
1 **Odour reducing microbial-mineral additive for poultry manure**
2 **treatment**

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13
14 **Abstract:** Poultry production systems are associated with emissions of odorous volatile
15 organic compounds (VOCs), ammonia (NH₃), hydrogen sulfide (H₂S), greenhouse gases,
16 and particulate matter. Development of mitigation technologies for these emissions is
17 important. Previous lab-scale research on microbial-mineral treatment has shown to be
18 effective for mitigation of NH₃, H₂S and amines emissions from poultry manure. The aim
19 of this research was to assess the effectiveness of surface application of a microbial-
20 mineral treatment for other important odorants, i.e., phenolics and sulfur-containing
21 VOCs. Microbial-mineral litter additive consisting of 20% (w·w⁻¹) of bacteria powder
22 (six strains of heterotrophic bacteria) and 80% of mineral carrier (perlite-bentonite) was
23 used at a dose of 500 g·m⁻² (per ~31 kg of manure). Samples of air were collected in two
24 series, 4 and 7 days after application of additives. An odor profile of the poultry manure

25 was determined using simultaneous chemical and sensory analysis. Reduction levels of
26 VOCs determined on Day 4 was between 31 and 83% for mineral adsorbent treatment
27 and in the range of 9 and 96% for microbial-mineral additive, depending on the analyzed
28 compound. Reduction levels on Day 7 were considerably lower than on Day 4, suggesting
29 that the odorous VOCs treatment efficacy is relatively short. There was no significant
30 difference between treatments consisting of microbial-mineral additive and mineral
31 carrier alone.

32

33 **Keywords:** Odour Mitigation, Poultry Manure Additive, GC-MS-Olfactometry, Volatile
34 Organic Compounds.

35

36 **1 Introduction**

37 Global growth in poultry production, especially in Europe where Poland is the largest
38 producer of poultry meat within EU (2014) [1], is strongly linked with livestock odour
39 that has become a nuisance for people living in surroundings of poultry houses [2] as well
40 as a problem for animal health [3]. There have been many studies on methods for
41 mitigating emissions of livestock odour, however, most of the papers focus on the swine
42 production [4, 5]. Methods of emission mitigation from poultry manure are less studied.
43 The reported methods on mitigation of emissions from swine manure rely on addition of
44 different adsorbents to the manure, amendment with yeast strains, or use of filtration and
45 biofiltration beds [6, 7, 8, 9, 10]; however, there have been some evaluations of bacterial
46 additives [11, 12], mineral additives or air filtration [13, 14, 15, 16, 17] in mitigation of
47 emission from poultry manure. Research by Matusiak et al. [18] has shown that the
48 microbial-mineral litter additive (MMLA) consisting of mineral carrier and bacteria

49 powder can be effective at reducing NH₃, amines and H₂S emissions from poultry manure.
50 Nonetheless, more comprehensive assessment of the MMLA is needed for treatment of
51 odour-generating compounds such as phenolics and sulfur-containing compounds.

52

53 Thus, the aim of the study was to evaluate the effectiveness of an innovative MMLA in
54 mitigating emissions of odorous volatile organic compounds (VOCs) from poultry
55 manure under conditions simulating a typical poultry house environment (air
56 temperature, ventilation rate, stocking density, and amount of manure generated). Our
57 working hypothesis was that the MMLA treatment performs as well as other additives
58 previously reported for other key odorants of concern [18] and that the microbial
59 treatment significantly improves the performance of MMLA treatment mix.

60

61 **2 Materials and methods**

62 The research was carried out at the Air Quality Laboratory in the Department of
63 Agricultural and Biosystems Engineering at Iowa State University. Microbial-mineral
64 litter additive (six strains of heterotrophic bacteria on a perlite-bentonite carrier) [18] was
65 tested under laboratory conditions. The MMLA, consisting of a mineral carrier and
66 bacteria powder, was added topically to nearly fresh manure from laying hens in order to
67 reduce the emissions of odorous VOCs. The manure (with cake-like consistency) was
68 accumulated in a layers house for 3 days and used for the trials directly after collection.

