Waste Management 56 (2016) 63–70

Contents lists available at ScienceDirect

Waste Management

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

Impacts of adding FGDG on the abundance of nitrification and denitrification functional genes during dairy manure and sugarcane pressmud co-composting

Qunliang Li *, Xiaobo Guo, Yanyu Lu, Guangchun Shan, Junhao Huang

School of Chemistry and Chemical Engineering, Guangxi University, 530004 Nanning, China

ARTICLE INFO

Article history:
Received 30 March 2016
Revised 5 July 2016
Accepted 5 July 2016
Available online 12 July 2016

Keywords:
Composting
Flue gas desulphurization gypsum
Nitrification
Denitrification
qPCR

ABSTRACT

To investigate the impacts of flue gas desulphurization gypsum (FGDG) amendment on the nitrification and denitrification during composting, dairy manure and sugarcane pressmud co-composting with FGDG (CPG) and without FGDG (CP) were conducted in this work. The physico-chemical parameters and the copies of nitrification and denitrification functional genes with real-time quantitative polymerase chain reaction (qPCR) during composting were analyzed. FGDG amendment displayed an inhibitory effect on the copies of *16S* rDNA and delayed the occurrence of the highest gene copies of *amoA* during composting. The *nxrA* gene copies was inhibited by FGDG amendment during the mature phase. The addition of FGDG increased the relative content of *narG* and *nirS* during composting, contributing to more NO_3^--N being reduced to NO_2^--N. The *amoA* showed significant negative correlation with OM and NH_4^+-N, and positive correlation with NO_3^--N. The *nxrA* displayed a negative correlation with temperature. These results demonstrated FGDG amendment significantly affected the copies of nitrification and denitrification functional genes, which changed the nitrogen flux of composting. Taken together, these data shed an insight into FGDG amendment affecting the nitrogen transformation during composting on a molecular level.

1. Introduction

Composting is a centuries-old and effective technique for utilizing the organic solid wastes (Gajalakshmi and Abbasi, 2008). During the composting, microorganisms convert large amounts of crop residues, manure, organic municipal wastes and industrial organic by-products into humified end-products, namely compost. Nitrification and denitrification are two key processes involved in the nitrogen (N) transformation during composting, and a large amount of gaseous nitrogen was released during the processes, i.e. NH_3 and N_2O. These emissions not only contribute to serious environment problem, but are responsible for the nitrogen loss in compost. The N loss via NH_3 is the main way, accounting for 24–77.4% of initial N of compost mixture (Martins and Dewes, 1992; Beck-Friis et al., 2001). In addition, 0.2–9.9% of initial N was released as N_2O, a by-product of nitrification and the intermediate product of denitrification.

Considerable ammonium (NH_4^+-N/NH_3) was produced in the processes of mineralization (or ammonification). The ammonia oxidation is the first step of nitrification, in which the ammonia-oxidizing archaea and bacteria (AOA and AOB, respectively) oxidize NH_3 to hydroxylamine (NH_2OH) with the catalysis of ammonia monooxygenase (*amoA*). Then the hydroxylamine was further oxidized to nitrite (NO_2^--N), catalyzed by hydroxylamine oxidoreductase (*hao*). During the second step of nitrification, namely, the nitrite oxidation, the nitrite oxidizing bacteria (NOB) oxidized the nitrite into nitrate (NO_3^--N) via nitrite oxidoreductase (*nxrAXB*). Nitrite or nitrate generated during the nitrification would usually be reduced by denitrifiers during the denitrification. The denitrification consists of four reduction steps, namely, NO_3^--N → NO_2^--N → NO → N_2O → N_2, which are catalyzed by nitrate reductase (*narG*), nitrite reductase (*nirK*, *nirS*), nitric oxide reductase (*norB*) and nitrous oxide reductase (*nosZ*), respectively (Ji et al., 2013; Van den Akker et al., 2011, 2008).

