Co-composting of Wheat Straw and Food Waste with and without Microbial Agent

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Abstract: In China, the treatment of agriculture residue and food waste is of great concern. Aerobic composting is gaining increasing attention because it can improve both organic waste recycling and soil remediation. The aim of this study was to evaluate the composting process of the mixture of wheat straw and food waste, and the effect of microbial agent on the degradation process. Results showed that typical temperature variation curves were observed with peak values at 65.8℃ and 60.5℃ with and without inoculation, respectively. VS, DOC, NH_4^+-N and C/N decreased over the composting process, while pH, EC, NO_3^--N and GI showed an opposite trend. The microbial community diversity was analyzed and *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Ascomycota* enriched in the compost. At the end of the composting process, the maturity was indicated by the finial C/N ratio and GI which reached 11.26±0.84 and 144.68±14.95% with inoculation. Inoculation had a positive effect on composting performance but is less economic.

1 INTRODUCTION

As a big agricultural country, China produces abundant of biomass wastes including agriculture residues, forestry waste, livestock manure and food waste annually. Among these biomass resources, wheat straw represents a large portion in crop residues which could be used for land application (Zhu et al., 2020). Wheat straw can be utilized as fuel for household cooking, silage for livestock, and material for mulching. However, most of the straws are burned in the open field since it is cost effective and convenient, resulting in serious environmental problems such as greenhouse gases and harmful smoke generation and a waste of biomass resources. At present, the main technologies to treat and recycle agriculture wastes are anaerobic digestion (AD) and aerobic composting (AC) (Li et al., 2011; Qian et al., 2014). Since methane rich biogas can be produced and seldom maintenance is required during the AD process, biogas plants have been largely constructed and employed for biomass conversion. Nevertheless, crop straws which is rich in lignocellulose and resistant to degrade is always co-digested with other easily biodegradable substrates (Lehtomäki et al., 2007). Problems related to crop straws in AD such as raw material floating, low degradation rate and difficult discharging are quite annoying. In addition, large amount of biogas slurry and biogas residue is produced and extra efforts on post-treatment are necessary to eliminate the negative effects for further land application (Wang et al., 2016).

Compared to AD, AC can convert crop straws into organic fertilizer directly in a sustainable and environmental friendly way which has been recommended in recent years (Bernal et al., 2009). AC is a process that breaks down organic wastes and produces CO_2, water, mineral ions and stabilized organic matters under certain conditions. The product is beneficial for soil amendment and plant growth. The process is induced by the activities of various microbial communities and influenced by factors

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such as substrate properties, C/N ratio, moisture content, temperature, pH value, bulk density, oxygen supply and raw material size (Hubbe et al., 2010). Nowadays, AC has been widely applied in the treatment of various kinds of organic wastes (Wei et al., 2017). Usually, straws are dosed as supplements and co-composted with livestock and poultry manures or sewage sludge (Meng et al., 2019). Studies on AC of wheat straw as the bulking material are still lacking. In this study, straws were used as the main composition of the compost and food waste was added to promote the degradation process. Wheat straw have high C/N ratio, low moisture content and porous structure while food waste has the opposite physicochemical properties. The mixture of these two kinds of materials can provide more balanced nutrient, proper moisture content and better ventilation condition for the microorganisms to carry out the composting process.

Accordingly, the present study aimed to evaluate the treatment effect of co-composting of wheat straw and food waste, and explore the effect of selected microbial agent on the degradation performance. The forms and distribution characteristics of carbon and nitrogen were determined along with other conventional parameters. Species succession among the microbial communities was investigated during the composting process. Besides, the evaluation of the compost maturity and quality was performed.

