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研究报告

Decreasing ammonia emission from chicken manure by microbe and litter material

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Abstract: Some de-odorizing microbes and litter materials were combined to decrease ammonia emission from chicken manure and minimize its dangerous effect on environment. The de-odorizing microbes (F468, M1−M9) could significantly decrease ammonia emission from chicken manure and F468 was the optimal. The ability of F468 to decrease ammonia emission could not be improved significantly by mixture with other de-odorizing microbe (M1−M9), and some microbes hindered its ability, therefore the method of application of single microbe (F468) was preferred. Ammonia was not efficiently decreased by adding litter material such as wheat bran, wheat straw and cottonseed bran. Ammonia emission was abundantly decreased by mixing litter materials with F468, such as 88% (*W/W*) ammonia loss was retrieved within 0−5 d by adding 5% (*W/W*) F468 and 10% (*W/W*) wheat straw. In general, the application of de-odorizing microbe and straw to decrease ammonia emission from chicken manure not only minimized its dangerous effect on environment, but also represented an alternative practice of open air burning of straw.

Keywords: Ammonia, Odor, Litter, Microbe

应用微生物与秸秆降低鸡粪氨气释放量

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摘　要: 为减轻大量禽畜废弃物中氨气流失对环境的污染，研究和优化了微生物与秸秆等辅料对氨气释放量的影响。结果表明，F468、M1−M9 等除臭微生物能显著降低氨气的释放量，其中 F468 是最优微生物，其它微生物与 F468 的配伍并没有显著增强 F468 降低氨气释放量的能力，有些微生物还

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降低了其能力，因此选择单一微生物法降低氨气释放量。单独添加辅料对降低氨气的释放影响较小，辅料与微生物的配伍可大量降低氨气的释放量。5%的 F468 与 10%的秸秆配伍在 1−5 d 降低 88%的释放量。应用微生物与秸秆不仅降低氨气挥发对环境的危害，也是秸秆资源化利用的有效途径之一。

关键词: 氨气, 臭气, 辅料, 微生物

With the development of intensive livestock industry, it became the main source of organic solid waste and odorous gases[1−2]. Among the 136 odorous gases from livestock industry, ammonia presented the greatest risk to environments[3]. Ammonia emissions led to malodor problems and volatilized ammonia tended to be oxidized by various oxidants in the air to produce nitrous oxides, which are widely recognized as the major contributors to the eutrophication of waters and acidification of soil. For its dangerous effects on environment, ammonia emission must be decreased substantially from livestock industry[4−6]. Application of microbes is one of the most promising and economical strategies for removing environmental pollutants[7−8] and ammonia loss could be decreased by microbes[9−11].

In our previous studies, some de-odorizing microbes, (F468, M1-M9) were isolated and could decrease odor from chicken manures[9−10]. In this study, those de-odorizing microbes and some litter material were combined to decrease ammonia emission from chicken manure.

1 Experimental

1.1 Microbe

The de-odorizing microbes (F468, M1−M9) were isolated according to the method described by Chen et al[9−10].

1.2 Medium

Dextrose agar medium (g/L)[12]: 200 potato extracts, 20 dextrose (glucose), and 20 agar.

Liquid fermentation medium (g/L)[12]: 10 glucose, 10 sucrose, 3.5 yeast extract, 1.5 $(NH_4)_2SO_4$, 0.75 KH_2PO_4, 0.1 NaCl, 0.3 $MgSO_4·7H_2O$, 0.03 $FeSO_4·7H_2O$, 0.05 $CaCl_2·2H_2O$, 0.02 $CuSO_4·5H_2O$, pH 6.5.

1.3 Chicken manure

Chicken manures were collected from the Hennery of Nanjing Agriculture University. The manures were loaded to 1 000 mL Erlenmeyer flask, in which a 20 mL beaker containing 2% (*V/V*) H_2SO_4 was laid to absorb ammonia[9].

1.4 Litter material

The wheat bran, wheat straw and cottonseed bran were collected from the cattle farm of Nanjing Agriculture University. The wood chip was shaved from the wood of *Populus alba*. All the litter materials were dried and shattered by a lawnmower into 0.5−1 cm^2 pieces.

1.5 Culture

Subculture: De-odorizing microbes (F468, M1−M9) were washed from potato dextrose agar medium and diluted successively with sterile water until the separate colony was formed when the diluted microbe was cultured on potato dextrose agar medium at 25 °C for 32 hours. When this subculture cycle was accomplished, the separate colony was used for next subculture cycle.

Fermentation culture: De-odorizing microbes (F468, M1−M9) were washed from the potato dextrose by sterile water and inoculated in the optimized fermentation liquid medium on 150 r/min shaking bed at 25 °C for 32 hours until its growth curve reached the stationary phase.

1.6 Ammonia analysis

Samples were loaded to 1 000 mL Erlenmeyer flask, in which a 20 mL beaker containing 2% (*V/V*)

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H_2SO_4 to absorb ammonia was laid, and then the flask was sealed as quickly as possible. Every 5 day, all the 20 mL beakers were taken out for Ammonia analysis and a new 20 mL beaker containing 2% (*V/V*) H_2SO_4 was laid. Ammonia was determined by the national standard method [13].

