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Controlling Hydrogen Sulfide Emissions during Poultry Productions

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Abstract

Hydrogen sulfide (H₂S) and other volatile sulfur compounds (VSC) have received a great deal of attention as gaseous emissions associated with poultry productions. These compounds, especially H₂S, have low odor thresholds (10 ppb) and when managed improperly, higher concentrations of H₂S negatively affect humans, poultry, and the environment. Primarily, odor emissions during poultry production depend on determinants such as sulfur containing compounds (cysteine and methionine) in feed and biological processes associated with their use/production. Post feeding, as manure accumulates and during storage, anaerobic decomposition of amino acids into intermediate sulfur-containing compounds that ultimately form VSCs. To manage poultry waste properly, it is important to have an understanding of determinants of H₂S emissions, associated microorganisms, as well as their interactions. Promising areas of research to reduce odor emission include feed supplementation (additives, prebiotics, and probiotics); manure manipulation (pH, moisture, and its microbial population); housing types; ventilation rates; and biofilters. The most promising singular methods to reduce 100% H₂S emissions are probiotic supplementation in feed, sawdust in manure, or a biofiltration system. Where cost and equipment availability may be prohibitive, combined methods (assuming additive effects) of fibrous byproducts and manure moisture control via microorganisms or oil addition can reduce 100% emissions as well. More investigations should focus on these single or combined methods in commercial poultry production.

Keywords: Poultry; Volatile sulfur compounds (VSCs); Hydrogen sulfide (H₂S); Probiotic supplements; Biofilters

Abbreviations:

CO₂: Carbon Dioxide; H₂S: Hydrogen Sulfide; H₂SO₄: Sulfuric Acid; NH₃: Ammonia; Ppb: Parts Per Billion; Ppm: Parts Per Million; S: Sulfur; SO₂: Sulfur Dioxide; SO₄²⁻: Sulfate; SRB: Sulfate-Reducing Bacteria; VSCs: Volatile Sulfur Compounds

Introduction

In 2017, the National Agricultural Statistics Service reported that 7.73 billion table eggs were produced by 311 million layers in the US [1]. An increase in egg production is needed for the higher demand in the US and emerging economies around the world. High egg production is accompanied by a high accumulation of manure leading to complaints from neighbors living in close proximity to layer operations. Hydrogen sulfide (H₂S), one of the volatile sulfur compounds (VSCs), has received a great deal of attention as one of the gaseous emissions associated with animal feeding operations because of its low odor threshold (H₂S=10 ppb) and its negative impacts on human and animal health and the environment.

The critical negative effects of H₂S on humans and the environment have been the subject of other reports [2-6]. Here, we focus on known effects of H₂S on poultry, on determinants of H₂S during poultry production, and various methods for control or prevention.

Negative effects for poultry

Only three studies of direct H₂S toxicity on poultry have been published. Klentz and Fedde [7] studied the respiratory response of White Leghorn chicken to acute concentration of H₂S (0, 0.05%, 0.2%, 0.3%, and 0.4%). At 0.05%, there were no significant differences in tidal volume and respiratory frequency compared to the control group. At 0.2% and 0.3%, birds had an increase in respiratory frequency but returned to normal within 30 minutes after H₂S exposure. All birds died within 15 minutes at 0.4% H₂S inhalation; this is equivalent to 4000 ppm. The researchers noted that chickens are less sensitive to H₂S than mammals, 500-1000 ppm leading to death. In the same study, they also examined the response of intrapulmonary CO₂ receptors to varying H₂S concentration (0.035-0.1% H₂S). H₂S caused an increase in intrapulmonary CO₂ receptors' discharge frequency and an increase in vertical sternal movements. This increase in discharge frequency inhibited carbonic anhydrase in the central respiratory neurons which led to apnea [7].

Kocaman et al. [8] observed that the concentrations of CO₂, NH₃, H₂S, relative humidity, and temperature in winter and spring are significantly different from summer and fall. Moreover, researchers found that an increase in CO₂

(950.0-1623.1 ppm), NH_3 (10.5-16.46 ppm), and H_2S (1.75-7.0 ppm) in poultry houses can decrease the feed conversion ratio (from 1.79 to 2.18 kg feed consumed/kg egg produced). The effect seems to be caused by a combination of the different gases and the condition of the poultry house rather than the effect of a single component.

Another study assessed the effect of only H_2S on the performance of broiler chicken. Each treatment was in an environmentally controlled chamber with 0 mg/kg H_2S in weeks 0-6 as a control; 3 treatments ranging from 2, 4, and 8 mg/kg H_2S during weeks 0-3; and 3, 6, and 12 mg/kg of H_2S during weeks 4-6. Results showed that H_2S had negative effects on broiler performance, resulting in an increase in production cost. From weeks 0-3, mean daily intake and the feed:gain increased as H_2S concentration increased. The highest concentration of H_2S (12 mg/kg) resulted in a significant decrease in carcass yield and a significant increase in the rate of water loss in breast and thigh. This result correlated with a decrease in pH values of breast and thigh. The researchers suggested that there should be less than 2 mg/kg of H_2S in the broiler houses from weeks 0-3 and less than 6 mg/kg of H_2S from weeks 4-6 for healthy broiler production [9]. Overall, researchers examine negative effects of NH_3 on poultry have found a detrimental effect of H_2S alone or in combination with other gases when it reached 1.75-7.0 ppm.

Determinants of H_2S emissions

One approach to control H_2S is to understand the role of primary determinants such as S-containing amino acids, associated biological processes, microorganisms, and resulting interactions.

Amino acids in feed: Methionine is one of 13 essential amino acids required for growth of poultry [10,11]. Due to low methionine in plant products, it is the only amino acid that must be synthetically produced in a form of DL-methionine or methionine hydroxyl analogue (MHA) to add to poultry diets [12,13]. Of 18.8% crude protein, 0.38% is methionine. Thus, the total S required by chicken is approximately 4.5% of the total protein [10]. Chavez et al. [14] investigated the effect of different methionine sources (liquid MHA, DL methionine, dry MHA, and sodium methioninate aqueous solution) and concluded that various methionine sources gave rise to the different odor profiles, varying concentrations of H_2S , COS, CH_3SH , CH_3SSCH_3 , and $\text{CH}_3\text{S}_3\text{CH}_3$.

