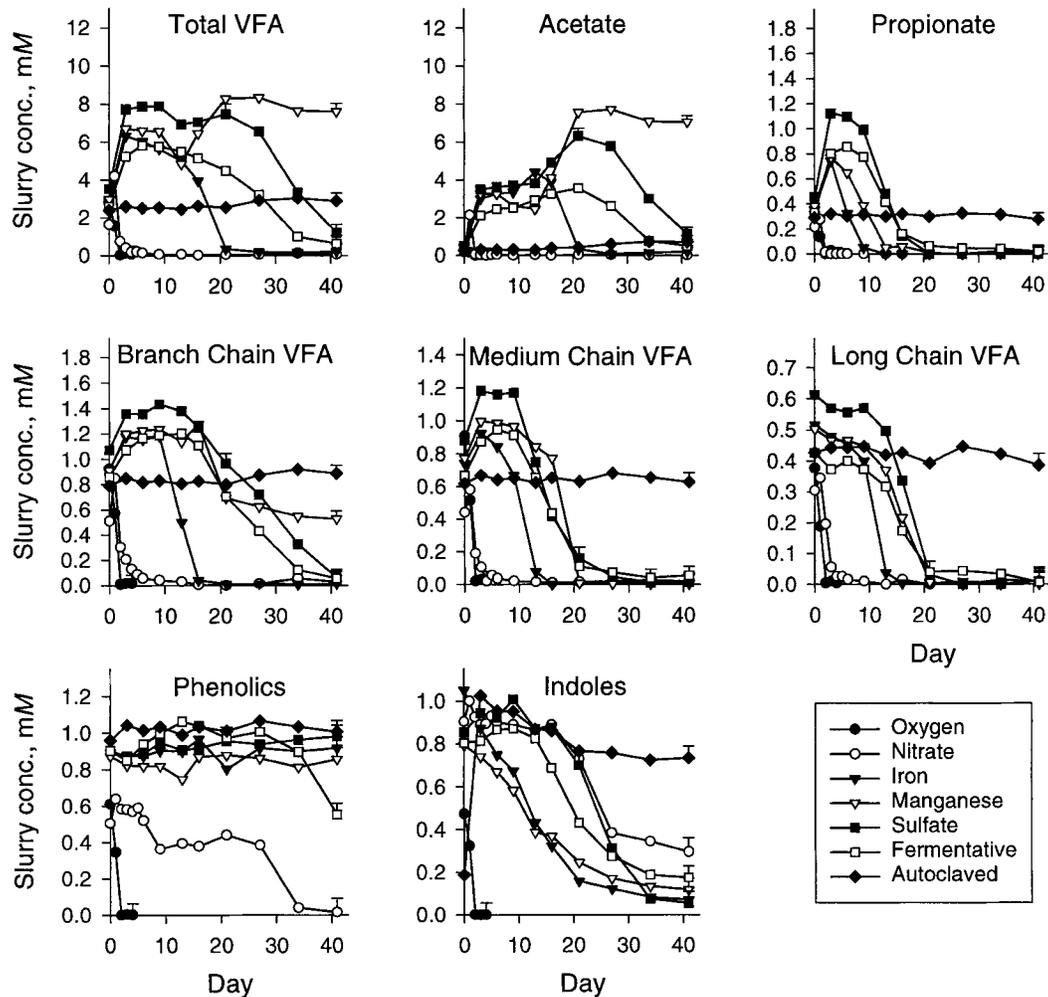
The persistence of individual compounds and groups of compounds was also dependent upon treatment. For the purpose of this study, individual and groups of odorous compounds were considered consumed/removed when the concentration did not differ from zero ( $P > 0.05$ ). All odorous compounds in the oxygen treatment were rapidly removed within 2 d from the slurries (Fig-

ures 2 and 3, filled circles). Similarly, most odorous compounds were removed in the nitrate treatments within 5 d (Figures 2 and 3, open circles). The exceptions in the nitrate treatment were indoles and phenols, showing only a slow loss from the slurries. A closer examination of individual phenols documented that cresol was completely removed within 5 d for both soils, whereas the complete consumption of phenol only occurred in ditch soils (Figure 3, open circles). Pen soils showed only a very slow consumption of phenol when nitrate was available.

