Waste Management 80 (2018) 10–16

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Effects of sulphur and *Thiobacillus thioparus* 1904 on nitrogen cycle genes during chicken manure aerobic composting

Yusheng Lu, Wenjie Gu *, Peizhi Xu, Kaizhi Xie, Xia Li, Lili Sun, Hangtao Wu, Chaohong Shi, Dan Wang

Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences/Key Laboratory of Plant Nutrition and Fertilizer in South Region, Ministry of Agriculture/Guangdong Key Laboratory of Nutrient Cycling and Farmland Conservation, Guangzhou, Guangdong 510640, China

ARTICLE INFO

Article history:
Received 27 March 2018
Revised 14 August 2018
Accepted 28 August 2018

Keywords:
Thiobacillus thioparus
Sulphur
Nitrogen cycle genes
Nitrogen loss
Composting
Gene expression

ABSTRACT

Severe nitrogen (N) loss is a barrier for composting treatment. Since N transformation during composting is closely related to nitrogen loss, the impacts of adding sulphur and *Thiobacillus thioparus* 1904 to N transformation during composting were investigated in this work. Physicochemical properties and the expression of genes encoding N-related proteins were analysed to evaluate microbiological processes associated with N dynamics. The results indicated that (1) sulphur addition reduced the pH and cumulative NH_3 emission, and decreased N losses by 44.23%, while no significant differences were observed in the expression of N cycle-associated genes compared with the control treatment; (2) the application of *T. thioparus* 1904 increased NO_3^--N content, reduced N loss by 28.20%, and significantly enhanced the expression of *ammonia monooxygenase A* (archaeal *amoA;* AOA) and *nitrite oxidoreductase A* (*nxrA*) during the mature phase; (3) the combined application of sulphur and *T. thioparus* 1904 significantly affected the expression of functional genes related to nitrification and denitrification, which contributed to a reduction in accumulated NH_3 emission, an increase in NO_4^+-N content, and a decrease in N losses by 70.94%. Expression of *ammonia monooxygenase A* (bacterial *amoA;* AOB), *nxrA* and *nitrous oxide reductase Z* (*nosZ*) genes in the combined treatment was positively correlated with NO_3^--N, whereas expression of AOA and accumulation of NH_3 were negatively correlated with NO_3^--N. These results indicate that the combined application of sulphur and *T. thioparus* 1904 had a significant regulatory effect on N cycle genes and effectively reduced the N loss during composting.

1. Introduction

Global livestock and poultry manure is predicted to reach 39.92 million tons (nitrogen content) in 2030 (FAOSTAT, 2017). Aerobic composting is an effective resource recycling method that can ease environmental pressure (Wong et al., 2017). Aerobic composting, consisting of organic matter biodegradation, mineralisation and humification, forms stable products via the participation of microbes (Bernal et al., 2009). In the composting process, NH_3 and N_2O emission losses can reach 24–77.4% and 0.2–9.9% of the total nitrogen (N) content, respectively (Li et al., 2016). Nitrogen loss during composting is affected by aeration, C/N ratio, humidity, temperature, pH, microorganisms and other factors (Bueno et al., 2008; Huang et al., 2004). Improving N retention and composting efficiency could significantly enhance nutrient recycling of livestock and poultry manure (Goyal et al., 2005).

Previous studies found that adding sulphur could improve compost quality and reduce N loss, as well as produce acidic compounds that reduce the pH, which broadens compost application (García-de-la-Fuente et al., 2007; Mari et al., 2005; Roig et al., 2004). Previous research (García-de-la-Fuente et al., 2011) revealed that sulphur oxidising bacteria (SOB) can lower the pH of compost by converting sulphur to SO2- 4under aerobic conditions. Other research showed that adding both sulphur and *Thiobacillus thioparus* not only reduces compost pH, but also significantly increases the content of NO_4^+-N and NO_3^--N (Gu et al., 2011). Thus, sulphur and *T. thioparus* affect both compost pH and nitrogen transformation. Furthermore, nitrate is utilised as an electron acceptor to inhibit sulphate reduction and significantly reduce poisonous sulphur gas emission (Zang et al., 2017). Much research in recent years has focused on the interactions between nitrogen and sulphur during sewage and sludge treatment (Fdz-Polanco et al., 2001; Kamp et al., 2006; Reyes-Avila et al., 2004). However, there has been little research on the mechanisms of nitrogen and sulphur in composting.

* Corresponding author.
E-mail address: guwenjie0818@163.com (W. Gu).

https://doi.org/10.1016/j.wasman.2018.08.050
0956-053X/

The biological processes involved in nitrification and denitrification during composting are largely associated with nitrogen transformation (Chiumenti, 2015; Maeda et al., 2011). Enzymes related to the nitrogen cycle such as ammonia monooxygenase encoded by *amoA*, including ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA), nitrite oxidoreductase (*nxrAXB*), nitrate reductase (*narG*), nitrite reductase (*nirK, nirS*), nitric oxide reductase (*norB*) and nitrous oxide reductase (*nosZ*) are important molecular indicators for evaluating N transformation and N loss (Angnes et al., 2013; Chen et al., 2014; Jarvis et al., 2009). Reverse transcription quantitative PCR (RT-qPCR) can be used to measure changes in mRNA expression of nitrogen cycle genes following addition of sulphur and *T. thioparus*, and knowledge of the correlations between gene expression and physicochemical properties could help us better understand the effects of sulphur and *T. thioparus* addition on N transformation during the aerobic composting process.

