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Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting

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ABSTRACT

The nitrogen turnover bacterial (NTB) agent, which is closely related to nitrogen turnover, was comprised of a bacterial consortium of ammonifiers, nitrobacteria and *Azotobacter* in this study. The three constituents of the bacterial consortium were added to pig manure and wheat straw mixtures in different doses and at different times, and subsequently composted to investigate their effects on nitrogen transformation and maturity. Throughout the period, the total N loss was 35–56%, 10.7–22.7% of which consisted of NH_3, and 18–35% of the initial organic carbon was degraded. Adding the NTB agent prolonged the thermophilic stage by one to six days compared to the control. The lowest N loss (35%), the highest degradation rate of organic carbon (35%) and the greatest increase in total nitrogen content (36.1%) occurred in the inoculation with 1% NTB agent at the beginning of composting. However, adding 1% NTB agent after the thermophilic stage and 3% NTB agent at the beginning of composting had no positive effect with respect to retaining nitrogen or accelerating the maturation process. Therefore, the inoculation with 1% NTB agent at the beginning of composting was effective for reducing N loss and promoting maturity.

1. Introduction

There were approximately 209.3 million tons of pig manure in China in 2009, generated by the intense and concentrated activity of the livestock industry (Huang et al., 2011; Tian et al., 2012). This large amount of animal manure must be managed urgently using appropriate disposal practices, or it could lead to many unpleasant environmental consequences, such as odorous and gaseous emissions, soil and water pollution (Kafle and Kim, 2013; Zhang et al., 2014). Composting is a common and effective method for treating agricultural solid waste, such as pig manure, and the product can be used as organic fertilizer (i.e., compost) or organic agricultural amendments (Albrecht et al., 2010; Karak et al., 2013; Kim et al., 2008; Zhang et al., 2014). During the composting process, microorganisms inherent to the material transform degradable organic matter into a humus-like product (Sarkar et al., 2010). However, low efficiency and undesirable compost quality were often caused by an insufficient quantity or poor biodegradability of the indigenous microbial community (Xi et al., 2007).

In recent years, exogenous microorganisms have received considerable attention because of their positive effects on the quality and maturity of the compost. Sarkar et al. (2010) reported that the biodegradation process became more efficient when the composting was inoculated with thermophilic bacteria (*Geobacillus* strains) in the thermophilic stage during vegetable waste composting. Zhang et al. (2013) found that inoculation with *Phanerochaete chrysosporium* increased the pile temperature, enhanced the substrate utilization and, thus, improved the compost. Similar results were also found in the agricultural waste composting by Zeng et al. (2010). Wang et al. (2011) demonstrated that *Penicillium expansum* could significantly increase the humus contents and, promote lignocellulose degradation, and it exhibited 1.5 times higher germination than the control. Zeng et al. (2007) observed that composting inoculated with *white-rot fungus* could be successfully processed and exhibited reduced bioavailability of lead (Pb). Hachicha et al. (2012) also found that inoculation with *Trametes versicolor* could increase the degree of aromatization of humic acids. Furthermore, inoculation with complex microorganisms was more effective for improving the composting process than the simplex inoculation (Vargas-Garcia et al., 2006; Wei et al., 2007; Xi et al., 2012). Wei et al. (2007) observed that inoculation with complex microorganisms (i.e., *Bacillus casei, Lactobacillus buchneri* and *Candida rugopelliculosa*) improved the degree of humification and accelerated the maturation process, and an increase in the humification indices was also reported by

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Vargas-Garcia et al. (2006) in the composting of horticultural wastes inoculated with *Bacillus shackletonni*, *Streptomyces thermovulgaris* and *Ureibacillus thermosphaericus*. By inoculating ammonia-oxidizing bacteria, *Nitrobacter*, and *Thiobacillus* in raw materials, Xi et al. (2012) observed the complex microorganisms significantly improved composting efficiency and quality.

Nitrogen (N) loss, however, can be severe when the decomposition of organic materials with high N contents (Gabhane et al., 2012). The N loss approached 16–74% of the initial nitrogen content, and was accompanied by decline in the value of the compost as fertilizer, as well as complaints about malodor (Gabhane et al., 2012; Ren et al., 2010; Tiquia and Tam, 2002). Therefore, inoculation with exogenous microorganisms to decrease N loss was also essential for improving compost quality. Unfortunately, many researchers focused on the effects of simple exogenous microorganisms, such as lignocellulose, on composting (Nair and Okamitsu, 2010; Sarkar et al., 2010; Wang et al., 2011; Zeng et al., 2007; Zhang et al., 2013), and there is little information on the impacts of complex microbial communities on the composting process (Ruimin, 2011; Vargas-Garcia et al., 2006), and nitrogen turnover in particular. In theory, the microbial physiological groups, ammonifiers, nitrobacteria, and *Azotobacter*, control the ammoniation, nitrification, and nitrogen fixation processes, respectively. Hence, it is necessary to study the impacts of these microbes on the composting process for keeping nitrogen and reducing the composting time. In addition, it has been demonstrated that the inoculated time can significantly affect the composting process. Some studies have reported that inoculation with microorganisms during the initial stage could increase the pile temperature, facilitate organic carbon degradation, and accelerated the composting process (Nakasaki et al., 2013; Wang et al., 2011; Wei et al., 2007; Zeng et al., 2007). However, some researchers believed that inoculation after the thermophilic stage could accelerate the thorough degradation of cellulose and lignin, improve maturity, and be the key to the second fermentation (Hachicha et al., 2012; Zeng et al., 2010; Zhang et al., 2013). Given the increasing awareness of the significance of inoculation, it is necessary to further elucidate the real effects of inoculation time on composting.