The iron treatment proved to be the next most effective, with all VFA and indoles removed within the incubation period (Figures 2 and 3, filled inverted triangles). Individual VFA were rapidly consumed, but some were removed faster than others. Propionate was consumed by d 6, followed by medium-chain VFA, long-chain VFA, and branched-chain VFA consumed by d 13, 13, and 16, respectively. Phenolic compounds showed no decrease in the iron treatments.



**Figure 2.** Accumulation and consumption of various odorous compounds and groups of odorous compounds across different treatments in the cattle feedlot pen soils. Standard error of the LS mean ( $n = 3$  for oxygen, pen nitrate, iron, manganese, sulfate [except d-21 ditch], fermentative, and autoclaved samples;  $n = 2$  for ditch nitrate and sulfate d-21 ditch sample) for each compound is reported on the last data point for last day sampled.



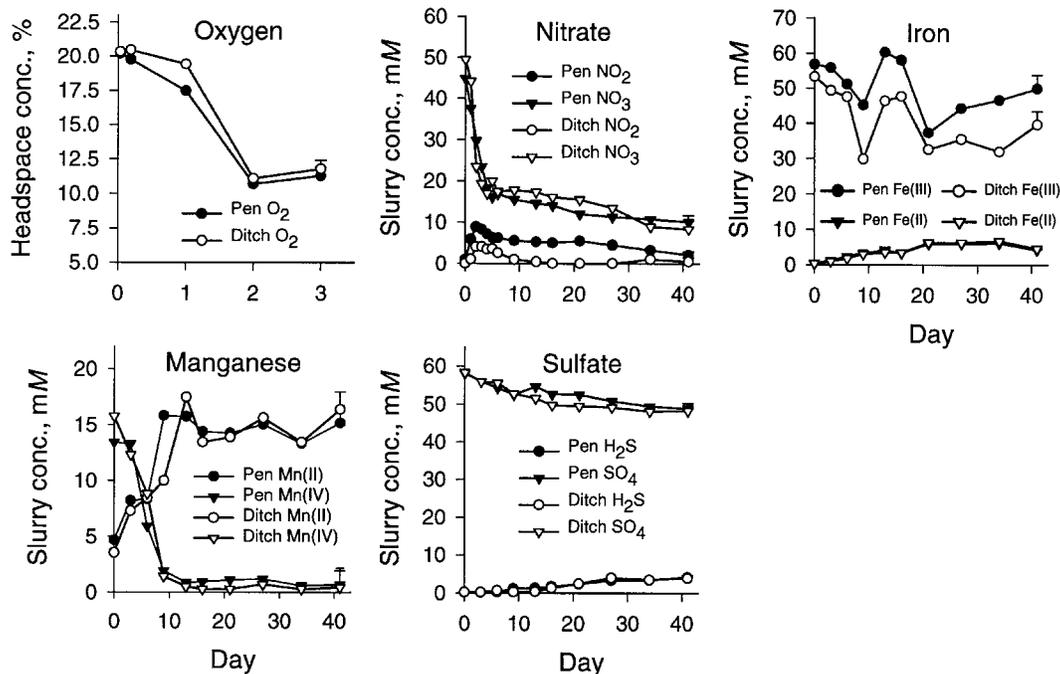
**Figure 3.** Accumulation and consumption of various odorous compounds and groups of odorous compounds across different treatments in the cattle feedlot ditch soils. Standard error of the LS mean ( $n = 3$  for oxygen, pen nitrate, iron, manganese, sulfate [except d-21 ditch], fermentative, and autoclaved samples;  $n = 2$  for ditch nitrate and sulfate d-21 ditch sample) for each compound is reported on the last data point for last day sampled.

The sulfate treatment was only slightly better at removing odorous compounds than the fermentative treatment (Figures 2 and 3, filled squares vs open squares). There were no major differences between the two treatments in the persistence of VFA (Table 2). However, the peak concentrations of VFA were significantly greater in the sulfate than in the fermentative treatment. The sulfate treatment did have a shorter persistence of indole than the fermentative treatment.

