RESEARCHREPORT
ENVIRONMENT

Title: Evaluation of Boric Acid and Sodium Tetraborate to Reduce Ammonia and Hydrogen Sulfide Emissions from Swine Facilities - **NPB #04-147**

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Abstract: Boric acid (BA) and sodium tetraborate (STB) were evaluated for their ability to inhibit ammonia and hydrogen sulfide emissions from swine wastewater and manure slurry in in vitro incubations. Addition of either 1% BA or STB inhibited ammonia and hydrogen sulfide emissions by almost 100 % from wastewater and manure slurries over 7 days of incubation. Concentrations of BA and STB as low as 0.0625 % were effective in treating wastewater. Other possible benefits for using boron include a reduction in malodor of the manure slurry, control of enteric pathogens and inhibition of mold growth. Boron, as commercial borax, is an economical treatment, costing about $1.77 per kg. A quantitative risk assessment study should be conducted to evaluate the benefit to risk possibilities for all factors for using boron to treat swine manure slurry.

Introduction: Odorous emissions from swine farms are the most readily detected, yet probably the most difficult to control of environmental pollution problems. Ammonia and hydrogen sulfide emissions have risen sharply, with more intensive livestock farming (Chang et al, 2001). Exposure to high emissions of ammonia and hydrogen sulfide concentrations is a major health issue for humans. Chronic exposure to these gases will also adversely affect the health and performance of pigs. The National Pork Board has identified means of controlling ammonia and hydrogen sulfide production in swine facilities as a research priority area.

One of the key reactions involved in the release of ammonia from swine manure slurry is the hydrolysis of urea by the bacterial enzyme, urease. Urea accounts for more than 50% of the nitrogen in swine manure slurry which is excreted via the urine. Many enteric bacteria possess urease activity, including pathogens such as **Escherichia coli, Actinobacillus pleuropneumoniae, Salmonella, Klebsiella, Clostridium, Streptococcus and Staphylococcus**. Hydrogen sulfide is produced during storage of swine manure slurry by sulfate reducing bacteria (**Desulfovibrio**), which uses the enzyme, hydrogenase to produce hydrogen ions for reducing sulfate to sulfide. Although there are a number of effective treatments to control ammonia and hydrogen sulfide emissions from livestock waste (Rideout and Fan, 2001), many are either too toxic, uneconomical to use, or not environmentally friendly for sustained use.

Boric acid (BA) and sodium tetraborate (STB) have been used for years as an insecticide, fungicide, herbicide, and bacteriocide, but are virtually non-toxic to humans, animals, birds, fish, aquatic invertebrates, beneficial insects and have a low impact on the environment. The LD50 toxicity of borax is about the same as table salt (e.g. 3,000 mg/kg body weight). Boron, as found in BA and STB, is an effective specific inhibitor of bacterial urease and hydrogenase (Hausinger, 1987). To our knowledge, BA and STB have not been studied to control ammonia and hydrogen sulfide emissions from swine facilties.

Objectives:

The **goal** of this project was to determine if boric acid (BA) and sodium tetraborate (STB) are effective in controlling ammonia and hydrogen sulfide emissions from swine facilities. The specific **objectives** of the first year of this project (2004-05) were:

(1) Determine the minimum effective concentration of BA and STB to inhibit ammonia and hydrogen sulfide emissions in waste water and manure slurry from finishing and nursery units.
(2) Determine the frequency of application of BA and STB needed to reduce ammonia and hydrogen sulfide emissions from stored swine manure slurry in a pull plug handling system.

Materials and Methods:

Boric acid and sodium tetraborate. Boric acid (H3B03) was reagent grade (J.T. Baker Co., F.W. 61.83) and sodium tetraborate (Na2B407 ● 10H20) was commercial grade, purchased from a grocery store (20 Mule Team Borax, Dial Corporation, Scottsdale, AZ, F.W. 381.28). Boric acid is a weak acid (Ka = 5.8 X 10 –l0), slightly soluble in water (5.5 g/100 g at 25 C), and contains 17.5 % boron. Sodium tetraborate decahydrate (borax) is very soluble in water and contains 11.35 % boron. Initial studies did not take into account this difference in boron content of BA and STB, but equivalent levels were calculated for all subsequent experiments of this study.