69

70 **2.1 Experimental conditions**

71 The treatment containers (six 1 L glass jars, 64.5 cm² of surface area) were filled, on the
72 Day 1 of each trial with, 200 g of nearly fresh manure from laying hens mixed with water

73 (representing two weeks of manure accumulation). Additional water was added to the
74 manure (25 mL of water per 100 g of manure) to prevent the manure from drying out and
75 to facilitate manure homogenization. All treatment containers were simulating a scaled-
76 down surface of poultry manure (~4 cm of manure depth) with controlled airflow over
77 the surface matching regular ventilation conditions.

78

79

<Figure 1>

80

81 Airflow through the containers was 12 exchanges per h, which was lower than the airflow
82 expected in a typical poultry house (approximately 27 exchanges per h [19]) due to
83 apparatus limitations. This lower airflow provided higher concentrations of VOCs and
84 improved the sensitivity of gas measurements while simulating a more environmentally
85 adverse situation (e.g., lower air exchanges during cool season). The inlet air (~200
86 mL·min⁻¹) was filtered through an activated carbon scrubber and humidifier. The
87 temperature and relative humidity of the air were measured after the air passed through
88 the humidifier, and were respectively 22 °C and 99%. In a single trial 3.2 g of the MMLA
89 (which corresponds to the dose of 500 g of MMLA per 1 m²), consisting of an 80% (w·w⁻¹)
90 mineral perlite-bentonite carrier and 20% of bacteria powder, was added topically to
91 the manure in two treatment containers. Concentration of bacteria powder was adopted
92 on the basis of previous work [20]. Two treatment containers were treated with perlite-
93 bentonite carrier only (2.56 g) to evaluate the influence of the mineral absorbent (MA)
94 alone. Two treatment containers were control (200 g of poultry manure only). The trials
95 lasted for 7 days and were carried out 3 times.

96

97 2.2 Sampling and chemical analysis

98 Manual solid phase microextraction (SPME) with StableFlex 50/30 μm
99 divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco,
100 Bellefonte, PA) was used for extraction of odorous compounds from the headspace inside
101 the treatment containers on Day 4 and Day 7 of each trial. An optimal sampling time of
102 30 min was determined during preliminary experiments, according to the procedure
103 described by Cai et al. [17]. The sampling procedure was as follows: airflow was stopped
104 for a single container by closing inlet and outlet of the treatment container, conditions
105 inside the treatment container were allowed to stabilize for 10 min and gas sampling was
106 conducted for 30 min. Collected gas samples were analyzed using a gas chromatograph
107 – mass spectrometry – olfactometry (GC-MS-O) (Microanalytics, Round Rock, TX,
108 USA). The GC-MS-O system components, acquisition software and basic GC oven
109 programs are described in detail by Cai et al. [21].

110

111 Odorous VOCs were tentatively identified on the basis of comparative analysis of the
112 determined mass spectrum and the National Institute of Standards and Technology
113 (NIST05) MS library. During the GC separation, aroma and odour intensity of individual
114 compounds were measured simultaneously, with the use of a sniff port and AromaTrax
115 10.1 software. Evaluation of air purification effectiveness was made on the basis of the
116 relative reduction value (RRV, %) determined for each tentatively identified odorous
117 VOC. The RRV was calculated similarly to the formula given by Cai et al. [17] as the
118 ratio of the difference between the control and treatments mean (of 6 replicates) peak
119 height counts of the tentatively identified odorous VOCs. Peak heights were used for

120 comparison instead of peak areas, to improve integration of asymmetric or low
121 chromatographic peaks.

122

123 2.3 Statistical analysis

124 Due to the high statistical dispersion, 180 outliers were removed from a total of 720
125 values using Q-Dixon test. Practically, the lowest and the highest measured peak height
126 for each compound, for each trial was an outlier. Data were tested for normality with
127 Shapiro-Wilk test and no set of values showed normal distribution. Statistical
128 comparisons between the treatments were done via Kruskal-Wallis test using GraphPad
129 Prism 5.04 (Prism for Windows, GraphPad Software Inc., La Jolla, CA). Differences
130 among treatment means were tested for significance using the Dunn's test. Differences
131 were considered significant at $P < 0.05$.