The manganese treatment produced a very unusual pattern of VFA accumulation and consumption in both soils tested (Figures 2 and 3, open inverted triangles). During the first 9 d of the manganese treatment, odor production and consumption were similar to the iron treatment, with rapid production followed by a short period when acetate concentrations remained unchanged. However, acetate concentrations that stabilized between d 3 and 9 markedly increased after d 13. These dynamic changes in acetate in the manganese treatments indicate that a secondary fermentation occurred.

The unusual behavior of the manganese treatments is likely attributable to the complete consumption of available oxidized manganese by d 13 (Figure 4). Electron acceptors in all of the other treatments were in excess throughout the course of incubation. The complete consumption of oxidized manganese was quite surprising based upon the stoichiometry of Mn(IV) as an electron acceptor. Organic matter and odor compound decomposition in the manganese treatments consumed a disproportionate amount of electron acceptor (1.2 mmol Mn(IV)/g soil) compared with other electron acceptors; particularly Fe(III), which consumed only 0.3 mmol Fe(III)/g soil. On a molar basis, oxidized iron accepts one electron, whereas oxidized manganese accepts two electrons. It is possible that an abiotic manganese-reducing process may account for the imbalance, but we have no evidence to support or reject this hypothesis.

Fermentative conditions probably best reflect the conditions found in highly organic cattle feedlot pen soils. Inorganic electron acceptors, such as oxygen, ni-



**Figure 4.** Concentration of oxidized and reduced electron acceptors during slurry incubation for pen and ditch soil samples. Standard error of the LS mean for each compound is reported on the last data point for last day sampled.

trate, oxidized metals, and sulfate, are not generally available, and areas of the pen that receive the largest amounts of feces and urine would quickly become anaerobic with the production of large quantities of odorous compounds. A plausible solution to decrease feedlot odors would be to manipulate the microbial environment to minimize the accumulation of odorous compounds in the fresh waste during decomposition and limit the duration of these compounds relative to those accumulated during a strictly fermentative process. Special consideration also needs to be given to ensure that the odorous compounds do not have increased volatility due to lower pH. The oxygen, nitrate, and iron treatments all accumulated significantly less VFA during the incubation compared with the fermentative treatment (Table 3). Furthermore, VFA in these treat-

ments persisted for a shorter period of time. Sulfate and manganese treatments actually accumulated significantly more VFA than the fermentative treatment, and VFA lasted as long as in the fermentative control. The pH of the slurries at peak VFA accumulation and at the end of incubation also favor oxygen and all the alternative electron acceptor treatments compared with the fermentative treatment, which had significantly lower pH (and increased VFA volatility) than the other treatments.

Based upon these findings, the best treatment for cattle feedlot manure would involve the complete aeration of feedlot soils and manures. In practice, this would be impossible to attain because the demand for oxygen outweighs the diffusive flux of atmospheric oxygen into wet feedlot soils and ultimately drives the feedlot sys-

**Table 3.** Effect of electron acceptors on VFA production, persistence, and pH in cattle feedlot pen samples<sup>a</sup>

Treatment	VFA increase $\mu\text{mol/g soil}^b$	VFA increase relative to fermentative treatment, %	Range of VFA persistence, d <sup>c</sup>	pH	
				At peak VFA accumulation	At final sampling
Oxygen	0 ± 0 <sup>v</sup>	0	2	7.4 ± 0.0 <sup>v</sup>	7.6 ± 0.0 <sup>v</sup>
Nitrate	10 ± 16 <sup>v</sup>	4	4–5	7.6 ± 0.1 <sup>vy</sup>	8.0 ± 0.1 <sup>x</sup>
Iron	171 ± 13 <sup>x</sup>	75	16–21	7.4 ± 0.0 <sup>v</sup>	8.1 ± 0.0 <sup>x</sup>
Manganese	350 ± 38 <sup>y</sup>	153	>41	8.1 ± 0.0 <sup>x</sup>	8.3 ± 0.0 <sup>y</sup>
Sulfate	308 ± 14 <sup>y</sup>	134	>41	7.5 ± 0.0 <sup>y</sup>	8.1 ± 0.0 <sup>x</sup>
Fermentative	229 ± 13 <sup>z</sup>	100	>41	6.8 ± 0.0 <sup>z</sup>	7.4 ± 0.0 <sup>z</sup>

<sup>a</sup>Within a column, means without a common superscript letter differ ( $P < 0.05$ ).