Biological process associated with sulfur amino acids: There are two possible pathways to form H_2S and CH_3SH in animals. These pathways are bacterial degradation of the S-containing amino acids and bacterial reduction of sulfate ions (SO_4^{2-}) (Figure 1). The digestive pathway is for the pig, a monogastric animal like chicken [15].

Microorganisms anaerobically decompose S-containing amino acids (cysteine/cystine and methionine) forming intermediate S-containing compounds that ultimately form H_2S and other VSCs [16-19]. The enzyme responsible for this pathway is methionine gamma lyase, which is present in some organisms from archaea to bacteria to plants [20]. H_2S , along with pyruvate and NH_3 , can also be released from the enzyme cysteine desulfhydrase,

catalyzing the α , β -elimination of L-cysteine [21]. Some of the *Lactobacillus* species, such as *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, and *Lactobacillus brevis*, were found to produce H_2S by this pathway if grown on peptone iron, triple sugar iron, Sulfide-Indole-Motility agars [22].

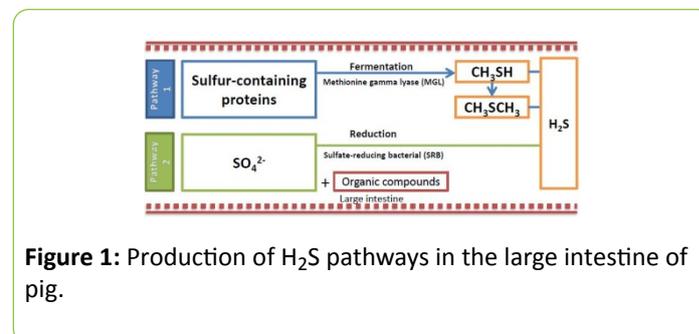
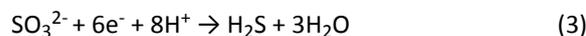
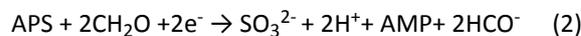


Figure 1: Production of H_2S pathways in the large intestine of pig.

More specific bacterial degradation of complex organic matter is through a branch of strictly anaerobic genera of *Deltaproteobacteria*, sulfate-reducing bacteria (SRB) such as *Desulfovibrio* [23], *Desulfobacter* [24], *Desulfococcus*, and *Desulfonema* [25]. This pathway has also been found in *Campylobacter* [26], *Escherichia coli* [27-30], and *Salmonella* [31-33]. These SRB use hydrogen and organic compounds for growth while reducing SO_4^{2-} to $\text{H}_2\text{S}/\text{HS}^-$ in the process. These redox reactions (1-3), where CH_2O represents a generic organic carbon compound, depict the outcome.



SRB may also use other volatile fatty acids such as acetate, propionate, butyrate, and lactate which serve as the final electron acceptor during cellular respiration, providing energy and promoting the growth of these bacteria [25,34,35].

There is evidence of H_2S production in the cecum of the chicken via microorganisms [36]. Gong et al. [37] identified bacteria present in the mucosa of chicken ceca using 16S rRNA. They found the chicken cecal environment to be highly diverse having butyrate-producing bacteria, which are closely related to *Fusobacterium prausnitzii*, one of the largest groups among 116 cloned sequences. They also identified other bacteria such as Clostridia, *Enterococcus cecorum*, *Escherichia coli*, Lactobacilli, and Ruminococci. Basic et al. [38] reported their findings on proteins in *Fusobacterium spp.* that are involved in the production of H_2S from cysteine. The most abundant enzyme detected was cysteine synthase which is involved in cysteine metabolism. Endogenous H_2S production occurs as a reversible reaction of cysteine synthesis. Serine sulfhydrase, isolated from chicken liver, is known to catalyze the reversible reaction between H_2S and serine to produce cysteine and water [39]. Ultimately, when investigating ways to negate the negative effects of H_2S , it is important to account for naturally occurring endogenous sources.

Feed amendment with byproducts: Many investigators have assessed strategies to lessen production of H_2S during storage of manure at its source (feed). These strategies include control of

dietary S amino acids by addition of various byproducts and inclusion of phytobiotics, prebiotics, or probiotics to minimize the amount of leftover S in manure.

When investigating the effect of feed manipulation on reduction of H₂S, literature of swine and broilers dominate that of layers. Results of swine research demonstrated the potential of reducing dietary S-containing amino acids and SO₄²⁻ to reduce H₂S emissions [40]. Kendall et al. [41] provided reduced crude protein (from 11.5% crude protein in the control to 8.25% in the treated groups) diets with 5% soybean hulls, high-available phytate corn, phytase, and reduced mineral SO₄²⁻ for six weeks to determine the effect on pig growth performance, NH₃, H₂S, odor examined, and nutrient excretion. There was a reduction in concentration of both NH₃ (48.7%) and H₂S (48%) at week six.

Jiao et al. [42] supplied varying amount of dietary methyl sulfonyl methane (MSM, at 0%, 0.05%, 0.10%, and 0.20%) in order to examine the effect on broiler performance and gas emission. They found a linear trend for H₂S reduction (P=0.09) with greater addition of MSM in the diet.

The effect of different dietary fat sources was evaluated for growth performance, excreta microbiology and noxious gas emissions in broilers. The two fat sources were halal tallow and a combination of tallow and lard. The investigators found no significant difference in H₂S reduction between the two fat sources during the 5-week study [43]. Researchers examined the same parameter for different treatments. The four treatments were (1) chicken fat, (2) tallow, (3) tallow and lard, and (4) pork fat/lard. Soybean was the control. NH₃, H₂S, and SO₂ emissions were significantly lower in diets with soybean oil and chicken fat compared to others [44].