132

133 **3 Results and discussion**

134 A total of 15 VOCs were tentatively identified in the analyzed gas samples emitted from
135 the treated/untreated poultry manure. The compounds along with their matching
136 percentage of identity assignment in the MS spectral database, GC column retention times
137 and mean peak height are shown in the Table 1. Significant differences between
138 treatments were noted only for dimethyl disulfide on Day 7, where mineral adsorbent
139 (MA) alone was showing better deodorizing effect. Statistically significant differences
140 between control group and microbial-mineral litter additive (MMLA) treatment were
141 determined for methanethiol, dimethyl sulfide, 2-butanone, 2-pentanone, s-methyl
142 thioacetate and methyl ethyl disulfide on Day 4 of treatment. Statistically significant
143 differences between control group and MA treatment were noted for s-methyl thioacetate

144 on Day 4 of treatment. Relative reduction of target VOCs in comparison to the control
145 group on Days 4 and 7 are shown on Figures 2 and 3, respectively.

146 <Table 1>

147

148 <Figure 2>

149

150 <Figure 3>

151

152 In this study, obtained reduction levels of tentatively identified VOCs content determined
153 on Day 4 ranged between 31 and 83 % for the MA treatment and between 9 and 96 % for
154 MMLA treatment, depending on the analyzed compound. Reductions on Day 7 ranged
155 from -243 to +65% (MA) and -219 to +59% (MMLA) depending on the analyzed
156 compound. Negative values indicate increase in content of particular VOC. The increase
157 of VOCs content can be explained by decrease in adsorption ability over time (sorber
158 saturation effect) and also by re-emission of VOCs adsorbed within first days after
159 application of MMLA.

160

161 Results can be compared with prior research testing various methods of odor reduction
162 from livestock buildings, including deodorization of manure. Deodorization of poultry
163 manure with bacterial additives has been carried out by Matusiak et al. [18] and reduction
164 between 58% and 73% has been reported after day 4 of treatment. The 58% to 73%
165 reduction corresponds to the ~40% adjusted reduction, considering the drop of VOCs
166 content in the control group itself (as an effect of treatment time). Biofiltration conducted
167 at the reproductive hen farm reduced VOCs containing sulfur by 51% (max. 70%), while

168 for aldehydes and ketones the reduction ranged between 62 and 99% [22,23]. Cai et al.
169 [17] reported, that average reduction of the odor was between 51 and 67% after topical
170 application of zeolite on the poultry manure. Opaliński et al. [24] filtered the air polluted
171 by poultry manure through aluminosilicates adsorbents and the reduction of NH₃ and
172 VOCs emissions ranged between 58 and 84%. Considering the above, it can be assumed
173 that a mineral adsorbents could have sufficient deodorizing potential.

174 It is also worth noting that concentration of bacteria powder in the MMLA used in this
175 research was 20%, which is economically unjustified at this point of development of the
176 litter additive. There were no significant differences between MA and MMLA treatments
177 at the investigated concentration of bacteria. Thus, mineral adsorbent alone appears to be
178 responsible for major deodorizing effect in the investigated additive, at least during the
179 first week after its surficial application to manure.

180

181 3.2 Aroma profile of the manure

182 A total of 14 aromas have been detected and recorded in the analyzed gas samples. The
183 aromas represent a panelist's response to separated odorants eluting from GC column and
184 a sniff port. Aromas along with the compounds corresponding to them are shown in the
185 Table 2. An example aromagram is shown in Figure 4.

186 <Table 2>

187

188 <Figure 4>

189

190 By comparison of GC column retention times of tentatively identified VOCs with
191 retention times of aroma events it can be assumed that: *animal/fecal, hay/straw,*

192 *characteristic, fecal, mushroom, sauerkraut* aromas can be associated with *methanethiol*,
193 *dimethyl sulfide, s-methyl thioacetate, dimethyl disulfide, 3-octanone, dimethyl trisulfide*,
194 respectively. All aromas of the sulfur-containing compounds present during the analysis
195 are reported to be rotten- or fermented-like [25] and occur in manure matrixes [21, 26].

196

197 Parcsi and Stuetz [27] analyzed gas samples from different poultry houses and identified
198 6 odorants with the use of olfactory detection port, which two of them (dimethyl disulfide
199 and dimethyl trisulfide) were consistent with the odorants reported in this study. The
200 effectiveness in aroma reduction in comparison to the control group on Day 4 and Day 7
201 ranged between -250% and +100% and is shown in Figures 5 and 6, respectively.

202

203 <Figure5>

204

205 <Figure 6>

206

207 Influence of the investigated additives on the reduction of aroma intensities was not
208 statistically significant because the evaluation was limited to one panelist; however,
209 a general observation was made, that the MA treatment had better effect in reducing the
210 aroma intensity compared to MMLA which also was confirmed after smelling the
211 headspace of treated manure directly, at the end of a trial.