Sources of waste water and swine manure slurries. Waste water from the liquid – solid manure separation handling system of the finishing facility at the MSU Swine Research and Teaching Center was collected in the morning when the system was first flushed. Swine manure slurry containing both urine and feces from the nursery facility was also collected from the MSU Swine Research and Teaching Center. Stored swine manure slurries and waste water (i.e. brown water) from six commercial swine farms were collected and transported to MSU. Waste water and slurries were collected in plastic milk jugs and transported to the Microbiology Laboratory of the Department of Animal Science.

Detection of ammonia and hydrogen sulfide. Ammonia concentrations were measured in the headspace gases of the incubation flasks using Drager ammonia tubes (5-70 ppm, Drager Safety AG & Co., Lubeck, Germany) and a hand held bellows type pump. Hydrogen sulfide concentrations were measured in the headspace gases of the incubation flasks using a Jerome hydrogen sulfide analyzer (model 631-X, Arizona

Instruments LLC, Tempe, AZ) and Drager hydrogen sulfide tubes (2-200 ppm, Drager Safety & Co., Lubeck, Germany).

Experimental protocol. Incubations were made in 2 liter Erlenmeyer flasks, containing a magnetic stirrer bar, covered with a sheet of parafilm paper and sealed with a rubber band. In later incubations, the parafilm paper was replaced with a rubber stopper to minimize gas losses during the incubation and daily opening of the flasks to take necessary ammonia and hydrogen sulfide readings. Initially 800 ml of slurry was incubated, but in later experiments, a volume of 200 ml was found to be sufficient for measuring gas emissions. Flasks were agitated for 3 min with a magnetic stirrer, then momentarily opened for sampling of the headspace gases using either a Drager tube or Jerome hydrogen sulfide analyzer. Incubations were done at room temperature in a fume hood.

Artificially Prepared Swine Manure Slurries. Fresh urine, not contaminated by feces, was collected in the morning, from sows as soon as it was excreted. Fresh feces were recovered from the pens of the same animals. About 300 g of feces was thoroughly mixed in 600 ml of distilled water and a watery slurry (2:1 v/v) was prepared. In 2 liter Erlenmeyer flasks, BA and STB were added at various percentages to the final volume (200 ml). Fresh urine (150 ml) was then added to the flasks and stirred for 3 min. Flasks were then inoculated with 50 ml of the fecal slurry, rubber stoppered, and stirred for an additional 3 min. Zero time ammonia readings were taken with Drager ammonia tubes and at daily intervals for 7 days.

Results:

The results which are being reported are for the first year (2004-05) of a two-year project, which is still ongoing (2005-06).

Inhibition of ammonia emissions from swine manure slurry. The production of ammonia in incubation flasks containing artificially prepared swine manure slurry (combination of urine and feces) with either 0.00, 0.0625 %, 0.125 %, 0.25 %, 0.50 % or l.00 % BA is shown in **Table 1.** and graphically in **Fig 1.** Adding increasing amounts of BA, up to 0.5% in the incubations, delayed the hydrolysis of urea to ammonia by 48 to 144 hours (i.e. 2-6 days) in comparison to the untreated control flask. With addition of 1% BA, there was virtually complete inhibition (> 95 % of control) of ammonia release from urea up to 7 days of incubation (**Fig. 1**).

STB was also effective in inhibiting urea hydrolysis to ammonia as shown in **Table 2**. Addition of 0.00, 0.0625 %, 0.125 %, 0.250 %, 0.50 % and 1.00 % STB to artificially prepared swine manure slurry resulted in a delay of 24 to 120 hours (i.e. 1-4 days) in ammonia release in comparision to the untreated control flask (**Fig. 2**). Similar to BA, STB at an addition of 1 % almost completely inhibited (> 94 % of control) ammonia release from urea in the swine manure slurry for up to 7 days of incubation (**Fig. 2**).