2 METHODS AND MATERIALS

2.1 Feedstocks and Microbial Agent

Wheat straw were purchased from a farm of Jiangsu Province, China. The straw was air-dried and smashed to 1-3 cm. Food waste was collected from the canteen of one research institution. Bones, plastic bags, napkins and other raffles were picked out and leachate was drained. Collected food waste was shredded by a food grinder into mushy mixture and stored at -20℃ before use. The characteristics of the feedstocks are shown in Table 1. Microbial agent mainly containing *Chelatococcus composti, Bacillus thermoamylovorans, Aspergillus fumigatus, and Aspergillus niger* was prepared and the concentration of each strain was about 109 cfu/mL.

Table 1: Basic physicochemical properties of the feedstock.

Parameter	Wheat straw	Food waste
Input in each reactor (kg)	2.2±0.1	2.5±0.1
Water content (%)	10.24±0.23	80.57±0.34
Volatile solid content (%)	94.83±0.13	87.25±0.91
TC (%Dry weight)	50.16±0.21	40.24±0.31
TN (%Dry weight)	1.12±0.11	2.96±0.13
C/N ratio	44.84	14.13

2.2 Experimental Apparatus and Tests

Composting was conducted in plastic bins of 30 L valid capacity with 46.5 cm in height and 32 cm in diameter. The compost bins were covered with insulating cotton to retain metabolic heat. An annular aeration pipe was placed at the bottom of the bin and 1 L/min aeration rate was set through an aerator pump for the composting process. A perforated polyvinylchloride tray was installed above the aeration pipe to support the compost and distribute the air uniformly. The structure of the composting reactor can be referred to Zhang et al (2021). Firstly, wheat straw was placed in a large-size plastic drum and certain amount of distill water was added to obtain the water content at around 65%. Then food waste was added to adjust the C/N ratio of mixed substrates to 32-35. Substrates were mixed thoroughly by hand to assure the maximum homogeneity.

For the inoculated treatments, a concentration of 0.5% (dry weight basis, w/w) microbial agent was introduced. Treatments with identical substrate composition but no microbial agent were served as the control groups. About 7.2 kg mixed substrate was placed in each compost bin occupying 85% of the volume. The experiment lasted for 30 days and samples were collected every 4 days for parameter analysis. Turning and mixing was conducted manually every 7 days to break any lumps formed and ensure the optimal aeration in the system.

2.3 Analytical Methods

Temperature was measured daily by a thermometer inserting at three locations in the compost bins (surface, 10 cm depth; core, 25 cm depth; and bottom, 38 cm depth), and the average temperature was recorded. The water content and volatile solid (VS) content were measured according to Standard Methods (APHA, 1998). Total carbon and total nitrogen content of dried samples were analyzed by an auto elemental analyzer (Vario MACRO cube, Elementar, Germany). About 5.0 g of fresh sample was extracted with 50 mL deionized water (1:10 w/v ratio) using a shaker at 100 rpm for 2 h followed by centrifugation at 10000 rpm for 10 min. Then the supernatant was measured for pH and electrical conductivity (EC) by a pH/EC meter (Orion VERSA STAR Pro, Thermo Fisher Scientific, USA). The absorbance of supernatant at wavelengths of 465 nm (E4) and 665 nm (E6) was measured using a spectrophotometer (UV-2600, Shimadzu, Japan). The supernatant was then filtered through 0.45 μm filter membranes. Dissolved organic carbon (DOC) and nitrate (NO_3^--N) were measured by a TOC/TN analyzer (TOC-L, Shimadzu, Japan) and an ion chromatography (ICS-5000+, Thermo Fisher, USA), respectively. Ammonium nitrogen (NH_4^+-N) was analyzed via a continuous flow analyzer (Syslyzer III, Systea, Italy).

For microbial community determination, about 0.3-0.5 g fresh samples were stored at -20℃ for genomic DNA extraction and 16s rRNA/18S rRNA amplification. The V3-V4 hypervariable-region was amplified with the bacterial universal primer sets: 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Primers of 18S rRNA: 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTCACCTCT-3'), were used to analyze fungal communities. All the sequencing analysis was conducted by Shanghai Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China) on an Illumina Miseq platform. All the pairs of sequences were clustered into Operational Taxonomic Units (OTUs) based on a ≥97% identity threshold by the SILVA database.

