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Insight into Effects of Initial Moisture Content on the Heat-Up of Sewage Sludge Composting During Mesophilic Phase

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ABSTRACT

The effects of initial moisture content (55%, 60% and 65%) on the heat-up of sewage sludge (SS) composting during the mesophilic phase were investigated. Monitoring results showed that low moisture content (55%) increased the heating rate, high initial moisture content (60% and 65%) significantly improved the activities of cellulase and peroxidase during the mesophilic phase. Furthermore, although high moisture content improved the diversity of bacteria during composting, there were no significant differences in the microbial structure during the process of succession. So, rather than inhibiting the activity of the bacterial population, the negative effect of high moisture content (60 and 65 per cent) on composting temperature heat up was attributable to the difficulty of heating produced by the specific heat capacity of water. The correlation index between initial moisture content and microorganisms was relatively low, while the temperature was the largest environmental factor affecting the bacterial community. This conclusion provided a hint to make an association between initial moisture content and composting temperature-rising stage..

INTRODUCTION

Sewage sludge (SS) is a by-product of municipal wastewater treatment plants, and the production of municipal SS is steadily growing as sewage treatment expands (Ma et al. 2019). Composting is one of the most widely used biological treatment processes to achieve biodegradation and stabilization of organic matter in sludge while reducing the negative impact of organic matter in sludge (Zhou et al. 2018). The initial qualities of the material, such as moisture content (MC), bulking agents, organic matter content (OM), bulk density, C/N ratio, pH value, and process-related elements, such as aeration mode and flow rates, all have an impact on the process' performance (Zhou et al. 2014). Initial MC has attracted wide attention due to water's ability to dissolve organic matter, participate in microbial metabolism, evaporate away heat and regulate composting temperature. Appropriate MC is the basis of biological decomposition, too high MC can lead to anaerobic conditions, while persistent anaerobic incomplete decomposition can produce odor and phytotoxicity.

As the driving force in the composting process, microbes play a vital role in the degradation of organic matter. Unlike agricultural composting, which aims to produce organic fertilizer, the primary goal of SS composting is to dewater, deodorize, and reduce the volume and mass of the SS (Zhou et al. 2017). As a result, the higher the sludge mixing ratio in the composting mixture and the larger the SS treatment capacity of each composting tank in actual production, the higher the initial moisture content of the sludge composting process. At the same time, a higher SS mixing ratio can effectively reduce the proportion of excipients and operation costs (Zhou et al. 2018). As a result, it is widely anticipated that the initial MC of the compost mixture would be increased as much as possible to improve treatment efficiency and lower the cost of composting operations in composting facilities (Zhou et al. 2014, Zhou 2017).

Water not only provides a carrier for soluble nutrients needed by composting microorganisms but also provides a medium for chemical and biological reactions during composting (Hamoda 1998). In the composting environment, MC below 40% or above 65% also limits the growth of microorganisms. At the same time, existing references and experience demonstrate that a high MC (65%) can have negative consequences such as a lower heating rate and thermophilic phase duration. As a result, the proportion of sludge mixtures in actual sludge composting projects is often increased in summer, and the compost has a relatively high MC. The proportion of sludge mixtures is lowered in the winter, as is the MC of the compost.

During composting, bio-heat is crucial for temperature-rising, and it is produced via the microbial degradation of the compounds (e.g., complex carbon, cellulose, hemicelluloses, and proteins) in the material. As a result, the temperature may be used to not only represent microbial metabolism but also to screen bacteria for OM transformation (Ma et al. 2019).

Generally speaking, microbial activity produces heat and promotes the heating of the pile, air blast, and water evaporation cause heat loss (Cai et al. 2016). Insufficient microbial heat generation or a high specific heat capacity of water are the two explanations for the difficulties of heating the pile with a high initial MC at the same ventilation rate (Cai et al. 2016). However, the causes of MC on composting heat up, whether high specific heat capacity of water or diverse microbiological activity cause different heat up effects, are not yet obvious in principle, and even more so in practice.

As a result, it's intriguing and crucial to investigate beginning MC during the temperature-rising stage of SS composting. Gaining a better understanding of the succession of microbial communities and initial MC will considerably improve composting efficiency.