Sharma et al. [45] found that providing similar calculated digestible methionine plus cysteine (7.3 g/kg in wheat and canola seed diet vs. 7.0 g/kg in wheat-corn without canola seed control diet) resulted in a higher concentration of CH₃SH from the diet with canola seed compared to that of the control. The researchers suggested that the significant difference was likely due to difference in moisture content. Higher moisture content produces more CH₃SH caused by increased anaerobic degradation. Sharma et al. [46] also reported a significant positive correlation between litter moisture with CH₃SH, H₂S, CH₃SCH₃, trimethyl amine, phenol, indole, and skatole.

When Wu-Haan et al. [47] fed diets contained 6.9% of CaSO₄ zeolite mixture to layers, they reported an increase in H₂S concentration from laying hens manure at different ages. The average H₂S daily emissions over three weeks for the treated diet was significantly (P< 0.01) higher (4.08 mg/bird) than that of the control diet (1.32 mg/bird). Researchers suggested that the acidifying effect of CaSO₄ contributed to the increase in H₂S emissions. Findings from another study showed that zeolite in poultry manure lowered the concentration of other volatile compounds but increased VSCs. The decrease in pH caused the noted change [48].

Wu-Haan et al. [49] investigated distillers dried grain plus soluble (DDGS), a byproduct of corn from the beverage and alcohol industries, for its capacity to reduce H₂S emissions. They investigated the effect of varying amounts of DDGS (0, 10, and

20%) in the diet on air emissions and laying hens performance. Each diet contained 0.22, 0.27, and 0.42% of S, respectively. Adding DDGS to the diet showed no significant effect on layer performance but a significant reduction in emissions. Daily emissions of NH₃ and H₂S from 21- to 26-week-old laying hens decreased at the 20% DDGS inclusion rate.

Chlorine dioxide has been investigated as a dietary supplement to reduce gaseous emission without affecting broiler performance. Addition of 0.05% and 0.1% chlorine dioxide resulted in an antimicrobial activity against *Escherichia coli* (in ileum and cecum) and *Salmonella Typhimurim* (in cecum). The reduction in these two SRB may explain the reduction in H₂S emissions, significantly lowered at three hours of fermentation with 0.05% chlorine dioxide. The emissions of CH₃SH were significantly lowered starting at the 0 hour of fermentation for both 0.05% and 0.1% chlorine dioxide [50]. The highest H₂S reduction rate (at 62.5%) of the feed additive was addition of 0.05% chlorine dioxide.

Microorganism supplementation as feed amendment:

Animals' microflora need to be stable in order for improvement in feed efficiency and effective dietary nutrients utilization. Feed supplement, such as phytobiotics, prebiotics, and probiotics should be considered for stabilization or improvement of the microflora community [51].

Use of phytobiotics: Phytobiotics, or phytochemicals, are herbs, spices and plant extracts (essential oil) used in human traditional medicine [52]. In recent years, phytobiotics have been used as alternatives to antibiotic growth promoters for beneficial effects such as higher feed intake, anthelmintic (antiparasitic), antimicrobial, coccidiostatic, and immunostimulating properties [53].

A dietary phytochemical feed additive, extracted from Korean pine, has been reported to significantly reduce NH₃ emissions but no significant difference was found for total CH₃SH, H₂S, and acetic acid. However, there was a significant positive correlation for reduction of all excreta gas emissions and higher phytoncide supplementation [54].

A byproduct of *Punica granatum L.* (pomegranate) has been used to investigate the effect of growth performance, noxious gas emissions, and economic efficacy in broilers. NH₃ and H₂S reduction were both significant, but not SO₂. Optimal reduction of NH₃ (37%) was found with 2.0% byproduct whereas optimal reduction of H₂S (86%) was found with 0.5% byproduct [55]. The same group of researchers investigated the effect of this byproduct on growth performance, fecal microbiology, and noxious gas emissions in broilers. Broilers were fed varying amounts (0, 0.5, and 1%) of the byproduct for 35 days. Both levels of byproduct significantly reduced NH₃ emissions at 12, 24, and 48 hours. Significant H₂S reduction was observed in 0 hour with 1% byproduct. Significant CH₃SH reduction was observed at 0, 3, and 48 hours of incubation [56].

A combination of exudates of *Lactobacillus plantarum* - fermented *Gynura procumbens*, *Saccharomyces cerevisiae* - fermented *Rehmannia glutinosa*, and *Bacillus licheniformis* - fermented *Scutellaria baicalensis* were investigated for their effect on broiler performance. Diets included varying amounts

(0, 0.05, 0.1, and 0.2%) of the fermented product for 35 days. NH_3 emissions were significantly lower compared to the control diet. Investigators found no significant reduction in both H_2S and total CH_3SH emissions but a significant linear correlation between the reduction of H_2S and total CH_3SH and the amount of fermented product added [57].

Use of prebiotics: Prebiotics are non-digestible food ingredients that promote the growth of the host's beneficial microflora [58,59]. Zhao et al. [60] explored the effect of levan fructan supplementation on broiler performance microflora and excreta noxious gas emissions. NH_3 was significantly lowered by the addition of the supplement at 0.25% and 0.50% fructan ($P < 0.013$). The higher amount of fructan did not improve the emission reduction efficiency. H_2S and acetic acid gas emissions were not significantly different from that of the control diet.

Supplementation of lactulose, a non-digestible carbohydrate used in stimulating the growth of Lactobacilli improves broiler performance, contrary to finding of Zhao et al. [60]. NH_3 , H_2S , and acetic acid gas emissions were decreased ($P < 0.05$) in diets with 0.1% and 0.2% lactulose compared to the control diet. As well, only the 0.2% lactulose diet had a significant increase in excreta Lactobacilli and a significant decrease in *Escherichia coli* compared to the control diet [61]. While many researchers have investigated the use of prebiotics in broiler productions, others have examined the use of probiotic as discussed below.

