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Phospholipids fatty acids analysis of microbial communities in sewage sludge composting with inorganic bulking agent

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ABSTRACT

Phospholipids fatty acids (PLFAs) in sewage sludge were analyzed to evaluate the variation in the microbial communities during 100 d composting. Pumice was used as an inorganic bulking agent to eliminate the influences of conventional organic materials on the PLFAs analysis result of the sludge. The results showed that the maximal PLFAs content of 407.41 μg g^{-1} VS was presented on Day-10 and the minimal PLFAs content of 207.49 μg g^{-1} VS was detected on Day-100. Bacteria and fungi were mainly the micro-organisms in the thermophilic stage. The number of bacteria distinctly reduced during the curing stage, whereas the abundances of gram-positive bacteria (14:0 iso, 16:0 iso, 17:0 anteiso), pseudomona (18:1 ω7c), actinobacteria (18:0 10-methyl), and fungi (18:3 ω6c (6, 9, 12)) tended to increase simultaneously. Protozoa were detected in the raw sludge and the matured sample, but not presented within the theromophilic period. Culture-dependent method was introduced to verify the results of microbial populations. The numbers of bacteria and fungi reached maximum (3.38×10^{12} and 3.31×10^{9} CFU g^{-1} d s, respectively) on Day-10 and then reduced. The number of actinomycetes increased gradually and reached 3.24×10^{7} CFU g^{-1} d s, on Day-100. The results indicated that PLFAs technique is an efficient and convenient method to investigate the variation in the microbial communities during sewage sludge composting.

Keywords: Sewage sludge; Compost; Phospholipids fatty acids; Microbial community; Inorganic bulking agent

1. Introduction

Sewage sludge is the main by-product produced in wastewater treatment process. A large number of hazardous substances are transferred to the sludge, causing the sludge to contain large amounts of organic contaminants, pathogens, parasites, and heavy metals. Without appropriate treatment, sewage sludge will threaten the urban environment and human health. Composting is an effective way to realize sludge land application as the fertilizer and organic amendment [1]. The composting process is a thermophilic biotreatment driven by dynamic changes in microbial community [2,3]. Successful composting process could provide the microbial community with its basic needs:

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moisture, oxygen, and temperature [4]. The change in sludge properties is closely related to the microbial community. Therefore, a better understanding of microbial community dynamics is important to improve the composting process and evaluate the production quality.

A variety of methods have been used so far to investigate the change in microbial community during composting. Traditional microbiological methods based on bacterial culture techniques were applied in early studies, but these methods had the complex operation and long test period [5]. Due to the complex composition of sewage sludge, the ecological factors such as temperature, moisture, pH, substrate characteristics, and mass transfer conditions changed significantly during the composting process. The morphology, quantity, and activity of the microbial communities in the sludge were consequently affected by these factors [6]. Because of isolation, culture, identification, and many other difficulties, the traditional microbiological methods are difficult to provide a systematic description of the complex ecosystem. Therefore, more direct and rapid methods are required to valuate microbial community in the composting process.

Growth-independent modern biochemistry and molecular techniques are developed to meet the above requirements. In the field of molecular biology, four main methods are used to analyze the succession progress of microbial populations: denaturing gradient gel electrophoresis (DGGE) [7,8] and single-strand conformation polymorphism [9,10] based on polymerase chain reaction (PCR) amplification and electrophoretic separation; microarray technologies based on molecular hybridization [11]; fluorescence *in situ* hybridization (FISH) based on fluorescence probe [12,13]; phospholipids fatty acids (PLFAs) based on the databases of microbial characteristic fatty acids and the gas chromatographic separation technology [14,15]. Limited by specific primers, PCR-DGGE cannot detect fungi and bacteria simultaneously, and the quantitative effect is poor [16]. Although the microarray technology has higher throughput, the biochip is expensive. Moreover, it is difficult to find a suitable biochip for sludge composting ecosystem. The FISH method cannot conduct comprehensive analysis for composting systems due to the limitation of the probe types.

