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Particle-scale visualization of the evolution of methanogens and methanotrophs and its correlation with CH_4 emissions during manure aerobic composting

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Methane (CH_4) emissions are a major environmental concern in composting facilities. Therefore, this study initially visualized the dynamic distribution and quantity of methanogens and methanotrophs in composting particles during manure aerobic composting using fluorescence *in situ* hybridization–confocal laser scanning microscopy (FISH–CLSM) and quantified their correlation with CH_4 emissions. The visualization results showed that methanogens existed inside the particles, while methanotrophs clustered in the outer layer; a facultative anaerobic zone existed in between. The quantification results of integral optical density of methanogens and methanotrophs per unit particle area (U_{gen} and U_{oxi}, respectively) indicated that, in the cooling phase, CH_4 generation and oxidation could still be high and could strike a balance if the initial organic matter content of composting materials is high, while both could be extremely low if the content is low. A strong linearity between U_{gen} obtained by FISH–CLSM and methyl-coenzyme M reductase copy number obtained by quantitative polymerase chain reaction analysis ($R^2 = 0.88$) was observed, which justified the effectiveness of the FISH–CLSM method and demonstrated that macro-scale CH_4 emissions were essentially an accumulation of particle-scale CH_4 emissions. CH_4 emissions were equal to $3.3297 \times 10^7\ U_{gen} - 3.1814 \times 10^6\ U_{oxi} - 3902.9900$ ($R^2 = 0.98$). Overall, the results showed that methanogens exerted more influence on CH_4 emissions than methanotrophs. Combining these results with CH_4-generation and -oxidation kinetics may help illustrate CH_4-emission mechanisms, improve particle-scale CH_4-emission models, and thereby provide theoretical guidance for operation optimization and emission reduction in composting processes.

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1. Introduction

According to the latest World Meteorological Organization (WMO) Greenhouse Gas Bulletin, due to increased emissions from anthropogenic activities, the globally averaged concentration of methane (CH_4), corresponding to the CH_4 concentration in the atmosphere, reached a new maximum peak of 1853 ± 2 ppb in 2016 (World Meteorological Organization, 2017). The global warming potential of CH_4 is ~25 times that of carbon dioxide (CO_2) on a 100-year time scale and only ranks behind CO_2 in its greenhouse gas contributions during composting (Bong et al., 2016). As manure has a relatively high content of organic carbon, carbon losses from CH_4 emissions during the aerobic composting of manure could account for 6% of the total carbon content of composting mixtures (Denes et al., 2015). Therefore, there is a need to clarify CH_4-emission mechanisms involved in manure aerobic composting, a deep understanding of which would facilitate the design of an environment-friendly and cost-effective composting strategy.

Since CH_4-emission mechanisms are considered to be a biological process dominated by methanogens and methanotrophs (Haug, 1993), some research has been devoted to the investigation of the ion of methanogens and methanotrophs during aerobic composting. The CH_4-emission mechanisms during composting have been reported to mainly include CH_4 generation in anaerobic zones and CH_4 oxidation in aerobic zones of the composting mixture (Cayuela et al., 2012; D'Imporzano et al., 2008; Maulini-Duran et al., 2014). Hence, visualizing the distribution and quantity of methanogens and methanotrophs in the composting mixture would be important in elucidating the CH_4-emission mechanisms that occur during manure aerobic composting.

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As is known, large composting piles such as pilot-scale reactors and windrows often have a hot anaerobic core surrounded by cooler microaerobic and aerobic zones. As the basic unit of composting piles, composting particles have also been proved to have anaerobic and aerobic zones (Ge et al., 2014; He et al., 2000; Jiang et al., 2011; Ro et al., 1998), which could be a starting point for illustrating the coupling of CH_4 generation and oxidation (D'Imporzano et al., 2008; Ge et al., 2016a; Maulini-Duran et al., 2014). Therefore, basic research to explore the CH_4-emission mechanisms at a particle scale is required, which might provide theoretical insights into interpreting macro-scale CH_4 emissions.

Several studies have employed fluorescence *in situ* hybridization–confocal laser-scanning microscopy (FISH–CLSM) to understand bacterial interaction on biofilms. Kim et al. (2012) reported the distribution of methanotrophs in biofilms and quantified the effect of the distribution on the biofilm volume using FISH–CLSM. Van Wey et al. (2012) used FISH–CLSM to observe bacterial distribution in biofilms and illustrate the correlation of this distribution with the transfer kinetics of nutrients. Jiang et al. (2009) characterized the distribution of sulfate-reducing bacteria in biofilms using FISH–CLSM and then simulated the specific growth rate of the sulfate-reducing bacteria.

Considering its utility, as shown by previous studies, we used the FISH–CLSM method to visualize the dynamic distribution and quantity of methanogens and methanotrophs in composting particles, as well as quantifying the correlation between their evolution and CH_4 emissions. The reliability of this method was examined by quantitative polymerase chain reaction (qPCR) data. This study is expected to enhance our understanding of CH_4-emission mechanisms that occur during manure aerobic composting, and provide methodologies and data for the design and optimization of composting strategies that comply with stringent environmental regulations.

