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研究报告

Reducing N Nutrient Losses and Enhancing Innocuous Process of Chicken Manures by Microbe

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Abstract: De-odorizing microbe F468 was used to reduce N nutrient losses and enhance the innocuous process of chicken manures. From its 1st to 80th subculture cycle, F468 showed stable ability to reduce NH_3 emission from chicken manures. The total N nutrient losses were reduced by 79% by the addition of F468, compared with that without F468. Moreover, F468 could accelerate the innocent process of chicken manures by reducing *Ascaris* eggs and *Fecal coliforms*. Without adding F468, it took 25 days and 20 days for the dead rate of *Ascaris* eggs and the number of *Fecal coliforms* in chicken manure to reach the national standard of NY884-2004, respectively. While adding F468, it took 15 days and 10 days for the dead rate of *Ascaris* eggs and the number of *Fecal coliforms* to reach the national standard, respectively.
Keywords: NH_3 emission, N nutrient losses, Manure, Microbe, Innocuous process

应用微生物降低鸡粪 N 营养元素损失，促进其无害化进程

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摘　要: 为减轻大量禽畜废弃物对环境的污染，将其资源化为优质的无害化有机肥料，研究了微生物 F468 对降低鸡粪 N 营养元素损失和促进其无害化进程的影响。结果表明，继代 80 次，F468 降低鸡粪氨气挥发的能力无显著变化，能降低鸡粪 79%的 N 元素损失。F468 还能通过降低蛔虫卵和粪肠杆菌的数量，促进鸡粪的无害化进程。不添加 F468，蛔虫卵和粪肠杆菌达到我国无害化标准(NY884-2004)的时间分别需要 25 d 和 20 d。加入 F468 后，蛔虫卵和粪肠杆菌达到我国无害化标准的时间分别需要 15 d 和 10 d，均比不加微生物提前 10 d 达到我国无害化标准。

关键词: 氨气挥发, N 营养元素损失, 微生物, 禽畜废弃物, 无害化

With the development of intensive livestock industry, it became the main source of organic solid waste, and proposed considerable challenges on environment, such as NH_3 emissions to atmosphere, microbial pathogens to water and heavy metals contaminations to soil[1−2]. To solve these problems, an envi-

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ronment-acceptable and energy-efficient approach was to transform those manures into fertilizers by composting, which can recycle the available nutrients[1,3–4]. However, manure composting often encountered nutrient losses and malodor problems. N nutrient losses led to eutrophication of fresh water, and NH_3 emissions led to odor complaints and acid deposition[1–2,5]. In addition, manure-based fertilizers should have been gotten innocuous treatments and sufficient pathogen inactivation[6–8] before using for crop production.

In our previous studies, a strain of de-odorizing microbe, F468, was isolated and identified as *Trichoderma vivid*[9], which could reduce 67% NH_3 emission from chicken manures. Based on its optimized fermentation process[10], F468 was applied to reduce the N losses and enhance innocuous process of chicken manures.

1 Experimental

1.1 Chicken manures

Chicken manures were collected from the Hennery of Nanjing Agriculture University. The manures were loaded to 1000 mL Erlenmeyer flask, in which a 20 mL beaker containing 2% (*V*/*V*) H_2SO_4 was laid to absorb NH_3[9].

1.2 Strain

F468 was isolated and identified by Chen[9].

1.3 Medium

Potato dextrose agar medium[10]: Potato extracts 1000 mL (200 g slice potatoes), 20 g/L dextrose (glucose), and 20 g/L agar.

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

1.4 Culture method[10]

Subculture: Spores from one colony of F468 were washed from potato dextrose agar medium and diluted successively with sterile water until the separate colony was formed when the diluted spore 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: Spores of F468 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.

1.5 Analyses methods

NH_3, NH_4^+-N and NO_3^--N were measured by the method described in the document[11].

Total carbon and total nitrogen content was determined by the methods according to the standard of NY 884-2004[12].

The death rate of *Ascaris* eggs was determined by the method according to GB/T 19524.2-2004[13].

The number of *Fecal coliforms* was determined by the method according to GB/T 19524.1-2004[14].