Use of probiotics: Probiotics are live microorganisms that, when ingested, may benefit the host by improving digestion [62]. Because in commercial poultry production chicks are separated from layers, the opportunity to transfer microorganisms from the layer's feces to young chicks to improve their digestion is reduced. Other possible microorganisms that can be offered to chicks to serve a similar function have been proposed. Mainly, *Lactobacillus* species have been used as probiotics because they are predominantly found in chicken's crop epithelial cells [63]. *Lactobacillus* organisms, endogenous in chicken as well as humans, are ubiquitous in nature. Research results provided information on the intestinal benefit of single-strain probiotics (*Bacillus cereus*, *licheniformis*, and *subtilis*; *Enterococcus faecium*; *Pediococcus acidilactici*; *Lactobacillus farciminius*, *rhamnosus*, *casei*, and *Plantarum*; *Streptococcus infantarius*; and *Saccharomyces cerevisiae*) for livestock and poultry [64]. *Lactobacillus rhamnosus*, alone, has been reported to reduce H_2S production *in vitro* under both aerobic and anaerobic conditions [65].

There is little information about *in vivo* investigations on the effect of probiotics in poultry production; that which is available has focused on broilers. Jeong and Kim [66] determined the effect of spore supplementation of *Bacillus subtilis* on broiler performance and noxious gas (H_2S and NH_3) emissions. Diets contained 0, 300, and 600 mg of *Bacillus subtilis*/kg feed at 1.0×10^9 cfu/g. There was no significant effect on the reduction of H_2S . In another study, these investigators ascertained the effect of astaxanthin (a carotenoid pigment produced by a yeast species, *Phaffia rhodozyma*) on the same parameters and found an insignificant reduction of H_2S [67]. Zhang and Kim [68] determined the effect of probiotic (*Enterococcus faecium*) and

two levels of energy (2,700 or 2,800 kcal/kg, metabolizing energy) on Hy-Line Brown layers. They also found no significant reduction in H_2S emissions or total CH_3SH from freshly collected manure which was allowed to ferment for 30 hours in a sealed container before sample collection from the headspace.

In contrast, Lan et al. [69] investigated the effect of *Enterococcus faecium* on growth performance, excreta microbiota shedding (Lactobacilli and *Escherichia coli*), and noxious gas emissions in broilers. They used varying amounts (0, 0.05, 0.10, and 0.20%) of *Enterococcus faecium* in the diets amounts. At day 7, only H_2S emissions were significantly ($P < 0.001$) reduced compared to that of the control diet, but the amount of *Enterococcus faecium* did not have a significant effect. At day 35, emissions of NH_3 , H_2S , and total mercaptans were significantly reduced compared to the control at 0.20% level ($P = 0.002$, 0.001, and 0.013, respectively). Lactobacilli were not significantly increased but *Escherichia coli* were significantly lowered at 0.10% and 0.20% *Enterococcus faecium* at day 7. At day 35, Lactobacilli were significantly increased at all levels with significantly lower level of *Escherichia coli* at 0.05% and 0.20%.

Zhang et al. [70] found a significant reduction in H_2S concentration (37.9%) using only 10^5 cfu/kg of *Bacillus subtilis* alone compared to the control diet. Additionally, Sharma et al. [71] found a significantly lower H_2S concentration (up to 29.9% reduction) in the litter from birds fed high crude protein with probiotic (*Bacillus subtilis*) added compared to other diets (high crude protein alone, high crude protein with antibiotic, and high crude protein with saponin at 26, 24, and 23 for starter, grower, and finisher diets, respectively). However, the decrease in H_2S concentration was not significantly different from that of the low crude protein diet (at 21, 19.5, and 18.4 for starter, grower, and finisher diet, respectively). The researchers noted the correlation ($r = 0.482$, $P < 0.01$) between H_2S and moisture content.

Ahmed et al. [72] determined the effect of *Bacillus amyloliquefaciens* on growth performance, cecal microflora, NH_3 , and H_2S emissions of broilers provided with varying amounts of probiotic (0, 1, 5, 10, and 20 g/kg) for 35 days. The results showed a negative linear and quadratic effects on fecal emissions of H_2S ($P < 0.001$) with an optimum effect at 5g/kg of feed. Other results also suggested a positive effect on bird health.

Multistrain probiotics (*Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici*, and *Lactobacillus salivarius*) isolated from intestinal tract of healthy adult chicken have been used as supplements to improve broiler growth responses, digestivity, and cecal microflora composition [73]. Reportedly, the use of multistrain probiotics is more effective than single strain supplementation [74]. A combination of *Lactobacillus species* (*Lactobacillus casei*, *brevis*, *buchneri*, and *plantarum*) was shown to significantly reduce the malodor from the broiler house. VSCs such as CH_3SCH_3 and $\text{CH}_3\text{S}_3\text{CH}_3$ were decreased [75]. An *in vitro* study showed that *Lactobacillus plantarum* and *rhamnosus* have antimicrobial activity against *Clostridium Perfringens*, bacteria known to reduce sulfite to the sulfide ion [76-78].

The effect of probiotics (*Bacillus*, *Lactobacillus*, *Streptococcus*, *Clostridium*, *Saccharomyces*, and *Candida* species) at a rate of 3 g/kg feed (10^{7-8} cfu/g) on broiler performance and odor was investigated. Detection of gaseous compounds was performed by holding inspection tubes (Gastec Co., Japan) one meter above the ground. Investigators found a reduction in NH_3 , H_2S , and CH_3SH in both male and female broilers compared to the control. They concluded that these bacteria had a beneficial effect for overall broiler performance [79].

In contrast, Zhang and Kim [80] found no significant reduction of H_2S in manure fermented for 1, 3, and 5 days with the use of spray-dried spore-forming bacteria at 2×10^8 viable spores/kg of *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Clostridium butyricum* in the diet.

An insignificant effect of multistrain complex probiotics was observed in a different study by Balamuralikrishnan et al. [81]. The researchers used two different commercially available types - Probiotic A [*Bacillus coagulans* (1×10^9 cfu/g), *Bacillus licheniformis* (5×10^8 cfu/g), *Bacillus subtilis* (1×10^9 cfu/g), and *Clostridium butyricum* (1×10^8 cfu/g)] and Probiotic B [*Bacillus coagulans* (1×10^9 cfu/g), *Bacillus licheniformis* (5×10^8 cfu/g), and *Bacillus subtilis* (1×10^9 cfu/g)].

