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Exploring the mechanisms of decreased methane during pig manure and wheat straw aerobic composting covered with a semi-permeable membrane

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ABSTRACT

It is very important to reduce methane production and emission during aerobic composting. In this study, the effects of covering with a semi-permeable membrane during pig manure and wheat straw composting were investigated. Two laboratory-scale composting reactors were used: the membrane covered treatment (treatment A) and the control treatment (treatment B). Composting in treatment A effectively improved the oxygen utilization rate and decreased methane emissions by 22.42% relative to the control treatment. Quantification of functional genes and Pearson rank correlations showed that the *mcrA* and *mcrA/pmoA* gene abundances were significantly positively correlated with temperature and negatively correlated with the interstitial oxygen concentration, and that the *pmoA* gene abundance was positively correlated with the carbon: nitrogen ratio and moisture content. Therefore, increasing the aeration rate and optimizing the carbon: nitrogen ratio and moisture content will decrease methane emissions. Together, the results demonstrate that coverage membrane could be a novel strategy for reducing methane emissions during composting.

1. Introduction

The amount of pig manure generated in China has increased dramatically with the rapid development of intensive farming. Manure can cause hygiene hazards and odor pollution, if not properly treated (Guo et al., 2012). Aerobic composting is an important way of treating animal manure with a low investment cost when compared with other waste treatment technologies (Su et al., 2016), but the uneven distribution of oxygen in a composting pile can cause greenhouse gases to be produced. Methane (CH_4) emissions from compost are second only to carbon dioxide (CO_2) and can account for up to 2–3% of the total carbon (C) content of the composting material (Ge et al., 2016; Hao et al., 2004; Maeda et al., 2013). The global warming potential of CH_4 is 25 times higher than the global warming potential of CO_2 (Maeda et al., 2013; Wen et al., 2014). Therefore, reducing CH_4 emissions has become a research focus. CH_4 emissions during composting can be minimized by adding a bulking agent, using an aeration system, adding certain chemicals, or by covering the composting material with mature compost (Chowdhury et al., 2014; Jiang et al., 2015; Maeda et al., 2013; Wang et al., 2014; Wen et al., 2014; Yang et al., 2015; Yang et al., 2013). However, methods of decreasing CH_4 emissions by achieving a uniform oxygen distribution and the mechanism involved are not well understood.

An aerobic composting technique that involves covering with a semi-permeable membrane has been developed (González et al., 2016; Levis and Barlaz, 2011; Levis et al., 2010). In this technique, the bottom of the composting pile is aerated in a well-controlled manner, and this, combined with the semi-permeable membrane on top of the pile, allows a uniform and appropriate oxygen concentration to be maintained to ensure that aerobic fermentation occurs. The semi-permeable membrane consists of three layers of material: the middle layer is expanded polytetrafluoroethylene (e-PTFE), which is sandwiched between two layers of solid polyester film. The e-PTFE is the most important part, and previous studies have shown that e-PTFE can recover ammonia (NH_3) from swine manure, where NH_3 permeates through a microporous hydrophobic membrane and was captured and concentrated in a stripping solution on the other side of the membrane (Garcia-Gonzalez and Vanotti, 2015; Masoud Samani Majd and Mukhtar, 2013; Vanotti et al., 2017; Vanotti et al., 2016). NH_3 emissions were decreased by approximately 30% by covering with a

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semi-permeable membrane during composting because of the presence of a water film (Ma et al., 2017; Sun et al., 2016). In previous studies it has been found that emissions of odorous and volatile organic compounds can be decreased by 90% using a membrane-covered composting system (Schmidt et al., 2009). However, there have been no studies on the effect of membrane-covered composting systems on CH_4 emissions and the mechanisms involved.

CH_4 is generated through microbial activity in strictly anaerobic environments (Sharma et al., 2011; Takai, 1970). Methyl-coenzyme M reductase, a key enzyme involved in CH_4 generation, can catalytically reduce a methyl group linked to coenzyme M to form CH_4. The *mcrA* gene that encodes methyl-coenzyme M reductase is present in all known methanogens, so *mcrA* can be used as a target gene when assessing the methanogenic mechanism (Ellermann et al., 1988). CH_4 is oxidized to CO_2 by methane-oxidizing bacteria during aerobic composting, and this is catalyzed by methane monooxygenase. There are two forms of methane monooxygenase, one (called soluble methane monooxygenase) dissociated in the cytoplasm and the other (called particulate methane monooxygenase) bound to cell membranes in particles. Almost all known methanotrophs except for *Methylocella* contain the *pmoA* gene, which encodes the key polypeptide (PmoA) of particulate methane monooxygenase. The *pmoA* gene is widely used to study methanotroph diversity in environmental media (Holmes et al., 1999; Murrell et al., 1998). The mechanisms involved in CH_4 generation by microorganisms and CH_4 emissions during membrane-covered aerobic composting have not yet been described.

