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Identification of Effective Microbes for Ammonia and Odor Removal from Animal Manure

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Abstract

In intensive livestock husbandry, the issue with ammonia (NH_3) emissions and odors is still only partially solved. The main reason is the insufficiently detailed knowledge of processes taking place in animal wastes due to the large number of involved microorganisms. The emitted NH_3 is directly implicated in the formation of $PM_{2.5}$ ("fine particles") in the atmosphere, water eutrophication and soil acidification, contributes indirectly to N_2O emissions, has an adverse impact on animal health and productivity and decreases manure fertilizing value. The development of technologies for control of emissions from livestock operations requires investigations on mechanisms of these reductions, in particular with respect to microbial additives for manure treatment. A number of microbial strains of great practical importance for mitigation of animal excreta odor are studied. The mechanisms of inhibition of ammonia production and odors include: 1) assimilation of ammonia and odorous components; 2) oxidation of ammonia to non-volatile nitrogen compounds (nitrification); 3) reduction of manure pH by organic acids production; 4) antimicrobial activity against ammonifying and odor-generating microorganisms. The effects of different microbial communities and proportions on odor removal should be studied in order to develop efficient odor eliminators.
Keywords: effective microbes, ammonia, odor removal, animal manure

Резюме

Проблемът с емисиите на амоняк (NH_3) и миризми при концентрираното отглеждане на селскостопански животни е все още частично решен. Основна причина за това е недостатъчно задълбоченото познаване на процесите в животинските отпадъци, поради участието на голям брой микроорганизми в тези процеси. Емитираният NH_3 директно участва във формирането на $PM_{2.5}$ ("фини частици") в атмосферата, еутрофикацията на водите и подкисляването на почвите, косвено допринася за емисиите на N_2O, повлиява неблагоприятно здравето и продуктивността на животните, и намалява наторяващата стойност на тора. Развиването на технологии за контрол на емисиите от животновъдните дейности изисква изследвания на механизмите на тези редукции, особено по отношение на микробно-базираните добавки за третиране на тора. Проучени са редица микробни щамове с висока приложна стойност за обезмирисяването на животински екскременти. Механизмите на инхибиране производството на NH_3 и миризми включват: 1) асимилация на амоняка и компонентите на миризмата; 2) окисление на амоняка до нелетливи форми на N (нитрификация); 3) намаляване на pH на тора чрез производство на органични киселини; 4) антимикробна активност срещу амонифициращи и генериращи миризми микроорганизми. Ефектите на различни микробни комбинации и пропорции върху премахването на миризмата трябва да бъдат проучени, за да се разработят ефикасни обезмирисители.

Introduction

Microorganisms play a major role in the generation and mitigation of bad odors. In odor generation, volatile organic compounds are normal end products or intermediate products of anaerobe-induced fermentative degradation of fecal substances (Zhu, 2000). About 168 compounds associated with odors generated from livestock farming are identified: ammonia (NH_3), amines, volatile fatty acids (VFAs), sulfur-containing compounds, skatole, phenol, alcohol, and carbonyls (O'Neil and Phillips, 1992). Given that microbial activities are the origin of the bad odor, the understanding of mi-

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croflora features is essential for the development of efficient odor control techniques (Zhu, 2000).

Bioadditives containing microorganisms have attracted a significant attention due to their feasibility and relatively low price (Haga, 1996) and are able to propose a long-term solution for odor and ammonia emissions control (McCrory and Hobbs, 2001). In many instances however, neither their microbial composition nor the mechanisms for malodor mitigation are clear (Kuroda *et al.*, 2004; Sasaki *et al.*, 2006) so they are developed without in-depth understanding of microbiological processes occurring in animal wastes (McCrory and Hobbs, 2001). Furthermore, the studies are mainly focused on reduction of VFAs as main malodorous elements in fresh animal feces yet only few studies have been devoted to microorganisms reducing ammonia and sulfur compounds (Kuroda *et al.*, 2004).