Various types of physical, chemical and biological additives were applied to the composting materials to facilitate the composting process, and reduce the loss of carbon and nitrogen. The additives have been extensively studied, such as coal fly ash, wood ash, bauxite, natural zeolites and kaoline (Belyaeva and Haynes, 2009; Koivula et al., 2004; Kurola et al., 2011; Villaseñor et al., 2011; Wong et al., 1997; Zambrano et al., 2010). Fernández-Delgado Juárez et al. (2015) observed adding wood ash could strongly influence the microbial activity and composition. Wei

* Corresponding author.
E-mail address: liqunliang231@163.com (Q. Li).

http://dx.doi.org/10.1016/j.wasman.2016.07.007
0956-053X/

et al. (2014) reported biochar addition could significantly affect the microbial community diversity. C. Wang et al. (2013) noted that biochar additives significantly altered the abundance of denitrifying bacteria, which lowered N_2O fluxes during the maturation phase. Flue gas desulphurization gypsum (FGDG) is a by-product from coal-fired power stations, and is rich in essential or beneficial mineral nutrients and low contents in heavy metals (S. Wang et al., 2013). Although, FGDG as a novel amendment was applied to composting to reduce the total N loss (Tubail et al., 2008; Guo et al., 2016), it is an open question whether and how FGDG amendment affects the microorganism communities in composting, especially the nitrifiers and denitrifiers communities. We hypothesized that the FGDG additives might affect the variation of nitrifiers and denitrifiers communities, which in turn influenced the transformation of nitrogen during composting. The impacts of adding FGDG on the copies of nitrification and denitrification functional genes during composting was firstly studied, which would achieve a further understanding on the effect of FGDG on the nitrifiers and denitrifiers communities during composting.

Though the diversity of bacterial communities during the composting process has been studied extensively using culture-dependent approaches, less than 1% of the total microorganism in the composting environment were culturable using the current techniques. Molecular biology tools such as qPCR might provide an indirect evidence for the major microbiological processes involved in N cycle during composting without having to isolate and cultivate microorganisms (Angnes et al., 2013), biofilter (Yang et al., 2014), wetland (Chon et al., 2011), marine sediments (Čuhel et al., 2010). Angnes et al. (2013) analyzed the copies of *amoA*, *narG*, *nirS*, *nirG norB* and *nosZ* with qPCR to evaluated N dynamics during swine slurry composting. Wang et al. (2015) assessed the *amoA*, *nirS*, *nirK*, *narG*, *napA*, *nosZ* via qPCR to study the treatment performance and nitrogen transformation process in a trickling filter.

The objective of this work was to investigate the impact of adding FGDG on the copies of nitrification and denitrification functional genes during composting with qPCR. The correlation among physico-chemical parameters and copies of these functional genes were statistically analyzed. The results obtained from this study are expected to achieve a further understanding on FGDG affecting the nitrogen transformation during composting on a molecular level.

2. Materials and methods

2.1. Composting procedure

The composting experiments were conducted for 50 d, at the School of Chemistry and Chemical Engineering, Guangxi University, from October 26 to December 15, 2014 (Guo et al., 2016). The dairy manure was obtained from the dairy farm at the Guangxi University. The sugarcane leaf and the sugarcane pressmud were collected from the Nanxu sugar factory (Nanning, China). Sugarcane leaf was cut into 10–20 mm pieces to adjust the initial C/N ratio of the composting materials and used as a bulking agent. The sugarcane pressmud is a by-product from sugar refinery, which is rich in content of organic carbon and nitrogen, and has been utilization as a carbon source for composting (Satisha and Devarajan, 2007; Kumar et al., 2010). FGDG was obtained from Nanning coal-fired power plant (China). The pH value of FGDG was 7.60, and no nitrogen was detected in FGDG. The main characteristics of the raw materials were analyzed (Table 1).