The PLFAs technique has the prominent performance on simple operation, quantitative effects, and effectiveness in getting a large amount of biological information at the same time [17]. Because some specific fatty acids are only present in certain species of microbial cells, the variation in microbial communities could be studied by measuring the contents of various fatty acids during this process. Although PLFAs analysis has been widely applied in the field of soil microbial system, few literatures reported the application of the PLFAs technique on the research of the sludge composting. In addition, organic bulking agents are also biodegraded during the composting treatment. Because of the huge difference in the substrate properties, the microbial community structures are quite different between the sludge particles and organic bulking agent. Moreover, many species of organic bulking agents with the different biodegradation properties have been used in the previous composting studies. Therefore, adding organic bulking agent greatly disturbed our understanding on the variation in the microbial community in the sludge particle.

The purpose of this study is to investigate the variation in microbial community through the analysis of the type and content of fatty acids. In this study, an efficient and convenient method, the PLFAs technique, was adopted. Pumice was introduced as an inorganic bulking agent. The interference on the determination of microbial population from other organic matters could be avoided when organic bulking agents, such as sawdust and straw, were used. The change in the influence factors, including temperature, moisture, pH, volatile solid (VS), and dissolved organic carbon (DOC) were simultaneously monitored. According to the result of PLFAs analysis, this work provided a better understanding of the relationship between the microbial community and the ecological factors in the sludge composting system.

2. Materials and methods

2.1. Feedstock composition and composting process

The dewatered sludge used in this study consisted of primary and secondary sludge collected from a municipal wastewater treatment plant (Harbin, China). The initial characteristics of sewage sludge are shown in the Table 1. Pumice was introduced to this study as the inorganic bulking agent, which had the high porosity (71.8–81%) and low volume weight (0.34–0.49 g cm^{-3}), and high compressive strength (14–38 kg cm^{-2}). 4 kg of sludge and 3.2 kg of pumice were mixed in a 50 L reactor and then composted for 100 d. The aeration rate was controlled at 0.04 m^3 min^{-1} during the first 20 d and reduced to 0.01 m^3 min^{-1} from Day-20 to Day-100. The composting materials in the reactor were turned over each 5 d over the entire period. The chemical components and the physical features of the pumice have been introduced in the previous study [18], and the composting progress was detailed in previous study [19].

Table 1
Initial characteristic parameters of sludge samples

Parameter	Moisture content	Volatile solid	pH	Total C	Total N	C/N
Value	76.9 ± 0.59%	55.49 ± 0.44%	7.08 ± 0.12	36.03 ± 0.36%	2.65 ± 0.28%	13.62 ± 0.48

2.2. Wet chemistry measurements

The sludge collected on Day-0, Day-10, Day-20, and Day-100 of composting represented the samples at the beginning, middle, the end of the active phase and the end of the curing phase, respectively. The pumice particles were removed by screening from each sample before the chemical measurements. Thus, the residual matter left in samples was the composted sludge. The moisture content of the sludge sample was determined by the weight loss at 105℃ for 24 h in a drying oven [20]. The VS of the sludge was determined by measuring the loss of dry solid mass on ignition at 550℃ for 5 h [21]. The composting temperature in the reactor was monitored by three thermocouple probes. The aqueous extract from the sludge samples at different composting phases was used to determine DOC and pH value. 5 g of sludge sample and 50 ml of deionized water were mixed and oscillated at 120 r min^{-1} for 24 h, then the aqueous mixture was centrifuged at 10,000 rpm for 10 min and filtrated through a 0.45 μm membrane filter. The composted sludge samples were dissolved in the distilled water with the mass ratio of 1:10 (sludge: water) to determine the pH value. Dresbøll's method was adopted for scanning electron microscopy (SEM) detection [22]. The samples were step-dehydrated in the ethanol solutions of 50, 75, 90, 95, and 100%, then freeze gasification dehydrated, and atomized, sprayed with gold. Specimens were examined in the scanning electron microscope (JSM-5610LV).

The value of moisture content, VS, pH, and DOC content were presented by using mean ± standard deviation. Means were calculated as an average of three parallel samples. The standard deviation of these values was calculated using Excel (Microsoft, 2003).