212

213 Economic analyses are ultimately needed for scale-up to farm applications. Material and
214 labor costs, feasibility of practical farm-scale application, biosecurity, toxicology, manure
215 quality and land application concerns have to be considered. To date, most published

216 literature involving economics of gaseous emissions from livestock operations are
217 focused on swine production [5,28]. It is recommended that economic analyses are
218 performed to estimate full scale application costs.

219

220 **4 Conclusion**

221 The microbial-mineral litter additive and mineral adsorbent (MMLA and MA,
222 respectively) were tested in lab-scale experiments for its potential to treat odorous VOCs
223 emissions from poultry manure within 7 days of its surficial application. Conditions under
224 which the study was carried were simulating scaled-down conditions occurring in a
225 typical poultry house in terms of ventilation rate and amount of manure per area.
226 Reduction level of tentatively identified VOCs content determined on Day 4 was between
227 31 and 83% for the MA treatment and in the range of 9 and 96% for MMLA treatment,
228 depending on the analyzed compound. MMLA and MA treatments efficacies were
229 considerably lower on Day 7 than on Day 4, and the increase in content of some VOCs
230 was observed likely due to saturation of the adsorbent and re-emission of VOCs. There
231 were no significant differences between treatments consisting of MMLA and MA. Thus,
232 based on these initial and short term (1 week) screening tests, there appears to be no
233 justification in enrichment of mineral adsorbents with investigated bacteria strains.
234 Additional research with either (a) longer treatment time, (b) optimization of bacteria
235 concentration, and/or (c) farm-scale research with poultry present during the experiment
236 is recommended. Economic analyses will also be needed to estimate full scale application
237 costs.

238

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329 hydrogen sulfide gas emissions. *Atmospheric Environment*, 2017, 150: 313-321. doi:
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- 331

332 **Figure Captions**

333 **Figure 1.** Laboratory-scale system for controlled studies of surficial treatments of
334 poultry manure and mitigation of odour and gaseous emissions at Iowa State University.

335 **Figure 2.** Comparison of % reduction in target gas emissions, Day 4.

336 **Figure 3.** Comparison of % reduction in target gas emissions, Day 7.

337 **Figure 4.** Gas sample comparison of aromagram (bold line) and chromatogram (thin
338 line) resulting from a simultaneous chemical and sensory analyses. An image generated
339 from AromaTrax software by a panelist assessing separated compounds eluting from the
340 sniff port.

341 **Figure 5.** Odour intensity reduction associated with target compounds determined
342 by panelist at the sniff port of GC-MS-O, Day 4.

343 **Figure 6.** Odour intensity reduction associated with target compounds determined
344 by panelist at the sniff port of GC-MS-O, Day 7.

346 **Table 1.** Effect of investigated treatments on selected tentatively identified VOCs (mean, n=6).

