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# Effect of temperature on bacterial emissions in composting of swine manure

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ABSTRACT

Swine manure was subjected to laboratory scale composting in order to quantify bioaerosols, i.e., airborne culturable bacteria and endotoxin, in the exhaust gas, which provided details on the effect of temperature on bacterial emissions. The concentration of airborne bacteria reached 31,250 colony-forming units (CFU)/$m^3$ during the thermophilic stage of composting, and positively correlated with the temperature profile of the compost pile. Initially, the endotoxin concentration was 1820 endotoxin units (EU)/$m^3$, but it decreased exponentially as the composting process proceeded. The temperature can be an excellent indicator of bacterial emissions during the composting process, indicating that the composting process requires a consistently high temperature to ensure sanitization of both compost and bacterial emissions. The cumulative emission data showed that emission factors was 11.2–13.5 CFU/g dry swine manure and that of endotoxin was 0.5–0.9 EU/g dry swine manure. The bacterial diversity in the bioaerosol was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis, revealing the presence of various gram-negative bacterial consortia.



## 1. Introduction

Composting has been considered an attractive option for treating organic wastes because it causes less environmental pollution and the final product can be used beneficially (Iyengar and Bhave, 2006). Hence, a large-scale composting is commonly applied for diverting sewage sludge, livestock manure, and other organic wastes from landfills and incinerators (Bernal et al., 2009; Domingo and Nadal, 2009; Kim et al., 2008).

Airborne contaminants, which include dust, microorganisms, microbial volatile organic compounds, and odor compounds, can be released during the operation of composting facilities (Domingo and Nadal, 2009; Fischer et al., 2008; Ko et al., 2008; Lehtinen et al., 2013). In particular, airborne microorganisms are much likely to be emitted by any handling of composting materials because microbiological activity is fundamental to the composting process (Sykes et al., 2007; Taha et al., 2005). Airborne microorganisms are generally referred to as bioaerosols, which are airborne particles that are of biological origin (Sanchez-Monedero and Stentiford, 2003). Inhalation of bioaerosols can cause a variety of inflammatory, hypersensitivity, and allergic responses in the lungs (Bunger et al., 2007; Herr et al., 2003), especially in sensitized individuals. Bioaerosols also include endotoxin, mycotoxin, and insect parts that can have adverse health effects as well (Millner, 2009). Endotoxin, which is used synonymously with the term lipopolysaccharide, is a major constituent of the outer cell membrane of gram-negative bacteria, and can be released into the environment after destruction of the bacterial cell. Thus, measuring the endotoxin concentration is very useful to investigate environmental safety and occupational hygiene (Deacon et al., 2009; Sykes et al., 2011).

Moreover, it has been previously reported that the exposure levels of bioaerosols at workplaces that handle organic waste materials, i.e., organic waste disposal plants (Lavoie et al., 2006; Nikaeen et al., 2009; Pankhurst et al., 2012), are relatively higher than those in other types of workplaces. In a full-scale municipal waste composting facility, airborne bacteria were detected at concentrations up to $10^4$ colony-forming units (CFU)/$m^3$ (Byeon et al., 2008) and $2.5 \times 10^5$ CFU/$m^3$ (Pankhurst et al., 2011). Though the number of bacteria generated is site-specific (Sanchez-Monedero et al., 2005), but it could be, at least to some extent, related to any operational or environmental parameter. Thus, key factors for successful composting such as temperature, moisture, aeration, and nutrients should be appropriately controlled (Kim et al., 2008). Lab-scale studies have been previously performed to investigate the effects of these factors on the emissions of $CO_2$, volatile organic compounds, and odor (Barrena et al., 2009; Pagans et al., 2006a,b). To the best knowledge, the effects of operational environmental

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parameters on bioaerosol emissions during the composting process have been not studied so fat.

The objective of this study was to investigate the effect of the compost temperature, which is one of the operational parameters, on the concentrations of airborne bacteria and endotoxin in the exhaust gas during swine manure composting. By using a generation profile, emission factors were estimated to provide a representative value that relates the quantity of bioaerosol released into the atmosphere with an activity associated with swine manure composting. In addition, microbial diversity of airborne bacteria was investigated by an analysis by denaturing gradient gel electrophoresis (DGGE) of the polymerase chain reaction (PCR) amplified V3 region of the 16S rDNA.

## 2. Methods and materials

### 2.1. Raw materials for composting

Dewatered swine manure samples were obtained from a pilot farm in Suwon city, Korea. The waste and volatile suspended solids (VSS) contents in the swine manure samples were about 76 wt% and 10 wt%, respectively. The pH of the sample was 7.5. Sawdust was used as bulking agent to provide an adequate porosity to the swine manure. The apparent diameter and density of sawdust were 10 mm and 66.7 kg/m$^3$, respectively. The raw composting mixture consisted of swine manure and sawdust in a ratio of 6:4 (v/v), and was put into the composting reactor with a total volume of 0.04 m$^3$. The moisture content of the mixture was initially adjusted to 60% and maintained at >50% during experimental period. The carbon to nitrogen (C/N) ratio was initially 23.1.

