Journal of Water Sustainability, Volume 6, Issue 4, December 2016, 113-124

The Evaluation of Small Scale Composting of Human Feces for Household Application

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ABSTRACT

In this study, in order to evaluate the efficiency of small scale composting of human feces based on the household application, 14-day batch experiments were conducted under ambient temperatures with sawdust as bulky matrix. The results showed that microbial communities changed significantly with the biomass decreasing in the first day, probably due to the die out of the pathogens existed in human feces. Meanwhile, the non-soluble organic matters was hydrolyzed to soluble fatty acids and resulted in the sharply decline of pH. However, with the adaption of microorganism, biomass increased fast in the 2nd day, and SCOD was degraded effectively accompanied with pH rising above 8.5. After available SCOD was depleted, temperature of compost decreased to ambient temperature, biomass and microbial communities tend to be stable. The total nitrogen loss during composting process was 15% and mainly resulted by ammonia gas emission, however the total phosphorous could be retained in compost completely. Although there was no thermophilic phase could be observed during composting process, the typical pathogens of feces die out in the first few days, meanwhile the seed germination index reached 93% at the end of composting, indicating the compost was manure. In addition, the *Pseudomonas putida* and *Pseudomonas panipatensis* showed strong signal in manure compost, both of them could function as soil inoculant and be exploited for soil bioremediation. Therefore, this small scale aerobic composting could achieve effective removal of organic matter and nutrient retention, the safe and stable final product could be used as fertilizer and soil conditioner. It would benefit the rural area where the feces could be treated in household scale and utilized for agriculture in-situ.

Keywords: Aerobic composting; human feces; nutrient conversion; bacterial community

1. INTRODUCTION

Each year, one person produces 50 kg of feces, which contain approximately 5.7 kg of nitrogen, 0.6 kg of phosphorus and 1.2 kg of potassium (Wolgast and vatten, 1993). However, from the hygienic point of view, human feces should not be used as fertilizer or soil conditioner before it was sanitized due to the presence of possible pathogens. Compared with other technologies, aerobic composting which is widely used in bio-toilet system has been recognized as an applicable method for sanitary disposal of human feces in rural areas, and areas with water shortages (Cordova and Knuth, 2005; Tønner-Klank et al., 2007; Zavala et al., 2005). There are three main reasons: first, many rural areas do not have drainage pipelines and sewage treatment

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DOI: 10.11912/jws.2016.6.4.113-124

systems, decentralized feces treatment systems are more appropriate (Gao et al., 2015; Wang and Zhou, 2008); second, composting toilets require little to no water and can therefore disconnect the toilet from both the water supply and wastewater infrastructure (Vickers, 2001); third, organic waste could be converted into substances of safe and stabilized states during composting process, the final products can also be used as a fertilizer and applied in-situ without contamination and avoid transportation (Anand and Apul, 2014; Guo et al., 2012; Zorpas and Loizidou, 2008).

For household composting, the volume of compost could not be too large as of centralized composting. Björklund (2002) pointed out that if the volume of compost is small and heat loss is fast, the peak temperature is normally 10-15˚C higher than the ambient temperature, the compost probably keep mesophilic condition through composting process without obvious thermophilic condition. Although pathogens die out under thermophilic condition more effectively than under mesophilic condition. Many studies pointed out that temperatures between 35˚C and 40˚C maximized microbial diversity and are optimum for degradation of organic in the composting process (Stentiford, 1996). Saludes et al. (2007) also reported that composting at 37˚C exhibits high microbial activity due to degradation of complex organic compounds. Therefore, it was meaningful to investigate the small scale composting for household application, although the temperature was maintained in mesophilic range.

In order to know the complex interactions of microorganisms involved in aerobic composting, the molecular biological methods such as PCR-DGGE could be used to obtain the useful information of the variation of microbial communities during aerobic composting reactors (Maeda et al., 2010; Tang et al., 2007). The dominant species of microorganisms have been found to be highly dependent on the composting process (Takaku et al., 2006). Therefore, it is necessary to investigate the characteristic of microbial communities and the relationship between microorganism and environmental change during composting process.

In this study, in order to evaluate the efficiency of small scale composting based on the household application, batch experiments were conducted under ambient temperature for the disposal of human feces with sawdust as bulky matrix. Organic degradation, nutrient retention and compost maturity were investigated under the assistance of PCR-DGGE and microbial sequencing.

