Feasible strategy for the nitrogen fixation and humification quality improvement by spent mushroom substrate as conditioning agent

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Abstract: There is an overlooked problem which increasing microbial abundance while reducing nitrogen loss during composting. This study investigated the viability of spent mushroom substrate (SMS) as conditioners in the aerobic composting of kitchen waste (KW) with cattle manure (CM). The variation of temperature, pH, C/N, organic matter, cellulose, hemicelluloses, germination index (GI), and microflora structure were explored to evaluate the potential in accelerating maturity and nitrogen fixation by SMS addition. The results showed that the addition of SMS accelerated the heating rate, prolonged the high temperature time, and decreased organic matter, hemicellulose, and cellulose by 17.49%, 23.61%, and 18.62%, respectively. The GI reached 105.86% with SMS addition, while 74.17% was found in control treatment after composting. SMS changed the microbial community composition and increased the species abundance. *Proteiniclasticum*, *Clostridium* XI and *Azomonas* were dominant bacteria, which increased the retention of nitrogen, promoted organic matter degradation and reduce compost time. The study can provide a feasible strategy for nitrogen fixation in the field of organic waste recycling.

Keywords: spent mushroom substrate, conditioning agent, nitrogen fixation, humification quality, aerobic composting
DOI: 10.25165/j.ijabe.20241703.7790

Citation: Ai S, Zhang Y B, Zheng G X. Feasible strategy for the nitrogen fixation and humification quality improvement by spent mushroom substrate as conditioning agent. Int J Agric & Biol Eng, 2024; 17(3): 235–240.

1 Introduction

With the rapid acceleration of urbanization and the rapid development of intensive livestock farming, urban kitchen waste (KW) and livestock excreta are increasingly generated[1,2]. The amount of KW generated in China is estimated to be 48-60 million t per year[3], with an annual growth rate of 5%-10%. The rapid development of animal husbandry has directly led to a significant increase in the production of livestock manure, which has become the main source of production of agricultural organic solid waste in China[4]. Composting is regarded as an effective resource utilization option owing to the microbial activity with a variety of special functions that enable organic waste to be biodegraded and transformed into valuable humus. Therefore, aerobic composting is an effective way to realize the KW resourceful treatment, but also a main mean to achieve the harmless and resource utilization of poultry manure and life garbage. However, high moisture content, low C/N ratio and harmful gas emission will not only lead to a decline in compost quality, but also lead to environmental problems. Moreover, the key problem to influence the compost quality is nitrogen loss. In view of the present situation, there is a significant motivation to search for a suitable substance for the improvement of the final quality of the compost.

The application of bulking agents and microbial amendments was widely used to enhance the efficiency of composting due to the high moisture content and low C/N ratio of KW and livestock excreta as composting materials. A bulking agent with a relatively high C/N ratio might be applied to balance the C/N ratios of KW and CM during composting. Also, bulking agents play a very important role in preparing the first composting mixes, which are used to make spaces between the particles, add air, control the amount of moisture in the waste, and give microorganisms a carbon source. Maize straw is a widely available and cost-effective bulking ingredient[5]. Hanajima[6] reported that maize straw can decrease the amount of leachate produced and improve compost maturity. Based on microorganisms as a key driving force in the process of compost biotransformation, inoculation of microorganisms has become a direct choice to change the metabolic activity of microorganisms. However, increasing microorganisms' activity will cause nitrogen loss, which could reduce fertility of compost products. Therefore, it is worth thinking about that how to improve microbial activity while reducing nitrogen loss.

Spent mushroom substrate (SMS) is a kind of cultivation waste that is discarded after the harvesting of edible mushrooms. The amount of SMS increases gradually with the progress of the mushroom industry. The traditional landfill disposal approach can cause serious environmental contamination, and phytotoxic compounds and active organic matter in SMS may cause hygiene hazard problems during agricultural utilization. SMS is rich in nutrients and fungus degrading cellulose and hemicellulose, as well as better air permeability, which make it an ideal microbial fertilizer carrier. Currently, several studies have been conducted on the composting of KW with SMS[7], but few studies have been reported on the use of SMS as a conditioning agent for the co-composting of

Received date: 2022-07-13 **Accepted date:** 2024-05-06
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KW with CM. Besides that, an issue that is frequently disregarded is how to increase microbial richness while decreasing the nitrogen loss during composting.