MATERIALS AND METHODS

Composting Materials

Dewatered SS was collected from Wulongkou municipal wastewater treatment plant (Zhengzhou, China). Sawdust was pinewood particles (particle size 1-2 mm). Sewage sludge and sawdust were homogenized to obtain a mixture at a ratio of 3:1 (w/w, fresh weight) as the main raw materials. To alter the initial MC of composting, three distinct piles were set up with a varying dosage of water: piles A, B, and C were added with water at a dosage of 0, 10, and 30 kg, respectively. The physicochemical properties of the experimental materials are given in Table 1.

Composting Process

Composting was conducted in three separate but identical reactors for 30 days. The reactors had a height of 120 cm, an inner diameter of 60 cm, and an effective volume of 280 L. They were made from polyethylene and covered with a 3 cm thick rubber board for thermal insulation. On the top of each reactor was a removable lid with a small hole (2 cm in diameter), and at the bottom of the reactor were uniformly distributed holes (1 cm in diameter). Temperature sensors were embedded in a metal bar and placed at heights of 20, 40, and 60 cm, with the temperature probe recording and storing temperature data once every minute.

Physicochemical Analysis

During the mesophilic phase (temperature-rising stage), triplicate samples were obtained from each pile at a depth of approximately 50cm on days 0, 1, 2, 3,5, 7, and 10 and stored at -4°C until analysis (Fig.1).

The MC of raw materials was determined by drying the samples at 105°C for 24 h. The volatile solid (VS) content was determined by measuring the loss of dry-solid mass after ignition at 550°C in a muffle furnace for 24 h. Using a combination pH meter, the pH was measured at a ratio of 1:5 after 30 minutes of shaking equilibration. Phenol disulfonic acid colorimetry is used to detect nitrate-nitrogen concentration. The activities of two microbial enzymes (cellulase and peroxidase) were determined at each sampling time. The activities of peroxidase and cellulase activity were measured according to the method of Ghose (1987).

Microbial DNA was collected from each pile on days 2, 5, and 10 during the mesophilic phase using a FastDNA soil rotation kit (MPBIO, USA) according to the trend of temperature change to determine bacterial diversity during the composting process. The concentration of the final DNA was measured using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). Primer 338F was used to amplify the V3-V4 hypervariable sections of the bacterial 16S rRNA gene (ACTCCTACGGGAGGCAGCAG).

Statistical Analysis

The average values and standard deviations of the data were calculated using Microsoft Excel software (Version 2016, USA) and figures were generated using OriginPro (Version 9.4, USA). The least significant differences (LSD) among the mean values during composting were calculated at a

Table 1: Physicochemical properties of the raw materials.

	Moisture content [%]	pH	Total organic carbon [%]	Total nitrogen [%]	Weight [kg]	Bulk density [g·cm^{-3}]
Sewage sludge	75.21	8.12	28.11	2.05	-	-
Sawdust	2.12	5.55	52.82	0.41	-	-
Pile A	56.75	8.74	49.32	1.32	120.00	428.57
Pile B	60.07	8.63	48.66	1.50	130.00	464.29
Pile C	65.40	8.60	48.97	1.49	150.00	535.71

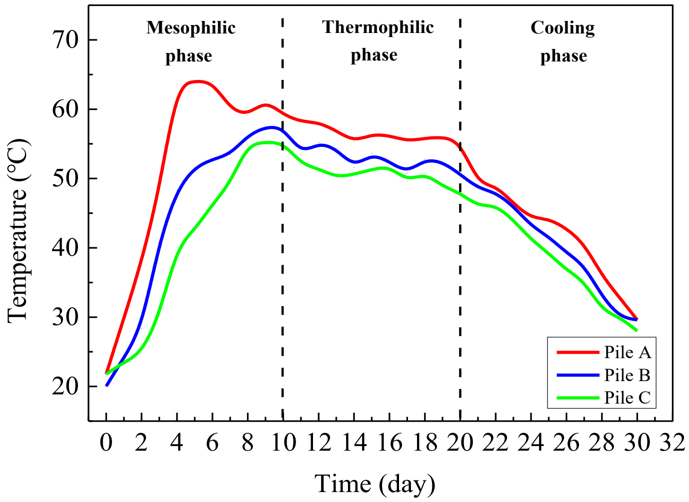

Fig. 1: Temperature of the material in the three composting piles throughout the composting process.

confidence level of $p< 0.05$ using SPSS software (Version 24.0, USA).