2. METHODS AND MATERIALS

2.1 Feed stocks

The human feces used in this study were collected from the university campus, and stored at -20˚C for later use. Sawdust used as bulky matrix was collected from a local timber processing plant. Due to its high porosity, the sawdust was considered as an optimal bulky, it could provide sufficient void space for water retention and drainage, and air ventilation, thus providing a proper aerobic environment for microbial growth in the composts (Zavala et al., 2004). In addition, the sawdust was almost non-biodegradable at a normal temperature and could keep a stable state after being put into operation in a composting reactor for several months (Bai and Wang, 2010). The Physicochemical properties of the feces and sawdust were showed in Table 1.

2.2 Experiment procedure

In this study, human feces and sawdust were mixed with a dry weight ratio of 1:4 which was found to be a proper composition for maintaining the aerobic composting condition (Bai and Wang, 2010). In order to evaluate the

performance of small scale composting, the total dry weight of the mixture was 3 kg. After adjusting the moisture content to 60%, the mixture was filled into a composting reactor made of steel with an inner diameter of 30 cm and length of 45 cm. In order to keeping the composting mixture in a homogenous condition, intermittent mixing was provided at a frequency once per 4 hour with 1 min forward agitation followed by 1 min reverse agitation. The generated ammonia gas emitted from compost and was captured by the sulfuric acid solution for quantification by chemical analysis. Before final emission, the exhaust air went through the sodium hydroxide solution for the absorption of carbon dioxide.

Composting process was conducted in early summer, the average ambient temperature was around 20-28˚C. For each batch experimental run, the duration time of composting was 14 days, since many researchers pointed out that 10-12 days were found to be a time frame for organic and inorganic substances in the composts to reach a stable concentration (Bai and Wang, 2010; Zavala et al., 2005). About 30 g of compost samples (equivalent to a dry weight about 15 g if 50% moisture content was accounted) were collected daily from the reactor after well mixing and stored at -20˚C until analysis. For evaluating the quantity of the exhausted ammonia gas, liquid samples were collected from the sulfuric acid solution (0.5 mol/L, 6 L) in minutes, hours, or daily according to the rate of NH_3 gas generation.

2.3 Chemical analysis

The temperature of compost was detected by a programmable logic controller (PLC) with three thermo sensors set at different locations inside the reactor. The moisture content of a compost sample was determined by measuring the moist loss after drying in an oven at 105˚C for 24 h. The seed germination index (GI) was analyzed according to Zucconi et al. (1981). Taking COD as an indicator of the fecal organic content, it was measured using a modified closed reflux, colorimetric method by directly digesting the compost sample (Zavala et al., 2005). Total nitrogen and total Kjeldahl nitrogen (KN) of the compost sample were also directly analyzed by a modified alkaline potassium persulphate digestion - spectrophotometric method and Kjeldahl method (APHA, AWWA and WEF, 1995). For evaluating the soluble components in the compost sample, 5 g of compost samples was stirred vigorously in 50 mL distilled water for 30 min and after removing the suspended substances by a 0.45 μm filter, the soluble COD, ammonium (NH_4-N), nitrites (NO_2-N), and nitrates (NO_3-N) were analyzed by standard methods (APHA, AWWA and WEF, 1995). The pH of the solution was taken as a measure of the acidity and / or alkalinity state in the composting reactor. The concentration of the ammonia gas absorbed by the sulfuric acid solution was measured by colorimetric method. The biomass was analyzed by phosphatide method according to Yu et al. (2002). All the analysis results were finally expressed as g/kg on dry weight basis and the quantity attributed to fecal source was calculated by subtracting that from the sawdust from the total value.

Table 1 Physicochemical properties of the human feces used in this study

Item	Moisture Content (%)	Total COD (g/kg)	Soluble COD (g/kg)	N_{total} (g/kg)	$N_{organic}$ (g/kg)	$N_{inorganic}$ (g/kg)
Feces	82.3	1473.4	415.6	56.28	47.22	9.06
Sawdust	12.1	1165.8	29.4	1.50	1.35	0.15

2.4 Microbial community

2.4.1 DNA extraction

Total DNA was extracted from 1 g samples using a commercial DNA extraction kit (Biomiga, Soil gDNA kit) following the manufacturer's instruction after the sample was pretreated by PBS. The extracted DNA sample was stored at -20˚C until further use.

2.4.2 PCR-DGGE

Microbial community analysis was performed using the PCR-DGGE method targeting the 16S rRNA gene. The primer set used in this study was 338F and 518R (A GC clamp was attached to the 5' end of the forward primer for DGGE-PCR). The PCR amplification was performed using 25 μL PCR reaction mixtures consisted of 2.5 μL of 1×buffer, 2 μL of dNTP, 0.4 μM of each primer, 2 μL of template DNA, and 0.25 μL of Taq polymerase (Takara, Code DR001A). Touch-down PCR protocol was as follows: initial denaturation at 94˚C for 5 minutes; 20 cycles of denaturation at 94˚C for 30 s, annealing at 65˚C (the annealing temperature reduced 1˚C every 2 cycles) for 30 s, and extension at 72˚C for 45 s; 15 cycles of denaturation at 94˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 45 s; the final extension at 72˚C for 7 min; and cooling at 4˚C.