Microbial assimilation of ammonium nitrogen is believed to be one of most important steps for ammonia removal by manure treatment (Nakai *et al.*, 1999; Nakai, 2001; Sasaki *et al.*, 2002, 2004b). Nitrogen assimilation or immobilization is the inclusion of NH_3 into organic compounds through a bacterial process. It may occur in both aerobic and anaerobic environments, but it is greater in aerobic conditions (Kermarrec, 1999). Most bacteria used in additives are obligate aerobes (Zhu, 2000). Although the correlations between NH_3 and odor are not definitively established, the attempts for reduction of NH_3 may have a corresponding impact on odor generation (Ullman, 2005).

Microbial isolates with high potential for deodorization of animal feces

In earlier studies, Ohta and Ikeda (1978) have investigated the possibilities for swine manure deodorization in a laboratory setting through *Streptomyces* – a genus from a group including a large spectrum of bacteria termed *Actinomycetes*. The optimum conditions of manure deodorization were determined as pH 8.6–10; temperature 35-40°C; humidity 42-63%; and the minimum amount of inoculum – 2 g bacterial culture per 10 g fresh faeces. Two species (*Streptomyces griseus* and *Streptomyces antibioticus*) demonstrated a strong potential with this respect. The amount of VFAs with up to six carbon atoms was significantly reduced after 48-hour treatment and reduction of specific bad odors was found out. The metabolism of actinomycetes was outlined as an important source of bioactive products and enzymes (Bull *et al.*, 1992; Ryckeboer *et al.*, 2003). In another study of Ohta and Kuwada (1988), the counts of actinomycetes and thermophilic bacteria increased during bovine faeces deodorization. The optimum conditions were determined as pH 7–9; temperature 30-40°C; humidity 35-52%; with minimum inoculum amount of 10% (w/w). To achieve the necessary porosity, rice hull, cut rice straw, or sawdust were added to the mixture of faeces and bacterial culture. Sulfur compounds e.g. H_2S and low-molecular weight VFAs were almost not detected in deodorized faeces. Some of deodorizing isolates were identified as *Corynebacterium* spp., *Flavobacterium* spp., *Streptomyces* spp., and *Thermoactinomyces* spp. Hayashida *et al.* (1988) selected five obligately aerobic strains of actinomycetes, identified as *Streptomyces antibioticus*, *S. puniceus*, *S. nigrifaciens*, *Thermoactinomyces vulgaris*, and *Thermomonospora viridis*. The strains grew preferably in non-sterilized fresh swine feces without additives. Fecal VFAs were completely assimilated by actinomycetes within 24 h (initial humidity of 65% and pH 8.0-8.5). All five strains used VFAs as a carbon source; in particular, *T. viridis* assimilated *n*-valeric acid, whereas *Thermoactinomyces vulgaris* assimilated *n*-butyric acid. The treatment of poultry manure turned out to be very difficult due to the presence of large amounts of uric acid. Nevertheless, about 50% of uric acid was degraded by these five strains. The thermophilic *T. viridis* strain (50-55°C) grew predominantly in swine and poultry manure and had a leading role in practical composting.

Other efficient microorganisms able to degrade completely all VFAs types are *Acinetobacter calcoaceticus*, *Alcaligenes faecalis* and *Arthrobacter flavescens* (Bourque *et al.*, 1987; Jolicoeur and Morin, 1987). Fitriyanto *et al.* (2017) isolated an ammonium-oxidizing bacterium identified as *Arthrobacter nitroguajacolicus* (genus *Arthrobacter*). The isolate was obtained from soil samples in an area with high ammonia emissions from a poultry farm and was shown able to grow in a medium with high ammonium concentration (including cow urine). The strain oxidized 26.86 mg L^{-1} ammonium during 48-hour cultivation at optimum growth temperature of 30 °C, by transforming ammonium-N to nitrite and nitrate (nitrification). Matusiak *et al.* (2013) investigated the morphological and physiological features and enzymatic potential of *Bacillus subtilis subsp. spizizenii*, *Bacillus megaterium*, *Pseudomonas* sp., *Psychrobacter faecalis*, *Leuconostoc mesenteroides*, *Streptomyces violaceoruber* and *Candida inconspicua*, originating from various environments – soil, silage and poultry manure. The tested microorganisms developed mainly in aerobic conditions within a temperature range from 10 to 44°C. All actively removed volatile compounds from poultry manure (dimethylamine, trimethylamine, hydrogen sulfide, isobutyric acid) with reduction level from 11 to 52%. *Pseudomonas* sp., *B. subtilis*, *P. faecalis* and *S. violaceoruber* were the strains able to reduce nitrogen compounds.