Thus, the objectives of this study were to (1) evaluate the effects of bacterial agents related to nitrogen turnover on nitrogen loss and maturity, and (2) explore the optimal doses and inoculated time in pig manure composting.

2. Materials and methods

2.1. Composting materials and reactor

Pig manure (PM) and wheat straw (WS) were used as the raw materials. The basic characteristics of them, respectively, are as follows: total organic carbon (TOC) was 359.0 and 419.6 g kg^{-1}; total nitrogen (TN) was 27.9 and 5.0 g kg^{-1}; the water content was 70.9% and 14.5%; and the C/N ratio was 12.8 and 83.3. Wheat straw was cut into 3–5 cm fragments.

The experiment was carried out in five self-built, aerated static compost reactors. The dimensions of each bin were 0.65 m × 0.5 m × 0.4 m with a volume of approximately 0.09 m^3. The installation of the reactor is shown in Fig. 1. The outer wall of the reactor was insulated with 0.08 m of plastic foam, which could maintain the thermo-energy produced during composting.

2.2. Exogenous microbial agent preparation

The NTB agents were comprised of ammonifiers, nitrobacteria, and *Azotobacter*. The three bacteria were isolated from mature cow dung, mature chicken manure, and mature pig manure compost, respectively, using a specific method of isolation medium for each strain. The isolated strains were stored at 4 °C. Before inoculation, every strain was cultivated in Luria–Bertani (LB) culture (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl 10 g L^{-1}, agar 15 g L^{-1}, pH 7.4–7.6); then, the microbial lawn of the bevel was washed in 100 mL LB culture solution using 5 mL sterile water and cultivated for 12 h. The concentrations of the ammonifiers, nitrobacteria, and *Azotobacter* strains were above 1×10^{10}, 1×10^5, and 1×10^5 CFU mL^{-1}, respectively. Finally, the three solutions of strains were combined at the volume ratio of 1:1:1 for the experiment.

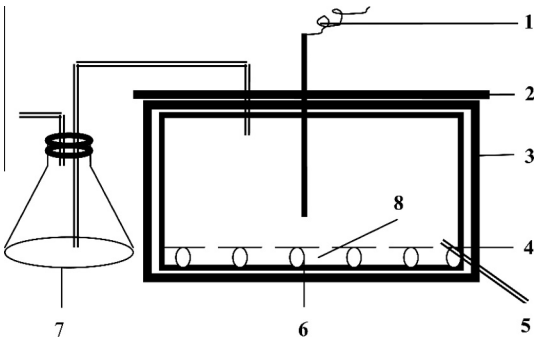

Fig. 1. Compost reactor (1) intelligent thermometer; (2) lid; (3) insulating layer; (4) sieve plate; (5) air pump; (6) holder; (7) surge flask; (8) buffer layer.

2.3. Composting treatment design

The experimental design included five treatments: (1) The control treatment (CK) was composted with approximately 21 kg fresh pig manure and 2 kg wheat straw mixture. (2) Treatment 1 (T1) was composted with the addition of 1% LB (10 mL kg^{-1} fresh compost) culture medium solution to the mixtures, which was the same as the CK mixture, at the beginning of composting (i.e., on day 1). (3) Treatment 2 (T2) was composted with the addition of 1% NTB agent to the same mixture as CK at the beginning of composting. (4) Treatment 3 (T3) was composted with the addition of 1% NTB agent to the mixture same as CK after the thermophilic stage (i.e., on day 12). (5) Treatment 4 (T4) was composted with the addition of 3% (30 mL kg^{-1} fresh compost) NTB agent to the mixture same as CK at the beginning of composting.

All the composting materials were turned after inoculation to spread the microbial agent. The water content of the mixtures was then adjust to approximately 65%, and approximately 80 L of each mixture was put into the reactor. Air was pumped from the bottom of the composter with an air flow of approximately 60 L min^{-1} during the composting until the 35th day, and the frequency of ventilation was twice per day at 9 a.m. and 3 p.m. for a duration of 30 min.

2.4. Sampling and analytical methods

Temperature was monitored every day by an intelligent PID temperature controller thermocouple thermometer XMT616 (Shanghai Renzhong Instrument and Electric Appliance Co., Ltd) before ventilation in the middle of the composting mass; the ambient temperature was monitored simultaneously. Samples were collected at 0, 8, 13, 20, 25, 36, and 45 days according to the changes of temperature. Before sampling, the pile was thoroughly turned manually. Sampling was performed in triplicate, mixing material from each of five points on diagonals into every sample, such that the results would be representative of the conditions of each treatment. The collected samples were divided into two parts. One part