| compound | R _T /min | spectral match with MS database | day 4 | | | | | | | | day 7 | | | | | | | |
|---------------------------|---------------------|---------------------------------|--------|--------|--------|--------|--------|--------|--------|---------|--------|--------|--------|--------|--------|--------|-------|---------|
| | | | C | | MA | | MMLA | | SEM | P-Value | C | | MA | | MMLA | | SEM | P-Value |
| | | | H | SD | H | SD | H | SD | | | H | SD | H | SD | H | SD | | |
| 1. methanethiol | 2.80 | 86 % | 12723 | 9125 | 7244 | 4430 | 3516 | 1104 | 1698 | 0.0294 | 4126 | 1225 | 5339 | 1916 | 6444 | 3176 | 468 | 0.2269 |
| 2. dimethyl sulfide | 3.17 | 93 % | 100273 | 71083 | 18316 | 17684 | 3555 | 971 | 16984 | 0.0463 | 15884 | 16852 | 11845 | 11439 | 16268 | 11947 | 2917 | 0.6387 |
| 3. 2-butanone | 4.15 | 74 % | 72537 | 53920 | 12340 | 11897 | 2879 | 311 | 12589 | 0.0151 | 16463 | 17514 | 14830 | 16224 | 13967 | 9853 | 3074 | 0.8671 |
| 4. 2-butanol | 5.22 | 79 % | 16139 | 6138 | 3502 | 2444 | 14640 | 19361 | 3503 | 0.0961 | 13108 | 15861 | 10728 | 9628 | 7567 | 4230 | 2294 | 0.9828 |
| 5. 2-pentanone | 5.60 | 59 % | 15271 | 9187 | 4170 | 3129 | 2298 | 1098 | 2154 | 0.022 | 2655 | 1695 | 4304 | 3228 | 6456 | 3864 | 706 | 0.1572 |
| 6. s-methyl thioacetate | 6.37 | 93 % | 8170 | 3478 | 1690 | 774 | 1413 | 442 | 1002 | 0.0066 | 3516 | 3716 | 2636 | 1980 | 1436 | 946 | 551 | 0.4263 |
| 7. dimethyl disulfide | 7.25 | 94 % | 800227 | 790731 | 513895 | 513207 | 129163 | 126608 | 148947 | 0.2443 | 19666 | 20920 | 6995 | 1875 | 62689 | 46311 | 8056 | 0.0225 |
| 8. methyl ethyl disulfide | 9.55 | 88 % | 15019 | 16269 | 9489 | 10108 | 1304 | 227 | 3160 | 0.0423 | 1001 | 249 | 3438 | 4942 | 1634 | 396 | 736 | 0.1389 |
| 9. 2-heptanone | 10.87 | 81 % | 9830 | 7951 | 6797 | 6849 | 1991 | 948 | 1670 | 0.2963 | 2404 | 1820 | 1822 | 1028 | 3246 | 1817 | 337 | 0.2758 |
| 10. 3-octanone | 13.32 | 93 % | 18672 | 17429 | 8445 | 8031 | 1834 | 729 | 3327 | 0.0716 | 3036 | 1409 | 4208 | 3260 | 3150 | 1796 | 488 | 0.8176 |
| 11. dimethyl trisulfide | 14.07 | 95 % | 441651 | 484344 | 112144 | 100126 | 50238 | 19648 | 80870 | 0.1129 | 15804 | 14597 | 5569 | 4293 | 38127 | 29594 | 4846 | 0.0718 |
| 12. 2-acetylfuran | 14.48 | 54 % | 12110 | 10497 | 5762 | 5490 | 2650 | 1919 | 1933 | 0.1661 | 1676 | 853 | 3040 | 3049 | 1966 | 890 | 436 | 0.8043 |
| 13. phenol | 21.87 | 94 % | 287710 | 173152 | 136910 | 82087 | 209976 | 132015 | 34648 | 0.3295 | 143685 | 132660 | 109257 | 144833 | 156696 | 149677 | 28073 | 0.5458 |
| 14. 4-methylphenol | 23.15 | 94 % | 28404 | 22409 | 10343 | 5932 | 11713 | 7108 | 3753 | 0.3505 | 12585 | 11991 | 13431 | 13327 | 6345 | 2967 | 2312 | 0.7562 |
| 15. 4-ethylphenol | 24.58 | 94 % | 65738 | 63272 | 25621 | 19660 | 24479 | 17635 | 10164 | 0.5195 | 41488 | 35413 | 34562 | 26047 | 28151 | 18146 | 5646 | 0.8190 |

R_T, retention time; C, control group; MA, mineral adsorbent; MMLA, microbial-mineral litter additive; H, mean peak height; SD, standard deviation; SEM, standard error of the mean;

348
349

Table 2. Summary of sensory analyses on aromas and odors emitted from poultry manure.

| A _T / min | odor | corresponding compound |
|----------------------|----------------|------------------------|
| 2.89 | animal/fecal | methanethiol |
| 3.20 | hay/straw | dimethyl sulfide |
| 6.24 | characteristic | s-methyl thioacetate |
| 6.80 | sweet | - |
| 7.37 | fecal | dimethyl disulfide |
| 8.97 | characteristic | - |
| 9.24 | sweet/berry | - |
| 13.36 | mushroom | 3-octanone |
| 14.12 | sauerkraut | dimethyl trisulfide |
| 16.07 | burnt bacon | - |
| 16.23 | soil/dusty | - |
| 18.97 | sour/dusty | - |
| 19.37 | burnt bacon | - |
| 19.92 | rotten eggs | - |

A_T, detection time ('time stamp') when panelist detected an odor while performing simultaneous chemical and sensory analyses using GC-MS-Olfactometry;

350