2.3. PLFAs analysis

Sherlock Microbial Identification System (SMIS) combined with gas chromatograph (GC) was adopted to determine the mass variation in the PLFAs with 9–20 carbon in the sludge during the composting process. The pretreatment method of samples and the standard analytical procedure provided by MIDI Company (USA) were adopted to analyze the types and contents of fatty acids in sludge samples of different periods [23,24]. Four reagents were used in the pretreatment procedure: (i) 45 g NaOH + 150 ml methanol + 150 ml deionized water, (ii) 375 ml 6.00 N HCl + 275 ml methanol, (iii) 200 ml n-hexane + 200 ml MTBE (Methyl tertiary butyl ether), and (iv) 10.8 g NaOH + 900 ml deionized water. Sample preparation processes included the saponification, methylation, extraction, and alkaline wash. Saponification was to dissolve the cell walls and release microbial fatty acids. Methylation was to produce fatty acid methyl esters and then improve its separation efficiency in the gas chromatography. Extraction was to dissolve methyl fatty acid in organic solvents. Alkaline wash is to remove the free fatty acids and residual reagents. Microbial PLFAs in the pretreated samples were quantitatively analyzed by the Agilent 6890 GC and SMIS.

2.4. Dilution plate method

1 g of sludge sample and 50 ml of sterile normal saline (0.9%) were mixed. The mixture was oscillated for 2 h at 4℃. 100 μl of bacterium suspension was uniformly coated on the plate medium. Beef extract peptone [25] (3 g beef extract, 10 g peptone, 18–20 g agar, 1 L water, pH 7.2–7.4), Czapek's medium [26] (3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 1 g KCl, 0.01 g $FeSO_4$, 20 g sucrose, and 20 g agar in 1 L water), and Gause's synthetic medium [27] (20 g soluble starch, 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g NaCl, 0.01 g $FeSO_4$, 10 g agar, and 1,000 ml distilled water) were used to cultivate bacteria, fungi, and actinomycetes, respectively.

3. Results and discussion

3.1. Temperature and VS

The variation in composting temperature could be divided into the fast rising, the slow decline, and the steady stages during the 100 d test (Fig. 1). The temperature rapidly increased from the ambient temperature to a high level above 50℃ during the first 4 d. Sufficient substrate promoted the microbial growth and reproduction. The biochemical heat was produced to increase the system temperature. In turn, the microbial metabolism, substrate biodegradation, and biochemical heat production were accelerated because

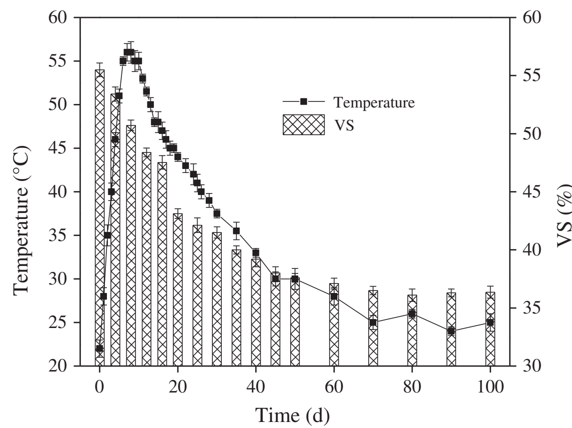

Fig. 1. Variation in temperature and VS of the sludge during composting.

of the increase in the temperature [28]. As the balance between heat production and heat dissipation was built, the temperature reached the top on Day-7. With the decrease in easily degradable compounds in the sludge, the growth of biomass and the activity of micro-organism were limited. As a result, the temperature fell below 45˚C on Day-20.

The VS content of the sludge declined rapidly during the initial period, and meanwhile the decreasing rate of the VS curve reduced with the treatment time. The initial VS content of raw sludge was as high as 55.49%, and afterward decreased to 43.12% on Day-20 and 36.36% on Day-100, respectively. This indicated that a large amount of organic components remained in the sludge after first 20 d of composting, but their biodegradation rate was significantly reduced. Because of the diverse chemical structures of the organic components in the sludge, these components had the substantial differences in biodegradation kinetics.