2 Results and Discussion

2.1 Effects of subculture and inoculation volume on NH_3 emission from chicken manures

For successful application in the manure compositing, F468 should have stable ability to reduce NH_3 emission during subculture and its inoculation volume should be optimized. Effects of subculture and inoculations on NH_3 emission were show in Figure 1 and in Figure 2, respectively.

As shown in Figure 1, from 1st to 80th subculture cycle, the ability of F468 to reduce the loss of NH_3 was stable. At its 1st subculture cycle, 70% (*W*/*W*) NH_3 was retrieved by adding F468, and 67% (*W*/*W*) NH_3 was retrieved at its 80th subculture cycle.

As shown in Figure 2, with inoculation volume increasing from 1% to 3%, NH_3 emission was reduced significantly. The inoculation volume of 3% decreased 64% NH_3 losses. While inoculation volume increased from 4% to 7%, NH_3 emission stay in a rather stable trend, therefore the optimal inoculation volume of F468 was settled as 3%.

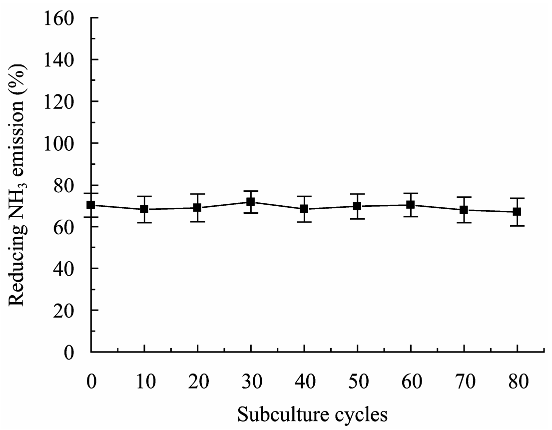

Fig. 1 Effect of subculture cycles of F468 on NH_3 emission

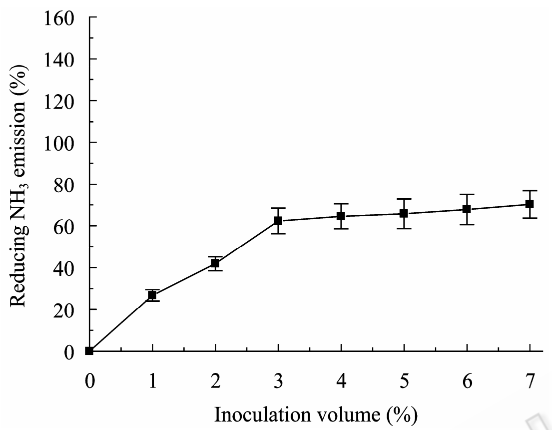

Fig. 2 Effect of inoculation volume of F468 on NH_3 emission

2.2 Effect of F468 on N nutrient losses from chicken manures

Large losses of manure nitrogen in the form of NH_3 or nitrous oxide to atmosphere contributed to air pollution and decreased the fertilizer value of manures[15], thus reducing N nutrient losses had become another important criterion for agronomic utilization of livestock manures[4–5]. The effect of F468 on N nutrient losses from chicken manures was showed in Table 1.

Table 1 Effect of F468 on losses of N nutrient in chicken manures

	Before treatment	Without F468 at 15^{th} d	With F468 at 15^{th} d
Carbon organic (g/kg)	386.0c	365b	284a
Total N (g/kg)	24.5c	9.8a	21.5b
NH_4^+-N (g/kg)	1.4a	6.2c	3.6b
NO_3^--N (mg/kg)	0	134.45a	178.74 b

Note: Values followed by the same letter in the same column were not various significantly according to the Least Significant Difference Test at 5% level.

As shown in Table 1, F468 could reduce total N nutrient losses from chicken manures significantly. With the inoculation of F468, total N nutrient losses were reduced by 79%, compared with that of without F468, while the carbon organic losses increased by 385%.