The degradation rates of VS were calculated as follows:

$$\text{Degradation Rate} = \frac{(A_i \times C_0 - A_0 \times C_i)}{(A_i \times C_0)} \times 100\%$$

where A_0 is the ash content of the original material of compost, A_i is the ash content of the compost on the day i, C_0 is the VS content of the original material of the compost, and C_i is the VS content of the compost on the day i.

RESULTS AND DISCUSSION

Dynamic of Physicochemical Parameters

Temperature changes: Initial MC has the greatest impact on temperature, which is widely considered as one of the most important parameters in the composting process (Zhou 2017, Ma et al. 2019). According to the change of temperature in the composting process, we divide composting processes into three phases, the rising temperature stage (0-10 days), the thermophilic stage (11-20 days), the cooling phase (20-30 days). Three piles reached the highest temperatures of 64.05°C, 57.44°C, and 56.21°C on day 5, day 10, and day 10, respectively (Fig. 1). Among them, Pile A had the highest heating rate, and Pile C had the lowest heating rate. Different initial MC causes significant changes in heating effects, which could be because water has a higher specific heat capacity than other composts (Cai et al. 2016). Heat transfer from water results in a slower heating rate and a longer composting cycle. The high MC may also cause anaerobic conditions as a result of water plugging, which will inhibit and terminate the composting process (Tiquia et al. 1996). As a result, high-throughput sequencing analysis for microbial succession during the composting process is required to better understand the effects of early MC on the mesophilic phase of SS composting, particularly insight into the microorganisms' water response mechanism during SS composting.

Changes in moisture content: Water not only provides a carrier for soluble nutrients needed by composting microorganisms but also provides a medium for chemical and biological reactions in composting (Hamoda 1998). And MC affects microbial activity, as well as the physical structure, in the composting process, and thus has a central influence on the biodegradation of organic materials (Makan et al. 2013). As shown in Fig. 2a, the MC of the three piles showed a gradual decline. On day 10, the MC of Pile A, Pile B, and Pile C decreased from 55.49%, 60.30% and 65.06% to 50.98%, 56.61% and 62.25%, respectively, down 4.51%, 3.69% and 2.81%, respectively. Because the loss of water is primarily the consequence of water evaporation, which is directly related to composting temperature and ventilation pore, Pile A's rapid heating and high temperature resulted in the biggest decrease in MC. The greater the evaporation of water with constant ventilation and ventilation pores, the higher the temperature (Cai et al. 2016, Ma et al. 2019).

Changes in volatile solids: VS is an important energy source for microbes (Jain et al. 2015). As shown in Fig. 2b, the VS of the three piles of treatments showed a gradual decline. The VS of Pile A, Pile B, and Pile C decreased from the initial 83.83%, 82.97%, and 82.20% to 74.31%, 76.11%, and 78.31% during the mesophilic phase, respectively. The VS degradation rates of the three piles were 11.3%, 8.2%, and 4.7%, respectively. Among them, the degradation rate of VS in Pile A was the highest, while that in Pile C was the lowest. This may be due to the premature dehydration of the compost due to the low MC in the composting process, which made the heap physically stable, but biologically unstable (Makan et al. 2013).

Changes in pH: The pH strongly affects microbial activity during composting and the degradation process can be enhanced by pH control (Gajalakshmi & Abbasi 2008). As shown in Fig. 2c, the changing trend of pH in the three piles was similar, showing a trend of first decreasing and then rising. The pH of Pile A, Pile B, and Pile C decreased from the initial 8.64, 8.63, and 8.60 to 8.5, 8.54, and 8.57 on day 3, and then gradually increased to 8.79, 8.75, and 8.67 on day 10. There was no significant difference in pH among the three piles during 0 to2 days, but the pH in Pile C during 3 to10 days was significantly lower than that in Pile A and Pile B. This may be due to the partial anaerobic reaction of high moisture content in Pile C, which results in low pH.

Changes in NO_3^--N: The changing trend of NO_3^--N content in the three treatments was similar (Fig. 2d). NO_3^--N in Pile A, Pile B, and Pile C increased gradually from initial 0.83 $g.kg^{-1}$, 0.86 $g.kg^{-1}$ and 0.82 $g.kg^{-1}$ to 2.5 $g.kg^{-1}$, 2.6 $g.kg^{-1}$ and 1.4 $g.kg^{-1}$ during mesophilic phase, respectively. There was no significant increase in NO_3^--N content in the three piles during the mesophilic phase. However, until the thermophilic phase and the cooling phase of composting, the NO_3^- -N in Pile A and Pile B began to rise rapidly, while the NO_3^--N in Pile C increased relatively slowly. This is due to the rapid degradation of organic matter into NH_4^+-N during the mesophilic phase, and the transformation of NH_4^+-N to NO_3^--N during the thermophilic phase and cooling phase (Gajalakshmi & Abbasi 2008). However, due to the high MC in Pile C, the local anaerobic reactions in the bioreactor lead to the low NO_3^--N content (Makan et al. 2013).