3.2. Moisture, pH, and DOC

Moisture is an important environmental variable in the composting system. Water provides a medium for the transport of dissolved nutrients required for the microbial metabolism [29]. Many investigations reported that suitable moisture content of 50–60% was proposed for efficient composting [30]. The drying effect generated by the high temperature and mechanical ventilation caused intensive water evaporation in the system. As shown in Table 2, the initial moisture content of the sludge was as high as 76.9%, and afterword gradually decreased to 62.0% on Day-20, which was still in the optimal range for microbes. Based on the mass calculation, approximately 61.8% of water in the raw sludge was released during this period. Therefore, the dramatic change in the moisture occurred in the survival environment of the microbes. Consequent change in pore structure inside the sludge particles would influence oxygen transfer efficiency in the microenvironment. Moreover, water evaporation took away a lot of heat from the system which had the effect of temperature regulation.

The pH value increased from 7.1 to 8.4 over the first 5 d and reached 8.8 on Day-10, then gradually decreased to 7.6 on Day-100. The protein substances in the sludge were rapidly degraded by micro-organisms during the initial period. Due to the low C/N ratio (6–9), the proteins hydrolysis released a large amount of ammonia into the liquid phase, which caused a rapid increase in the pH value during the initial period. Afterward, the pH of the sludge extract slowly declined as the ammonia emission and the reduction of proteins content under the high temperature condition.

The variation in the soluble substances content reflected the substrate condition of the microbes in the sludge during composting process. The DOC content was considered as a useful parameter to evaluate the production maturity in the composting of different materials [31]. The low DOC content in the materials could significantly reduce the growth rate and the activity of the heterotrophic microbes [32]. In this study, the DOC concentration of the raw sludge was as high as 136.3 gC $(\text{kg VS})^{-1}$, which provided adequate substrate for microbial proliferation. However, the DOC decreased sharply to 108.3 gC $(\text{kg VS})^{-1}$ on Day-5 and reached a level of 45.7 gC $(\text{kg VS})^{-1}$ on

Table 2
Change in characteristic parameters of aqueous extract of the sludge samples during the 100 d composting period

Samples	Moisture content (%)	pH	DOC (gC $(\text{kg VS})^{-1}$)
Day-0	76.9 ± 0.59	7.08 ± 0.12	136.3 ± 2.35
Day-5	70.5 ± 0.49	8.4 ± 0.15	108.3 ± 3.22
Day-10	68.8 ± 0.68	8.79 ± 0.18	96.0 ± 1.79
Day-20	62.0 ± 0.45	8.35 ± 0.08	45.7 ± 3.08
Day-100	41.5 ± 0.32	7.98 ± 0.21	32.6 ± 2.15

Day-20 due to the rapid growth of microbial quantity. After that, the DOC content slowly decreased and limited the growth of the heterotrophic microbes. At the end of curing stage, the DOC content of the Day-100-sample reached 32.6 gC $(kg VS)^{-1}$. Wu et al. [33] suggested that 10–17 g kg^{-1} could be recommended as a threshold level indicating maturity. Combined with the VS content (36.36%) on Day-100, the DOC content was 11.85 g kg^{-1}. It indicated that the compost stabilized.

3.3. Total PLFAs content

The total content of the PLFAs in the sludge samples collected at the different phases is shown in Fig. 2. The total amount of the PLFAs increased firstly (from 309.34 μg g^{-1} VS on Day-0 to 407.41 μg g^{-1} VS on Day-10) because of the sufficient substrate and the increasing temperature. Although the high moisture of the raw sludge probably affected the oxygen diffusion in the matrix, the rising PLFAs content showed that the high moisture did not greatly limit the increase of microbial population in this period. The increase of the total PLFAs in the thermophilic phase has been reported in other sewage sludge composting studies [34,35]. With the increase in microbial population and the reduction of organic substrate with incubation time, the number of micro-organisms in unit mass of organic matter reached the maximum on Day-10. Meanwhile, the substrate content became the limiting factor for microbial growth, after that the total amount of the PLFAs in the sludge began to decrease. The total amount of the PLFAs decreased from the maximum (407.41 μg g^{-1} VS) on Day-10 to 207.49 μg g^{-1} VS on Day-100.