The inhibition of ammonia release by BA and STB treatments is illustrated by the reactions in Drager ammonia tubes used to quantify ammonia emissions in the headspace of incubation flasks (**Photo 1**). These reaction tubes show that over time the inhibitory effect of BA and STB on ammonia emissions decreased as a function of its concentration in the incubation flasks, with the exception of the 1 % level of addition, which remained inhibited even after 7 days (**Photo 1**).

Reduction of odor from swine manure slurry. Although a controlled odor evaluation was not conducted, a clear improvement in the odor of the BA and STB treated swine manure slurry was noted. The odor of the untreated control swine manure slurry had a very pungent, highly objectionable ammoniacal smell, while the 1 % BA and STB treated swine manure slurry was virtually odorless, due to the inhibition of the hydrolysis of urea to ammonia. A similar improvement in the odor of stored swine manure slurry with the inhibition of hydrogen sulfide emissions was also noted.

BA vs STB comparison. When comparing the results of BA and STB on controlling ammonia emissions from swine manure slurry, it would appear that on an equivalent boron basis, STB is slightly more effective than BA. This is suggested by the more effective inhibition at the 0.50 % treatment with STB in comparison to the 0.50 % treatment with BA (**Figs 1 and 2**). STB is more water soluble than BA, and its other chemical properties (i.e. non-ionic detergent) maybe more effective for inhibiting the ureolytic bacteria.

Inhibition of hydrogen sulfide emissions from wastewater and nursery slurry. The inhibition of hydrogen sulfide emissions from wastewater by BA treatment is shown in (**Table 3**). Wastewater from the liquid-solid separation system of the MSU finishing facility was diluted 1:10 v/v with distilled water and 200 ml was incubated with 0.00, 0.0625, 0.125, 0.250, 0.500 and 1.00 % BA for 7days. Concentrations of hydrogen sulfide were relatively low, but increasing amounts of BA inhibited the emission of hydrogen sulfide from the wastewater. With 1 % addition of BA, hydrogen sulfide concentration was almost completely inhibited (**Fig. 3**).

The inhibitory effect of BA was more clearly evident when it was use to treat nursery slurry, which exhibited a higher hydrogen sulfide activity than wastewater due to its higher solids content (**Table 4**). Hydrogen sulfide emission in the untreated nursery slurry control flask peaked at 100 ppm after 96 hours of incubation. Addition of 0.0625% and 0.125 % BA were not effective in controlling hydrogen sulfide emissions up to 120 hours of incubation. In contrast, the addition of 1 % BA almost completely inhibited (99.93 %) hydrogen sulfide emissions from the nursery slurry at 96 hours, with 0.5 % BA showing a slight rebound (30 ppm) in hydrogen sulfide emission after 7 days (**Fig.4**). The observed decrease in hydrogen sulfide concentrations in all flasks after 120 hours could be due to loss of the gas, with the opening and closing of the flasks to take readings with the Jerome analyzer.

The inhibition of hydrogen sulfide emissions from wastewater by STB treatment is shown in (**Table 5**). Wastewater from the liquid – solid separation system of the MSU finishing facility was diluted 1:10 v/v with distilled water and 200 ml was incubated with 0.00, 0.0625, 0.125, 0.250, 0.500 and 1.00 % STB for 7 days. Hydrogen sulfide emissions in the untreated wastewater control flask peaked at 5 days at 38 ppm. All treatments with STB, even as low as 0.0625 %, substantially decreased hydrogen sulfide emissions in wastewater by 24 hours of incubation and emission levels remained low for up to 7 days (**Fig. 5**).