Sasaki *et al.* (2005) evaluated the ammonia assimilation ability of microorganisms isolated during cattle manure composting and their distribution in the microbial community. The isolates exhibiting high ammonia consumption from the medium incubated at 37°C were highly similar to *Enterobacteriaceae*, *Brevibacteriaceae*, *Xanthomonadaceae*, *Pseudomonadaceae*, and *Actinomycetales*. The isolates obtained from incubation at 55°C were identified as thermophiles or endospore-forming microorganisms. Nakasaki *et al.* (1985) noted that thermophiles grew through catabolism but not through anabolism during the high-temperature composting stage. In the research study of Sasaki *et al.* (2005), *Bacillus* isolates were thermophilic but nevertheless were not able to assimilate actively ammonia during the anabolism stage of composting. *P. citrea* and *Streptomyces* spp. were also identified as prevailing composting actinomycetes. During composting of swine, cattle manure and sewage sludge, Sasaki *et al.* (2009) demonstrated that members of genus *Bacillus* and *Bacteroidetes* were mainly detected after the thermophilic stage, regardless of the fact that bacteria belonging to various types were also present during the early composting stage. In a study on the microbial community in a wastewater lagoon of a dairy cattle farm, Sasaki *et al.* (2004a) identified isolates characterized with highest ammonia assimilation ability at 10 and 37°C as *Janthinobacterium lividum* and *Bacillus* sp., respectively. Ammonia-assimilating organisms existed as non-dominant species in the lagoon microbial community.

Lactic acid bacteria

The treatment of swine manure with *Lactobacillus plantarum* and soluble carbohydrates reduced substantially the ammonia evaporation by 34.6–92.4%, with greatest reduction for *L. plantarum* + 10% sucrose, in combination with manure pH decrease to about 4.0. The reduction of pH resulted in higher NH_4^+-N content (5.3–17.5%) in treated manure. In addition, the treatment with *L. plantarum* reduced VFAs associated with malodor (valeric acid, 12.3-47.7%; *iso*-valeric, 3.5-23.8%) and main microbial odor producers, with greatest reduction in the *Eubacteria* counts (34.1%) being achieved with the combination *L. plantarum* + 10% sucrose (Huang *et al.*, 2006). In a study performed by McCrory and Hobbs (2001), the combination of *L. plantarum* and glucose reduced swine manure pH from 8 to 6. From pig feces, Kim and Park (2006) isolated a bacterial strain identified as *L. plantarum* with excellent denitrifying ability under anaerobic conditions. Its inoculation in nitrate-supplemented minimal medium (MM-NO_3^-), resulted in rapid reduction of nitrate concentration after 20-hour incubation by 93.8% and nitrite production declined to almost zero during the experiment. These results demonstrated that the isolate could reduce nitrate to nitrogen gas without accumulation of nitrite under anaerobic culture conditions. After 50-hour incubation of the strain, the great amount of acetic acid in fresh piggery slurry was reduced by about 40%. The *n*-butyric, *n*-valeric, and *iso*-valeric acids decreased gradually, while *iso*-butyric acid and capronic acids were eliminated at a significant extent in the beginning of treatment. The efficiency of NH_3 removal attained a peak of 98.5% following 50-hour incubation. The H_2S concentration was not altered. The *Lactobacillus* strain isolated by Yan *et al.* (2017) could reduce NH_3, H_2S and odor intensity by more than 30% in 36 days' treatment of swine manure.

Alam *et al.* (2021) assessed the odor reduction potential of four bacterial species isolated from fresh swine feces. One of species was identified as *Enterococcus faecium*. Bacterial cultures were anaerobically incubated for 12 and 24 h. With *E. faecium,* no NH_3 and H_2S concentration were detected on the 24th hour of incubation, while the lowest total volatile fatty acids, the highest lactate and moderate butyrate concentrations, as well as the lowest total biogenic amine, histamine, ethylamine, putrescine, methylamine, and cadaverine were measured. Kim *et al.* (2022) isolated two strains (L12I and 12III), identified as *Pediococcus acidilactici*. The strains decreased manure pH (swine, cattle, poultry, and sawdust at ratios of 50, 10, 10, and 30%, respectively) and inhibited the growth of hyper-ammonia-producing bacteria (HAB) with urease activity (*Clostridium aminophilum* and *Proteus mirabilis*). Ammonia emission reduction demonstrated by L12I and 12III was 23.58% and 38.00% respectively. The strains were additionally able to improve manure quality by preservation of total nitrogen and urea contents. The total N of controls, L12I, and 12III decreased by 22.16, 14.93, and 10.27% respectively vs the initial content, whereas urea reduction percentages were by 62.18, 45.57, and 45.00%.

