

## Study on microbe during thermophilic aerobic composting for sanitary disposal of human feces

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**Abstract.** During aerobic composting for sanitary disposal of human feces, compost was used as organic fertilizer with full of nitrogen. It would be favorable to hold more nitrogen in compost if change of biomass and the community structure—mainly ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), which was with the characteristics of nitrogen transformation, was found out during thermophilic aerobic composting of human feces. In this study, 14-days batch experiment were conducted using a closed aerobic composting reactor with sawdust as the bulky matrix to simulate the condition of a composting reactor for sanitary disposal of human feces. Attention was paid to the change of biomass from temperature. Under a controlled condition of temperature at 60°C, moisture content at 60%, and continuous air supply. Biomass was analyzed by Phospholipid fatty acids (PLFA). AOB and NOB were studied by fluorescence in situ hybridization (FISH). It shown that: during thermophilic composting, biomass reached to max at 7th day firstly and decreased obviously from 7th to the end of composting. AOB and NOB were decreased during composting and almost missed at the end of composting. The study showed that: biodegradation of Norg may have been hindered due to lack of ammonifying bacteria (such as AOB and NOB) activity and good for nitrogen holding during thermophilic composting, thermophilic composting could decrease fecal nitrogen loss, keep high organic nitrogen content in the composts for better fertilizer utilization.<sup>1</sup>

**Keywords:** Composting for human feces; PLFA; FISH; AOB; NOB; Nitrogen holding; Fertilizer

### 1. Introduction

Aerobic composting has been recognized as a suitable technology for sanitary disposal of human feces in a dry toilet system<sup>[1]</sup>. It draws attention especially from regions and areas where provision of sufficient water for toilet flushing is difficult due to water shortage<sup>[2]</sup>. The operational temperature for the commercial bio-toilet of this kind has thus been recommended as 50-60°C through an automatically controlled heating system<sup>[3]</sup>.

Many studies have so far been conducted on the characteristics of aerobic composting for sanitary disposal of human feces. Attention is mainly given to the process of biodegradation in which organics and fecal nitrogen are decomposed or transformed under the action of microorganisms, and the effect of pathogen inactivation which is the most important issue from the sanitary viewpoint. A thermophilic condition ranging from 50°C up to 65°C have been recognized to be optimum both for obtaining the best biodegradation effect<sup>[4]</sup> and effective removal of *E. coli* and other pathogens<sup>[5-11]</sup>. As the composting products can be utilized as fertilizers, it would be favorable if the composting condition could be well controlled for holding fecal nitrogen as far as possible in the composts. In different studies using sawdust as bulky matrices for feces composting, nitrogen losses are reported in a wide range from less than 50% to as high as 94%<sup>[7,12-15]</sup>. However, the process of transformation and loss of fecal nitrogen during aerobic composting is still a topic for detailed investigation.

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On the basis of previous findings that high percent fecal nitrogen could be well held in the composts in a prototype composting toilet operated under thermophilic condition<sup>[7,15]</sup>, the current study used a specially designed batch composting reactor to investigate the microbe of compost. Attention was mainly paid to the biomass of microbe of compost and ammonifying bacteria (such as AOB and NOB) activity in the reactor and lost from the reactor.

## 2. Materials and methods

### 2.1. Experimental device and Experimental operation

The experimental device used in this study is a closed composting reactor as shown in Fig. 1. The reactor chamber is a polymethyl methacrylate cylinder of 4.32 dm<sup>3</sup> net volume (inner diameter: 10 cm, height: 55 cm) with an outer jacket space as water bath for thermo control. A hand-driven shaft with agitation plates (not shown in Fig. 1) is mounted horizontally in the reactor for mixing. An air diffuser is set at the bottom of the reactor for introducing a constant air flow through the air supply unit. An exhaust pipe at the top is connected to a water cooled condensing unit where vapor is condensed and gas is led to a sulfuric acid solution to absorb the exhausted ammonia from the reactor.