Activities of peroxidase: Peroxidase is the most intensively studied extracellular enzyme of white-rot fungi, which can oxidize the lignin polymer (Wu et al. 2017). As shown in Fig. 2e, the changing trend of peroxidase was different among the three piles. The content of peroxidase in Pile A increased first and then decreased. The maximum content of peroxidase was 136.74 μmol $(h*g)^{-1}$, which appeared on day 5. However, Pile B and Pile C decreased first and then increased, from 141.6 μmol $(h*g)^{-1}$ and 205 μmol $(h*g)^{-1}$ on day 0 to 126.16 μmol $(h*g)^{-1}$ and 126.75 μmol $(h*g)^{-1}$

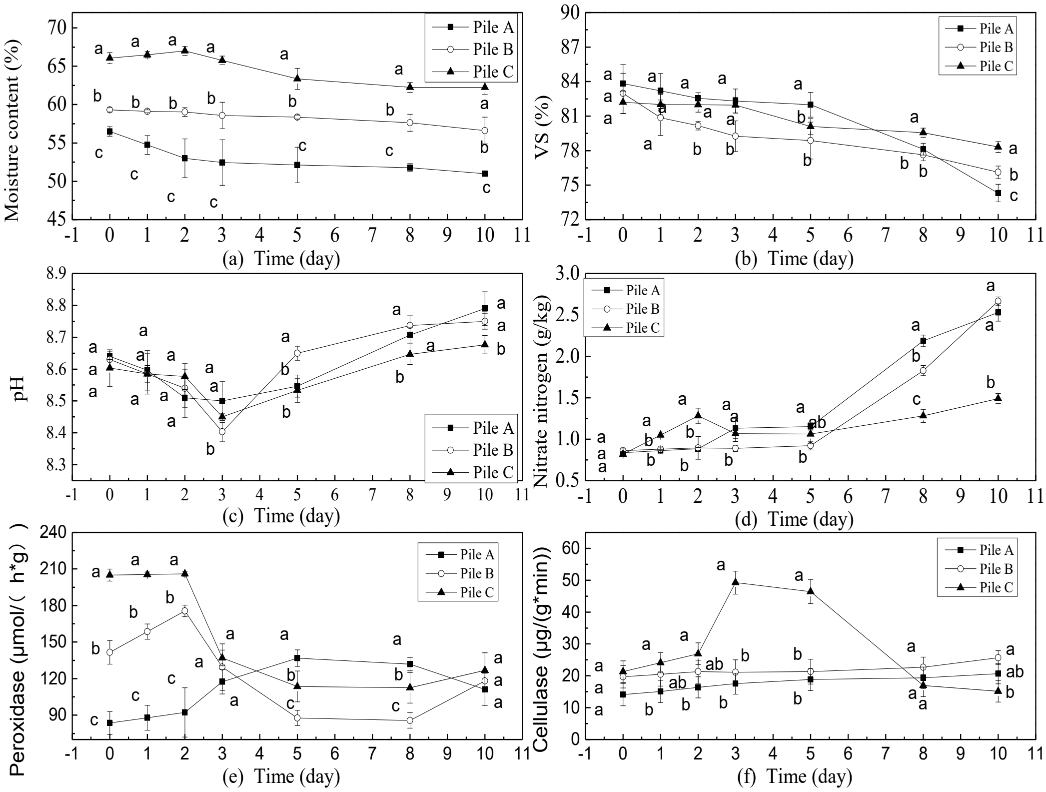

Fig. 2: Moisture content, VS, pH values, NO_3^--N content, peroxidase content, and cellulase content of three composting piles during the mesophilic phase. Note: Significance (*) is considered at $P < 0.05$.

on day 10, respectively. The content of peroxidase in Pile B and Pile C was significantly higher than that in Pile A during the first three days, but the content of peroxidase in Pile A was significantly higher than that in Pile B and Pile C during day 5 to day 8. It may be deduced that high MC promotes the rise of peroxidase activity in the early stages of the mesophilic phase, which may help the compost biodegrade (Hu et al. 2019).