A phytotoxicity test was performed by seed germination with Chinese cabbage seeds. The 1:10 aqueous extract of compost was prepared and 6 mL of the extract was added into a sterile petri dish (90 mm in diameter) containing a filter paper. About 20 Chinese cabbage seeds were placed on the filter paper and incubated in the dark at 25℃. In contrast, 6 mL deionized water was used as the control experiment. After incubation for 48-72 h, the numbers of germinated seed and root length were measured and recorded. Germination index (GI) was calculated according to the reference (Zucconi, 1981).

2.4 Statistical Analysis

Statistical analyses were performed in duplicate samples and the average values with standard deviation were reported. The data were processed to a one-way analysis of variance (ANOVA) using IBM SPSS statistics ver. 22.

3 RESULTS AND DISCUSSION

3.1 Temperature Variation during the Composting Process

Temperature variations in all composting bins were monitored and showed in Figure 1. The ambient temperature varied within a narrow range from 25.5℃ to 29.0℃. In experiments with inoculation, the temperature increased rapidly to the thermophilic value (>50℃) after 4 days' composting. A further increase was observed and temperature reached its peak at 65.8±1.0℃ on day 5. The thermophilic stage lasted for 7 days when most potential pathogens, pets and weed seeds were likely to be killed. Then temperature of composts showed a downward trend to around 40℃ followed by a maturation phase when the temperature ranged from 39.2℃ to 31.5℃ until the end of experiment. In the control treatments, temperature variations experienced similar mesophilic, thermophilic, cooling and maturation phases. The maximum temperature reached 60.5±1.0℃ on day 8 and the duration of thermophilic stage was 5 days.

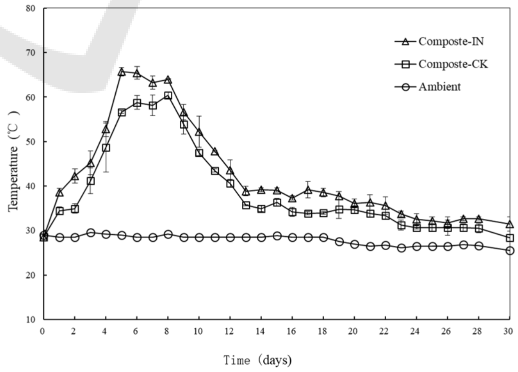


Figure 1: Temperature variation during the composting process. Compost IN means composting experiment with inoculum; Compost CK means control experiment.

In this work, though the main component was wheat straw which is rich in lignocellulose and recalcitrant to decomposed (Yu et al., 2007), the presence of easily degradable organic materials supplied by food waste contributed to initiate the bio-process successfully. Metabolic heat was released significantly by the activity of microorganism in the organic matter decomposition process. In the control tests, the temperature increased rapidly during mesophilic and well maintained in thermophilic phase with the effect of endogenous microorganisms. However, slightly lower temperature values were observed during the whole experiment as well as a shorter thermophilic duration time in the control groups compared those in tests with exogenous microbial agent.

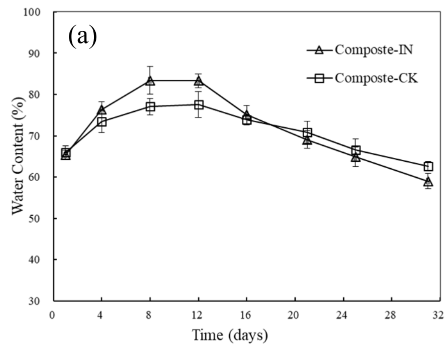
3.2 Changes of Moisture, pH, EC and E4/E6