DGGE was performed on the D code Universal Mutation Detection system. PCR products were run on polyacrylamide gels (8% w/v) containing a linear formamide/urea gradient from 40% to 60%. The gels were run for 14 h at constant voltage of 70 V and 60˚C in 1×TAE buffer, then stained with gel red for 30 min, and photographed using a UV transilluminator.

The selected DGGE bands were excised using sterilized cutter blades and washed twice using sterile water, then incubated with 50 μL sterile water at 4˚C for 24 h. The eluted DNA from excised DGGE bands were re-amplified with the primer set without the GC clamp. The PCR products were purified and cloned using pMD19-T vector system, and then sequenced by the commercial biological industry. These sequences were identified by comparing with the reference database in GenBank using the BLAST program. Redundancy analysis (RDA) was used to test the influence of the environmental factors on bacterial community.

3. RESULTS AND DISCUSSION

3.1 Evaluation of organic removal efficiency and nutrient recovery during aerobic composting

3.1.1 Characteristics of organic degradation

For household composting, it is difficult to achieve a thermophilic temperature during composting process due to the small scale and fast heat loss (Björklund, 2002). As shown in Fig. 1a, the change of temperature during aerobic composting could be divided into 3 stages. In the first 2 days, the temperature increased to the peak of 38.1˚C which was 10˚C higher than initial temperature. In this stage, the soluble COD increased from 410.7 g/kg to 431.2 g/kg (Fig. 1b), indicating some non-soluble fraction of feces was hydrolyzed to soluble fraction. Meanwhile, pH decreased from 6.47 to 5.44 as illustrated in Fig. 1c, probably due to the formation of fatty acids (Sundberg et al., 2004). However, with the utilization of soluble COD by microorganism and the decomposition of nitrogen, pH increased beyond 8 in the 2nd day. Then the temperature fast decreased to ambient temperature in the next 4 days, accompanied with the decrease of soluble COD. Subsequently, the temperature was in stable state, the soluble COD and pH also maintained around 100 g/kg and 8.7, respectively.

Compared with other studies, although the

maximum temperature of the compost could not reach thermophilic condition, the trend the temperature change was similar with traditional composting process. In this study, soluble COD was used to indicate the degradation of organic matter, since only soluble matter could be effectively utilized by microorganisms. It was clear that mesophilic condition could also achieve a significant hydrolysis in which organic matter could be converted into soluble and biodegradable organic molecule, follow by effective biodegradation. It was probably resulted by the high microbial diversity and activity for organic degradation during composting process (Saludes et al., 2007; Stentiford, 1996).

3.1.2 Evaluation of nutrient retention and compost maturity

In composting process, the retention of fecal nutrients such as nitrogen and phosphorous were considered as the evaluation of fertilizer efficiency. In this study, the concentration of nitrate and nitrite was very low throughout the composting process, therefore, they were not illustrated in Fig. 2a. The concentration of total nitrogen declined almost linearly in the first 7 days, and tended to be stable subsequently. The nitrogen loss was only 15%. In the first 2 days, a slight increase of ammonium could be observed, resulted by the ammonification of organic nitrogen. After the pH increased beyond 7, ammonia gas emitted fast with ammonium decrease, since the emission of ammonia gas could be considered as a conversion of ammonium into gaseous ammonia under alkaline condition. In aerobic composting, the main nitrogen loss was caused by the ammonification of organic nitrogen and the emission of ammonia gas. Due to the lower temperature compared with other studies, the nitrogen loss was not so significant, which was beneficial for nitrogen retaining.