Activity of cellulase: The types of cellulolytic microorganisms present in the mixture influence cellulase activity, which catalyzes the hydrolysis of cellulose to D-glucose (Castaldi et al. 2008). The three heaps' cellulase change trajectories were substantially different (Fig. 2f). The cellulase content in Pile A and Pile B increased gradually, reaching the maximum on day 10, respectively. The cellulase content in Pile C increased first and then decreased, reaching its maximum on day 3. This may be due to the fact that high MC can increase the solubility of cellulase substrate, and stimulate the production of cellulase enzymes.

Evolution of Bacterial Community

Effects of moisture content on bacterial diversity: The coverage index was commonly used to describe the depth of sequencing, which presented results that accurately reflect the depth of sequencing samples (Li et al. 2015). During composting, the excellent coverage index of all samples was greater than 0.98 (Table 2), indicating that the sequencing data was appropriate. The ace index was used to measure the diversity of bacterial communities, with a higher value indicating greater diversity (Liu et al. 2017). The Shannon index was chosen to reflect the degree of bacterial community diversity, and the greater Shannon index indicated the higher diversity of the bacterial community (Huang et al. 2013, Liu et al. 2017). On day 2, the Shannon index of Pile B was significantly higher than that of Pile A and Pile C. On day 5, the Shannon index of Pile A was the lowest, while that of Pile C was the highest, and that of Pile B was in the middle. On day 10, the Shannon index of Pile B was significantly higher than that of Pile A and Pile C, and the Shannon index of Pile A was the lowest. According to the trends of the Shannon index in the three piles (Table 2), the results showed that the microbial diversity was the lowest when the initial moisture content was 55% and the highest when the initial moisture content was 60%, which was most conducive to bacterial reproduction and degradation of organic matter.

Effects of moisture content on bacterial community structure: The dynamic of bacterial community at the genus level is shown in Fig.3. On day 2, *Pseudomonas*, *Acinetobacter,* and *Candidatus_Microthrix* were the dominant bacteria in the three piles. Pile C (26.0%) had the highest proportion of *Pseudomonas* and Pile B (11.4%) had the lowest proportion of *Pseudomonas*. *Pseudomonas* is correlated with organic acids synthesis and with a concomitant drop in pH (Kiymaci et al. 2018). Pile C (19.5%) had the highest percentage of *Acinetobacter* while Pile B (10.9%) had the lowest percentage of *Acinetobacter.* Acinetobacter not only plays an important role in the rapid degradation of OM in compost but also relates to the metabolism of carbon and nitrogen. Pile B (11.5%) had the highest proportion of *Candidatus_Microthrix* and Pile A (8.1%) had the lowest proportion of *Candidatus_Microthrix.* The genus *Candidatus_Microthrix* was related to lipid metabolism by using long-chain fatty acids as a carbon and energy source (Levantesi et al. 2010). On day 5, *Planifilum*, *Bacillus,* and *Candidatus_Microthrix* were the dominant bacteria in the three piles. *Planifilum,* a thermophilic genus within Thermoactinomycetaceae (Yu et al. 2018), accounted for the highest proportion (23.3%) in Pile A and the lowest proportion (0%) in Pile C, the reason is that the temperature of Pile B and Pile C was relatively low on day 5 (Fig. 1). *Bacillus* accounted for the highest proportion (17.7%) in Pile A and the lowest proportion (0.5%) in Pile C. *Bacillus* can degrade protein and starch, was known as competent cellulose-degrading bacteria. *Candidatus_Microthrix* accounted for the highest proportion (13.5%) in Pile C and the lowest proportion (2.4%) in Pile A. On day 10, *Planifilum*, *Candidatus_Microthrix,* and *Thermobifida* were the dominant bacteria in the three piles. *Planifilum* accounted for the highest proportion (22.4%) in Pile A and the lowest proportion (0%) in Pile C. Thermobifida has been stated to be effective on cellulose and hemicellulose degradation while secreting hemicellulases and cellulases (Zhang et al. 2015). *Candidatus_Microthrix* accounted for the highest proportion

Table 2: Bacterial alpha diversity index.