### 2.2. Composting reactor

The laboratory scale experimental set-up was composed of a reactor made of a stainless steel cylinder, whose internal diameter was 0.35 m and height was 0.85 m. The total volume of the reactor was 0.08 m$^3$. The reactor was placed in a tank made of stainless steel, in which high density polyurethane was used as thermal insulation to prevent heat loss from the compost pile. A schematic diagram of the reactor is presented in Fig. 1. Dried purified air was evenly supplied to the reactor through a perforated plate. The aeration rate was set at 2.5 L/min. The air outlet was positioned on the top plate of the reactor to allow sampling of the exhaust gas for analysis. Temperatures of the composting materials were monitored during the composting period using two sensors located at 40 cm and 50 cm from the bottom of the reactor. Temperatures were automatically averaged and recorded every 4 h. Two replications of the experiment were conducted. The results presented in this paper, except for data in Table 1, correspond to one replication. Differences in the temperature profiles between the two experiments were as low as 20%. The experiments were terminated when the composting temperature was near room temperature. The system was operated at room temperature, i.e., at about 20 °C.

### 2.3. Analytical methods

#### 2.3.1. Bioaerosol quantification

The generated bioaerosol was sampled a the scheduled time points by connecting a Biosampler (SKC Inc., USA) to the reactor, containing 50 mL of a 0.6% NaCl solution added prior to sampling. The exhaust gas sampling was carried out for 30 min to yield a sample volume 70 L. After sampling, the volumes of the solutions were measured to evaluate evaporative loss and the solutions were kept in a thermostat-controlled cold box until measurement of airborne bacteria or endotoxin. For the measurement of airborne

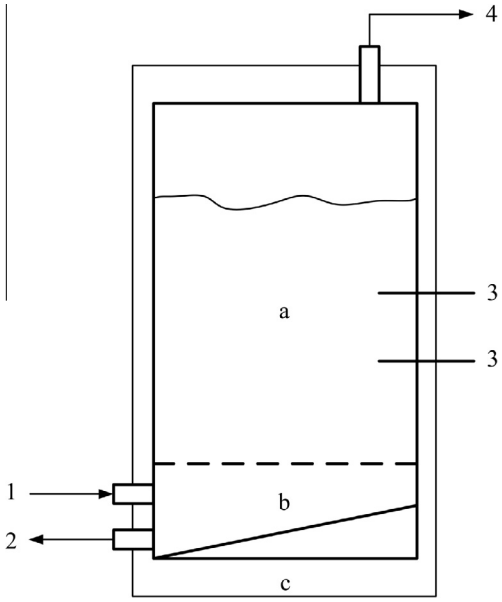


**Fig. 1.** Schematic diagram of the composting reactor: air inlet (1); leachate outlet (2); temperature sensor with recorder (3); air outlet and air sampling with a Biosampler (4); composting volume (a) and air supply and leachate collection (b) section was partitioned with a perforated plate; thermal insulation (c) was installed to prevent heat loss. The total volume of the reactor was 0.08 m$^3$ with 0.35 m internal diameter and 0.85 m height.

bacteria, the solutions were transferred to the sterilized clean bench. Tryptic soy agar (Hanil Komed Co., Korea) was inoculated with 200 μL sample solution and cultured in an incubator for 2 days at 38 °C. The colonies were counted, and the value was divided by a known volume of sampled air. The result was expressed as colony forming unit (CFU)/m$^3$. The endotoxin measurement of the extraction solution was performed using the limulus amebocyte lysate (LAL) test (Vmax, Molecular Devices Corp., USA). The result was expressed as endotoxin unit (EU)/m$^3$. Control values were subtracted from the sample values. All analyses were performed in triplicate. The data presented in this paper correspond to the geometric mean of one set of data values.

#### 2.3.2. Molecular biological analysis

To analyze the complexity of the microbial community in the bioaerosol generated from the composting pile, DNA was sampled from all bacterial colonies grown on plates used in this study in order to obtain a sufficient amount for this analysis.

DNA extraction: 0.25 g of bacterial colonies in 5 mL of sterile water was vortexed for 3 min, and the supernatant was centrifuged at 13,000 rpm for 5 min. A DNA extraction kit (Power Soil DNA Isolation Kit; MoBio Laboratories, USA) was used for the extraction of DNA from the sample.

PCR amplification: The PCR primers 10f and 1400r were used to amplify the 16S rDNA gene from the extracted DNA template. The primers 341f and 518r were used to amplify the bacterial V3 region of the 16S rDNA. The sequences of the primers used were as follows: 10f, AGA GTT TGA TCM TGG CTC AG; 1400r, AGC GGC GGT GTG TAC AAG; 341f, CCT ACG GGA GGC AGC AG; and 518, ATT ACC GCG GCT GCT GG. PCRs were performed on an UVIgene™ thermocycler. The reaction mix contained 10×h-Taq buffer (5 μL), 10 mM dNTP mix (1 μL), each primer (2 μL), 5×Band doctor (10 μL), h-Taq (0.5 μL), and DNA template (2 μL); sterile deionized water was added to a final volume of 50 μL. The following PCRs conditions were applied: 95 °C for 9 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 35 °C for 1 min, and extension at 72 °C for 2 min; and a final extension at 72 °C for 10 min. PCR