Hence, in this study, the aerobic composting method was used to investigate the transformation characteristics and the varieties of microbial population diversity of the mixed aerobic fermentation using KW and CM as the main mixtures and SMS as the bulking agent. The objective is to improve microbial activity while reducing nitrogen loss, evaluate the potential in accelerating maturity and stability by the SMS to adjust the appropriate C/N during aerobic composting. It will provide an essential technical basis for expanding the range of available conditioning agents for the composting of KW and CM and improve the degree of waste resource utilization.

2 Materials and methods

2.1 Experimental materials procedure

KW was taken from the student canteen at Northeast Agricultural University. CM and maize straw were obtained from Harbin Xiangfang farm, China. SMS was taken from the Baoshan mushroom factory, Shanghai, China, whose main component was cotton seed hulls. The SMS was added to the mixture of KW and CM for mixed aerobic composting, set as the test group. While the experimental group without the addition of SMS was set as a control, and a rigid plastic drum with a volume of 250 L was used as the composting reaction device. The initial mass of each composting group was the same, and the ratio of KW, CM, and corn straw was 2:4:1. The amount of conditioner added was based on the final adjusted moisture content range of 65%-70% and the C/N ratio range of 25:1-30:1. During the composting process, the composting piles were manually turned over every 7 d to make sure that the fermentation system got enough air.

2.2 Analysis methods

The treatments were operated for 15 d. The 2 points temperature of the compost center were monitored, and took the average value as the moment of compost temperature. For the determination of all the indices, solid samples weighing about 0.4 kg were collected (on Day 0, 3, 6, 9, 12, and 15) in triplicate using the multiple point sampling technique, and they were properly mixed before analysis. The total organic carbon (TOC) was measured by TOC analyzer[8]. Total nitrogen (TN) was measured using the Kjeldhal method[9]. The ANKOM220 fiber analyzer (Ankom Technology, USA) was used to determine the cellulose and hemicellulose content. According to the procedures described by Miao et al[10], the Fresh compost samples were mixed with distilled water (1:10 w/w ratio) and agitated for 30 min. The pH of the compost was calculated from the mixture. The extract was centrifuged, and the supernatant was collected and filtered through a 0.45 μm filter membrane to calculate the GI. The seed GI was utilized to determine the phytotoxicity of the compost. Twenty bok choy seedlings were spread on the filter paper in petri dishes (10 cm in diameter) and wet with 10 mL of the compost water extract. Three replicate dishes of each sample were incubated at 20°C for 3 d. The number of germinating seeds and root length were assessed, with distilled water serving as a control.

High-throughput sequencing was to determine the compost bacteria community composition. Compost samples cultured after composting were sent to Sangon Biotech Co., Ltd. (Shanghai, China) for high throughput. The data were obtained from the project report.

3 Results and discussion

3.1 Changes of temperature during composting

In a composting system, temperature is crucial to the life activity of microorganisms, and its change is the characteristic of the degradation process of organic matter. Meanwhile, a long-term high temperature could effectively kill pathogenic bacteria and other harmful microorganisms. From Figure 1a, it can be seen that the compost pile with the addition of SMS heated up faster during the composting process, with the highest temperature reaching 64.5°C and maintaining above 50.0°C for 5 d. The high temperature period ended on the 9th day and lasted for 7 d in total, which reached the requirement of harmlessness and the treatment effect was more ideal. In contrast, the compost pile without the addition of SMS was slow to warm up during the composting process, and the maximum temperature was only 50.7°C, which was maintained above 50.0°C for 2 d. The high temperature period ended on the 9th day and lasted for 6 d. Among them, there was a small sawtooth-shaped fluctuation on the 7th day (Figure1a) due to the temperature dropping after turning the pile, but at the same time, oxygen was delivered to the compost heap during turning and the temperature increased slightly afterwards. The test and control groups all showed the three-stage temperature changes that the piles reached their highest points, started to go down, and then stayed the constant temperature for the last 2 d of composting, illustrating that the addition of SMS as a conditioning agent for the composting of KW mixed with CM was better than no addition of SMS. Both in the time of reaching the high temperature and the duration of the high temperature period, which may be related to the action of indigenous microorganism SMS. In general, keeping the temperature of the pile between 50°C and 55°C for 5-7 d was important to kill pathogenic bacteria in the pile and make sure that the compost passed the sanitary index and was ready[11].