Many studies have stated that the optimal water content for AC is 50-60% (Mohee and Mudoo, 2005). However, the initial water content in this study was adjusted slightly higher at 65% with lignocellulose-rich and porous structure substrates. A rise in the water content was observed in the early stage due to thriving microbial metabolic activities (Figure 2a). The water content peaked at 83.43±3.41 % on day 8 and at 77.63±3.12 % on day 12 with and without inoculation, respectively. Though high temperature enhanced the moisture evaporation, the generation of metabolized water was stronger than the moisture ventilation during the mesophilic and thermophilic stages. Moreover, moisture condensed on the lid of the bin and fell back to the mixture which also caused an increase in water level and the hydrothermal environment. Along with the constant ventilation forced by aeration and decreasing microbial decomposition rate, water content was reduced till the end of the composting to around 59.07±1.84 % and 62.75±1.17% with and without inoculation, respectively. No leachate was collected at the bottom of the bin.

Similar changes in pH were observed in inoculated and non-inoculated treatments (Figure 2b). Initially, pH was acidic and the value was around 4.5 in all bins. The low pH was attributed by amino acids and fatty acids which were produced from the easy-degraded organic matters from food waste such as carbohydrates, protein and fat. Along with the consumption of intermediate compounds (mainly organic acids) and the release of ammonia, pH increased immediately to 7.0-8.0 and then fluctuated around this value until the end of composting. With easy-degraded organic materials exhausted, complex lignocellulosic substrates were gradually degraded since the fiber surface has been soften in the humid and acidic environment. The compost samples from maturation phase were slightly alkaline (7.5-8.5) which were suitable for the growth of microorganism and plant seedlings (Bustamante et al., 2008).

EC indicates the salinity level of substrates and the possible phytotoxic effects. As illustrated in Figure 2c, EC showed a continuously increasing trend from 1.86±0.01 mS/cm and 1.61±0.11 mS/cm with and without inoculation, respectively, to about 3.50 mS/cm at the end of the composting process. The increase in EC was induced by the decomposition of complex organic matters into small molecule dissolved organic matters as well as the release of mineral ions. During the whole process, EC values with inoculation were higher than those in experiments without inoculation, suggesting that the degradation and mineralization of organic matters could be enhanced by the activities of both endogenous and exogenous microorganisms. Some researches have stated that a high EC (>4 mS/cm) related with high salt content had adverse effects on plant cultivation (Meng et al., 2019). In this study, EC of processed compost was found below 4 mS/cm which was within the prescribed limits of phytotoxicity.

The absorbance at 465 nm (E4) and 665 nm (E6) of aqueous extracts of compost were determined and E4/E6 ratio was described in Figure 2d. E4/E6 underwent an increase and the highest value reached to 8.04±0.32 and 7.23±0.71 with and without inoculation, respectively. Positive linear relationship between water soluble organic carbon and absorbance at 465 nm has been approved. Therefore, the increase in E4/E6 ratio indicated that more water soluble organic carbon existed via decomposition of organic compounds. Then E4/E6 ratio of the inoculation experiment and control experiment declined to 3.36±0.11 and 4.05±0.16 at the end of the composting, respectively. Information on condensation degree of humus with aromatic nucleus can be provided by E4/E6 ratio (Inbar et al., 1993). The decline in E4/E6 ratio was likely caused by the consumption of small molecule organic matters and the formation of humic substances.