Two strains of *Bacillus subtilis* were used in conjunction when challenging broilers with *Salmonella typhimurium* to understand the effect on performance, blood profiles, intestinal Salmonella concentration, and noxious gas emissions. The two strains of bacteria were as effective as using the antibiotic (virginiamycin) in lowering the intestinal concentration of Salmonella. However, only the NH_3 emissions were significantly lowered. CH_3SH , H_2S , and acetic acid emissions were not significantly different from that of the control [82].

Hossain et al. [83] investigated the effects of *Bacillus subtilis*, *Clostridium butyricum*, and *Lactobacillus acidophilus* on excreta noxious gas emissions in broilers. In this study, the probiotics were added to the feed. Diets were (1) control as antibiotic-free diet (2) 5 ppm enramycin (3) 5 ppm avilamycin (4) 0.1% probiotic and (5) 0.2% probiotic. Investigators found no significant effect on reduction of H_2S concentration.

Manure, manure pH, and manure amendment: As noted above, research on feed manipulation can reduce H_2S emissions in manure. Other researchers have focused on direct manipulation of manure.

Lin et al. [84] quantified the source of S from three different laying hen houses (conventional cage, enriched colony, and aviary) to be about 91.7% from feed and 8.3% from water. Of the total, 67.8%, 25.9%, 6%, and 0.3% was deposited in manure, egg, air, and chicken as body weight, respectively. However, Wu-Haan et al. [85] reported 57.1% S after manure clean out at the end of a 3-week study. Less frequent clean-out time resulted in higher loss of S into the atmosphere. This conclusion is in agreement with results indicating that total reduced S concentration in the air was generally at its highest on storage days 30 to 35 [86].

Amino acid compounds have been found in animal manure [87-89]. Banwart and Bremner [90] investigated the origin of the

VSCs including H_2S , CH_3SH , COS, CH_3SCH_3 , CH_3SSCH_3 , and CS_2 , and found that all, except H_2S , are released from the decomposition of S-containing amino acids in soils treated with sludge, manure, dried or fresh plant materials. The release continues for up to 44 days. Investigators further noted that H_2S was not detected due to its quick sorption by soil, making detection in the air impossible [91]. This may also be a possible reason for low concentration of H_2S detected in other poultry houses.

More variety of VSCs was found in fresh manure than in old manure [86]. However, based on comments from a panel of 10 volunteers, the concentration of H_2S , or the rotten-egg odor, was more prominent in the dried manure than in fresh manure [92].

Gay et al. [93] compared the total reduced S, NH_3 , and other odor levels from various animal housing facilities and manure storage sites. Composting of laying hen manure ranked third in highest emissions of total reduced S compare to other types of animal manure storage units. The measurement ranged from 1.35 to 370.0 $\mu\text{g/s/m}^2$, having the highest variability (standard deviation=104 $\mu\text{g/s/m}^2$ for N=19). The researchers noted that the high variability may have been due to the differences in sampling sites, including factors such as diets, manure management, the design of the storage unit, seasons, and the ambient air temperature.

Manure pH and manure amendment can aid in removal of unwanted odors from poultry houses. Manure pH plays a crucial role in emissions of H_2S . The following equation derived by Xue et al. [94], shows the relationship between H_2S concentration and the H^+ .

$$\frac{[\text{H}_2\text{S}]}{[\text{H}_2\text{S}] + [\text{HS}^-] + [\text{S}^{2-}]} = \frac{1}{1 + \frac{K(\text{H}_2\text{S})}{[\text{H}^+]} + \frac{K(\text{H}_2\text{S})K(\text{HS}^-)}{[\text{H}^+]^2}}$$

Where $[\text{H}_2\text{S}]$ is free H_2S concentration (mol/L), $[\text{HS}^-]$ is HS^- concentration (mol/L), $K(\text{H}_2\text{S})$ is equilibrium constant for H_2S (mol/L), $[\text{S}^{2-}]$ is concentration of S^{2-} (mol/L), and $K(\text{HS}^-)$ is the equilibrium constant for HS^- (mol/L) [95].

The equation shows that higher pH may reduce H_2S emissions into the atmosphere [96]. Sharma et al. [97] modeled the effect of pH on the H_2S production by anaerobic sewer biofilm where multiple SRB species have been identified from the sewer biofilm [98]. The result of Sharma et al. [97] indicated that the maximum H_2S production was at physiological pH (6.5-7.5). The S^{2-} production was reduced outside of this range with up to 50% inhibition at pH 4.0 and pH 9.0. Free NH_3 inhibited the effect on H_2S production at high pH. The researchers were not able to determine the effect of low pH inhibition; however, they reported that acetic acid and other volatile fatty acids were not the cause.

A pilot-scale composting reactor showed the H_2S reduction potential when adding sawdust to manure to improve the biodrying process; however, certain conditions must be met. The temperature must be more than 30°C above the ambient

temperature. The moisture content should be between 30-40%. Exploring two conditions, the exhaust H₂S of manure with sawdust was shown to be below the detectable limit (0.1 ppm as measured by a Gastech portable detector) [99] compared to the manure without added sawdust (3-5 ppm).

Gutarowska et al. [100] proposed to use a mixture of six strains of bacteria and one yeast (*Bacillus subtilis subspecies spizizenii*, *Bacillus megaterium*, *Pseudomonas sp.*, *Psychrobacter faecalis*, *Leuconostoc mesenteroides*, *Streptomyces violaceoruber*, and *Candida inconspicua*) in the water as poultry manure deodorization. They found that the highest removal of volatile compounds (NH₃, H₂S, dimethylamine, trimethylamine, and isobutyric acid) was caused by *Bacillus subtilis subsp. spizizenii*, *Leuconostoc mesenteroides*, *Candida inconspicua*, and *Psychrobacter faecalis*. This surface application of bacteria removed NH₃ and H₂S from the exhaust gas by 20.8% and 17.5%, respectively. Moreover, there was a 45% reduction of protein and amino acids, particularly cysteine and methionine, after 24 hour of deodorization. A reduction of cysteine may explain the reduction in H₂S concentration [101].