3.2 Change of pH during composting

The initial pH in the SMS treatment and the CK treatment were 7.95 and 8.21 respectively. Changes of pH of the two treatments showed a similar trend, as shown in Figure 1b, firstly decreased then increased, and then decreased slightly. Macromolecular organic matter degraded to small molecular organic acids with composting proceed. The accumulation of organic acids resulted in a decrease in pH. Along with the fermentation process, organic acids were further decomposed into alkaline substances such as ammonia nitrogen and amines, making a rising pH. The highest pH value of the SMS group was 9.08 (9.06 of control), then finally declined to 9.03 (8.90 of control), all accorded with the standard of pH 8.0-9.0 of compost maturity.

3.3 Changes of C/N during composting

The ratio of C/N plays a key role in microbial metabolism and the synthesis of cellular substances. Carbon is known as the energy source of the cell, about 2/3 of which is consumed as carbon dioxide and another 1/3 is used for the synthesis of cytoplasm during microbial metabolism[12]. While nitrogen is mainly used in the synthesis of cell protoplasm for proliferation. Compost materials can be decomposed when the C/N ratio decreases to 15-20[13] in the aerobic fermentation process. As shown in Figure 1c, the C/N of both the experimental groups showed a decreasing trend as the composting progressed. The value of C/N ratio decreased from 25.05 to 13.37 in control group, from 28.96 to 16.23 in SMS group by the end of composting. T=(end C/N)/(initial C/N) was proposed to evaluate compost maturity. T value shows less than 0.6 indicating the compost reached maturity[14]. In this study, at the end of composting,