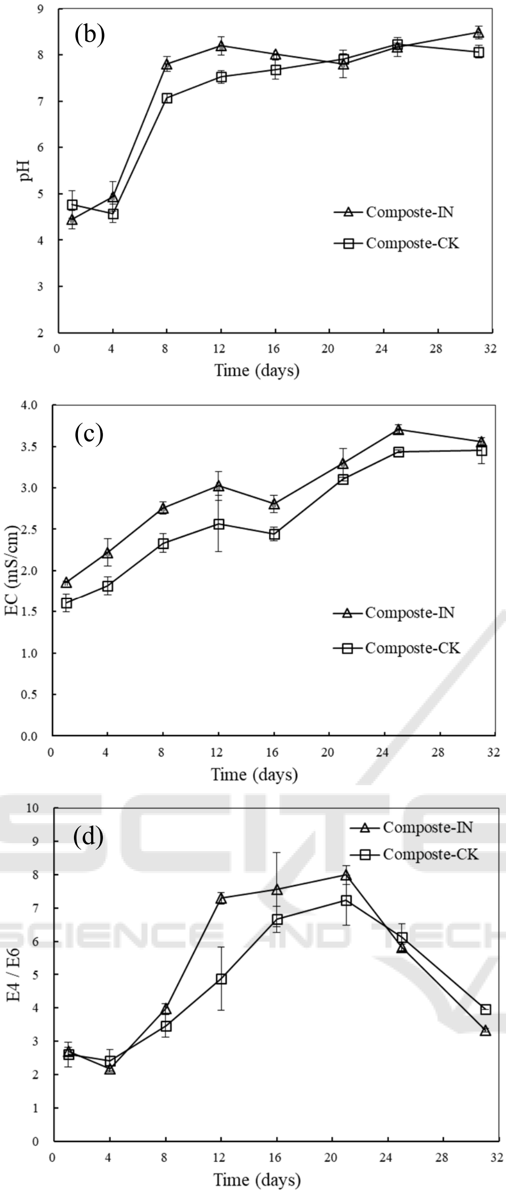


Figure 2: Changes in physicochemical parameters during composting.

3.3 The VS Content and DOC Evolution

The VS content decreased along with composting time for all treatments due to the loss of organic matters by microbial degradation (Figure 3a). The initial VS was around 93%. Overall, experiments with inoculation had a higher loss of VS content (27%) than that in the control (18%). A sharper decline during the mesophilic-thermophilic stage suggested that easy-degraded organic matters were mainly utilized. They were converted into small molecule dissolved organic matters which was consistent with the increase in DOC during the first few days (Figure 3b). The maximum values of DOC reached to 32.17±1.86 g/kg and 26.44±1.50 g/kg with and without inoculation, respectively. Since small molecule soluble organic matters were more available to microbes, decreases in DOC was observed with the depletion of soluble organic matters. Afterwards, the relative low degree of VS loss during the cooling and maturation stage was contributed by the huminification of recalcitrant decomposable compounds. The final VS contents of the composting mixtures were 67.07±1.22% and 74.53±1.96% with and without inoculation, respectively. At the end of the composting, the DOC contents in all treatment were around 10 g/kg which was identical to that in the previous work (Zhou et al., 2014) which conducted the co-composition of food waste and sawdust. Wider variation ranges were observed both in VS content and DOC with inoculation than those in the control. This suggested a higher metabolic activity of microorganisms with inoculation than that in the control throughout the entire composting process.

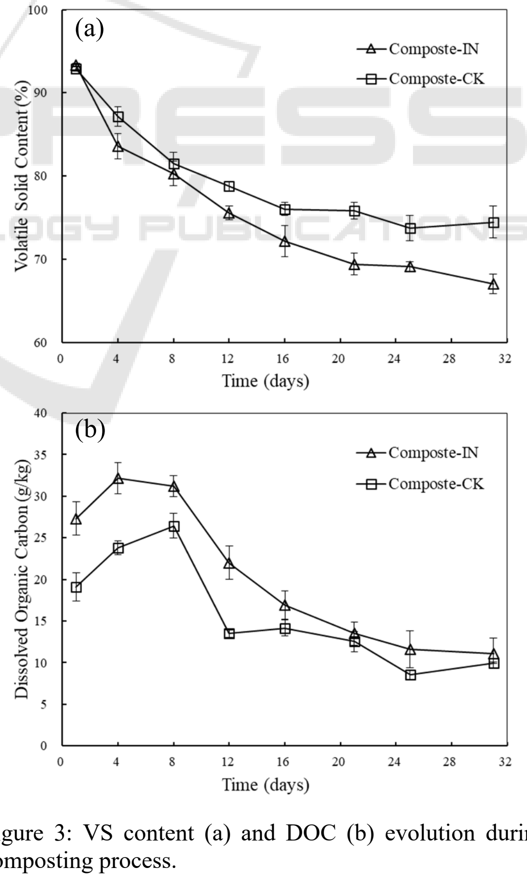
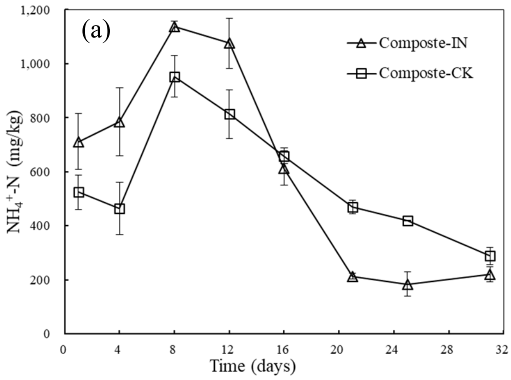