Yeasts

In their studies, Kim *et al.* (2004) have used three isolates for swine manure treatment : *Candida rugosa* – isolated from compost; *C. rugosa* and *C. maris* – isolated from soil. The three yeast isolates were inoculated on liquefied 10% pig slurry medium for 72 h at 30°C. With respect to VFAs, *C. maris* from soil demonstrated 100% reduction of butyric, *iso*-butyric, and *iso*-valeric acids. *C. rugosa* from compost showed 100% reduction of butyric and *iso*-butyric acids, whereas *C. rugosa* from soil: 100% reduction of propionic, butyric, and *iso*-valeric acids. Additionally, these yeasts reduced efficiently NH_4-N, soluble-N, and biological oxygen demand (BOD). The report of Kim *et al.* (2021) was aimed to evaluate the efficiency and mechanism of action of *Saccharomyces boulardii* for reduction of ammonia emissions from animal manure (mixture of 50% swine, 10% cattle, 10% poultry, 30% sawdust). For genomic quantification of ammonia-producing bacteria in manure, *Proteus mirabilis* was used. After 24-hour incubation, manure pH in controls was significantly increased; DNA concentration of *P. mirabilis* that increased manure pH via ammonia production, demonstrated 2.7-fold increase. The experimental variant (*S. boulardii*) showed considerable reduction of pH and of *P. mirabilis* presence, as well as significant reduction of cumulative ammonia emissions by about 25% for 35 days. The study data suggested that the changes of animal manure pH resulted not only from chemical activity of manure components, but also from environmental changes due to the replication and activities of microorganisms in manure.

***Bacillus* species**

Bacillus strains were reported to possess a high potential for ammonia assimilation through its conversion into bacterial cells and/or extracellular products as poly-γ-glutamic acid (PGA) as temporary ammonia depots (Pötter *et al.*, 2001; Hoppensack *et al.*, 2003; Kuroda *et al.*, 2004). The study of Pötter *et al.* (2001) concluded that a number of Gram-positive bacteria (*Bacillus licheniformis*, *B. subtilis*, *B. megaterium*, *B. anthracis*, *Sporosarcina halophila*, and *Planococcus halophila*) were able to synthesize polyamino acid PGA as capsular substance or water-soluble slime. *B. licheniformis* was shown to excrete up to 20 g/l PGA into the medium. The degradation of PGA from non-adapted microflora is very slow, as the polymer is markedly resistant to proteolytic attacks. The authors outlined the N:C ratio of 1:13.8 as optimal for growth and PGA production, with influence of the used carbon source added to the liquid manure. Hoppensack *et al.* (2003) isolated a PGA-producing *Bacillus licheniformis* strain and tested its ability to reduce NH_4^+-N in liquid manure through conversion of ammonia into biomass and PGA as serve form of nitrogen. In this study, the ammonia amount decreased substantially from 2.83 to 0.1 g/l^{-1}. Nakasaki and Ohtaki (2002) reported a reduction of ammonia emissions by 40% through utilization of a *B. licheniformis* strain at optimum temperature of 50°C during organic matter composting. In studies with poultry manure (Borowski and Gutarowska, 2008; Gutarowska *et al.*, 2014), the best efficiency for removal of selected volatile compounds was associated with genera *Bacillus* and *Pseudomonas*. The most active bacterial species were *B. licheniformis*, *B. subtilis*, *P. fluorescens* and *Pseudomonas* sp., found able to reduce N-NH_4 and sulfides by about 60-70%. Ma *et al.* (2021) reported about ammonia and hydrogen sulfide reduction rates in swine manure of 85.81 and 99.29%, respectively with application of *B. licheniformis*, and rates of 69.69 and 90.80%, respectively with *B. subtilis*. Seeking efficient bacterial compost accelerators for production of high-quality and well-balanced compost, Todorova (2013) used *B. subtilis* as bacterial additive, because being a spore-forming microorganism, it survived successfully the thermal composting stage. The layer manure and wheat straw compost produced with this additive was dark brown, loose, soft, with earthy smell and with significantly higher total N and total C contents (Todorova and Ivanova, 2013).