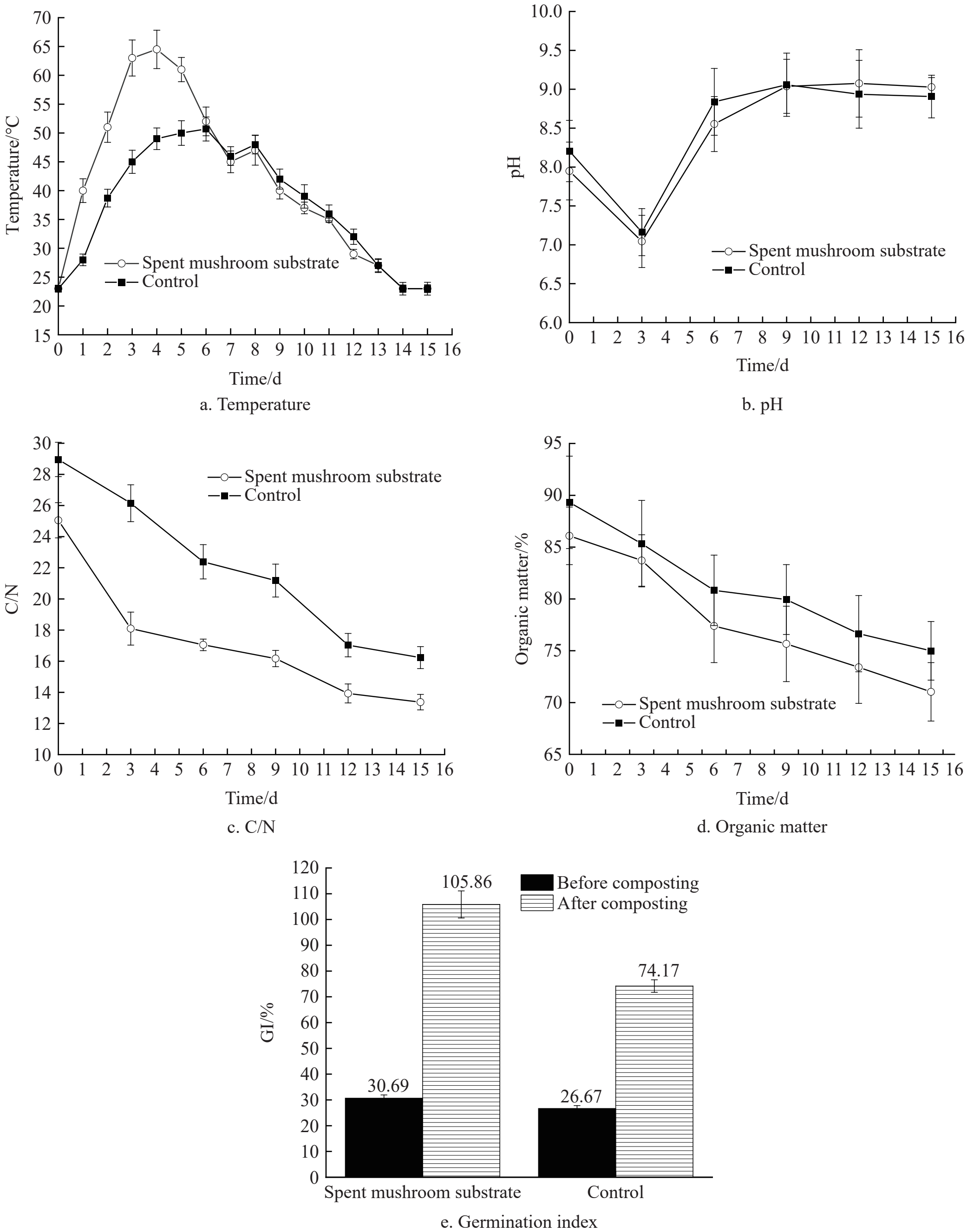


Figure 1 Changes in maturity indexes during composting period of CK and SMS group

the *T* values of the two experimental groups were 0.53 and 0.56, respectively, illustrating that both composts have reached maturity.

3.4 Changes of organic matter during composting

The initial pile contains a large amount of organic matter, but it was not suitable for direct use as fertilizer without biostabilization, and composting treatment was precisely the biological transformation process that stabilizes it[11,15,16]. Therefore, the change of organic matter content is one of the most important indicators to characterize the smoothness of composting[17]. The initial organic matter contents were 86.09% and 89.32% in control and SMS group, respectively. Figure 1d showed a decreasing trend of organic matter as the composting proceeded in both treatment groups, and the reducing extent leveled off slightly as well as the rate after the 6th day. At the end of the composting, the organic matter degradation rate of 15.06% and 14.33% in SMS group and control group were obtained, respectively. Higher organic matter degradation and lower C/N revealed that SMS prevented the nitrogen loss effectively, which the mechanisms of nitrogen retention will be explored in microbial communities.

3.5 Changes of GI during composting

The compost maturity is related to phytotoxic substances in the immature compost[18]. As shown in Figure 1e, the GI was 30.69% and 26.67% in SMS and control groups, respectively, due to a lot of inhibitory organic matter in initial composting stage. The toxic effect on the seeds is reduced with SMS addition. Harmful substances were decomposed with composting proceed, so the GI improved overtly in both groups. The GI reached 105.86% and 74.17% in SMS and control groups, respectively, and GI of SMS group was 42.7% higher than the control group, demonstrating its better humification level after composting. While 74.17% of GI of control group only could be considered as basically non-toxic.

3.6 Changes of cellulose and hemicellulose during composting

KW, CM, SMS, and maize stalks are rich in cellulose and hemicellulose[16]. Thus, the changes of the content of cellulose and hemicellulose in the composting process were analyzed in this study. From Figure 2a, there was a slightly decrease on the content of hemicellulose in two groups in the first 3 d, then decreased slowly in the last 6 d. At the end of composting, the content of hemicellulose in the SMS treatment and the CK treatment decreased from 19.82% and 23.69 % to 15.14% and 19.16%, respectively. The change trend of cellulose in two groups was the same as that of hemicellulose. At the end of composting, the content of cellulose in the SMS treatment and the CK treatment decreased from 26.96% and 28.02% to 21.94% and 23.17%, respectively (Figure 2b). These finding indicated that the SMS accelerated the degradation of cellulose and hemicellulose in the initial stage of composting and enhanced the compost maturity quality, which was consistent with the results by Padhan et al.[15]. Intrinsic flora of SMS are beneficial to the degradation of hemicellulose and cellulose[11]. In the later part of the thermophilic phase, hemicelluloses and cellulose decreased rapidly due to optimal environment provided by higher temperature. This result was in accordance with the study by Xu et al.[2], who reported that 70% of lignin was degraded when the temperature of the compost was around 50°C.