Figure 3: VS content (a) and DOC (b) evolution during composting process.

3.4 Changes in Ammonia Nitrogen and Nitrate Nitrogen

Figure 4 shows the changes in concentrations of NH_4^+-N and NO_3^--N in extract during the composting process. The concentration of NH_4^+-N followed a typical trend which NH_4^+-N increased during the early stage of the process, then decreased as the process progressed and reached a low level at the end of the composting process. The increase during the early days was due to the conversion of organic-N compounds into NH_4^+-N via ammonification (Gao et al., 2010). The amount of NH_4^+-N reached a peak at 1138.18±18.27 mg/kg and 952.86±77.55 mg/kg with and without inoculation, respectively, on day 8. Simultaneously, the volatilization loss of NH_4^+-N was enhanced by the high temperature. Then the NH_4^+-N concentrations declined and the final values were 220.24±28.26 mg/kg and 288.94±32.45 mg/kg in the inoculated and non-inoculated treatments, respectively. Values below the maximum limit of 400 mg/kg for NH_4^+-N content were recommended for mature compost in many researches (Luo et al., 2018). In this work, values blow 330 mg/kg in NH_4^+-N concentrations of finished compost were obtained.

The concentration of NO_3^--N was relatively low and steady during of first 12 days of the composting process. Less NO_3^--N was generated when little nitrification happened during thermophilic stage since the activity and growth of nitrifying bacteria was inhibited by high temperature and excessive amount of ammonia. Afterwards, the amount of NO_3^--N increased gradually when nitrifying bacterial turned from dormant to active physiological state. The final content of NO_3^--N reached 849.58±72.94 mg/kg and 385.63±41.71 mg/kg with and without inoculation, respectively. Usually, the production of nitrate rich compost is desired since NO_3^--N is a more favorable source of N to be absorbed than NH_4^+-N for plant cultivation (Sun et al., 2016).



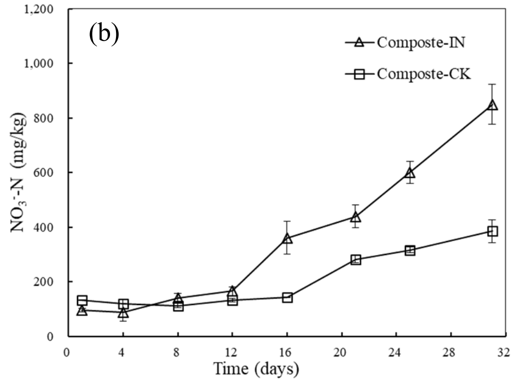


Figure 4: Changes in concentrations of NH_4^+-N (a) and NO_3^--N (b) during the composting process.

3.5 Changes in Microbial Community Structure

To evaluate microbial community diversity and dynamic changes during the AC process, samples were collected from original, thermophilic, cooling and maturation phases. Five phyla were dominant and accounted for more than 97% of the sequences which were *Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes* and *Ascomycota*. As composting processed, succession of the microbial community emerged induced by differences in environmental conditions and substrate composition.