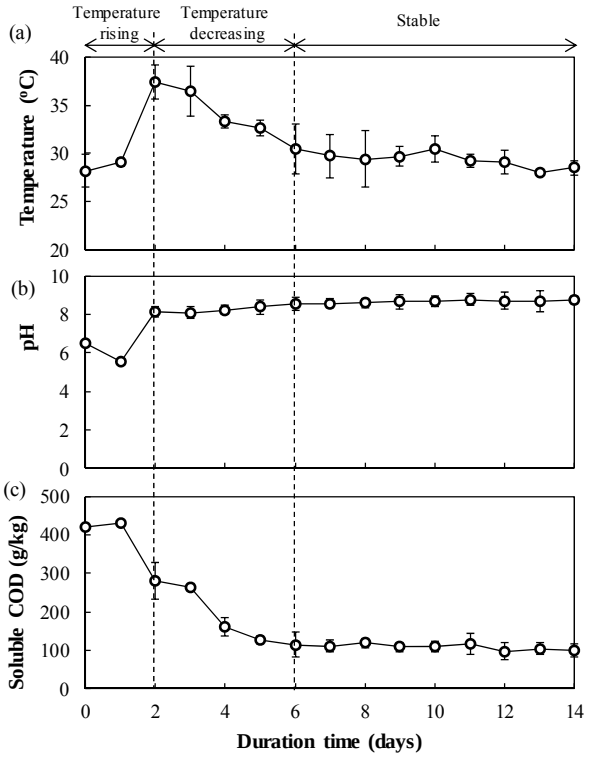

Figure 1 Variation of (a) temperature, (b) pH and (c) soluble COD during aerobic composting

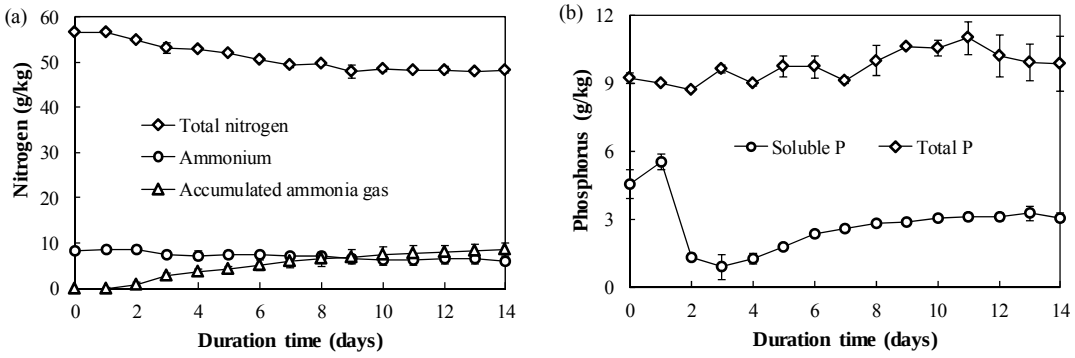

Figure 2 The variation of (a) nitrogen and (b) phosphorous during aerobic composting

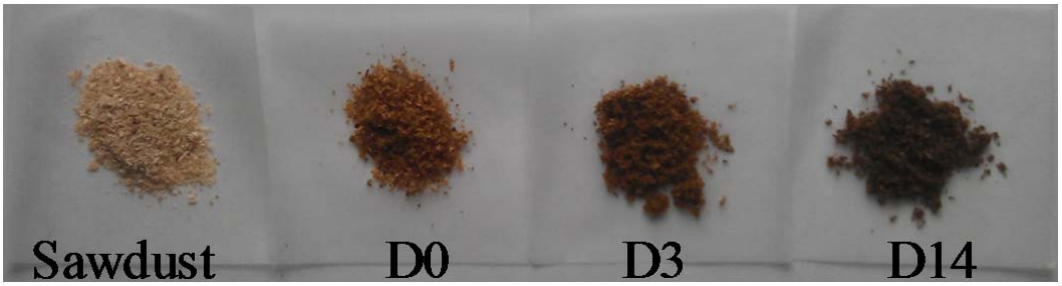

Figure 3 The change in color during aerobic composting

In Fig. 2b, the soluble phosphorous decreased from 5.51 g/kg to 1.32 g/kg rapidly in the 2nd day accompanied with the pH increasing. The main reason should be the formation of precipitate such as struvite under alkaline condition. However, the total phosphorous was almost stable during the aerobic composting, indicating it could be retained in compost completely.

For final composting product, maturity of compost should be evaluated. The color as an indicator of maturity of compost was shown in Fig. 3. The original mixture was yellow, while the color became yellowish brown after 3 days composting. At the end of composting process, the compost was dark brown which could was an indicator for manure compost. Meanwhile, the GI was also analyzed in order to evaluate the maturity of compost and toxicity to plants. In the 1st day, the germination index was lower than 10%, but it increased beyond 80% in the middle of composting process, and reached 93% at the end of composting. Yang et al. (2016) reported that if a GI value of compost is greater than 80%, it is considered safe for applications on land and indicates maturity of the compost.

Therefore, for this small scale composting based on the household application, the final manure compost could be used as fertilizer which contained a lot of nutrient.