Matusiak et al. [102] further investigated the deodorizing capacity for the same six strains of microorganisms, enriched with two species of *Lactobacillus plantarum*. Mixtures of microorganisms in water were sprinkled on poultry manure with and without *Yucca schidigera*. Poultry manure was aerobically incubated in a sealed chamber with a flow rate of 2 L/min. The highest reduction in H₂S concentration was the poultry manure with *Yucca schidigera* alone (64%), followed by poultry manure with microorganisms alone. This study also reported the benefit of the yucca extract to lower the concentrations of odorous compounds such as NH₃, dimethylamine, H₂S, isobutyric acid, and trimethylamine emitted from poultry manure. *Yucca* produces saponin that has been reported to inhibit microbial fermentation of protein [103].

Borowski et al. [104] reduced NH₃ and H₂S from the exhaust air by 94% and 60%, respectively, after 2 days of deodorization using a combination of bacterial species (*Pseudomonas fluorescens*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus megaterium*, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*) in manure. The most effective method was 20% spray-dried microorganisms onto perlite and bentonite (2:8 ratios by weight) stored at room temperature (22 °C) for at least 5 months.

A combination of spraying a water-oil mixture on manure and increased ventilation reduced H₂S emission by 32% (from 13.2 to 9.0 ppm). The mixture was added at a rate of 80% sunflower oil and 20% water. The spraying area was 0.5 L mixture for 100 m² floor area. In addition to reducing H₂S emissions, this method also decreased temperature, relative humidity, concentration of dust, CO₂, and NH₃ [105]. The oil component most likely affects other parameters, ultimately lowering H₂S emitted in the house.

Quebracho tannins also have the ability to reduce H₂S and methane gas emissions by reducing the number of SRB and their metabolic activities. Stored swine manure, somewhat representative of poultry, was used in the experiment. The

tannins can be sprayed over manure or mixed with the liquid used to remove manure [106].

Packaged bacteria that can be added to manure to reduce H₂S may become the norm in the future. Possibly, the combination of tannin-containing horticultural byproducts and packaged bacteria could be used to reduce SRB in manure.

Housing: Housing types are critical as they often determine how manure is removed or stored over long periods of time. The National Air Emissions Monitoring Study [107] was funded by the Animal Feeding Operation industry with the Environmental Protection Agency to provide information about emissions of particulate matter, NH₃, H₂S, and volatile organic compounds from industries for swine, broilers, laying hens, and dairy cows. Out of 25 sites, only five were poultry farms (3 layers and 2 broilers). The H₂S emissions data was collected from layer houses in North Carolina, Indiana, Kentucky, and California (Figure 2) [107].

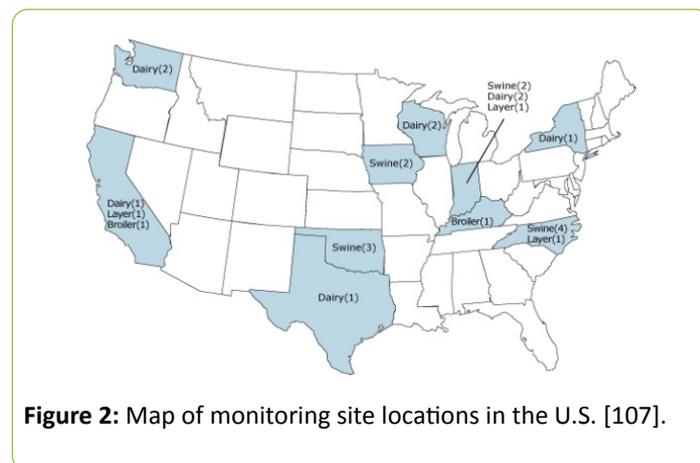


Figure 2: Map of monitoring site locations in the U.S. [107].

Lim et al. [108] reported a detectable amount of H₂S (0.02 ppm) was found in caged-hen high-rise layer houses. Layers were fed different types of feed to determine their efficacy in reducing NH₃ and H₂S. The control, a standard industry diet, produced 0.045 ppm of H₂S as a mean concentration over a 2-year period. This was well above the detectable limit, as low as 0.01 ppm. Researchers noted that most of the odor in poultry houses was from NH₃ and H₂S [109]. Almuhanha et al. [110] detected a lower average H₂S concentration of 6.05 µg/m³ and 8.6 µg/m³ (=0.01 ppm) for two broiler houses. The maximum H₂S concentration was 162.80 and 37.50 µg/m³. However, the results are not clear because the two housing conditions were not specified.



Figure 3: Poultry housing: high rise with reverse stair-step [112].

A more recent study monitored two different types of poultry housing and found that manure-belt housing (Figure 4) [113] was 92% higher in emissions per animal unit (AU) and 78% higher in emissions per hen compared to high-rise houses (Figure 3) [112] (Table 1) [111].

A report on poultry housing in South Korea stated that caged layer houses tend to have the highest levels of NH_3 and H_2S compared to layer houses with manure belts and broiler houses.



Figure 4: Poultry housing: battery cage with manure-belt [113].

The author suggested that the difference in ventilation system could be the cause of this trend. Caged layer houses use a mechanical ventilation system which is usually set below the recommended ventilation rate to lower cost, whereas, the manure belt and broiler house have natural ventilation [119].

Table 1: Daily means of H_2S emissions¹ from nine US laying hen houses using the same measurement.

State	Valid day (d)	Emission ($\text{gd}^{-1}\text{AU}^{-1}$)	Emission ($\text{mg d}^{-1}\text{hen}^{-1}$)	Reference	Type of house
Indiana	84	0.484	1.52	[107]	high-rise
Indiana	314	0.5	1.55	[115]	high-rise
Indiana	313	0.4	1.26	[115]	high-rise
California	614	0.396	1.33	[116]	high-rise
California	632	0.374	1.2	[116]	high-rise
North Carolina	656	0.206	0.623	[117]	high-rise
North Carolina	652	0.237	0.694	[117]	high-rise
Indiana	276	0.506	1.46	[118]	high-rise
Indiana	296	0.442	1.28	[118]	high-rise
Weighted mean		0.355	1.101	-	-
Indiana	634	0.679	1.95	[111]	Manure-belt
Indiana	624	0.685	1.96	[111]	Manure-belt
Weighted mean		0.682	1.955	-	-

¹ H_2S concentrations were recorded by pulsed fluorescence analyzers. Ventilation rates were calculated from the fan monitoring system. Adapted from Ni et al., [111].