<sup>b</sup>VFA increase equals peak VFA accumulation during the incubation minus initial concentration.

<sup>c</sup>Period of time until total VFA concentration  $\leq 0.025$  mM.

tem toward anaerobic conditions. An engineered system to aerate high oxygen-demand soils would be very expensive. The next most effective treatment for feedlot soils/manures would involve stimulating nitrate-linked denitrification of OM and odorous compounds on the feedlot surface. Logistically, this approach would be much easier: all that is needed is periodic nitrate surface application. However, application of nitrate to feedlot soils carries its own set of limitations, including a greater risk of N in ground and surface waters (Ciravolo et al., 1979; Ritter and Chirnside, 1990; Nolan et al., 1997), the need for frequent reapplication, the production of the potent greenhouse gas  $N_2O$ , and the psychological barrier of "fertilizing" manure. Stimulating microbial nitrification, the aerobic conversion of ammonia to nitrate, could fuel denitrification in the anaerobic soils. Unfortunately, this approach would decrease manure nutrient value (manure nitrogen is lost during the denitrification process) and produce  $N_2O$ , a potent greenhouse gas (Rogers and Whitman, 1991).

Given the potential problems of introducing oxygen into feedlot soils, nutrient loss and contamination, and greenhouse gas production associated with nitrate amendment, oxidized iron may be more economically effective and environmentally friendly. Compared with standard fermentation, iron produced lower concentrations of odorous compounds that persisted for a shorter time (Table 3). Organic matter (and odor) decomposition coupled to the reduction of oxidized iron may also limit N losses as available ammonia would be used for the growth of iron-reducing bacteria. Additionally, the production of nitrogenous greenhouse gases would be less than the production associated with large-scale nitrate application. Because reduced iron is rapidly oxidized in air (Ghiorse, 1989), iron can cycle between oxidized to reduced forms repeatedly as it is moved between the aerobic surface and the deeper anaerobic soils by cattle hoof action, as has been observed in benthic communities with active bioturbation (Lovely, 1995). Thus, one application of Fe(III) would last longer than a single application of nitrate, which would be rapidly converted to  $N_2$  gas. Sulfate amendment to feedlot soils would likely increase overall odor due to the formation of highly offensive  $H_2S$  (Figure 4) and similar production of VFA and aromatic compounds compared with the fermentative treatment. Manganese has the potential to be as good as iron amendment to feedlots, but the amount of manganese would need to be much larger based upon the concentrations used in this study.

In conclusion, we found that the addition of alternate electron acceptors to anaerobic feedlot waste incubations had a significant effect on the peak accumulations of odorous compounds and on the persistence of these compounds during decomposition. Different cattle feedlot soils produced the same pattern of odor compound consumption when presented with the same electron acceptors. The addition of oxygen to samples resulted in the quickest removal of odorous compounds with no build-up of VFA during decomposition. Anaerobic

respiration with nitrate and iron also showed promise for minimizing VFA production, limiting the duration of odorous compounds, and controlling VFA volatility during incubation, especially when compared with the fermentative control.

## Implications

Cattle feedlot odors are predominantly of microbial origin and are produced during the fermentative decomposition of animal manures. This research suggests that the amount and persistence of malodorous volatile fatty acids and aromatic compounds produced in anaerobic feedlot soils is dependent upon the quantity and type of electron acceptor present. The application of this concept to feedlot production facilities—providing alternate electron acceptors, such as nitrate or oxidized iron, wherever feedlot soils have become fermentative—may significantly reduce odor. Of course, larger-scale tests at the feedlot-pen level need to be conducted to determine the effectiveness of this proposed management strategy. Similarly, better control of anaerobic microbial processes may also benefit other types of confined animal production operations (i.e., swine) where odor is an issue.

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