Microbial relative abundance at the genus levels is shown in Figure 5. At beginning, as described in Figure 5a, the top 5 bacterial genera included *Leuconostoc*, *Weissella*, *Lactococcus*, *Lactobacillu* and *Pediococcus* whose relative abundance was 96.78%. On day 4, dominant bacteria switched to *Lactobacillus*, *Paenibacillus*, *Brevibacillus*, *Bacillus* and *Acinetobacter*. Their relative abundance were 74.83% and 75.20% in the control and inoculated experiments, respectively. *Lactobacillus* bacteria is always found in plant-derived raw material decomposing systems and can accelerate compost ripening (Li et al., 2020). *Bacillus* is thermotolerant and able to secrete various extracellular enzymes such as proteases, amylase and cellulases which contributes a lot in lignocellulosic degradation (Jurado et al., 2014). Afterwards, the relative abundance of the top 5 bacterial genera changed and their relative abundance decreased from 55.85% on day 12 to 27.04% on day 22 in the control groups, and from 47.21% to 18.36% in the inoculated groups. Along with composting processed, the bacterial community composition seemed more complicated and diverse which had a positive effect on the lignocellulosic substrate degradation.

Specially, fungi are actively involved in the degradation of lignocellullosic compounds via producing a broad variety of functional enzymes and physical destruction by the fungal hyphae (Yu et al., 2007). Fungal relative abundance at the genus levels is shown in Figure 5b. At day 0, the dominant fungal genera were *Candida* (83.35%), *Wallemia* (11.99%) and *Aspergillus* (3.64%). At day 4 in the inoculation and control experiment, *Aspergillus* as a thermotolerant fungus proliferated to 94.27% and 79.57, respectively. Later, the relative abundance of *Thermomyces* showed a dominant value. During the maturation phase, the major genera evolved to *Thermomyces* (84.94%), *Mycothermus* (10.30%), and *Myceliophthora* (4.25%) in the control groups while those values were 67.81%, 27.66% and 3.96% with inoculation, separately.

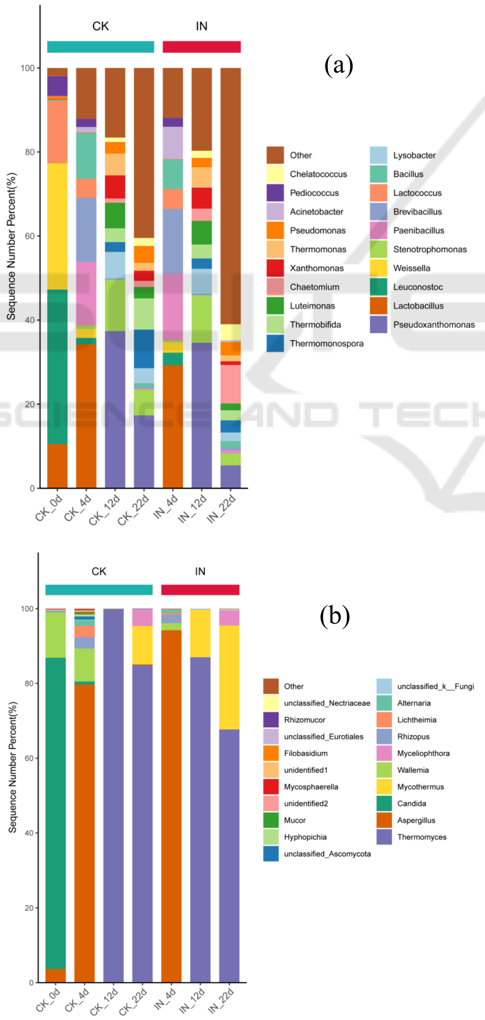


Figure 5: The genus composition of the bacterial community (a) and fungal community (b) at different stages of AC.

More diversity appeared during the maturation phase in the inoculation groups compared those in the control groups. Though the inoculated strains did not proliferated during the whole period, the richness and diversity of the microbial community increased with more metabolism pathways of substrates which led to the better composting efficiency.