3.2 Effect of organisms on composting process

3.2.1 Biomass variation

Aerobic compost is a biological treatment, microorganism play an important role in organic degradation. In this study, biomass was used to indicate the quantity of total microorganism. As illustrated in Fig. 4, the variation of biomass was in accordance with the change of temperature and COD concentration. The microorganism of the initial mixture was mainly introduced by human feces, the significant change of environment and the aerobic

condition prevented their survival in the composting process. Therefore, in the 1st day, the biomass declined slightly. Then, the biomass increased fast due to the growth of microorganisms which have adapted the composting condition. Meanwhile, the temperature rose to the maximum and the COD was degraded fast. With the available carbon resource being exhausted, the biomass declined accompanied with the decrease of temperature. From the 6th day, the biomass tended to be stable, meanwhile, the soluble COD was maintained at 100 g/kg, indicating there was no available carbon source could be used for microorganism growth, therefore, aerobic composting stepped into manure stage.

3.2.2 Microbial community

Detailed information of microbial communities could help to understand the microbial action for organic degradation during aerobic composting. Fig. 5 showed the DGGE bands pattern of 16S rRNA gene fragments amplified with the primer set 338F-518R. The DGGE bands marked by small letters represented the bacteria in initial mixture, while those marked by Arabic numerals represented the bacteria which appeared as the composting processes proceeded. The cluster analysis revealed that the microbial communities in the initial mixture and the 1st day were quite different. From the 2nd day, the microbial communities changed gradually and tended to be stable over time, this phenomenon was in accordance with the organic degradation and biomass variation. Meanwhile, the microbial diversity was also increased, since the mesophilic condition was favorable for microbial growth.

In order to know the relationships between environmental factors and microbial communities, redundancy analysis (RDA) was conducted using the sets of daily collected data. As shown in Fig. 6, the length of the arrows indicated the variance caused by the environmental factors in the composting reactors such as soluble COD, various forms of nitrogen, pH and moisture content, and the direction of the arrows indicated an increasing weighting of these factors. It was noticed that at bacterial communities underwent a quick change in the first 3 days, slight change in the following days, and tended to be stable after Day 7, that was in accordance with cluster analysis. The microbial community was strongly affected by environmental factors such as temperature, nitrogen content and COD, however, the influence from moisture content seemed to be less significant.