The inhibitory effect of STB was more clearly evident when it was used to treat nursery slurry which exhibited a higher hydrogen sulfide activity due to its higher solids content (**Table 6**). Hydrogen sulfide emission peaked in the untreated nursery slurry control flask at 140 ppm after 96 hours of incubation. In contrast, the addition of 1 % STB inhibited hydrogen sulfide emissions by 99.96 % (0.06 ppm) after 96 hours of incubation, and hydrogen sulfide emission levels remained low for up to 7 days of incubation (**Fig. 6**).

Evaluation of commercial farm wastewater and slurries. Wastewater and slurries were collected from six commercial swine operations (designated as **B,C, PS, RN, BS and BF**). **B** was from a large earthen storage used for a finishing operation. **C** was from a secondary earthen storage for a farrowing unit. **PS** was wastewater (e.g. grey water) collected from a storage tank which was being recycled from a earthen storage facility to flush under slats. **RN** was wastewater (e.g. black water, some solids) collected from shallow pits of a pull plug system to an earthen storage facility. **BS** was slurry (e.g. very thick sludge, high solids content) collected from a deep concrete storage pit for storage of manure from gestation and farrowing facility. **BN** was slurry (e.g. black water, some solids) collected from a pit under a modified open front building for growing-finishing pigs.

All of the collected commercial samples were very low in ammonia production due to their longer length of storage. There was considerable variation in the batches of waterwater and swine manure slurries collected from commercial swine farms with respect to hydrogen sulfide activity. Some slurries (e.g. high solids content) showed very high (> 50 ppm) hydrogen sulfide emissions (**Appendices, Experiments 10,11,12**), while others, especially recycled wastewater showed virtually no (< 1 ppm) hydrogen sulfide emissions (**Appendices, Experiments 9,10**). It took several days of incubation before some of the slurries produced any hydrogen sulfide, suggesting that the sulfate reducing bacteria had to adjust to the incubation conditions before resuming their activity (**Appendices, Experiment 10).**

Similar to the results obtained with the wastewater from the liquid-solid manure separation system of the finishing facility and manure slurry from the nursery facility of the MSU Swine Research and Teaching Center, addition of increasing amounts of BA and STB to commercial wastewater and manure slurries substantially decreased hydrogen sulfide emissions.

Microbiological Studies. The effect of BA and STB in inhibiting the growth of total coliforms, ureolytic and sulfate reducing bacteria was determined. One ml of slurry was removed from incubation flasks treated with varying amounts of BA and STB, and serially diluted in EC broth for the determination of total coliforms. The inoculated test tubes were incubated at 37 C, and monitored daily for growth for one week. Treatment with BA did not elicit much of a decrease in total coliform with increasing BA percentages in the incubation flasks (**Table 7**) . However, treatment with STB showed a 2 log decrease in total coliforms with 0.500 % and a 3 log decrease in total coliforms with 1.0, 3.0 and 5.0 % additions (**Table 7**). Ureolytic bacterial growth in urea broth medium was decreased by 1 to 2 log over the control with 5 % BA and STB respectively (**Table 8**). Sulfate reducing bacterial growth in TSI broth medium was reduced by 1 to 3 log over the control with 5 % BA and STB respectively (**Table 8**).

Inhibition of mold growth. Although boron has been reported to control mold growth, an unexpected observation during this study was the inhibition of mold growth by BA and STB in the incubations of the swine manure slurry. Over the period of incubation, untreated wastewater and swine manure slurry quickly developed a very thick, white layer of mold growth on the surface and walls of the incubation flasks (**Photo 2 and 3).** Addition of BA and STB, as a function of its concentration, appeared to inhibit the mold growth (**Photo 4**). Culturing the mold in potato starch broth medium with BA and STB also showed an inhibition in their growth. Mold growth in the untreated control flask was evident on the surface and bottom of the potato starch broth medium, but was absent in the flasks treated with a combination of BA and STB at 0.25 % or higher **(Photo 5**). A change in the color (e.g. grey-black to light brown) of the slurries was also noted with the addition of BA and STB (**Photo 5)**. Molds possess urease activity, so this could account for their growth inhibition. Further studies should be done to determine the benefit of this mold inhibition in swine manure slurries.