Hanajima *et al.* (2009) investigated the dynamics of bacterial community in the course of reduction of malodorous substances in aerated semi-liquid swine manure and used the NH_4-N:Total N ratio for evaluation of the extent of ammonium-N assimilation. The decreased ratio was partially due to NH_4-N fixation by *Bacillus* spp., whereas increased ratio was probably due to NH_4-N formed by decomposed bacterial cells and/or other nitrogen-containing organic compounds. Shimaya and Hashimoto (2011) isolated a thermophilic nitrifying bacterium from dairy cattle manure compost. The strain was classified as belonging to genus *Bacillus*, close to *B. halodurans*, but was identified as a new species. The effect of its inoculation for reduction of ammonia emissions during composting at 50°C through direct addition of bacterial culture was evaluated. The strain grew well in dairy cattle manure. According to the results, the strain oxidized NH_4–N and produced NO_2–N in the matter. After its supplementation, ammonia concentrations decreased by 35.9% over a week. Wang *et al.* (2019) tested various concentrations of *Bacillus stearothermophilus* (BS) during composting layer manure in an aerobic bioreactor with added sawdust for greater volume. The compost warming period was 2 days with BS and 4 days without BS. Ammonia emissions from the treatment with 8.00 g/kg BS were significantly lowed as compared to lower BS levels and control treatments, the ammonium-N and nitrate-N concentrations were significantly higher and pH – substantially reduced. The addition of BS altered the microbial community structure during the warming and high-temperature periods of composting by increasing the relative shares of lactic acid Bacillus and nitrification bacteria. Kuroda *et al.* (2004) set as a goal to isolate a thermophilic ammonia-tolerant bacteria for application in reduction of NH_3 emissions during swine manure composting. The isolated strain was classified as *Bacillus*, close to *B. pallidus*. Its addition resulted in increase in total nitrogen, lower ammonia emission and 40% reduction of nitrogen losses. In later experiments Kuroda *et al.* (2015) mixed an equal amount of water with the additive a day before its use, then the mixture was added to a strain concentration of over 10^7 CFU/g DM in the initial matter, and lower level of emitted NH_3 and lower nitrogen loss by 22% was detected. The additive amount for efficient reduction of NH_3 emission was 10.75 g, or 1/400 of the total weight of matter (3.6 kg swine manure; 0.6 kg sawdust; 0.1 L water). The strain may be steadily stored at a density of 10^9 CFU/g DM in a dried state for a long period at 10-30°C. The cells in the additive were under the form of endospores, which was convenient for preparation of the bioadditive and its practical application. After 1-year storage, 77% of the initial strain concentration was preserved. The phylogenetic dendrogram based on 16S rDNA sequence indicates that the strain is positioned closest to *B. thermolactis*.

The key to the utilization of bacterial cultures for livestock manure deodorization is when they become the predominant strain in manure (Grubbs, 1979). When *Bacillus* strains are used as bacterial additive (mainly during animal manure composting), they become predominant in the bacterial community, which is essential for their efficiency. This was observed in a number of studies (Kuroda *et al.*, 2004, 2015, 2017; Hanajima *et al.*, 2009; Shimaya and Hashimoto, 2011; Li *et al.*, 2012; Todorova, 2013). It was also demonstrated that *Bacillus* strains produced bacteriocins with strong antimicrobial activity against a broad spectrum of microorganisms (Lim *et al.*, 2011; Todorova and Kozhuharova, 2010; Sumi *et al.*, 2015). The first peptide antibiotic, bacitracin, produced by *Bacillus licheniformis* cultures, is widely applied in human and veterinary medicine, some other peptide antibiotics synthesized by different *B. licheniformis* strains were also investigated (Mendo *et al.*, 2004). It was reported that *B. amyloliquefaciens* produced an antimicrobial bacteriocin (barnase), which may reduce ammonifying *Clostridium perfringens*, *E. coli* and *Yersinia* in feces, thus reducing the release of NH_3 in this experiment (Lisboa *et al.*, 2006). Therefore, the reduction of ammonifying bacteria density is of key importance for reduction of NH_3 emissions (Kim *et al.*, 2014).