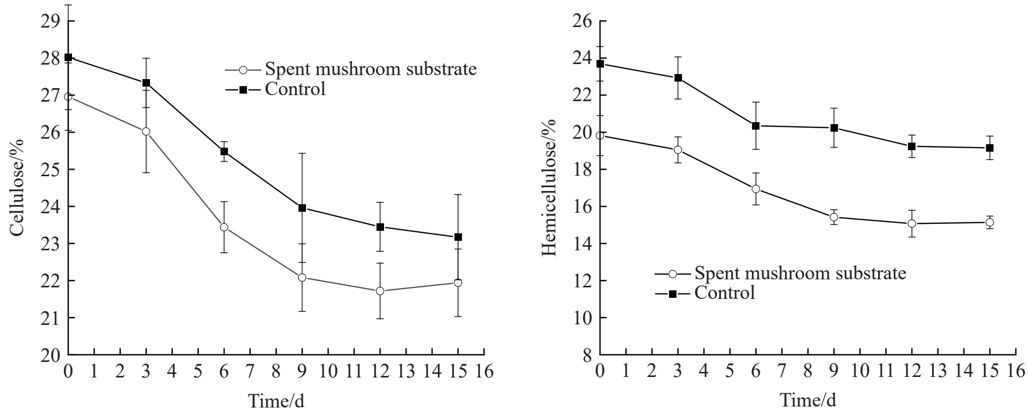


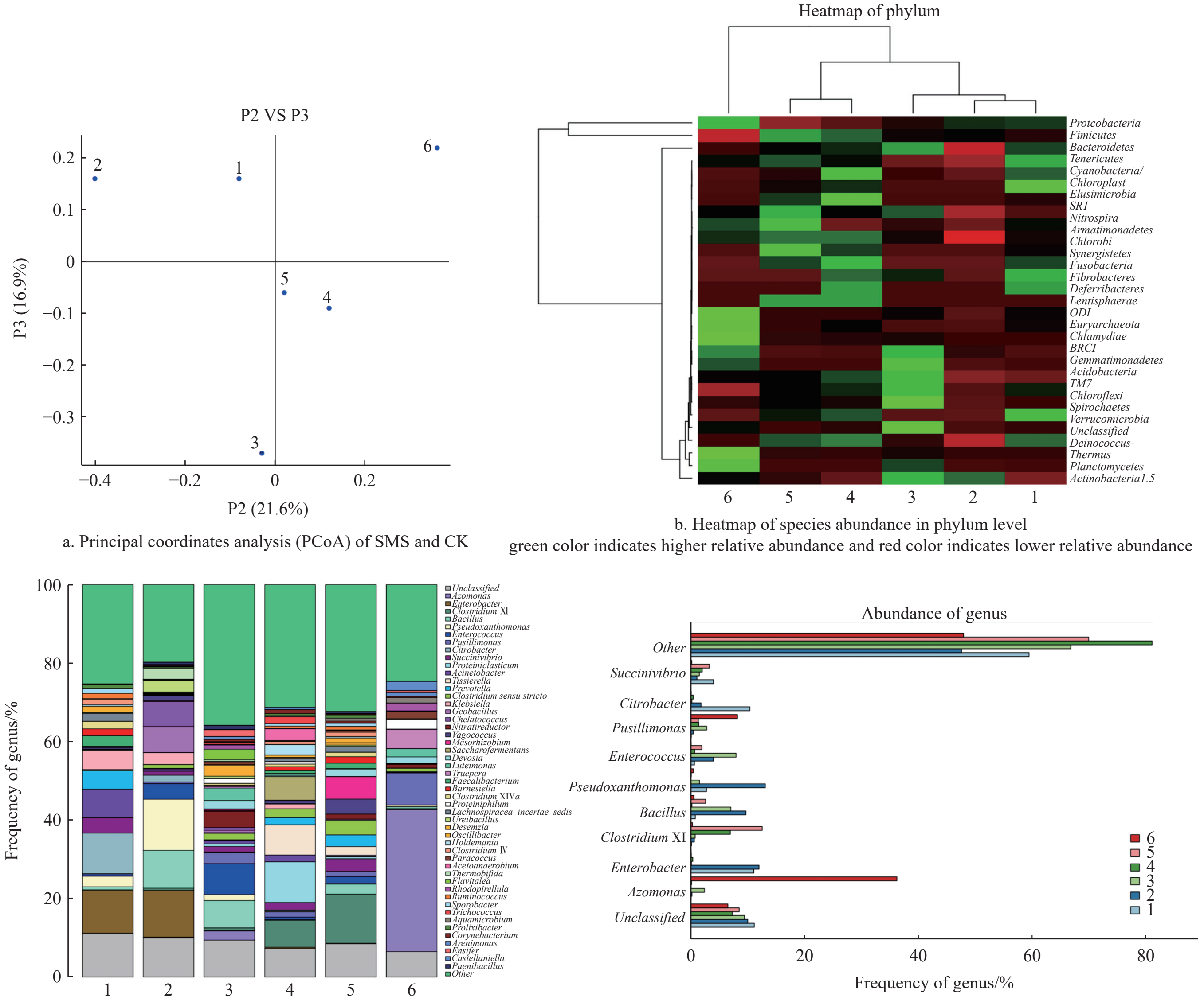
Figure 2 Changes of cellulose and hemicellulose during composting period of CK and SMS group