Discussion:

Several issues needed to be addressed before an accurate assay for determining the effectiveness of BA and STB in controlling ammonia and hydrogen sulfide emissions could be made**.** These issues are discussed below.

Need for agitation. Ammonia and hydrogen sulfide emission concentrations were initially measured in a static incubation system, since stored swine slurry is normally not agitated until it is removed. However, concentrations measured in the headspace gases of the incubation flasks were found to be too low and variable as to make an accurate determination of any BA and STB treatment effects impossible. One of the causes of this problem was that gases produced during the fermentation appeared to be entrapped in the solids matrix of the waste slurry and were only slowly released into the headspace resulting in considerable variability. This was a problem especially with the nursery slurries which contained high solids content then the wastewater. Secondly, hydrogen sulfide was partially dissolved in the liquid phase of the slurry, and could not be released without agitation. Agitation of the swine slurry yielded higher emission concentrations of hydrogen sulfide and a more accurate determination of actual production rates. Agitation of the slurry did not seem to diminish the ability of the sulfate producers to generate hydrogen sulfide.

Microbial activity. An early concern was whether the hydrogen sulfide emissions in the headspace gases released during agitation was due to active microbial activity of the sulfate reducing bacteria or either escape of the hydrogen sulfide entrapped in the solids matrix or dissolved in the liquid phase of the slurry. Partitioning of hydrogen sulfide between liquid and gas phase depends on the pH, initially dissolved hydrogen sulfide and temperature. At pH 7.0, hydrogen sulfide represents 50 % of dissolved sulfide in water, and its concentration increases as pH decreases. The pH of all the wastewater and slurries examined were over pH 7.0. Hydrogen sulfide concentration in water decreases as temperature increases (Sawyer and McCarty, 1967). Experimentation showed that hydrogen sulfide was actively being produced in the incubation flask by sulfate reducing bacteria, even when slurries were diluted with distilled water and maintained under less than strict anaerobic conditions. .

Fermentation time frame. Another problem that became evident during the study was that ammonia and hydrogen sulfide were not being produced in the same time frame during storage of swine manure slurry. Ammonia production occurred very soon (< 24 hr) after urine and feces were excreted by the pig, and very little ammonia production was detected in manure slurries stored for any appreciable length of time. Hydrogen sulfide, in contrast, was produced only after a longer period of time (>several days) during storage. This difference in time frame when ammonia and hydrogen sulfide were being produced necessitated that separate experiments needed to be conducted for measuring ammonia emissions using fresh urine and feces collected as soon as it was excreted by pigs.

Measuring methods. Accurately measuring ammonia and hydrogen sulfide emission concentrations in incubations within the measuring capabilities of the ammonia and hydrogen sulfide Drager tubes (2-200 ppm) and Jerome H2S analyzer (< 50 ppm) was another challenge. There was considerable variation in ammonia and hydrogen sulfide concentrations which necessitated that certain samples, such as nursery slurries containing both feces and urine and stored slurries with high solids contents be diluted with distilled water, so that they could be accurately measured. In some instances, both Drager tube and Jerome readings had to be used to measure the extreme ranges in hydrogen sulfide concentrations that were detected in incubation flasks.

Inhibition of ammonia and hydrogen sulfide emissions. Although there was considerable variation between different batches of wastewater and slurries, the results of this study clearly demonstrates that boron, in the form of boric acid and sodium tetraborate (borax) is highly effective in inhibiting ammonia and hydrogen sulfide production in stored swine manure slurry. The mechanism by which this inhibition occurs is believed to be due to the inhibition of the microbial enzyme, urease, which is involved in the hydrolysis of urea and to the inhibition of the microbial enzyme, hydrogenase, which is involved in the reduction of sulfate to sulfide. The pH of the wastewater and slurries were not appreciable affected by either BA or STB addition, so a pH effect is probably not involved in inhibiting the microbial activity.