3.6 Compost Maturity Evaluation with C/N and Germination Experiment

Organic carbon and nitrogen are commonly utilized by microorganisms for cell growth and metabolic activity during AC, which leads to variations in the C/N ratio. The loss of carbon and nitrogen is mainly in the form of CO_2 and NH_3 stripping. Since the degradation of carbon is faster than the release of nitrogen, the decline in the C/N ratio during the composting period is always observed (Zhou et al., 2014). In our work, the initial C/N ratio of the composting mixtures was around 33 which located within the appropriate levels for composting microbes (Figure 6a). The C/N ratio showed a downward trend and the final values dropped to 11.26±0.84 and 13.95±0.93 with and without inoculation, respectively. The final C/N ratio can be used to assess the compost maturity. Some studies stated a value equal to or less than 20 indicates a satisfactory maturation (Fourti, 2013).

Phytotoxicity determined by the germination experiment is also used to test compost safety and maturity (Yang et al., 2013). The response of Chinese cabbage to the toxicity of the compost water extract in term of GI is illustrated in Figure 6b. The GI values of all experiments dropped during the early stage of composting process. The lowest values reached about 10% which suggested a very toxic extract of the compost product. The seed germination was inhibited by the excessive toxic materials such as short chain volatile fatty acids and ammonia which was proved by the low pH (Figure 2b) and high NH_4^+-N content (Figure 4a). With the depletion of the toxic materials, the GI values rose significantly and reached to 144.68±14.95% and 92.64±12.27% at the end of composting with and without inoculation, respectively. Many reports have claimed that a more than 50% GI indicated the compost was phytotoxic-free while a more than 80% value indicated mature product (Luo et al., 2018). Values above 1 indicated a positive effect of finished compost on seed germination. Clearly, inoculation was helpful in enhancing the maturity and releasing of nutrients in accordance with the higher NO_3^--N concentration in compost extract.

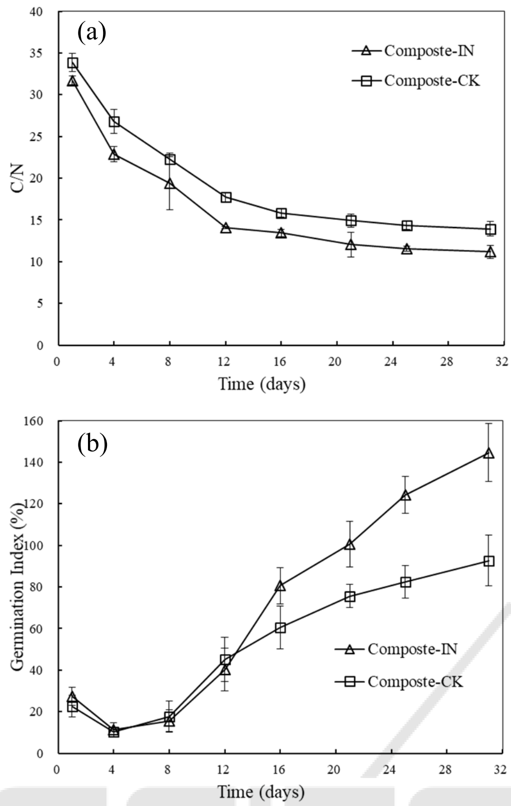


Figure 6: Changes in C/N (a) and germination index (GI) (b) during the composting process.

4 CONCLUSIONS

In all treatments, typical variation curves in parameters such as temperature, EC, DOC, C/N and the succession of microbial community were observed. Produced composts were found to be phytotoxic-free convinced by the germination experiment. When microbial agent was applied, better performance was obtained during co-composting of wheat straw and food waste proved by the relatively higher thermophilic temperatures, lower C/N ratio and higher GI value. Inoculation contributed to a more diverse microbial community and had a clear advantage in acceleration of the compost degradation, sanitation and maturation process. However, application of microbial agent is less economic. Without microbial agent, satisfied results could still be achieved because of the appropriate composting conditions such as nutrient adjustment, forced aeration and active endogenous microorganisms.

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