3.7 Changes in microbial community diversity during composting

The composting process is essentially a process of physiological metabolism and community succession of microorganisms. The changes of microbial communities are shown in Figure 3 from the compost pile of the warming, high temperature, and cooling periods in the CK (samples 1, 2, and 3) and SMS (samples 4, 5, and 6) group. The analysis of principal coordinates analysis (PCoA) demonstrated that P2 (21.6%) and P3 (16.9%) explained 38.5% (Figure 3a) of the variation in bacterial community composition among the SMS and CK group. The marked shift of bacterial community was presented due to the addition of SMS, and the heat map (Figure 3b) illustrated that the species abundance enhanced in phylum level with SMS addition, especially in warming and high temperature periods. From Figure 3c and Figure 3d, it can be seen that there are differences in the dominant microorganisms SMS at each stage of composting between the groups with and without the addition of SMS, and the microbial richness at each stage of composting is very high, with greater variation in microbial diversity at different periods of the same material and at the same period of time for different materials.

For the CK group, *Enterobacter* spp. and *Citrobacter* were presented in large quantities in the warming period of the compost and produced acid by fermenting glucose leading pH decrease, which was consistent with the change pattern of pH at the beginning of composting. Meanwhile, *Enterobacter* increase not only the pile temperature, organic matter degradation, as well as the relative content of total nitrogen, but also reduce the C/N ratio, promoting sequentially the decomposition of compost[19]. During the high temperature period, higher temperature provided a suitable ambient environment for the growth of *Pseudoxanthomona*, leading to the proliferation of *Pseudoxanthomonas*, while the degradation of organic matter in the early phase also provided sufficient metabolism for the growth of *Bacillus*. These strains have strong decomposition and transformation abilities for cellulose, hemicellulose and lignin[20], thus accelerating the bioconversion of refractory straw of piles. During the cooling period, more *Enterococcus* was presented with more organic acid production, leading to the pH decrease of the pile, which is consistent with the pH change trend at a later stage. In addition, the presence of large number *Bacillus* at this stage was beneficial to the decomposition of the mixtures. In comparison, in the warming period of SMS group, the dominant specie was *Proteiniclasticum*, a kind of sulfate-reducing bacteria, indicating great potential for nitrogen fixation by adding SMS *Clostridium* XI, *Bacillus*, and *Pseudoxanthomonas* acted synergistically to convert organic matters to a large amount of acid, making the pH drop and the substrates' acceleration degradation with the temperature increase of the pile, and also leading the transformation of harmful substances and harmlessness of the pile; In the high temperature period of compost, *Clostridium* XI proliferated and became the dominant bacteria in the genus stage by hydrolyzing sugars and proteins. During the cooling stage, *Azomonas* spp. grew rapidly, leading to the increase of the total nitrogen (TN) and the C/N ratio. Additionally, the presentation of *Pusillimonas* spp. was conducive to more substrates decomposition by utilizing petroleum hydrocarbons in the SMS group.