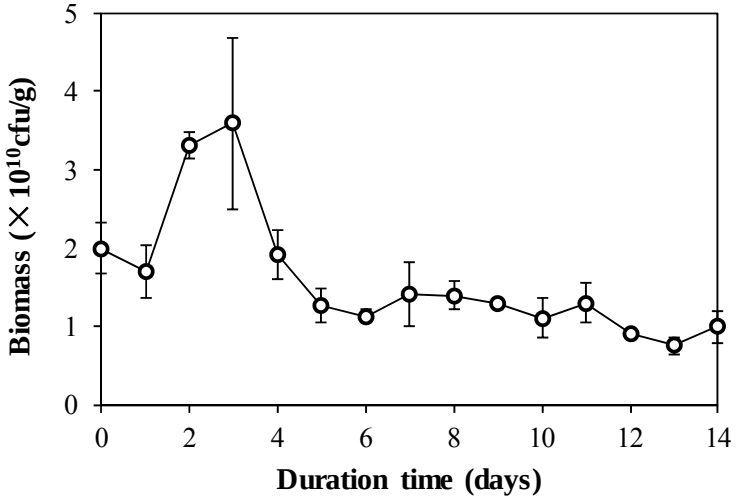

Figure 4 The change of biomass during aerobic composting

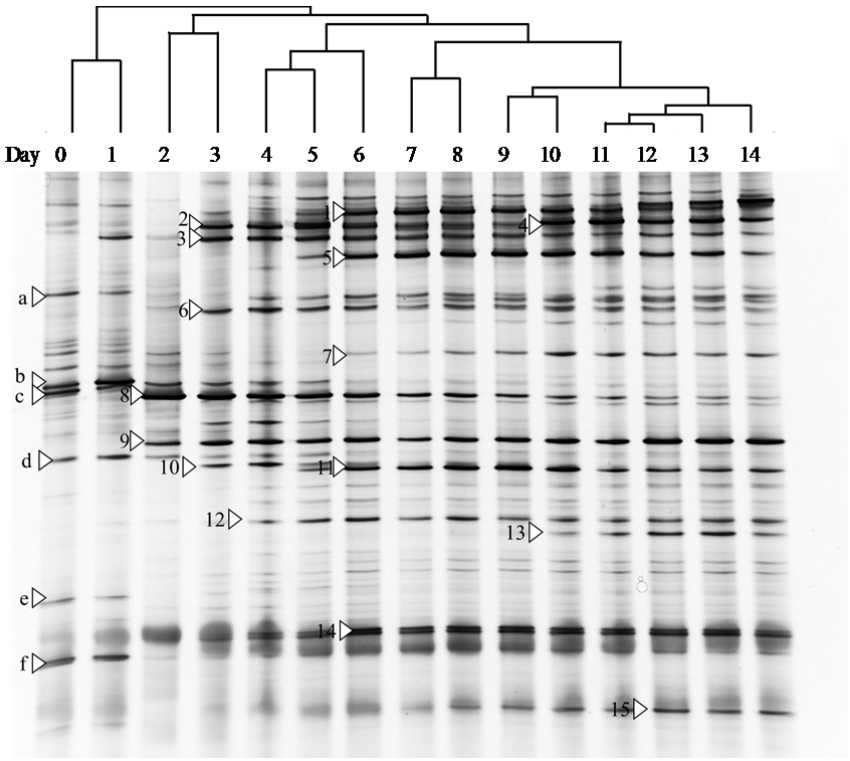

Figure 5 DGGE profile of 16S rRNA gene fragments with cluster analysis

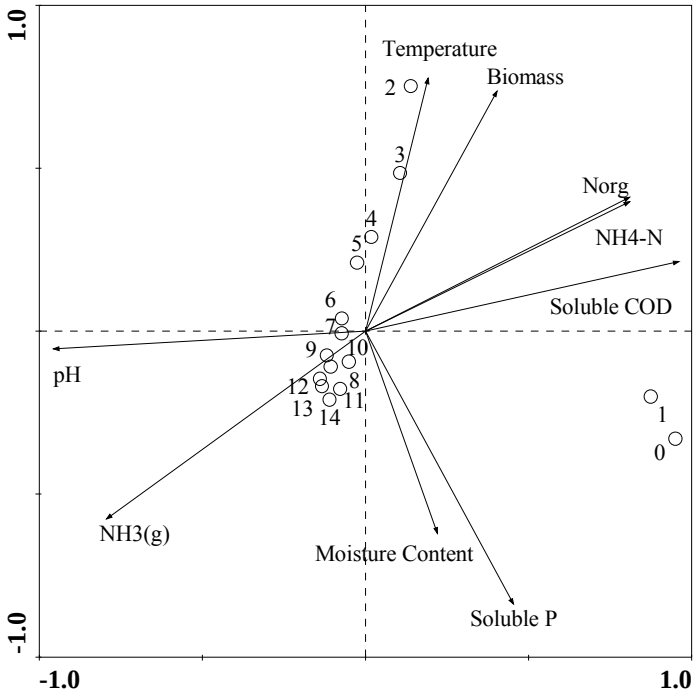

Figure 6 Redundancy analysis (RDA) of the data sets of bacterial communities and environmental factors for the cases of aerobic composting. Empty circles indicate the bacterial communities; numbers beside the circles indicate the day of sampling

Table 2 Sequence analysis of bands excised from DGGE gels derived from bacteria 16S rRNA extracted from compost samples

Band position	Closest sequence available in database	Similarity
a	*Enterococus faecalis*	100%
b	*Escherichia fergusonii*	100%
c	*Pseudomonas hussaini*	97%
d	*Pediococcus lolii*	100%
e	*Turicibacter sanguinis*	98%
f	*Pediococcus lolii*	100%
1	*Flavobacterium frigoris*	97%
2	Uncultured *bacterium* (municipal waste composting)	100%
3	*Flavobacterium anatoliense*	97%
4	*Pseudomonas formosensis*	100%
5	*Moheibacter sediminis*	95%
6	*Pseudomonas putida*	96%
7	*Pseudomonas* sp.	98%
8	*Acinetobacter variabilis*	99%
9	Uncultured *bacterium*	97%
10	Uncultured compost *bacterium* (municipal waste composting)	100%
11	*Pseudoxanthomonas suwonensis*	99%
12	*Pseudomonas panipatensis*	100%
13	*Erythrobacter luteus*	100%
14	Uncultured *bacterium*	100%
15	Uncultured *bacterium* (cow manure composting)	100%

The sequence information for the bands excised from DGGE gels was shown in Table 2. The dominant species identified from the initial mixture included the typical fecal pathogens such as *Escherichia* and *Enterococcus*. In addition, *Turicibacter sanguinis* is also an anaerobic bacterium was isolated from a blood culture of a febrile patient (Bosshard et al., 2002). Although the temperature did not reach the thermophilic range during composting, many of the pathogens eventually died out in the first 2 days with the increase of temperature, probably due to lack of an anaerobic condition for their survival. Band c exhibited strong signal till the day 5 were identified to belong to *Pseudomonas* sp. which could break down organic substances (Bastian et al., 2009; Zhang et al., 2012). Their disappearance almost coincided with the time when soluble COD tended to be unchanged as shown in Fig. 1c. The band d and f was closed to *Pediococcus lolii* which could produce lactic acid from glucose (Doi et al., 2009). The existence of these two bands in the first two days may be one reason for pH decrease.