Time	OTUs			Shannon			Simpson			Ace			Coverage		
	Pile A	Pile B	Pile C	Pile A	Pile B	Pile C	Pile A	Pile B	Pile C	Pile A	Pile B	Pile C	Pile A	Pile B	Pile C
DAY 2	722	749	719	4.30	4.40	4.10	0.039	0.036	0.050	885.5	942.7	924.2	0.9902	0.9912	0.9901
DAY 5	580	794	858	3.98	4.50	4.68	0.058	0.035	0.032	976.0	1012.3	1093.9	0.9904	0.9895	0.9884
DAY 10	607	766	890	4.11	4.79	4.60	0.055	0.021	0.032	985.6	961.1	1138.4	0.9905	0.9907	0.9878

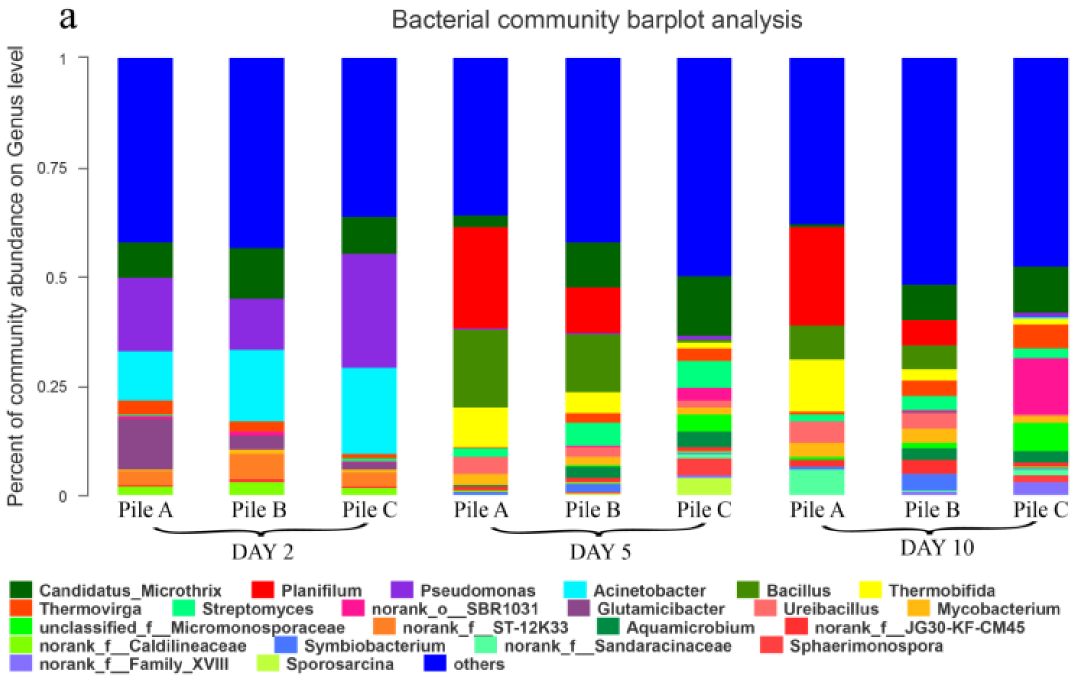

Fig. 3: Distribution of bacterial community compositions at the different composting times.

(10.7%) in Pile C and the lowest proportion (0.6%) in Pile A. *Thermobifida* accounted for the highest proportion (12.0%) in Pile A and the lowest proportion (1.2%) in Pile C.

CONCLUSIONS

The initial MC influenced the heat up during sewage sludge composting; a larger MC reduced the heating rate and the duration of the thermophilic phase. High initial MC considerably increased the activities of cellulase and peroxidase during the composting mesophilic phase. The high MC could slow down the heating process because water has a higher specific heat capacity than microbial activity, which produces insufficient heat. The impact of varied initial MC on microorganisms is primarily to alter the fraction of dominant strains rather than the species of dominant strains.

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REFERENCES

Cai, L., Chen, T.B., Gao, D. and Yu, J. 2016. Bacterial communities and their association with the bio-drying of sewage sludge. Water Res., 90: 44-51.

Castaldi, P., Garau, G. and Melis, P. 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. Waste Manag., 28(3): 534-540.

Gajalakshmi, S. and Abbasi, S.A. 2008. Solid waste management by composting: State of the art. Crit. Rev. Environ. Sci. Technol., 38(5): 311-400.