The SEM micrographs of the sludge samples showed that a large amount of bacteria existed and the bacillus had an absolute advantage (Fig. 3(a)). The number of micro-organisms in unit area increased obviously in the SEM micrograph during the thermophilic phase (Fig. 3(b)). At the end of the active phase, a large amount of fungi and actinomycetes were found in the micrograph, meanwhile the number of bacteria in unit area decreased (Fig. 3(c)). In addition, the effect of the moisture on the amount of micro-organisms was significant. At the end of curing stage, the amount of micro-organisms significantly reduced in the surface of sludge particle, and the change in the microbial communities arrived at a steady state (Fig. 3(d)).

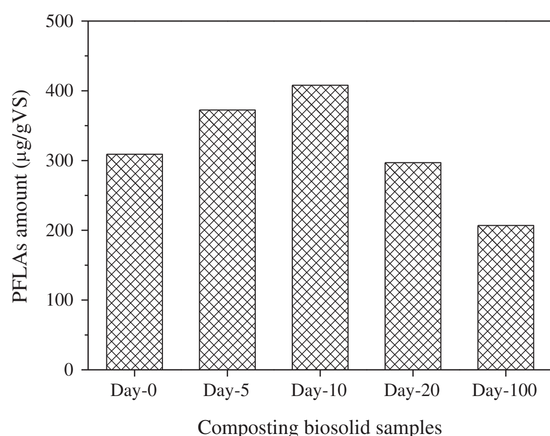

Fig. 2. Relative total content of PLFAs in the sludge samples of different composting phases.

3.4. Specific PLFA

The various PLFAs could be divided into three cases according to the change in trend of contents (Table 3). First, the PLFAs content continued to increase, including 18:1 ω9c, 18:3 ω6c (6, 9, 12), 14:0 iso, 15:0 iso, 16:0 iso, 17:0 anteiso, 17:0 iso 3OH, and 19:0 cyclo ω10c. It indicated the relative increase in the fungi and the thermophilic bacteria in the unit mass of sludge. The increase of PLFAs including 15:0 iso, 16:0 iso, 17:0 anteiso, and 17:0 iso 3OH has proved to provide a good indication of the thermophilic *Bacillus* sp. [34]. These micro-organisms maintained the high activity under the high temperature condition and played an important role in the sludge biodegradation at the thermophilic stage. The increase in fungi and thermophilic bacteria in the final materials showed that the low moisture (under 50%) and substrate level may benefit their competition. In addition, the contents of 18:0, 16:1 ω9c, and 16:0 anteiso were detected to decrease in the final production. 18:0 is one of the non-specific PLFAs occurring in a large number of bacteria, 16:1 ω9c is one of the specific PLFAs representing gram-negative bacteria [36].

Second, the PLFAs content increased in the temperature rising period, decreased in the high temperature period and revived in the curing period. They were 17:0 10-methyl, 18:1 ω7c, 15:0 anteiso, and 18:1 ω7c 11-methyl. This trend suggested actinomycetes, pseudomonas, and cellulomonas multiplied rapidly in the temperature rising period, but they might be greatly inhibited by the high temperature condition. The relative contents of these PLFAs in the final production were higher than that in the raw materials, meaning that these micro-organisms maintained high metabolic activity under the low moisture environment and played an important role in the degradation of the macromolecular substances such as cellulose and lignin. Especially, the actinomycetes is considered to be an index of compost maturity and its increase in