The bulky matrix used in this study was sawdust from a local timber processing plant. The human feces used in this study were collected from the university campus under the assistance of students. In order to keep the initial quality of the feces identical in different experimental runs, the collected substances were well mixed, divided into equal quantity stocks, and preserved at -20°C for later use. Batch experiment was conducted in a two-week composting period which was selected as a suitable duration by preliminary experiments for all the biodegradable matters in the feces to be decomposed under a thermophilic condition. 320 g of sawdust and 80 g of feces weighed on dry weight bases were added to the reactor so that their dry weight ratio was 4:1. The initial moisture content was adjusted using deionized water to 60% and during the composting period, the condensed water was periodically sent back to the reactor to maintain the moisture content. Air flow to the reactor was controlled at a rate of 1.6 dm<sup>3</sup>/min, and by cycling the water in the water bath through a thermo controlled heating system, the temperature inside the reactor was maintained at 60°C during the composting period.

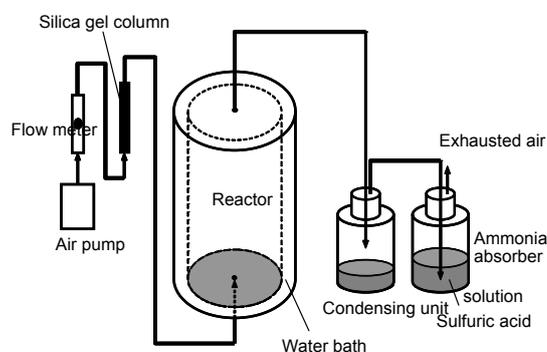


Fig.1 Diagram of the experimental composting reactor

### 2.2. Sampling and analytical methods

In the two-week composting period, mixture was sampled each day from the reactor after well mixing the substance in the reactor. The following analyses were conducted with each sample.

Nitrogen analysis was conducted regarding total nitrogen ( $N_{\text{tot}}$ ) by alkaline potassium persulfate digestion method<sup>[17]</sup>, inorganic nitrogen ( $N_{\text{ino}}$ ) as the sum of  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  by standard methods<sup>[18]</sup> after re-suspending, ultrasonic crusting, centrifuging, filtering the sample. Organic nitrogen ( $N_{\text{org}}$ ) content was obtained by calculating the difference between  $N_{\text{tot}}$  and  $N_{\text{ino}}$ .

Lipid is the main content of biological membrane and it decomposes soon after the cell dies. Therefore, the phosphorus in the lipid can be measured to denote the living biomass in a reactor. This method was used for this study in the following procedures<sup>[19,20]</sup>. Place the sawdust sample in a 100ml triangular flask. Add 19

ml extraction mix liquid(chloroform, methanol and water at a volume ratio of 1:2:0.8), shake for 10min, and then place for 12h. Add 5ml chloroform and 5ml water into the flask again and place for another 12h. Remove 5ml liquid from the chloroform layer which contains lipids to a 10ml scaled cuvette, and evaporate to dryness by water bath. Add 0.8ml 5% solution of potassium sulfate to the cuvette and water to a total volume of 10ml. Digest it at 121°C in a high-pressure stream sterilization pot for 30 min, and then measure the concentration of phosphate in the solution. The results were expressed as nmol p/g. 1nmol p approximately equal to 10<sup>8</sup> E.coli.

New technical approaches in molecular microbiology such as Fluorescence In Situ Hybridization (FISH) permit a new level of insight into the population structure and function of complex ecosystems like activated sludge<sup>[21-26]</sup>. Samples were fixed in 4% paraformaldehyde<sup>[21]</sup>. Ultrasonification (35 kHz, 5 min) was applied to the fixed samples from compost prior to hybridization in order to break up large solids. In situ hybridizations of cells were performed with fluorescently labeled. Oligonucleotide probes were obtained from AUGCT, China. Fluorescently labeled probe Nso1225(HEX) was detected for ammonia-oxidizing bacteria (AOB) and Fluorescently labeled probe Ntspa662 (FITC) for nitrite-oxidizing bacteria (NOB).