2. Materials and methods

2.1. Manure aerobic composting experiments and physicochemical analyses

Composting materials for this study included pig manure (10.0 kg) and chopped wheat straw (1.0 kg) collected from a livestock and poultry test site at the Chinese Academy of Agricultural Sciences (Changping, Beijing, China) and suburban areas of Beijing, respectively. Deionized water (1.0 kg) was added during the mixing of the materials to achieve an appropriate moisture content of 50–67% (Haug, 1993). The thoroughly mixed materials were divided into two mixtures with the same weight, and each mixture was loaded into a 15 L cylindrical reactor (0.40 m height × 0.25 m inner diameter) (Lü et al., 2008) to conduct two independent aerobic composting experiments (experiments A and B) that lasted for 16 days. Furthermore, an intermittent aeration strategy of 1 h on/1h off, with a rate of 0.35 L min^{-1}, was selected based on previous work (Ge et al., 2015b). Composting temperature was determined using a thermocouple (Pt100, Omega, USA) located in the middle of the composting mixtures, and the oxygen concentration (volume fraction) in the output streams from the reactors was measured at the end of the aeration phase using an oxygen sensor (O_2S-FR-T2-18X, Apollo Electronics Co., Ltd., Zhuhai, China). Gaseous samples of the input and output were collected in 1 L gas bags, and the CH_4 concentration in the output streams was determined by gas chromatography using a flame ionization detector (GC-2014, Shimadzu, Nagoya, Japan) and an Rt®-Q-BOND column (30 m long × 0.53 mm diameter, Restek, USA). Detailed operation conditions for gas chromatography were described previously (Ge et al., 2016a,b). Cumulative CH_4 emissions were estimated by the linear trapezoidal method using the "Trapz" function of mathematical software (MATLAB, MathWorks, USA) and expressed as carbon equivalents. The function is designed to integrate the integrand with the form of discrete data pairs (Wang et al., 2016). Briefly, the cumulative CH_4 emissions were calculated from the area under the time-profile curve for CH_4 concentration in the output stream from the composting reactors.

The condition of oxygen transportation in the composting mixtures mainly depends on the free air space (*FAS*) in the mixtures. *FAS* is defined as the ratio of the gas volume to the total volume of the mixture and calculated as follows (Haug, 1993; Wang and Ai, 2016):

$$FAS = 1 - \rho_m DM/(G_{DM}\rho_w) - \rho_m(1 - DM)/\rho_w \tag{1}$$

$$1/G_{DM} = OM/G_{OM} + (100\% - OM)/G_{ash} \tag{2}$$

where ρ_m is the density of the mixture, kg m^3; DM is the dry matter content of the mixture, %; G_{DM} is the specific gravity of the dry matter, kg m^3; ρ_w is the density of water, 1000 kg m^3; OM is the organic matter content of the mixture, %; G_{OM} is the specific gravity of the organic matter, 1000 kg m^3; and G_{ash} is the specific gravity of ash, 2500 kg m^3.

Sampling was conducted on days 0, 2, 4, 6, 8, 12, and 16; each time, each of the two mixtures was thoroughly mixed by hand before and after sampling. Considering that the mixtures were not homogenous, three samples were taken, one each from the upper, middle, and lower parts of each mixture. The moisture and organic matter contents of the samples were measured according to standard procedures (03.09-A and 05.07-A) provided in the Test Method for the Examination of Composting and Compost (TMECC) (U.S. Composting Council, 2002). The carbon to nitrogen ratio was calculated from total carbon and total nitrogen, which were determined by dry combustion using an elemental analyzer (Vario MACRO, Elementar, Hanau, Germany). All measurements were determined in triplicate.

The physicochemical properties of the initial composting mixtures in experiments A and B are listed in Table 1. The initial values of the moisture content, organic matter content, and carbon to nitrogen ratio were considered satisfactory composting conditions (Haug, 1993). Statistics showed that the organic matter content in experiment A was significantly higher than that in experiment B ($p < 005$), possibly due to human error that occurred during the division of the composting materials between the two reactors, as well as the heterogeneity of the raw materials.

2.2. Visualization of the evolution of methanogens and methanotrophs during manure aerobic composting using FISH–CLSM

2.2.1. Sample pretreatment

According to previous studies (Díaz et al., 2006; Guo and Zhang, 2014; Jupraputtasri et al., 2005; Kim et al., 2012) and the results of preliminary experiments, pretreatment of the composting samples was performed as follows. To maintain ionic and pH conditions,

Table 1
Physicochemical properties of the initial composting mixtures in experiments A and B.

Physicochemical properties	Experiment A	Experiment B	Significance level
Moisture content (%)[a]	56.17 ± 2.59	56.47 ± 2.84	0.897
Organic matter content (%)[b]	83.25 ± 1.35	79.25 ± 1.21	0.019
Total carbon content (%)[b]	42.02 ± 0.15	43.87 ± 2.19	0.216
Total nitrogen content (%)[b]	2.55 ± 0.21	2.78 ± 0.09	0.160
Carbon to nitrogen ratio[b]	16.56 ± 1.41	15.80 ± 1.00	0.489

[a] Measurements based on wet weight.
[b] Measurements based on dry weight.

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