Notably, how to increase the N conversion and reduce the N_2O emission in composting process has been the focus of attention. In this study, the abundance of *Azomonas* reached an astonishing 36.18% and the composting time reduced obviously with SMS addition. Combining the effects of each genus of bacteria during the composting process, it was observed that the microflora composition by adding SMS significantly changed, and the higher relative abundance of bacterials has appeared compared to control group. Moreover, the proliferation of *Clostridium* XI, *Bacillus* and *Pseudoxanthomonas* further accelerated the degradation of organic matter and crude fibers in SMS group, consequently, composting temperature, humification and decomposition were significantly



a. Principal coordinates analysis (PCoA) of SMS and CK

b. Heatmap of species abundance in phylum level
green color indicates higher relative abundance and red color indicates lower relative abundance

c. The relative abundance of major communities

d.the bacteria distribution map of each sample in the genus level

Note: Samples 1, 2, and 3 represent the heating period, high-temperature period and cooling period of the added CK group, respectively. Samples 4, 5, and 6 represent the heating period, high-temperature period and cooling period of the SMS group, respectively.

Figure 3 Microbial community variation during composting period of CK and SMS group

higher than control group without SMS.

4 Conclusions

SMS, as an additive, is a promising conditioning agent for enhancing the humification quality with KW, CM, and maize straw. Compared with control group, the temperature and GI was raised obviously, and the lower C/N ratio and higher degradation of organic materials were conducive to nitrogen retention in SMS composting group. The microbial composition showed significant change in species and relative abundance, especially *Proteiniclasticum*, *Clostridium* XI and *Azomonas* became dominant genera, leading to nitrogen fixation and compost time reduction. It is apparent that SMS addition is beneficial to humification and nutrient retention in aerobic fermentation of organic wastes. Our study will provide a feasible strategy for nitrogen fixation in the field of organic waste recycling.

Acknowledgements

This project was funded by the Key Research and Development Projects of Heilongjiang Province (Grant No. 2022ZX02C14), the Technological Project of Heilongjiang Province "the Open Competition Mechanism to Select the Best Candidates" (Grant No. 2022ZXJ05C01), and National Key R&D Program of China (Grant No. 2023YFD1501000)

[References]

[1] Zhang L X, Gao X Z, Li Y M, Li G X, Luo W H, Xu Z C. Optimization of free air space to regulate bacterial succession and functions for alleviating gaseous emissions during kitchen waste composting. Bioresource Technology, 2023; 387: 129682.

[2] Xu Z C, Qi C R, Zhang L X, Ma Y, Li J G, Li G X, et al. Bacterial dynamics and functions for gaseous emissions and humification in response to aeration intensities during kitchen waste composting. Bioresource Technology, 2021; 337: 125369.

[3] Chi C P, Chu S H, Wang B, Zhang D, Zhi Y, Yang X J, et al. Dynamic bacterial assembly driven by *Streptomyces griseorubens* JSD-1 inoculants correspond to composting performance in swine manure and rice straw co-composting. Bioresource Technology, 2020; 313: 123692.

[4] Qu H, Cao J, Wang P, Li R, Qi Z, Fu J, et al. Volatile organic compounds and dominant bacterial community during aerobic composting of vegetable waste and cow manure co-complexing. BioResources, 2022; 17(1): 1338–1353.

[5] Han Y, Liu W Y, Chang N, Sun L, Bello A, Deng L T, et al. Exploration of

β-glucosidase-producing microorganisms community structure and key communities driving cellulose degradation during composting of pure corn straw by multi-interaction analysis. Journal of Environmental Management, 2023; 325: 116694.