Ghose, T.K. 1987. Measurement of cellulase activities. Pure Appl. Chem., 59(2): 257-268.

Hamoda, M.F., Abu Qdais, H.A. and Newham, J. 1998. Evaluation of municipal solid waste composting kinetics. Resour. Conserv. & Recycl., 23: 209-223.

Hu, T., Wang, X., Zhen, L., Gu, J., Zhang, K., Wang, Q., Ma, J., Peng, H., Lei, L. and Zhao, W. 2019. Effects of inoculating with the lignocellulose-degrading consortium on cellulose-degrading genes and fungal community during co-composting of the spent mushroom substrate with swine manure. Bioresour. Technol., 291.

Huang, K., Li, F., Wei, Y., Chen, X. and Fu, X. 2013. Changes of bacterial and fungal community compositions during vermicomposting of vegetable wastes by *Eisenia foetida*. Bioresour. Technol., 150: 235-241.

Kiymaci, M.E., Altanlar, N., Gumustas, M., Ozkan, S.A. and Akin, A. 2018. Quorum sensing signals and related virulence inhibition of Pseudomonas aeruginosa by a potential probiotic strain's organic acid. Microb. Pathog., 121: 190-197.

Levantesi, C., Rossetti, S., Thelen, K., Kragelund, C., Krooneman, J., Eikelboom, D., Nielsen, P.H. and Tandoi, V. 2006. Phylogeny, physiology, and distribution of '*Candidatus Microthrix calida*', a new Microthrix species isolated from industrial activated sludge wastewater treatment plants. Environ. Microbiol., 8(9): 1552-1563.

Jain, S., Jain, S., Wolf, I.T., Lee, J. and Tong, Y.W. 2015. A comprehensive review on operating parameters and different pretreatment methodologies for anaerobic digestion of municipal solid waste. Renew. Sust. Energy Rev., 52: 142-154.

Li, R., Wang, Q., Zhang, Z., Zhang, G., Li, Z., Wang, L. and Zheng, J. 2015. Nutrient transformation during aerobic composting of pig manure with biochar prepared at different temperatures. Environ. Technol., 36(7): 815-826.

Liu, N., Zhou, J., Han, L., Ma, S., Sun, X. and Huang, G. 2017. Role and multi-scale characterization of bamboo biochar during poultry manure aerobic composting. Bioresour. Technol., 241: 190-199.

Ma, C., Hu, B., Wei, M., Zhao, J. and Zhang, H. 2019. Influence of matured compost inoculation on sewage sludge composting: enzyme activity, bacterial and fungal community succession. Bioresour. Technol., 20: 13-26.

Makan, A., Assobhei, O. and Mountadar, M. 2013. Effect of initial moisture content on the in-vessel composting under air pressure of organic fraction of municipal solid waste in Morocco. Iran. J. Environ. Health Sci. Eng., 10: 54.

Tiquia, S.M., Tam, N.F.Y. and Hodgkiss, I.J. 1996. Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. Bioresour. Technol., 55(3): 201-206.

Wu, J., He, S., Liang, Y., Li, G., Li, S., Chen, S., Nadeem, F. and Hu, J. 2017. Effect of phosphate additive on the nitrogen transformation during pig manure composting. Environ. Sci. Pollut. Res., 24(21): 17760-17768.

Yu, Z., Tang, J., Liao, H., Liu, X., Zhou, P., Chen, Z., Rensing, C. and Zhou, S. 2018. The distinctive microbial community improves composting efficiency in a full-scale hyperthermophilic composting plant. Bioresour.Technol., 265: 146-154.

Zhou, H., Shen, Y., Meng, H., Gao, D. and Chen, T. 2018. Effect of air temperature and aeration strategy on water removal during sewage sludge composting. Dry. Technol., 36: 1474-1480.

Zhang, L., Ma, H., Zhang, H., Xun, L., Chen, G. and Wang, L. 2015. *Thermomyces lanuginosus* is the dominant fungus in maize straw composts. Bioresour. Technol., 197: 266-275.

Zhou, H.B., Ma, C., Gao, D., Chen, T.B., Zheng, G.D., Chen, J. and Pan, T.H. 2014. Application of a recyclable plastic bulking agent for sewage sludge composting. Bioresour. Technol., 152: 329-336.

Zhou, J. M. 2017. The effect of different C/N ratios on the composting of pig manure and edible fungus residue with rice bran. Comp. Sci. Util., 25(2): 120-129.