N nutrient of livestock manures comprised of undigested dietary N, endogenous N and microbial N. NH_4^+-N is the most of the N nutrient form catabolized by microbe. NH_4^+-N was transformed to NO_3^--N or synthesized to organic N by microbe, and those forms of N nutrient (NO_3^--N and organic N) did not lead to N losses through volatizing easily. However, most of NH_4^+-N could not be transformed to NO_3^--N or organic N quickly and would be transformed to NH_3 volatizing to atmosphere, which leaded to N nutrient losses[16]. Our results showed that the inoculation of F468 can decrease the N losses. The microbe ecology of livestock manures may be changed by adding F468 and some potential loss N was utilized quickly by catabolizing some carbon organic source and transformed to stable microbe protein, in consequence, the total N of livestock manures was increased while the carbon resource was reduced.

2.3 Effect of F468 on *Ascaris* eggs in chicken manures

Parasite was often linked with livestock and its manures. Losses caused by parasitic diseases were higher than the losses inflicted by bacterial and viral diseases[17] in pig farming, and the commonly used indicator of parasite in livestock manures was *Ascaris* eggs. According to the innocent standard of NY 884-2004, the death rate of *Ascaris* eggs in livestock manures should be ⩾ 95%[12]. *Ascaris* eggs could recover after 53-347 days in manures[18], for example, the survival of *Ascaris suum* egg was above 80% after 12 weeks under anaerobic conditions[19]. The effect of F468 on the death rate of *Ascaris* eggs was showed in Table 2.

Without inoculating F468, it took 25 days for the death rate of *Ascaris* eggs to reach the national standard of livestock manures for innocent process (⩾ 95% NY 884-2004)[12], however, when F468 was added to livestock manures, *Ascaris* eggs decreased significantly. 15 days later, all *Ascaris* eggs were dead and reached the national standard for innocent process.

Table 2 Effect of F468 on the death rate of Ascaris eggs in chicken manures

The death rate of *Ascaris* eggs (%)	1st d	5th d	10th d	15th d	20th d	25th d
Without F468	0	22	48	65	87	100
With F468	0	55	76	100	100	100

Note: The *Ascaris* eggs death rate of national standard for innocuous process was ≤ 95% (NY 884-2004[12]).

2.4 Effect of F468 on *Fecal coliforms* in chicken manures

Most of the environmental problems caused by waste management have been focused on nutrient losses, malodor problems and air pollution. Microbes from manure were often on the priority list for control and remediation[20−21]. Numerous pathogenic enteric microbes in manures which infected livestock may contain human pathogens which infected infect humans[21]. Meanwhile, *Fecal coliforms* can grow in fresh manure and survive longer under cool, shaded conditions[22]. According to the national standard of livestock manures for innocent process NY 884-2004[12], the number of *Fecal coliforms* should be ≤ 10−100 cell/g. The effect of F468 on the change of *Fecal coliforms* in chicken manures was showed in Table 3.

Without F468, it took 20 days for *Fecal coliforms* to reach the national standard for innocent process (10−100 cell/g, NY 884-2004)[12]. After F468 was added to manure, the count of *Fecal coliforms* was reduced significantly. After 10 days, *Fecal coliforms* count was reduced to 60 cell/g and reached the national standard for innocent process. 20 days later, all the *Fecal coliforms* in chicken manure were dead.

Table 3 Effect of F468 on *Fecal coliforms* in chicken manures

The number of *Fecal coliforms* (cell/g)	1st d	5th d	10th d	15th d	20th d
Without F468	60000	7000	350	110	35
With F468	60000	420	60	0	0

Note: The *Fecal coliforms* national standard for innocuous process was 10−100 cell/g (NY 884-2004[12]).

3 Conclusions

From its 1st to 80th subculture cycle, F468 showed a stable ability to reduce NH_3 emission from chicken manures. After inoculation of F468, the total N nutrient losses were reduced by 79%, compared with that of the control. Moreover, F468 could accelerate the innocent process of chicken manures by reducing *Ascaris* eggs and *Fecal coliforms* significantly. After adding F468, it took 10 days in advance for the dead rate of *Ascaris* eggs and the number of *Fecal coliforms* to reach the national standard for innocent process, compared with that of not adding.

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