[6] Hanajima D. Collection of ear corn residue and its utilization as a bulking agent for cow manure composting. Animal science journal, 2020; 91(1): e13323.

[7] Qian X, Bi X H, Xu Y F, Yang Z W, Wei T T, Xi M J, et al. Variation in community structure and network characteristics of spent mushroom substrate (SMS) compost microbiota driven by time and environmental conditions. Bioresource Technology, 2022; 364: 127915.

[8] Kong W L, Sun B, Zhang J Y, Zhang Y T, Gu L K, Bao L J, et al. Metagenomic analysis revealed the succession of microbiota and metabolic function in corncob composting for preparation of cultivation medium for *Pleurotus ostreatus*. Bioresour Technol, 2020; 306: 123156.

[9] Wang M H, Liu Y, Wang S Q, Wang K, Zhang Y. Development of a compound microbial agent beneficial to the composting of Chinese medicinal herbal residues. Bioresour Technol, 2021; 330: 124948.

[10] Miao Y C, Li J J, Li Y, Niu Y H, He T H, Liu D Y, et al. Long-term compost amendment spurs cellulose decomposition by driving shifts in fungal community composition and promoting fungal diversity and phylogenetic relatedness. Environmental Microbiology, 2022; 13(3): e00323–22.

[11] Khalil S, Panda P, Ghadamgahi F, Barreiro A, Rosberg A K, Karlsson M, et al. Microbial potential of spent mushroom compost and oyster substrate in horticulture: Diversity, function, and sustainable plant growth solutions. Journal of Environmental Management, 2024; 357: 120654.

[12] Liu Q, Huang B, Hu S J, Shi Z W, Wu J, Zhang Y T, et al. Effects of initial corncob particle size on the short-term composting for preparation of cultivation substrates for *Pleurotus ostreatus*. Environmental Research, 2024; 248: 118333.

[13] Wang L X, Chang R X, Ren Z P, Meng X, Li Y M, Gao M. Mature compost promotes biodegradable plastic degradation and reduces greenhouse gas emission during food waste composting. Science of The Total Environment, 2024; 926: 172081.

[14] He X Q, Peng Z H, Zhu Y X, Chen Y F, Huang Y P, Xiong J P, et al. Wheat straw biochar as an additive in swine manure Composting: An in-depth analysis of mixed material particle characteristics and interface interactions. Waste Management, 2024; 176: 41–51.

[15] Padhan K, Patra R K, Sethi D, Mohanty S, Sahoo S K, Panda N, et al. Exploitation of cellulose degrading bacteria in bioconversion of agro-wastes. Chemosphere, 2024; 347: 140654.

[16] Wang S C, Long H C, Hu X R, Wang H, Wang Y C, Guo J M, et al. The co-inoculation of Trichoderma viridis and Bacillus subtilis improved the aerobic composting efficiency and degradation of lignocellulose. Bioresource Technology, 2024; 394: 130285.

[17] Chen Z X, Du Z L, Zhang Z Y, Wang G A, Li J. Dynamic changes in soil organic carbon induced by long-term compost application under a wheat-maize double cropping system in North China. Science of The Total Environment, 2024; 913: 169407.

[18] Zhang Y, Yang B Y, Peng S W, Zhang Z W, Cai S Y, Yu J X, et al. Mechanistic insights into chemical conditioning on transformation of dissolved organic matter and plant biostimulants production during sludge aerobic composting. Water Research, 2024; 255: 121446.

[19] Zainudin M H, Mustapha N A, Maeda T, Ramli N, Sakai K, Hassan M. Biochar enhanced the nitrifying and denitrifying bacterial communities during the composting of poultry manure and rice straw. Waste Manag, 2020; 106: 240–249.

[20] Zhang X J, Zhan Y B, Zhang H, Wang R H, Tao X L, Zhang L P, et al. Inoculation of phosphate-solubilizing bacteria (*Bacillus*) regulates microbial interaction to improve phosphorus fractions mobilization during kitchen waste composting. Bioresour Technol, 2021; 340: 125714.