Leonard et al. [120] investigated the air quality in a broiler house for 20 minutes each week of the production cycle and found no detectable H_2S using 10 ppb as the limit of detection. Broilers were raised in a wood-frame construction with earthen floors using short straw as litter. H_2S production, along with

gases such as CO_2 and NH_3 , were measured from three different commercial laying hen barns by the same group of researchers. Barn A was a single-story house with individually housed hens stacked in three levels. Manure belts were used to collect droppings which were conveyed and elevated once a week to a

manure spreader outside of the barn. Barn B was a double-story that had a deep-pit utilizing the lower half as the manure storage. Hens were lined up in three rows of doubly flat-deck cages (Figure 5) [114]. Manure was removed annually by a tractor with a front-end loader. Barn C, like Barn A, was a single-story unit with three levels of stair-step cages. The droppings were scraped from the shallow manure pit monthly to a cross-conveyor and elevated into a manure spreader. The researchers did not detect any H_2S in barn A or C and only 30 ppb from barn B. They noted that this concentration was very low however, workers should be cautious when working in the barns especially during manure clean-out [121].



Figure 5: Poultry housing: flat-deck [114].

Guarrasi et al. [122] compared the occupational exposure of H_2S in poultry, beef/dairy, and swine operations. They reported that poultry operations have the highest weighted mean H_2S concentration (0.33 ppm) among the three different animal facilities. Further, they compared two different types of housing. The floor-based housing had the highest weighted mean H_2S concentration (4.52 ppm) compared to the caged-based housing (0.04 ppm). The caged-based housing was a representative house for all layers whereas the floor-based housing represented the broiler operation.

Conclusions obtained from results of investigation indicate the concentration of H_2S found in poultry houses is relatively low. The danger is more pronounced when there is improper handling of wastes.

Ventilation rate and biofilters: As noted, proper handling of waste is critical when lessening the effects of H_2S on workers. If manure is stored for any length of times, the ventilation rate should be clearly monitored. One of the ways to reduce VSC emissions from poultry houses is to reduce the moisture content of the manure. A proper ventilation system can be used to control moisture and create appropriate indoor air quality [123]. Zhang et al. [124] investigated the combined effect of ventilation rate and the use of a super-plasma ionizing air purifier on the indoor air quality in broiler production. The different ventilation settings were 10X and 5X/h. The results showed that the 5X/h ventilation rate produced significantly higher concentration of H_2S than the 10X/h ventilation rate.

In addition to ventilation by fan, biofilters (Figure 6) [125] can be used to treat exhaust air in mechanically ventilated buildings

by blowing through a media covered with a biofilm (containing bacteria). A species of bacteria with the ability to remove H_2S from the exhaust air is *Pseudomonas putida*. Without causing acidification of the biofilter, this species of bacteria can convert H_2S to mainly elemental S, allowing the microorganism activity to continue without much monitoring. When immobilized with calcium alginate, it was reported to remove up to 96% of H_2S at 10 - 150 ppm with a flow rate of below 72 L/h [126].

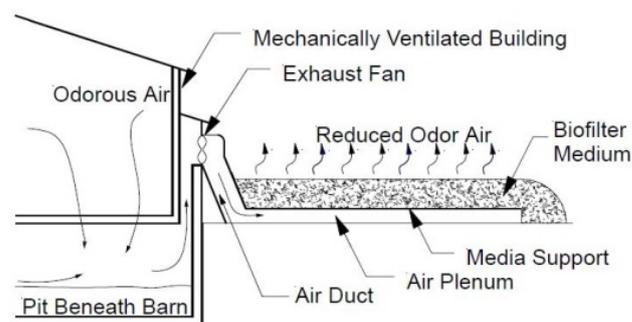


Figure 6: Schematic representation of biofiltration system where bacteria can be introduced into the media support for odor removal [125].

Laboratory-scaled research was designed to show the capacity of immobilized *Thiobacillus thioparus* as a biofilter to remove H_2S under low-concentration. High removal (97.5-98.0%) of H_2S can be achieved with temperatures between 20-37°C and the flow rates of the inlet H_2S concentrations are either 36 or 72 L/h. This species of bacteria oxidizes H_2S to SO_4^{2-} , elemental S, SO_3^{2-} , and S^{2-} [127]. Co-immobilization of *Thiobacillus thioparus* and *Nitrosomonas europaea* was also found to be effective in reducing both H_2S and NH_3 emissions. However, researchers found that H_2S lowered the removal efficiency of NH_3 but NH_3 had no effect on removal of H_2S [128]. Sercu et al. [129] used *Acidithiobacillus thiooxidans* and *Hyphomicrobium* VS in a two-stage biofiltration. Together, more than 99.8% H_2S removal efficiency was achieved.

Sun et al. [130] further investigated the effect of biofilters in the removal of H_2S with varying moisture content and reaction time, which is defined as the duration of contact time between air and biofilter media. When using compost/wood chips, the average removal rate of H_2S varied from 47% to 94% with moisture content of 50% and gas retention time of 20 seconds being the highest removal rate. As noted by Bohn [131], biofiltration remains a promising field of research due to its minimal maintenance/cost and high efficiency; however, to our knowledge, there is no published research to date on the prevalence of biofilter use in the poultry industry.

Summary of methods used to effectively reduce H_2S emissions

VSC and most especially H_2S produced during poultry production is harmful to humans, poultry, and the environment. Understanding the chemistry and biological processes for production of H_2S and employing mitigation processes from

input (feed) to biofilters is necessary to greatly reduce emissions. **Table 2** is an extensive summary of reported procedures (above 30% reduction) to reduce VSCs and H₂S

emissions discussed in this review. Thus, not all reduction methods are presented in the table.

Table 2: Summary table of H₂S mitigation strategies.