2 Results and Discussions

2.1 Decreasing ammonia emission by adding de-odorizing microbe

The successful bioremediation depended on the right microbe[8]. The effects of de-odorizing microbes (F468, M1−M9) on decreasing Ammonia emission were showed in Table 1.

Table 1 Decreasing ammonia emission from chicken manure by de-odorizing microbe
表 1 应用除臭微生物降低鸡粪中氨气释放

Microbe 微生物 (5%, *W/W*)	Time course changes of ammonia emission (μmol) 氨气的释放过程		
	1−5 d	6−10 d	11−15 d
0	61.0±3.8	38.1±2.2	18.3±2.1
F468	17.6±0.8	6.3±0.5	3.9±0.6
M1	32.1±2.4	22.1±1.1	12.8±0.8
M2	35.5±2.5	21.3±1.9	18.2±0.9
M3	40.2±3.2	18.3±0.9	15.2±1.2
M4	25.6±1.8	15.7±0.9	8.5±0.4
M5	33.7±2.0	20.8±1.8	12.6±0.9
M6	36.8±2.5	31.2±1.8	15.3±0.5
M7	26.5±1.8	14.8±1.1	9.8±0.5
M8	27.8±1.9	21.3±1.2	11.5±0.7
M9	27.3±2.0	17.5±1.0	11.8±0.2

Note: The values are means of three separate experiments ± standard deviation.

As shown in Table 1, all the de-odorizing microbes (F468, M1−M9) could significantly decrease Ammonia emission from chicken manure. Among de-odorizing microbes, F468 was the optimal and 71% (*W/W*) ammonia loss was retrieved. For bioremediation, mixed microbes were likely required and 40%−60% ammonia emission was decreased by Effective Microorganisms (one mixed microbial preparation)[14], and other de-odorizing microbes (M1−M9) were mixed with F468 to enhance its ability of decreasing Ammonia emission.

2.2 Decreasing the ammonia emission by adding mixed microbe

The effects of the mixed microbes on Ammonia emission were showed in Table 2.

As shown in Table 2, the ability of F468 to decrease ammonia emission could be improved by some other de-odorizing microbes, such as M2, which enhanced the ability of F468 from 71% to 79%, while some microbe such as M3 hindered its ability of F468 to decrease ammonia emission. Generally, the ability of F468 could not have been improved significantly by mixing with other de-odorizing microbes (M1−M9).

Table 2 Decreasing ammonia emission from chicken manure by adding mixed microbe
表 2 应用复合除臭微生物降低鸡粪中氨气释放

Microbe 微生物 (5%, *W/W*)	Time course changes of ammonia emission (μmol) 氨气释放过程		
	1−5 d	6−10 d	11−15 d
5% F468	15.6±1.8	7.2±0.5	6.9±0.7
2.5% M1+2.5% F468	17.2±1.2	8.7±0.2	6.3±0.3
2.5% M2+2.5% F468	12.3.±0.7	5.8±0.2	4.2±0.3
2.5% M3+2.5% F468	42.3±2.7	22.3±1.8	14.2±0.9
2.5% M4+2.5% F468	15.1±0.8	6.7±0.4	5.2±0.2
2.5% M5+2.5% F468	35.6±3.1	15.1±0.5	7.1±0.4
2.5% M6+2.5% F468	19.8±1.1	5.2±0.1	3.5±0.2
2.5% M7+2.5% F468	15.8±0.6	11.2±0.5	8.7±0.3
2.5% M8+2.5% F468	22.1±1.8	12.5±0.6	7.5±0.4
2.5% M9+2.5% F468	15.5±0.9	5.6±0.4	3.5±0.2

Note: The values are means of three separate experiments ± standard deviation.

Mixed microbes were preferred for decreasing ammonia emission[14], but microbial metabolism can produce toxic metabolites in some cases and stringent regulations have restricted microorganism application[7], and environmental regulation might specify more health and safety criteria for the application of mixed microbes than that of single, therefore single microbe was preferred in this research.

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2.3 Decreasing ammonia emission by adding litter materiel

The effects of litter material on Ammonia emission were showed in Table 3.

As shown in Table 3, cottonseed bran, wood chip, wheat bran or wheat straw could decrease the loss of ammonia to some extend, but its amount was below 25%. This result was also confirmed by other report that there is no obviously way to decrease N losses efficiently by adding litter material only[6].

Table 3 Decreasing ammonia emission from chicken manure by litter materiel
表 3 应用辅料降低鸡粪中氨气释放

Litter materials 辅料 (%, *W/W*)	Time course changes of ammonia emission (μmol) 氨气释放过程		
	1−5 d	6−10 d	11−15 d
0	54.0±1.8	31.1±1.2	22.3±1.1
7.5 cottonseed bran	47.3±2.5	28.1±2.8	17.3±1.9
5.0 wood chip	44.7±4.1	25.7±3.5	19.5±2.7
10.0 wheat bran	42.3±4.3	28.1±3.6	17.3±2.3
7.5 wheat straw	46.9±5.2	24.4±3.4	16.2±2.1

Note: The values are means of three separate experiments ± standard deviation.