### 3. Results and discussion

#### 3.1. General performance of the composting reactor for fecal nitrogen transformation

Variation of total fecal nitrogen, organic nitrogen and inorganic nitrogen(NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N) in the composting process were shown as Fig.2. It was noticed that N<sub>tot</sub> decreased quickly in the first day of composting and then decreased gradually in the following days. The trend of variation of N<sub>tot</sub> was similar to that of N<sub>ino</sub>, while the N<sub>org</sub> content was almost unchanged in the whole composting period, indicating that only N<sub>ino</sub> was involved in the nitrogen transformation process. A quick change of concentration was seen in the first day for each component. The fecal NH<sub>4</sub>-N which was the main component of the N<sub>ino</sub> (>90%) underwent a sudden decrease in the first day. The initial NO<sub>2</sub>-N, though its content was very low, almost dropped to nil after the first day while NO<sub>3</sub>-N increased by about the same amount from nil. In the following days, the NH<sub>4</sub>-N content gradually decreased and finally dropped to nil while NO<sub>3</sub>-N almost kept a constant value. The total nitrogen loss was 0.93 g or 17% of the initial N<sub>tot</sub>. Almost all the nitrogen loss was from the decrease of inorganic nitrogen content because organic nitrogen content was unchanged. As shown in Fig. 2, as the low initial content of NO<sub>2</sub>-N finally depleted and an almost equivalent content of NO<sub>3</sub>-N was finally gained, it could be understood that the fecal nitrogen loss was mainly from the depletion of NH<sub>4</sub>-N.

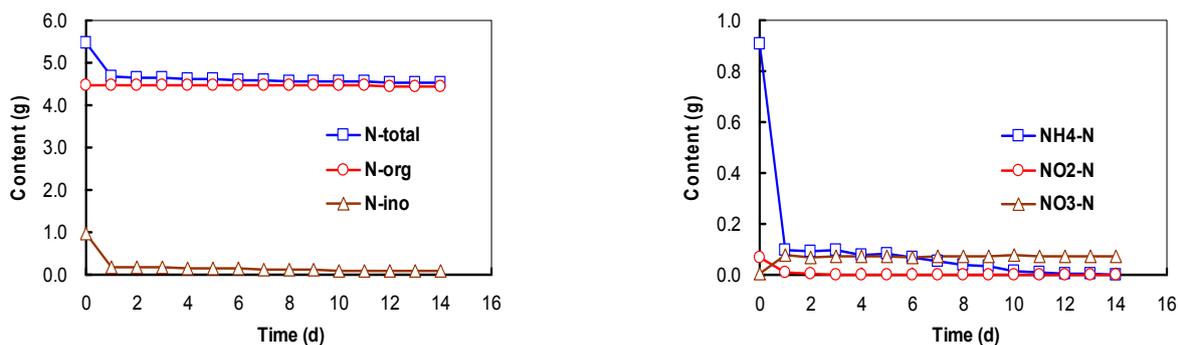
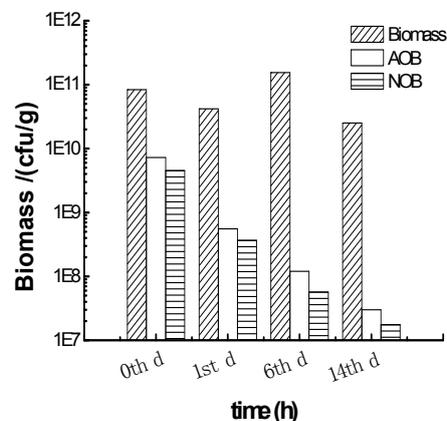
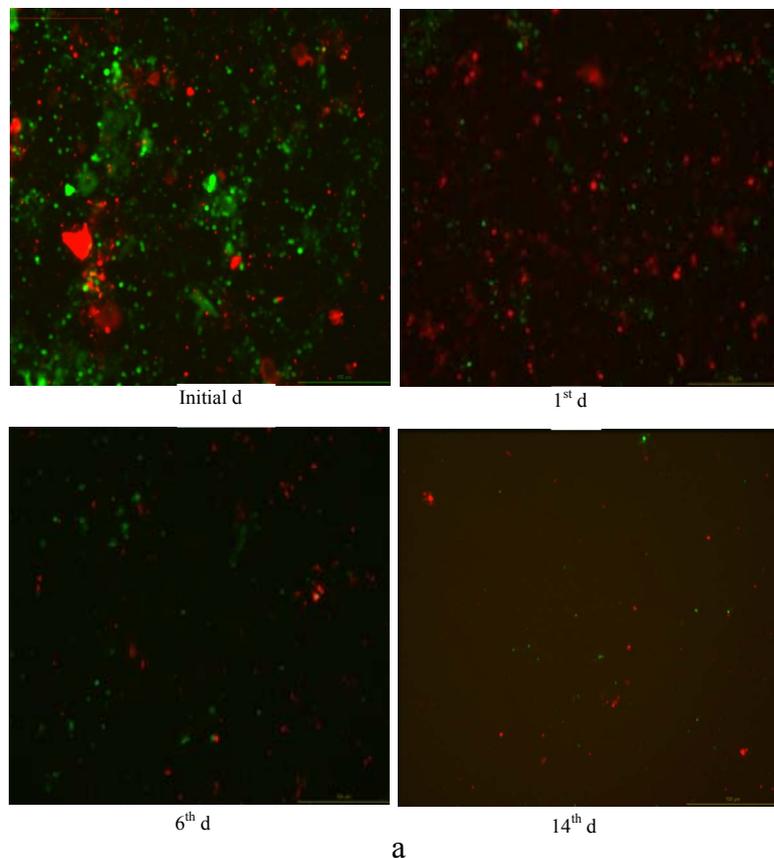