Determinant	Criteria	Conclusion	Quantifiable change	Reference
Pre-excretion strategies				
Dietary Fat Source	halal tallow vs. haram lard vs. chicken fat	decrease with chicken fat	49.0	[44]
Byproduct	DDGS ¹ (0, 10 and 20%)	decrease with 20% DDGS	58.0	[49]
Feed Additive	Chlorine dioxide (0.05, 0.1%)	decrease with 0.05% chlorine dioxide	62.5	[50]
Phytobiotics	<i>Punica granatum L.</i> (0, 0.5, 1.0, 2.0%)	decrease with 0.5%	86.0	[55]
	<i>Punica granatum L.</i> (0, 0.5, 1.0%)	decrease with 1.0%	33.0	[56]
Prebiotics	Lactulose (0, 0.1, 0.2%)	decrease with 0.1, and 0.2% lactulose	50.0, 52.9, respectively	[61]
Probiotics				
Single strain	<i>Bacillus amyloliquefaciens</i> (0, 5, 10, 20 g/kg feed)	decrease	87.7	[72]
Multistrain	<i>Bacillus</i> , <i>Lactobacillus</i> , <i>Streptococcus</i> , <i>Clostridium</i> , <i>Saccharomyces</i> , and <i>Candida species</i>	decrease	100 (up to 1 ppm lowered)	[79]
Post-excretion strategies				
Moisture	20% sawdust	decrease with increasing aerobic condition	100 (5 ppm lowered)	[99]
Manure Amendment	<i>Bacillus subtilis subsp. spizizenii</i> , <i>Bacillus megaterium</i> , <i>Pseudomonas sp.</i> , <i>Psychrobacter faecalis</i> , <i>Leuconostoc mesenteroides</i> , <i>Streptomyces violaceoruber</i> , enriched with two species of <i>Lactobacillus plantarum</i> and <i>Yucca schidigera</i>	decrease	64.0	[102]
	<i>Pseudomonas fluorescens</i> , <i>Enterococcus faecium</i> , <i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> , <i>Leuconostoc mesenteroides</i> , and <i>Lactobacillus plantarum</i>	decrease	60.0	[104]
	80% sunflower oil and 20% water mixture	decrease	32.0	[105]
Biofilter	<i>Pseudomonas putida</i>	decrease	96.0	[126]
	<i>Thiobacillus thioparus</i>	decrease	97.5-98.0	[127]
	<i>Thiobacillus thioparus</i> with <i>Nitrosomonas europaea</i>	decrease	95.0	[128]
	<i>Acidithiobacillus thiooxidans</i> and <i>Hyphomicrobium VS</i>	decrease	99.8	[129]
	compost/wood chips	decrease	47.0-94.0	[130]

¹DDGS- Distillers dried grain plus soluble

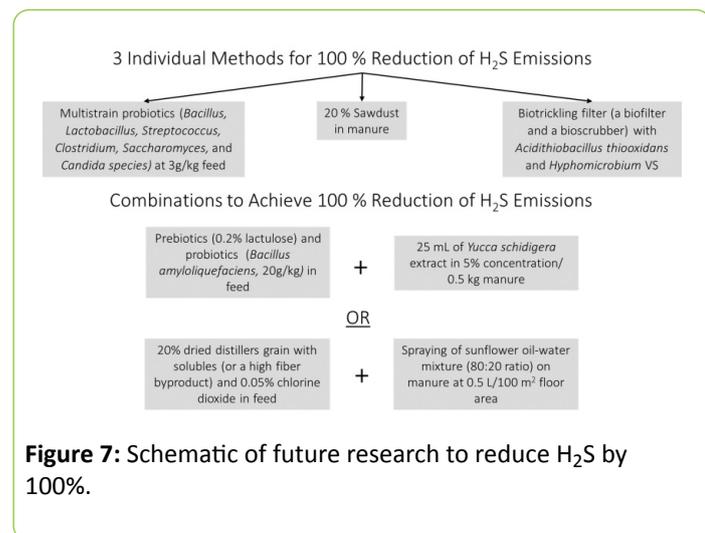
Table 2 was examined to determine recommendations for single or combined methods to achieve 100% reduction of H₂S. As shown in **Figure 7**, this can be achieved by feeding multistrain probiotics, treating manure with sawdust, or installing a "biotrickling filter," combining a biofilter and a bioscrubber, in the poultry house. A combination of *Bacillus*, *Lactobacillus*, *Streptococcus*, *Clostridium*, *Saccharomyces*, and *Candida species* used as a probiotic in feed will not only decrease the amount of H₂S released, but also promote and maintain a healthy gut microflora. Sawdust is used to lower the moisture content and to prevent the anaerobic decomposition of the undigested S-

containing amino acids. The biofiltration system using *Acidithiobacillus thiooxidans* and *Hyphomicrobium VS*, though proven only in the laboratory settings, relies on the ability of the selected strains of microorganisms to trap H₂S as H₂SO₄ in the exhaust fan before the air is released to the surrounding area.

In locales where resources may be limited, other combined methods to reduce H₂S emissions can be employed. The symbiotic effect of prebiotic(s) and probiotic(s) can promote the health of birds. If there is an additive effect, inclusion of 0.2% lactulose and 20 g *Bacillus amyloliquefaciens*/kg of feed will reduce H₂S, producing the desired effect of zero emissions. Or

5% concentration of *Yucca schidigera* extract added at a rate of 25 mL/0.5 kg manure can be used to eliminate H₂S emissions from manure.

If microorganisms are inaccessible, byproducts such as DDGS (20%) or perhaps other high fiber ones can be added to feed replace traditional corn-soybean meal. By supplying the antimicrobial activity of chlorine dioxide (0.05%) to the high fiber byproduct, an additional reduction of H₂S can be achieved. Manure can also be sprayed with sunflower oil and water to greatly reduce H₂S as well.



Conclusion

It is not likely that all possible ways to reduce H₂S will be used at the recommended levels. While research to control H₂S continues with broilers, more work should focus on their effects for laying hens especially during peak egg production at 30-40 weeks. With the recent advancement in microbial technologies, biofiltration and probiotics are promising areas of future research. Results of research, conducted in small laying hen houses under controlled conditions, can be scaled to industrial use, thereby affording more protection for animals, workers, and the environment, thus leading to a positive public acceptability of these very important agricultural operations.

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