Two composting experiments were conducted. 26 kg wet raw materials in CP were dairy manure + sugarcane leaf + sugarcane pressmud (15 + 1 + 10 kg) and 26 kg wet raw materials adding 2.6 kg FGDG in CPG were dairy manure + sugarcane leaf + sugarcane pressmud + FGDG(15 + 1 + 10 + 2.6 kg). 2.6 kg FGDG was added into the raw materials to get a target final rate of 10%, which was optimized in our pre-experiment (data not shown). The bulk density of CP and CPG were 430 and 477 kg/m^3, respectively. In the first stage of composting, the raw materials in CP and CPG were packed loosely into two open reactors. The reactor was a 60 L chemical drums with dimension of ∅400 mm × 620 mm (height) and covered with insulation material to preserve heat loss. In the second stage of composting (from 27 d), the CP and CPG were indoors to accelerate the moisture loss via natural ventilation. The compost was turned on 3, 6, 9, 12, 15, 21, 27, 33 and 40 days to provide a good aeration. For each sampling, three subsamples collected randomly from multipoints in compost after each turning, were used for physico-chemical analysis. The odd subsamples were pooled, mixed and kept at −20 °C (Zhang et al., 2011). The samples on 0, 3, 9, 15, 27, 33 and 50 d were used for qPCR analysis.

Table 1
Characteristics of composting materials.

Composting material	Moisture (%)	Kjeldahl N (%)	Organic matter (%)	C/N
Dairy manure	77.0 ± 0.4	2.05 ± 0.02	68.5 ± 0.8	19.5 ± 0.3
Sugarcane pressmud	59.0 ± 0.2	1.01 ± 0.01	45.0 ± 0.5	26.0 ± 0.5
Sugarcane leaf	14.9 ± 0.7	0.950 ± 0.01	72.7 ± 1.1	44.2 ± 0.6

2.2. Physico-chemical parameter analysis

Moisture content was determined by drying fresh samples at 80°Cuntil the mass loss in 24 h was less than 0.5% relative to the previous day (Zeng et al., 2012). The ambient temperature and compost temperature in the centre of the composting mixture were daily recorded by a temperature sensor at 10 AM (Zhang et al., 2011). After mechanically shaking the fresh sample in a water suspension at a ratio of 1:10 (w/v) at 200 rpm for 30 min, the pH and electrical conductivity (EC) were determined with a pH meter (PHS-25) and a conductivity meter (DDB-303A), respectively. The Kjeldahl N (KN) content was quantified according to the Kjeldahl digestion method (Barrington et al., 2002). NH_4^+-N, NO_2^--N and NO_3^--N of the sample were extracted with 1 mol/L KCl at a ratio of 1:10 (m/V). NH_4^+-N content was analyzed by the Indophenol Blue method and NO_2^--N content was measured by the sulfanilamide-NAD reaction (Guo et al., 2016). NO_3^--N content was analyzed by colorimetry (Zhang et al., 2009). Organic matter (OM) was measured by dry combustion of dried samples (550 °C for 5 h).

2.3. DNA extraction

DNA was extracted from 100 mg CP and CPG, respectively, using the PowerSoil® DNA Isolation Kit according to the manufacturer's instructions. Afterwards, extracts were stored in −20 °C until use. The DNA concentration and purity were checked by spectrophotometry (ND-1000, NanoDrop Tech.). DNA quality was analyzed on 0.8% agarose gel in tris borate EDTA (TBE 0.5×) buffer. The size DNA marker used was lambda-*Hind*III (Promega, America).

The nitrification and denitrification functional genes were quantified over time during composting, and primers sequence in Table 2 were used and synthesized by Sangon (Shanghai, China).

2.4. Standard curves

The target genes (i.e., *16S* rDNA, *amoA*, *nxrA*, *narG* and *nirS*) were amplified by PCR from the samples. PCR products of the expected size were ligated into the Peasy®-T1 Cloning Vector and trans-