In this study, two composting experiments were conducted using laboratory-scale membrane-covered aerobic composting reactors to investigate aerobic fermentation and anaerobic CH_4 generation and emissions. The abundances of methanogen and methanotroph bacterial communities were assessed using a real-time quantitative polymerase chain reaction (qPCR) method. Correlations between the physicochemical characteristics and functional genes were identified to attempt to explain the CH_4 generation, CH_4 emissions, and the mechanism through which microorganisms produce CH_4 during membrane-covered aerobic composting. The results will provide guidance for optimizing composting processes and decreasing CH_4 emissions.

2. Materials and methods

2.1. Membrane-covered aerobic composting

Two experiments were performed using laboratory-scale aerobic composting systems. One of experiments, treatment A, used a membrane-covered composting system, and the other, treatment B, used a control composting system with no membrane. Each reactor had a volume of approximately 95 L. A schematic diagram of a reactor is shown in Fig. 1. The membrane was Gore-Tex, and its parameters are as follows: average pore diameter of <0.2 µm, water resistance of >50 kPa, water vapor permeability resistance of <19.5 $m^2Pa\ W^{-1}$, and the air permeability of 1.5–6.5 $m^3\ m^{-2}\ h^{-1}$ (Sun et al., 2016).

Fresh pig manure was obtained from the Chinese Academy of Agricultural Sciences (Changping, Beijing), and wheat straw was collected from the China Agricultural University Experimental Station (Shang Zhuang, Beijing). The straw was cut into 3–5 cm long pieces. An appropriate moisture content (MC) and an appropriate carbon to nitrogen (C/N) ratio were achieved by mixing pig manure and wheat straw at a wet weight mass ratio of 18:1. The physicochemical properties of the raw materials and the mixture are shown in Table 1. Air was added to each reactor at a flow rate of 5.6 $L\ min^{-1}$ based on previously reported laboratory studies.

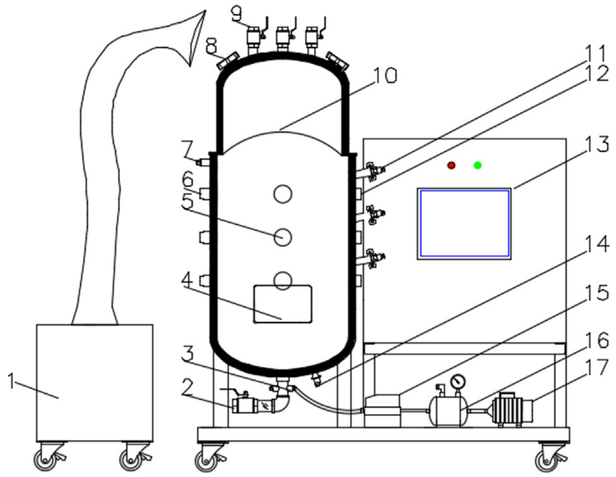

Fig. 1. Schematic of the membrane-covered aerobic composting system: (1) off-gas absorption, (2) filtrate collection, (3) gas inlet, (4) material outlet, (5) sampling opening, (6) built-in oxygen sensor, (7) built-in pressure sensor 1 (sub-membrane), (8) windows, (9) gas outlet, (10) membrane, (11) built-in pressure sensor 2 (in pile); (12) built-in temperature sensor, (13) automatic control system, (14) built-in pressure sensor 3 (gas inlet), (15) flowmeter, (16) air bag, (17) gas pump.

2.2. Sample collection and analytical methods

Approximately 500 g samples, one each from the top, middle, and bottom layer, were collected and mixed from each reactor on days 0, 3, 6, 9, 12, 15, 21, and 27 of the composting process. Part of each sample was stored at −20 °C for physicochemical analysis and the remainder was stored at −80 °C for microbial analysis (Zhang et al., 2015).

The volatile solids (VS) content and MC were measured using a standard method (TMECC, 2000). The total N and total C contents were measured using an elemental analyzer (Vario MACRO; Elementar, Hanau, Germany), and the C/N ratio was calculated.

2.3. Oxygen and CH_4 concentration measurements and calculations

The interstitial oxygen concentrations and CH_4 emission rates were determined every 12 h. The oxygen concentration was monitored using an oxygen sensor (4OXV; City Technology, London, UK). Samples of the outlet gas and of gas from beneath the membrane were collected in 500 mL air bags during the aeration process, and the CH_4 concentrations in the samples were determined using an Agilent 7890A gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The packed column and detector temperatures were 55 °C and 200 °C, respectively. The flow rates of hydrogen and air for the flame ionization detector were 40 and 400 mL/min, respectively. The CH_4 emission rate was defined as the difference between the CH_4 concentrations in the input and output gases (Ge et al., 2016) and calculated using the equation

$$E_{CH_4} = Q(CH_{4,out} - CH_{4,in}) \times 10^{-6}/V_m \times 60$$

where E_{CH4} is the CH_4 emission rate during aeration (in mol $CH_4 \cdot kg^{-1}\ VS \cdot h^{-1}$), Q is the aeration rate (in $L \cdot kg^{-1}\ VS \cdot min^{-1}$), $CH_{4,\ out}$ is the CH_4 concentration (in ppmv) in the outlet or sub-membrane gas during aeration, $CH_{4,\ in}$ is the CH_4 concentration (in ppmv) in the inlet gas during aeration (which is approximately 0 during aeration), 10^{-6} is the conversion factor of ppmv, V_m is the