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Research article

Relationship between maturity and microbial communities during pig manure composting by phospholipid fatty acid (PLFA) and correlation analysis

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ABSTRACT

The dynamic of microbial community plays vital role during composting. We therefore conducted a combined study on the maturity of compost (by pig manure composting with covering matured compost) and the successions of microbial communities (via phospholipid fatty acid (PLFA)). Our results showed that pH, electrical conductivity (EC), NH_4-N, and germination index (GI) were suitable indicators for compost maturity evalument. In addition, there was a closer correlation between maturity indexes (NH_4-N and GI) and the microbial compositions (as evaluated by microbial PLFA). The regression predicting model for NH_4-N used bacteria PLFA 15:0 and fungi PLFA 18:1ω9t ($R^2 = 0.98$, $P < 0.01$) and for GI used fungi PLFA 18:1ω9t and 18:1ω9, 12 ($R^2 = 0.94$, $P < 0.01$) as the evidences of good predictive ability. It also indicated that PLFA 18:1ω9t has a good relationship with the changes of NH_4-N and GI during the composting. Our results revealed the potential of using microbial PLFA for evaluating the maturity during pig manure composting.

1. Introduction

In recent years, microbial-based aerobic composting is a common and effective method for treating pig manure, and the final product (i.e., compost) can be used as soil conditioners or amendments fertilizer without adverse environmental effects (Bedada et al., 2016). When compost products are used for agricultural applications, the maturity and stability of the products should be taken into consideration. Immature compost not only would cause N starvation (Willson and Dalmat, 1986), but also results in the delay of plant growth and phytotoxic effects (Keeling et al., 1994). In addition, the offensive odor of immature compost is unbearable, and it would be harmful to human health (Kato et al., 2005). Therefore, as soil conditioners or amendments, it is crucial to evaluate the maturity of compost.

Compost maturity or stability is often determined by physical, chemical and biological parameters (Bernal et al., 2009) such as temperature, pH (de Bertoldi et al., 1983), electrical conductivity (EC) (Garcia et al., 1991), NH_4-N (Tiquia and Tam, 1998), NH_4-N/TN, C/N (Bernal et al., 2009), *T* value (the ratio between final C/N and initial C/N) (Itavaara et al., 1997), and seed germination index (GI) (Zucconi et al., 1981). Maturity is not judged by a single indicator, and therefore is best assessed by measuring two or more parameters of compost.

Furthermore, composting is a process involving the decomposition and transformation of biodegradable organic waste accomplished by the bioreaction of various microorganisms (Gómez et al., 2006). Therefore, microbial characteristics, such as phospholipid fatty acid (PLFA) analysis, 16S rDNA analysis, and quinone profiling, are used for assessing the maturity of compost. Among them, PLFA analysis is considered to reflect the actual condition of the microbial community because this analytical method is based on the

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extraction and quantification of phospholipids from whole microorganisms in the sample (Kato et al., 2005). As a result, PLFA profiling has been started using in composting process (Covino et al., 2016). The total amount of PLFA can be used as an indicator of viable microbial biomass, hence can help us to understand the succession of microbial populations during the composting process (Amir et al., 2008). Previous studies have also suggested that PLFA analysis can be used to evaluate compost maturity (Belete et al., 2013). Thus, PLFA analysis studies coupling correlation analysis and the quantitative characterization of maturity indexes as well as microbial community succession are needed to evaluate the maturity well during aerobic composting.

It has been demonstrated that covering composting piles with mature compost could effectively adsorb and oxidize NH_3 emitted from the composting (Luo et al., 2014; Maeda et al., 2010). Mixing mature compost with raw materials can improve inter-particle voids in compositing pile, thus increasing air permeability and relieving the transit and volatilization (Iqbal et al., 2010). In addition, mature compost has not only large amount of adsorption capacity for adsorbing NH_3 emissions, but also yield suitable environment for microbial growth in composting pile due to the large surface area and permeable pore space (Qiang et al., 2009). Thus, the studies of composting with covering mature compost have become a highlight of composting in recent years. Combined the analysis of PLFA, Kato and Miura (2008) reported that gram-positive bacteria and actinomycetes had positive and negative correlation to GI and NH_4-N respectively during the cattle manure composting covering with mature compost. It is worth mentioning that their work is the first time to quantify the correlation between the maturity index and the composition of PLFA. However, few studies has been performed to establish the prediction model between maturity and microbial community succession with the aim of evaluating the maturity well during aerobic composting.