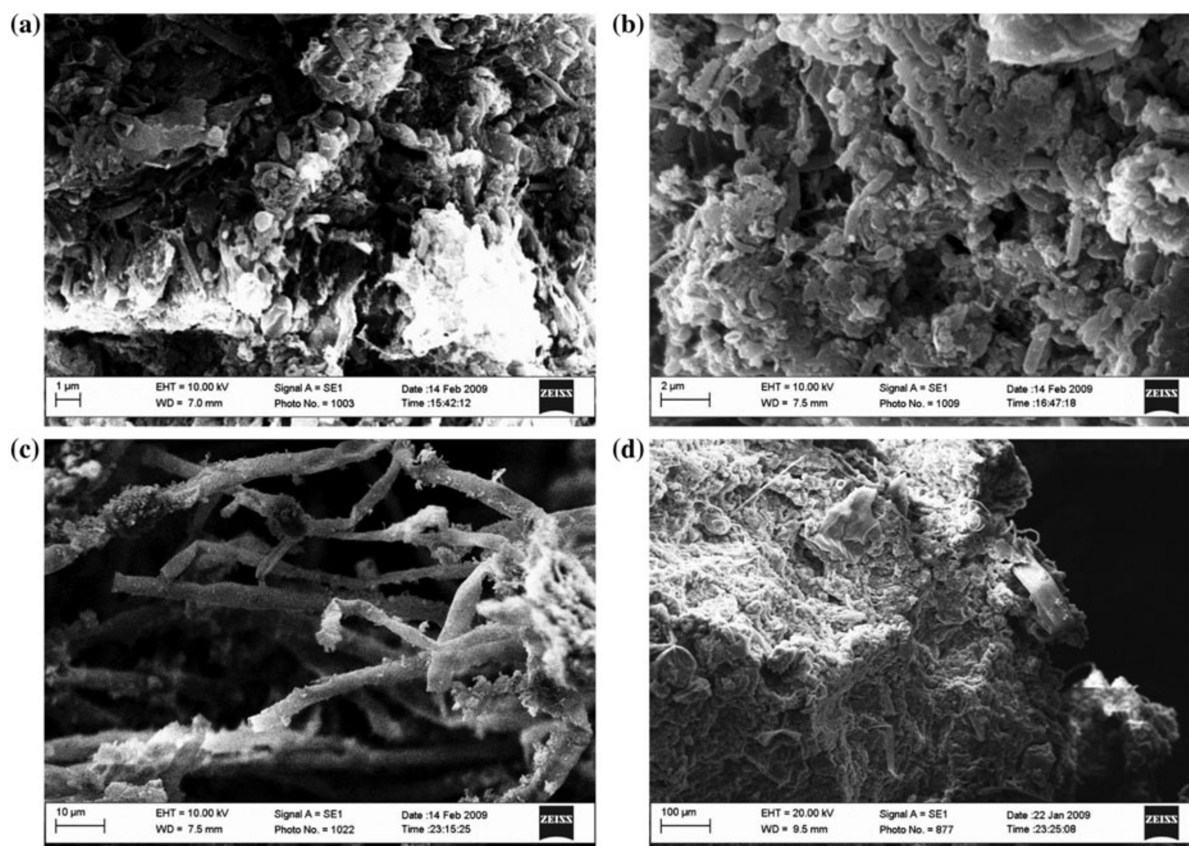

Fig. 3. The scanning electron microscopy (SEM) micrograph of the sludge samples at the different composting phases: (a) raw sludge, (b) sample after 10 d composting, (c) sample after 20 d composting, and (d) sample after 100 d composting.

the late phase of composting has been reported in other studies [37,38].

Third, the PLFAs were undetected in the active composting, including 20:4 ω6, 9, 12, 15c, 17:1 ω7c, and 16:0 10-methyl. These specific PLFAs representing protozoa, flavobacterium balustinum, and sulfate-reducing bacteria disappeared during the thermophilic stage and emerged in the final samples, which was possibly related to the strong inhibition of high temperature (50–60˚C) and high ammonia concentration. In addition, methane-oxidizing bacteria (16:1 ω5c) disappeared after 10 d, probably because the decrease in the moisture promoted oxygen transfer and oxidation—reduction potential (ORP) level inside the sludge and inhibited the anaerobic bioprocesses.

3.5. The change in microbial populations

The relative content of the specific PLFA in the sludge samples reflected the variation in specific microbial population during the composting process (Fig. 4). According to the previous studies [34,39], the sum of response value of gas GC to the PLFAs 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 16:0, 17:0, 17:0 iso, 17:1 ω7c, 17:1 ω6c, and 18:1 ω7c represented the amount of bacteria; the sum of response values of 18:1 ω9c and 18:3 ω6c (6, 9, 12) represented the amount of fungi; the sum of response values of 17:0 10-methyl, and 18:0 10-methyl represented the amount of actinomycetes; the response value of 20:4 ω6, 9, 12, 15c represented the amount of protozoa.