2. Materials and methods

2.1. Experimental design

Fresh chicken manure and mushroom residues (1:1 mixing ratio at 50% humidity) and composted aerobically for 21 days. Fresh chicken manure was provided by Zhongluotan town livestock and poultry farms in Baiyun District, Guangzhou, China, and mushroom residues were gathered at the edible fungus factory of Guangdong Xinghe Biotech Corporation in Dongguan City, China. The physical and chemical properties of fresh chicken manure and mushroom residues are shown in Table 1. Industrial sulphur powder was passed through a 0.15 mm sieve prior to use as a composting additive, and *Thiobacillus thioparus* 1904 from the German Collection of Microorganisms and Cell Cultures (DSMZ) was grown in fermentation medium containing (per L) 1.2 g Na_2HPO_4, 1.8 g KH_2PO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.1 g $(NH_4)_2SO_4$, 0.03 g $CaCl_2$, 0.02 g $FeCl_3$, 0.02 g $MnSO_4$ and 10 g $Na_2S_2O_3$, which was autoclaved at 121 °C for 15 min prior to use. *T. thioparus* 1904 cultures were centrifuged, and the bacterial pellet was resuspended to a cell density of 1×10^8 CFU ml^{-1}.

Four treatments were performed: (1) raw material (Control; T1); (2) raw material + 0.25% sulphur (net weight; T2), (3) raw material + 0.25% sulphur + 5% (v/w) *T. thioparus* 1904 inoculum (T3), (4) raw material + 5% (v/w) *T. thioparus* 1904 inoculum (T4).

2.2. Composting device

Composting reactors consisted of stainless steel cylinders with an effective volume of 100 L (Fig. 1) with sensors inside for temperature monitoring. Ventilation devices were automatically controlled at a ventilation rate (dry matter) of 0.2 $L{\cdot}(kg{\cdot}min)^{-1}$ and a ventilation frequency of 10 min intervals every 6 h.

2.3. Compost sampling and analysis

Composting samples (~500 g) were collected in triplicate at 0, 1, 3, 5, 7, 10, 14 and 21 days during the composting process from 35 to 45 cm below the surface of the reactor and thoroughly mixed. The physical and chemical properties of compost were analyzed according to previous methods (Gu et al., 2011). Fresh samples were used for the determination of pH, ammonium N and nitrate. A portion of each sample was passed through a 1 mm sieve for the determination of total N, and another portion was stored at −80 °C for total RNA extraction and N cycle gene analysis. Compost gas collection and analysis was performed as described previously by Luo et al. (2014), and ammonia gas was absorbed by boric acid solution and measured by H_2SO_4 titration.

2.3.1. RNA extraction and reverse transcription

Compost total RNA was extracted using a Soil RNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA) and a 0.5 g sample was dissolved in 100 μL Rnase-free water according to the kit instructions and stored at −80 °C. RNA quality and quantity were measured using an ultra-micro NanoDrop ND-1000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA was removed from compost total RNA using a PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). RT-qPCR was performed using random 6-mer primers (100 μM) to synthesise compost cDNA.

2.3.2. RT-qPCR

RT-qPCR was used to measure the expression levels of N cycling genes *16S* rDNA (bacterial and archaeal), *amoA* (bacterial and archaeal), *nxrA*, *nirK*, *nirS* and *nosZ*. RT-qPCR was performed on an Applied Biosystems 7500 instrument (Applied Biosystems, CA, USA) with SYBR Green assays. Primers used for gene analysis are shown in Table 2. Reactions (20 μL) followed the procedure outlined in the SYBR Premix Ex TaqTMII (Tli RNaseH Plus) kit (TaKaRa) and contained 10 μL of 2 × SYBR Premix Ex Taq II (Tli RNaseH Plus), 0.4 μL of each respective primer (10 μM), ROX Reference Dye II (50×) and 1 μL of cDNA template. All samples were analysed in triplicate. Gene expression data were calibrated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and expressed as fold-change (FC) relative to reference genes (bacterial and archaeal *16S* rDNA) using the formula fold-change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (C_{t,Target} - C_{t,16S\ rDNA})_{Time\ x} - (C_{t,Target} - C_{t,16S\ rDNA})_{Time\ 0}$.

2.4. Data analysis

Experimental data were manipulated using Microsoft Excel 2010, and one-way analysis of variance (ANOVA) and Pearson's correlation analysis were performed using R software.

3. Results and discussion

3.1. Physicochemical analysis

Variations in physicochemical properties during composting are shown in Table 3. At the end of composting, the pH of T1 and T4 was 8.82 and 8.63, respectively. However, the pH of T2 and T3 following sulphur addition was significantly decreased ($p < 0.05$). This could be due to oxidation of elemental sulphur, generating SO_4^{2-} and H^+, resulting in acidification, consistent with previous studies (García-de-la-Fuente et al., 2007; Gu et al., 2011).

The NO_4^+-N content was significantly increased in both T2 and T3 treatments. The highest content was achieved in T3 (4030.00 mg/kg), followed by T2 (3942.70 mg/kg; $p < 0.01$), both

Table 1
Physical and chemical properties of composting material.

Raw material	Moisture content (%)	C/N ratio	Organic matter (%)	Total N (%)	Total P (%)	Total K (%)
Fresh chicken manure	75.0	21.07	50.13	1.38	1.54	1.70
Mushroom residue	8.93	29.57	80.54	1.58	1.91	1.17