In this study, pig manure composting with covering matured compost was conducted to evaluate the maturity of compost and investigate microbial successions via PLFA analysis. Furthermore, the analysis of correlation between the indexes of maturity and PLFA microbial profile characteristics, followed by regression modeling analysis throughout the composting process, will be used to assess whether PLFA analysis is an indicator for evaluating the maturity of pig manure composting.

2. Materials and methods

2.1. Composting materials

Fresh pig manure (PM) and wheat straw (WS) collected from a swine farm and local cropland, respectively, were used as the raw materials for the present study of aerobic composting. Wheat straw was cut to lengths of 1.0–2.0 cm pieces, and used to adjust the water content and C/N ratio to suitable levels for composting. Mature compost (MC), which used the same raw materials in this study, was collected from the previous study (Jiang et al., 2015). The basic characteristics of PM, WS, and MC, respectively, are as follows: total organic carbon (TOC) was 359.0, 419.6, and 243.5 g kg^{-1}; total nitrogen (TN) was 27.9, 5.0, and 30.6 g kg^{-1}; the water content was 70.9%, 14.5%, and 19.5%; and the C/N ratio was 12.8, 83.3, and 8.0.

2.2. Composting experiments method and design

Self-built, aerated static composting reactors (0.65 m height, 0.50 m length, and 0.40 m width, about 90 L) were set up in this study, being covered with 0.08 m plastic foam for retaining the heat generated during composting. And the reactor consisted of, for instance, a sealed reaction chamber, sieve tray, holder, air pump, and temperature detector (Jiang et al., 2015).

Fresh pig manure and wheat straw were mixed at a ratio of 10.5: 1 by fresh weight. The pile of composting consists of 18 kg mixture of pig manure and wheat straw, and 5 kg mature compost. The basic material (18 kg mixture) used in each treatment was piled up layer-by-layer on the sieve plate of the reaction chamber but without subsequent compaction under the homogenous conditions. The 5 kg manure compost piled up on the metal frame above the under layer, rather than mixed with the mixture of pig manure and wheat straw. The water content of the stock material was initially adjusted to approximately 65%. No further adjustment in moisture was made throughout the composting period.

The temperature was monitored every day at a depth of 0.20 m using the thermometer function of a programmable temperature controller (XMT616) before aeration, while the ambient temperature was monitored simultaneously. Aeration was arranged by blowing fresh air (60 L min^{-1}) using an air pump through a perforated tube at the bottom of each box during the composting. After 45 days, the aeration was ceased because of the temperature of compost reached to ambient temperature.

Compost samples were collected seven times over the duration of the experiment (0, 8, 12, 20, 25, 36, 45, 75 days) according to the change of temperature after the start of the experiment. Part of samples were stored at −20 °C for PLFA, NH_4-N and NO_3-N analysis, and others were drying at 50 °C for TN and TOC analysis. The analysis of PLFA did not include the samples on 8 and 20 days.

2.3. Analytical methods

NH_4-N in compost-KCl extract was detected by NaOH distillation-H_2SO_4 titration. EC, pH and GI were measured on an aqueous extract obtained from the fresh samples of compost. The aqueous extract was obtained using the method described by Huang et al. (2004). EC was measured with an S30 EC meter (Mettler Toledo Instruments (Shanghai) Co., Ltd.) and pH was measured with a Delta 320 pH meter (Mettler Toledo Instruments (Shanghai) Co., Ltd.). Chinese pakchoi (*Brassica campestris* L. *ssp. chinensis Makino*) seeds were used for the GI measurement. Ten Chinese pakchoi seeds were distributed on filter paper (Hangzhou Whatman-Xinhua Filter Paper Co., Ltd.) in Petri dishes (0.1 m diameter) and moistened with 5 mL of the compost extract. Three replicate dishes for each sample of different stages were incubated at 25 °C for 48 h. The number of germinating seeds and their root lengths were measured. Distilled water was used as a reference. GI was used to assess phytotoxicity of the compost and calculated using Eq. (1)

$$GI(\%) = \frac{\text{Seeds germination in treatment } (\%) \times \text{Root length in treatment}}{\text{Seeds germination in control } (\%) \times \text{Root length in control}} \times 100\% \quad (1)$$