According to the variation in various PLFAs content in the samples, a large amount of information about the microbial community could be obtained. (i) The number of bacteria was significantly larger than other species in the thermophilic stage and the number difference among bacteria, fungi, actinomycetes, and protozoa was reduced in the curing period. This result suggested that the bacteria dominated the biodegradation of organic matters during active composting, while the synergy between various microorganisms acted significantly during the curing period. (ii) The number of fungi increased firstly and then decreased with the composting time, indicating that

Table 3
Relative content of PLFAs in the sludge samples of different composting phase

Microbial group	PLFAs	The abundances of the PLFAs (%)				
		Day-0	Day-5	Day-10	Day-20	Day-100 d
Aerobes G^+	14:0 iso	0.69	0.86	1.01	0.89	1.01
Aerobes G^+	14:0 anteiso	0.6	0.63	–	0.66	0.29
Bacterical in general	14:0	5.05	4.37	4.91	4.30	4.09
Aerobes G^+	15:0 iso	1.57	2.11	1.46	2.51	2.43
Aerobes G^+	15:0 anteiso	2.20	2.68	1.06	0.45	2.60
Bacteria G^+	16:0 iso	0.89	1.21	0.87	0.99	1.46
Bacteria G^+	16:0 anteiso	0.70	0.77	0.54	0.83	0.53
Bacteria G^-	16:1 ω9c	3.05	3.38	2.60	0.78	1.22
Methane-oxidizing bacteria	16:1 ω5c	0.97	1.04	0.95	–	–
Bacterical in general	16:0	25.49	20.83	24.03	25.82	12.94
Bacteria G^-	15:0 iso 3OH	1.09	0.99	–	–	–
Sulfate-reducing bacteria	16:0 10-methyl	–	–	–	0.92	2.19
Arthrobacter	17:0	1.76	–	–	0.69	–
Bacteria G^+	17:0 iso		1.38	–	–	1.48
Bacteria G^+	17:0 anteiso	0.97	1.24	0.99	1.23	1.34
Flavobacterium balustinum	17:1 ω7c	0.38	–	–	1.63	3.05
Bacteria G^-	17:1 ω6c	0.62	–	–	–	1.24
Actinobacteria	17:0 10-methyl	0.83	1.3	–	–	3.14
Fungi	18:3 ω6c (6, 9, 12)	1.3	1.46	1.56	1.40	2.56
Bacteria G^+	18:0 iso	0.83	0.89	0.59	0.93	–
Pseudomonas	18:1 ω7c	7.07	8.87	3.44	4.35	11.12
Fungi	18:1 ω9c	–	–	2.83	4.18	3.38
Hydrogenobacter	18:0	8.77	9.25	8.36	10.62	4.56
Cellulomonas	18:1ω7c 11-methyl	1.18	1.21	0.86	1.23	1.45
Bacteria G^-	17:0 iso 3OH	2.22	3.27	1.83	2.95	2.63
Actinomycetes	18:0 10-methyl	0.87	–	1.01	1.75	3.35
Burkholderia	19:0 cyclo ω8c	0.52	–	–	–	–
Bacteria G^-	19:0 cyclo ω10c	4.98	4.73	7.74	7.90	6.44
Bacterical in general	20:0	13.37	–	–	–	–
Protozo	20:4 ω6, 9, 12, 15c	2.15	–	–	–	3.42

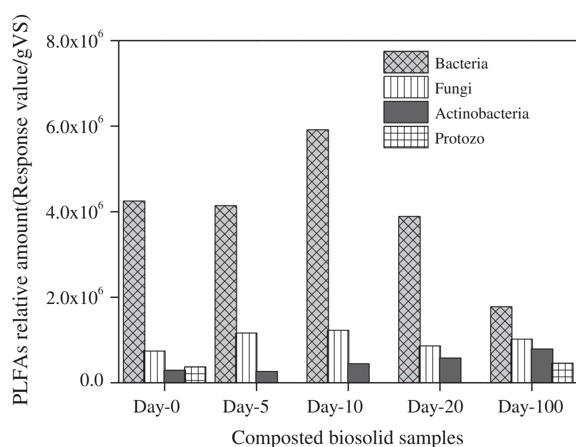

Fig. 4. The relative content of the PLFA from various microbial communities in the sludge at different composting phases.

some thermophilic fungi still had the metabolic activity in the range of 50–60℃. (iii) The PLFAs contents of the actinomycetes were detected to decrease intensively in the thermophilic period and gradually increased during the curing period. It showed that the high temperature could inhibit the actinomycetes, but the low moisture and poor substrate content benefited the ecological competition of the actinomycetes. (iv) Protozoa disappeared under the high temperature condition, and a small amount of protozoa appeared in the final materials, which demonstrated that the protozoa were more sensitive to the high temperature and contributed less to the sludge biodegradation in the thermophilic stage.