During composting process, a lot of bands appeared, most of them survived until the end of composting. Band 6 and 12 affiliated to *Pseudomonas putida* and *Pseudomonas panipatensis*, respectively, both of them could function as a soil inoculant and exploited for soil bioremediation (Gomes et al., 2005; Gupta et al., 2008). *P. putida* and *P. panipatensis* appeared from day 3 and day 5, respectively, and showed strong signal until the end of composting, therefore the final composting product may be valuable to use as soil

conditioner. Band 1 and 3 belonged to *Flavobacterium*, many species of the genus of *Flavobacterium* are able to hydrolyse organic polymers such as complex polysaccharides and probably play an important role in the uptake and degradation of organic matter (Bernardet et al., 1996). Band 11 affiliated to *Pseudoxanthomonas suwonensis* which is isolated from cotton waste composts and the optimal temperature is 30˚C (Weon et al., 2006). It appeared when the temperature of compost declined to 33˚C in this study. Band 2, 9, 10, 14 and 15 was identified as uncultured *bacterium*, most of them were isolated from municipal solid waste and cow manure composting process.

During this small scale composting process, the bacterium dominated in human feces almost disappeared completely due to the environmental change. Those appeared bacterium became dominant and play an important role in organic degradation. The high abundance of two typical microbes which was responsible for soil bioremediation indicated the high value of final compost as fertilizer and soil conditioner.

CONCLUSIONS

Although the temperature could not reach thermophilic level during small scale aerobic composting in this study, the organic matters could be degraded effectively under mesophilic condition. The nitrogen lost in form of ammonia gas when the pH increased above 7, while phosphorous was retained in compost completely. The PCR-DGGE analysis indicated the typical pathogens existed in feces died out in the first several day of composting, and typical soil bioremediation bacterium was found during composting process. The germination index reached 93% at the end of composting, indicating the final compost was maturity and safe for applications on land. This household scale composting could help rural area achieve an in-situ collecting-treatment-utilization system of human feces.

ACKNOWLEDGEMENT

This work was supported by Natural Science Foundation for Young Scientists of Xi'an University of Architecture and Technology, China (Grant No. QN1615) and National Natural Science Foundation of China (Grant No. 51608430).

REFERENCES

Anand, C.K. and Apul, D.S. (2014). Composting toilets as a sustainable alternative to urban sanitation–A review. *Waste Management*, 34(2), 329-343.

APHA, AWWA and WEF (1995). *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, USA.

Bai, F. and Wang, X.C. (2010). Nitrogen-retaining property of compost in an aerobic thermophilic composting reactor for the sanitary disposal of human feces. *Frontiers of Environmental Science and Engineering in China*, 4(2), 228-234.

Bastian, F., Bouziri, L., Nicolardot, B. and Ranjard, L. (2009). Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biology and Biochemistry*, 41(2), 262-275.

Bernardet, J.F., Segers, P., Vanca, M., Berthe, F., Kersters, K. and Vandamme, P. (1996). Cutting a Gordian Knot: Emended Classification and Description of the Genus Flavobacterium, Emended Description of the Family Flavobacteriaceae, and Proposal of Flavobacterium hydatis nom. nov. (Basonym, Cytophaga aquatilis Strohl and Tait 1978).

International Journal of Systematic Bacteriology, 46(1), 128-148.

Björklund, A. (2002). *The Potential of Using Thermal Composting for Disinfection of Separately Collected Faeces in Cuernavaca, Mexico*. Swedish University of Agricultural Sciences, International Office, Ultuna, SWE.

Bosshard, P.P., Zbinden, R. and Altwegg, M. (2002). Turicibacter sanguinis gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 52(4), 1263-1266.

Cordova, A. and Knuth, B.A. (2005). Barriers and strategies for dry sanitation in large-scale and urban settings. *Urban Water Journal*, 2(4), 245-262.

Doi, K., Nishizaki, Y., Fujino, Y., Ohshima, T., Ohmomo, S. and Ogata, S. (2009). Pediococcus lolii sp. nov., isolated from ryegrass silage. *International Journal of Systematic and Evolutionary Microbiology*, 59(5), 1007-1010.

Gao, H., Zhou, C., Li, F., Han, B. and Li, X. (2015). Economic and Environmental Analysis of Five Chinese Rural Toilet Technologies Based on the Economic Input-Output Life Cycle Assessment. *Journal of Cleaner Production*, 8(8), 91-98.