Compound microbial inoculants

The odor components are complex, so often a single strain is not able to remove them completely (Ma *et al.*, 2021). A complex microbial adsorbent composed of *B. megaterium*, *S. griseus* and *C. tripicalis* (+ rice hull and ceramic particles as carrier) has reduced ammonia concentration and odor intensity by more than 80%, and hydrogen sulfide concentration by more than 65% in swine, chicken and cattle manure (Ye *et al.*, 2008). During composting of swine manure + sawdust supplemented with 1% compound microbial agents (*Bacillus subtilis*: *B. licheniformis*: *Bacillus megaterium*: Yeasts = 1:1:1:2), the total nitrogen (TN) content in the experimental group was higher in the maturation phase, which was attributed to microbial inocula and their role in nitrogen preservation (Li *et al.*, 2020). A microbial complex of *B. flexus* and *L. casei* was reported to achieve an ammonia-nitrogen degradation efficiency of 53.19% in swine manure (Sun *et al.*, 2020).

B. stearothermophilus, *C. utilis*, and *B. subtilis* in ratios of 2:1:1 (A), 1:1:2 (B), 1:2:1 (C), and 1:1:1 (D), were tested to determine the best combination of the three microbial agents for ammonia reduction during fresh layer manure and sawdust (ratio 4.90:1) composting. The groups A, B, C, and D reduced ammonia emissions by 17.02, 9.68, 53.11, and 46.23%, respectively. The groups C and D demonstrated significantly increased nitrate-N concentrations and decreased pH values and ammonium-N concentrations. During compost warming and high-temperature stage (days 0-8), nitrate-N content was considerably greater in C and D treatments, meaning that added microorganisms were able to perform nitrification during the high-temperature stage. The total nitrogen loss rates for control, A, B, C and D were 38.66, 32.80, 37.97, 22.16, and 25.66%, respectively. Up to the third day of composting, the control had a significantly lower total nitrogen content than groups A, C and D, proving that microbial agents fixed nitrogen during the warming up stage. During the composting, the total bacterial number was substantially greater in groups C and D (Zhou *et al.*, 2019). Bajagain *et al.* (2022) developed and presented a novel biofoam technology consisting of spraying a surfactant foam on swine manure. Three NH_3-degrading isolates from swine manure: *Saccharomyces cerevisiae*, *L. lactis* subsp. *hordniae*, and *L. argentoratensis* were used, with respective NH_3 removal efficiencies of 45, 82, and 85% under a capped jar system and ambient temperature 22°C. Reduction efficiency increased at 30°C to rates of 49, 90, and 92%, respectively. The three strains were mixed in 1:1:1 ratio to evaluate their potential for NH_3 removal from the NH_3 source in an open jar. The tested microbial consortium removed completely NH_3 after 48 h. The concentration of NH_3, released from swine manure in an open reactor, with application of surfactant bacteria-loaded foam containing the microbial consortium was significantly reduced to 10 ppm (after 24 h) and 0 ppm (after 48 h).

Conclusion

During the aerobic degradation of livestock manure (composting), in particular during its thermophilic stage, nitrogen losses due to ammonia emissions are considerable. In free-range livestock production systems with prolonged accumulation of manure in the animal premises as farm litter, high ambient levels of released ammonia are also anticipated due to the constant deposition and mixing of urine and feces and the rapid hydrolysis of urinary nitrogen compounds. In such systems, the introduction of functional microorganisms with high potential for deodorization and assimilation of ammonia through its conversion into microbial biomass and extracellular products, or bioconversion to non-volatile nitrogen compounds seems an extremely efficient approach. Microbial additives are reproducible and may offer a long-term solution for control of emitted NH_3 and odors through enhanced biodegradability of manure, and additionally – for improvement and balancing of manure fertilizing value. The strong antimicrobial activity against a broad range of microorganisms, including ammonifying bacterial in animal feces is another beneficial mechanism of action of inoculated microflora, proved efficient in mitigation of manure ammonia emissions.

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