Fig.2 Variation of the components of fecal nitrogen in the composting process

#### 3.2. The biomass and the population structure of compost

Because the organic nitrogen content did not change in the whole composing process, it could be assumed that under the thermophilic condition, the nitrogen cycle related to microorganism activity might have been hindered. From a view point of fertilizer application, such a condition would be favorable for holding the organic nitrogen in the compost. However, at this stage it is still difficult to explain the mechanism of nitrogen holding in the aerobic and thermophilic composting process and further studies are needed from the viewpoints of microorganism activity and nitrogen cycle in the macro and micro environment in the composting reactor. The biomass reached to max at 6<sup>th</sup> day firstly and decreased obviously from 6<sup>th</sup> day to the end of composting during thermophilic composting. AOB and NOB were decreased during composting and

almost missed at the end of composting which was shown as Fig. 3. the biomass of AOB and NOB was decreased quickly during the aerobic and thermophilic composting of feces shown as Fig.4. The study showed that the biodegradation of Norg may have been hindered due to lack of ammonifying bacteria (such as AOB and NOB) activity and good for nitrogen holding during thermophilic composting, thermophilic composting could decrease fecal nitrogen loss, keep high organic nitrogen content in the composts for better fertilizer utilization. Biomass was analyzed by Phospholipid fatty acids (PLFA). The Biomass of compost during composting was shown as Fig. 4.



b  
Fig. 3 change of AOB and NOB during composting of feces  
Note: Green NOB; Red AOB.  
a FISH images of composts during 14-day composting of feces  
b change of biomass of AOB and NOB during composting of feces

#### 4. Concluding remarks

This study investigated the fecal nitrogen transformation process in an aerobic and thermophilic composting reactor using sawdust as bulky matrix. Under a condition of controlled temperature at 60°C and moisture content at 60%, more than 70% fecal organic removal was obtained while merely 17% fecal nitrogen was lost. The nitrogen loss occurred mainly in the first day with quick depletion of inorganic nitrogen. As a result of mass balance between the exhausted NH<sub>3</sub> gas and the fecal NH<sub>4</sub>-N content, it was understood that the conversion of ammonium into gaseous ammonia was the main reason for the nitrogen loss.

#### 5. Acknowledgement

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