Gomes, N.C.M., Kosheleva, I.A., Abraham, W.R. and Smalla, K. (2005). Effects of the inoculant strain Pseudomonas putida KT2442 (pNF142) and of naphthalene contamination on the soil bacterial community. *Fems Microbiology Ecology*, 54(1), 21-33.

Guo, R., Li, G., Jiang, T., Schuchardt, F., Chen, T., Zhao, Y. and Shen, Y. (2012). Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresource Technology*, 112 (58), 171-178.

Gupta, S.K., Kumari, R., Prakash, O. and Lal, R. (2008). Pseudomonas panipatensis sp. nov., isolated from an oil-contaminated site. *International Journal of Systematic and Evolutionary Microbiology*, 58(6), 1339-1345.

Maeda, K., Hanajima, D., Morioka, R. and Osada, T. (2010). Characterization and spatial distribution of bacterial communities within passively aerated cattle manure composting piles. *Bioresource Technology*, 101(24), 9631-9637.

Saludes, R.B., Iwabuchi, K., Kayanuma, A. and Shiga, T. (2007). Composting of dairy cattle manure using a thermophilic-mesophilic sequence. *Biosystems Engineering*, 98(2), 198-205.

Stentiford, E.I. (1996). Chapter 6 – Composting Control: Principles and Practice. In:*The Science of Composting*, Epstein, E. (Eds.). CRC Press, Boca Raton, USA.

Sundberg, C., Smårs, S. and Jönsson, H. (2004). Low pH as an inhibiting factor in the transition from mesophilic to thermophilic phase in composting. *Bioresource Technology*, 95(2), 145-150.

Takaku, H., Kodaira, S., Kimoto, A., Nashimoto, M. and Takagi, M. (2006). Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and -independent approaches. *Journal of Bioscience and Bioengineering*, 101(1), 42-50.

Tang, J.C., Shibata, A., Zhou, Q.X. and Katayama, A. (2007). Effect of Temperature on Reaction Rate and Microbial Community in Composting of Cattle Manure with Rice Straw. *Journal of Bioscience and Bioengineering*, 104(4), 321-328.

Tønner-Klank, L., Møller, J., Forslund, A. and Dalsgaard, A. (2007). Microbiological assessments of compost toilets: in situ measurements and laboratory studies on the survival of fecal microbial indicators using sentinel chambers. *Waste Management*, 27(9), 1144-1154.

Vickers, A. (2001). *Handbook of water use and conservation*. American Water Works Association, Denver, USA.

Wang, R.S. and Zhou, C.B. (2008). Ecological sanitation development in China: Potential, challenges and strategies. *Chinese Journal of Ecology*, 27(7), 1200-1206.

Weon, H.Y., Kim, B.Y., Kim, J.S., Lee, S.Y., Cho, Y.H., Go, S.J., Hong, S.B., Im, W.T. and Kwon, S.W. (2006). Pseudoxanthomonas suwonensis sp. nov., isolated from cotton waste composts. *International Journal of Systematic and Evolutionary Microbiology*, 56(3), 659-662.

Wolgast, M. vatten, R. (1993). *Om tankar i kretslopp*. Crenom HB, Uppsala. (in. Swedish).

Yang, L., Zhang, S., Chen, Z., Wen, Q. and Wang, Y. (2016). Maturity and security assessment of pilot-scale aerobic co-composting of penicillin fermentation dregs (PFDs) with sewage sludge. *Bioresource Technology*, 204, 185-191.

Yu, X., Zhang, X.J. and Wang, Z.S. (2002). phosphatide method of biomass determination during drink water treatment process. *Water and Wastewater Engineering,* (5), 1-5 (In Chinese).

Zavala, M.A.L., Funamizu, N. and Takakuwa, T. (2005). Biological activity in the composting reactor of the bio-toilet system. *Bioresource Technology*, 96(7), 805-812.

Zavala, M.A.L., Funamizu, N. and Takakuwa, T. (2004). Temperature effect on aerobic biodegradation of feces using sawdust as a matrix. *Water Research*, 38(9), 2406-2416.

Zhang, D.C., Mörtelmaier, C. and Margesin, R. (2012). Characterization of the bacterial archaeal diversity in hydrocarbon-contaminated soil. *Science of The Total Environment*, 421-422, 184-196.

Zorpas, A.A. and Loizidou, M. (2008). Sawdust and natural zeolite as a bulking agent for improving quality of a composting product from anaerobically stabilized sewage sludge. *Bioresource Technology*, 99(16), 7545-7552.

Zucconi, F., Pera, A., Forte, M. and Bertoldi, M.D. (1981). Evaluating Toxicity of Immature Compost. *Biocycle*, 22(2), 54-57.