Although urease inhibition by boron has been previously reported, this is the first demonstration of its application for controlling ammonia emissions from swine manure slurries. The use of boron to control hydrogen sulfide emissions, to our knowledge, has not been previously reported in the literature.

Depending on whether wastewater or slurries are being treated (i.e. higher solids content), addition of BA and STB below l% was shown to be effective. Wastewater containing very little solids could be inhibited in hydrogen sulfide emissions by as low as 0.0625% STB.

Initial data suggested that the acid and salt forms of boron might exhibit different properties in the swine manure slurry, but combination studies with equivalent boron levels indicate that both are about equal in their inhibitory effect on ammonia and hydrogen sulfide. Since borax is considerably cheaper in cost than boric acid, there is an economic advantage for using the hydrated salt form of boron.

Microbiological control. The microbiological data suggest that BA and STB are effective in inhibiting the growth of coliforms, ureolytic and sulfate reducing bacteria. However, the concentrations tested were very high, and the serial dilution methodology may have been too insensitive to detect changes in the total microbial population in the slurry. Additional indepth studies with pure cultures of coliforms, ureolytics and sulfate reducers as well as known enteric pathogens would be beneficial.

Other boron compounds. Besides boric acid and sodium tetraborate, other boron compounds might also be effective in inhibiting ammonia and hydrogen sulfide. **Sodium perborate**, also called perboric acid or metaborate peroxyhydrate is another form of borate that might be effective in inhibiting ammonia and hydrogen sulfide emissions from stored swine manure slurry. Perborate is an oxygen bleach agent used in powder detergent formulations, fabric dry bleaches, denture cleaners, automatic dishwater detergents and various laundry products. On contact with water, sodium perborate hydrolyses into hydrogen peroxide and borate. Further studies with sodium perborate and other boron compounds might be warranted.

Treatment of swine facility. During the second year of this project (2006), we will treat a swine facility (MSU Swine Research and Teaching Facility) with boric acid and borax and monitor ammonia and hydrogen sulfide emissions in the whole house. Ammonia must be controlled very quickly after manure is excreted, while hydrogen sulfide production occurs during longer term storage. This difference may dictate the location in the swine facility where the boric acid and borax should be applied for maximum effect. Logical sites for introducing boric acid or borax in the waste handling system would be either in recyled wastewater, under the slats or in the storage pits. It is unlikely that boric acid and borax will be toxic to pigs, but we will avoid any direct contact of the boron products with animals.

Quantitative Risk Assessment. While boron is not very toxic for animals, there is some concern about its application to field crops, since there is a narrow range between essentiality and toxicity for some plants. Technology for extracting boron by processing and reuse is an available option. A quantitative risk assessment (QRA) study (Hurd, 2006), should be done to determine the benefit to risk probabilities of all the factors involved for using boron to treat swine manure slurry.

Lay Interpretation

The experimental results of the first year of this study clearly demonstrates that boron, in the form of boric acid and sodium tetraborate (borax) is very effective in inhibiting the emissions of ammonia and hydrogen sulfide from wastewater and manure slurries from swine facilities. Addition of either 1 % boric acid or sodium tetraborate inhibited ammonia and hydrogen sulfide emissions from wastewater and manure slurries by almost 100 % for over 7 days of incubation. However, concentrations of boric acid and sodium tetraborate as low as 0.0625 % seem to be effective, depending on whether wastewater or manure slurries are treated. Additional benefits for using boron include a reduction in malodor of the manure slurry, control of enteric pathogens, and inhibition of mold growth. Economically, reagent grade boric acid cost $16.76 per kilogram, while commercial borax is $1.77 per kilogram. While boron is not very toxic for animals, there is a very narrow range between essentiality and toxicity for different plants should boron treated swine manure be applied to field crops. However, in boron deficient soils, addition of boron to livestock manure could improve its fertilizer value. A quantitative risk assessment study should be conducted to evaluate the benefit to risk probabilities for all factors for using boron to treat swine manure slurry.

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