To verify the microbial population results of PLFAs analysis, dilution plate method was implied. The populations of bacteria, fungi, and actinomycetes were counted on different plate mediums (Fig. 5).

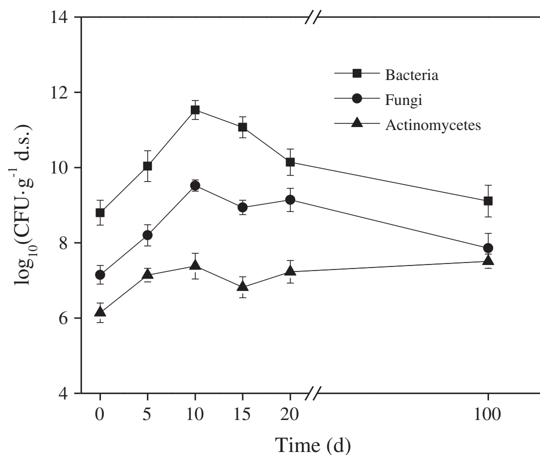

Fig. 5. Change in micro-organism populations during the composting process.

Bacteria were the dominant microbe during the composting. The number of bacteria increased from Day-0 to Day-10 and reached a peak of 3.38×10^{12} CFU g^{-1} d s. Such a high bacterial population could be found in In Hiraishi et al. [40] and Xi et al. [41]'s report. Similar phenomenon could be observed on the number of fungi, which increased to 3.31×10^{9} CFU g^{-1} d s, on Day-10. Only the number of actinomycetes kept increasing during the composting process and reached a peak of 3.24×10^{7} CFU g^{-1} d s, on Day-100. The variation in micro-organism populations was almost the same as the results of PLFAs analysis. Therefore, it was convincing to evaluate the variation in microbial communities by PLFAs technique.

PLFAs technique has been widely applied on the research of soil microbial community structure. Several researches have introduced this method into composting. Amir et al. [36] found the number of actinomycetes associated with compost stability increased at the end of composting of agro-industry sludge with different proportions of household waste. Steger et al. [42] used a conversion factor for PLFA to living microbial biomass to analyze the community structure in composting wheat straw. This method could provide information about quantitative changes of microbe on community level. However, researchers found that the same PLFAs were stated to indicate different groups. For instance, the PLFAs 18:1 $\omega 9$ is a relatively good indicator of fungi in some soils, but it is still present in some bacteria [43]. Therefore, caution must be taken in interpreting specific PLFAs. In general, PLFAs technique is a rapid and inexpensive method to evaluate the variation in microbial community structure in composting.

4. Conclusions

The variation in PLFAs content provided a large amount of information on the microbial community during the sludge composting. The number of microorganisms in unit mass VS in the sludge slowly decreased from the maximum (407.41 μg g^{-1} VS) on Day-10 to 207.49 μg g^{-1} VS on Day-100. Bacteria dominated absolutely (5.9×10^{6} g^{-1} VS) during the thermophilic stage of composting, and thermophilic fungi also play an important role in the same period. The numbers of bacteria and fungi on Day-10 were 3.38×10^{12} and 3.31×10^{9} CFU g^{-1} d s, respectively. The high temperature (above 50℃) significantly inhibited the actinomycetes, but the low moisture (under 50%) and poor substrate content benefited the ecological competition of actinomycetes. The number reached a peak of 3.24×10^{7} CFU g^{-1} d s, on Day-100. The number difference among bacteria, fungi, actinomycetes, and protozoa was obviously reduced in the mature sludge. PLFAs technique was an efficient and convenient way to evaluate the variation in microbial community during the composting of sewage sludge.

Acknowledgments

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