2.4 Decreasing the ammonia emission by adding F468 and litter materiel

The effects of F468 combined with litter material on ammonia emission were showed in Table 4.

As shown in Table 4, ammonia emission was abundantly decreased by mixing litter materials with F468. The optimal litter material was 10% wheat bran, with 10% (*W/W*) of which F468 can decrease ammonia loss by 94% (*W/W*) within 1−5 d.

2.5 The kinetics of ammonia emission after adding F468 and 10% wheat straw

Wheat bran was the optimal additive, but was rarely used because of its limited resource while wheat straw resources were abundant, and burring wheat straw had been prohibited by many countries, which would pollute atmospheres and had a statistically significant effect on asthma morbidity[15]. Therefore, application of straw not only decreased ammonia loss, but also represented an alternative practice of open air burning of straw. The dynamics of ammonia emission after adding F468 and 10% wheat straw was showed in Fig. 1.

Table 4 Decreasing ammonia emission from chicken manure by F468 and litter materiel
表 4 应用辅料+F468 降低鸡粪中氨气释放

Litter materials (%, *W/W*)+ F468 (*W/W*) 辅料+ F468	Time course changes of ammonia emission (μmol) 氨气释放过程		
	1−5 d	6−10 d	11−15 d
5% F468	13.6±1.3	7.8±0.7	5.9±0.6
10.0 cottonseed bran+5% F468	9.8±0.2	5.6±0.3	3.1±0.2
7.5 cottonseed bran+5% F468	7.7±0.7	2.3±0.2	3.3±0.6
5.0 cottonseed bran+5% F468	8.2±1.0	4.9±0.3	8.1±0.8
7.5 wood chip+5% F468	4.1±0.3	1.0±0.1	2.1±0.1
5.0 wood chip+5% F468	5.4±1.2	1.1±0.4	2.2±0.6
2.5 wood chip+5% F468	10.2±0.8	1.3±0.5	2.0±0.3
15.0 wheat bran+5% F468	3.2±0.2	3.1±0.1	0.3±0.1
10.0 wheat bran+5% F468	2.7±0.3	3.3±0.2	0.2±0.1
5.0 wheat bran+5% F468	6.2±0.4	3.1±0.4	2.4±0.2
15.0 wheat straw+5% F468	6.7±0.5	2.6±0.2	3.5±0.2
10.0 wheat straw+5% F468	5.7±0.5	1.3±0.1	1.5±0.2
7.5 wheat straw+5% F468	7.7±0.8	1.5±0.2	1.8±0.2
5.0 wheat straw+5% F468	7.2±0.6	1.7±0.1	1.7±0.3

Note: The values are means of three separate experiments ± standard deviation.

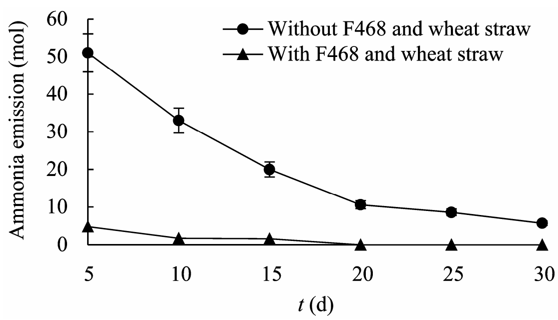

Fig. 1 Time course changes of ammonia emission from chicken manure
图 1 鸡粪中氨气释放过程

The kinetics of ammonia emission from chicken manure could help to apprehensively understand its emission pattern. As showed in Fig. 1, the majority of

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ammonia emission occurred at 0−10 d and its amount occupied above 93% of the total emission during 0−30 d. The optimal phage for decreasing ammonia emission was the period of 0−10 d. Nevertheless, all the efforts would be useless. By adding 5% (*W/W*) F468 and 10% (*W/W*) wheat straw, 88% ammonia loss could be retrieved within 0−5 d.

The process of successful bioremediation not only depended on having the right microbes, but also depended on the appropriate environment for remediation[8]. Litter materials was often added as bulking agents to improve the manures structure and enhance aeration, absorb excess liquids, and then decreased ammonia loss by providing microbe with extra energy source to balance the normally high N contend and increased the ratio between C and N[4]. F468 may produce some enzyme to make full use of litter materials, and turn some potential loss N as table microorganism protein or microorganism N, thus ammonia emission was reduced. This may be one mechanism of F468 to reduce ammonia emission. This may be one mechanism of F468 to reduce ammonia emission.

3 Conclusions

(1) The most optimum phage for decreasing ammonia emission from chicken manure was the period of 0−10 d.

(2) By adding F468 and 10% wheat straw, 88% (*W/W*) ammonia loss could be retrieved within 0−5 d.

(3) Application of microbe and straw not only decreased ammonia loss significantly, but also represented an alternative practice of open air burning of straw.

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