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Influence of inoculating microbes on municipal sewage sludge composting

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Abstract

The influence of Ilkompost and Micromix bacterial consortium inoculation during sewage sludge (SS) with wheat straw (WS) composting was assessed. The effect of inoculation on compost quality parameters such as pH, temperature, nutrient contents and C/N, bacterial and fungal population were determined. Compared to the control treatment, the temperature of piles and population of microorganism increased after inoculated bacterial consortiums at the beginning of compost. But, WS addition did not effect on compost quality parameters and microbial population. Fungal and bacterial population, the peak temperature, or heating rate, of Micromix bacterial consortium based on *Streptomyces pratensis, Bacillus mesentericus, Azotobacter chroococcum* inoculated treatments was clearly higher than that of Ilkompost bacterial consortium based on *Pediococcus pentosaceus, Streptomyces sindenensis, Bacillus megaterium* inoculated treatments.

Keywords: Sewage sludge, composting, inoculation, bacteria, fungi.

Introduction

Currently, with the development of industrialization and urbanization, the yield of sewage sludge (SS) in Kazakhstan sharply increases every year (Ospanov et al., 2022). SS is a by product of waste water treatment process, which contains lots of organic matter, plant nutrients (N, P, K, Ca, Mg, etc) and harmful components such as potential toxic heavy metals and pathogenic microorganisms (Delibacak et al., 2020; Kızılkaya et al., 2021). The use of SS in agriculture includes several operations that improve its efficiency in crop production, as compared to mineral fertilizers, which are related to the stabilising process before spreading (Shashoug et al., 2017; Jatav et al., 2021). These are principally aerobic digestion, anaerobic digestion, composting, liming, and pelletisation. SS has been globally applied as an effective and cost efficient process for its management and reuse (Wang et al., 2019a). However, SS cannot be composted alone owing to its high moisture content and poor air permeability (Zhou et al., 2014), and therefore must be mixed with bulking agents to reduce the moisture content and improve the free air space of composting materials (Ma et al., 2019a). Numerous studies have evaluated the effect of these bulking agents such as sawdust, straw, cotton waste, and matured compost to regulate the physicochemical properties of the matrix during composting (Fan et al., 2019; Nie et al., 2019; Ma et al., 2019b).

Numerous microbiological and physico-chemical techniques have been developed to characterize the agrochemical properties and the maturity of compost (Gülser et al., 2015). The previous studies indicated that the presence of more stable organic matter in the mature compost, which may be good indicators of SS composting with microbial inoculation. The results on the microbial inoculation of different composting processes can be found in the literature (Biey et al., 2000; Baheri and Meysami, 2002; Barrena et al., 2006; Kızılkaya et al., 2015). It seems clear that microbial inoculation can have a positive effect on composting (Tiquia et al., 1997; Bolta et al., 2003).

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In this study, inoculation microorganisms were used in SS composting in order to increase temperature in thermophilic phase and improve the composting process. The effect of two types of bacterial consortium (named Micromix and Ilcompost) inoculation on the microbial population and compost quality parameters during the composting was also determined.

Material and Methods

Preparation of bacterial consortium

To preparation a bacterial consortium, 65 different microorganisms were isolated from samples of sewage sludge, then 15 microorganisms were selected after assessing their agro-ecological properties: nitrogen-fixing ability, growth stimulation of seedlings; cellulose destruction, inhibitor possibilities of growth and reproduction of soil pathogens. Next, microorganisms that could interact without suppressing any strain in the consortium were united. As a result, 3 consortiums of microorganisms were created, one of them did not show good growth on a nutrient medium from sewage sludge, for the remaining two consortium, liquid nutrient media were tested for growth and development activities of microorganisms. As a result, two types of bacterial consortium (named Micromix and Ilcompost) were used in the study:

i. Micromix bacterial consortium based on *Streptomyces pratensis, Bacillus mesentericus, Azotobacter chroococcum.* The inoculated micromix bacterial consortium was comprised of the enriched ONB (Oatmeal based liquid nutrient medium) and the consortium was obtained from composting samples by ONB medium (28 g Oatmeal, 0.5 g NaCl, 0,01 g $FeSO_4$ in 1 L distilled water, pH 7,2).

ii. Ilcompost bacterial consortium based on *Pediococcus pentosaceus, Streptomyces sindenensis, Bacillus megaterium*. The inoculated Ilcompost bacterial consortium was comprised of the enriched WNB (wheat bran based liquid nutrient medium) and the consortium was obtained from composting samples by WNB medium (15 g weat bran, 18 g sucrose, and 0,01 g $FeSO_4$ in 1 L distilled water, pH 7,2).

The enrichment process of ONB and WNB were as follows. 5 ml fresh suspensions of microorganisms (concentration was 1×10^8 CFU/ml) was took out and injected into 250 ml ONB and WNB medium, then cultivated at the temperature (30 ℃) and shaking speed (100 rpm/min). Culture time was set to 7 days. After 7 days of cultivation, 10 ml inoculant was transferred into fresh 250 ml enrichment medium and cultivated at the same condition. The inoculant of enriched ONB and WNB was centrifuged and suspended in sterile water, then sprayed on the composting mixture. The concentration of the inoculant was 1×10^8 CFU/ml.

Composting materials and experimental design

The dewatered municipal sewage sludge from waste water (SS) and wheat straw (WS) were used as the raw materials for composting. The dewatered SS was collected from a municipal wastewater treatment plant in Astana province, Kazakhstan. WS was collected on the field of the Research and Production Center for Grain Farming named after A.I. Baraev. The main characteristics of the raw materials were shown in Table 1.

Table 1. Physicochemical characteristics of the raw materials.

	Organic matter, %	Organic C, %	Total N, %	Total P, %	Total K, %	C/N
SS	48.0	27.8	5.2	1.1	0.2	5.4
WS	80.0	46.4	0.6	0.2	0.7	77.3

SS: dewatered municipal sewage sludge from waste water; WS: Wheat straw

The moisture content of every pile (width: 40 cm, height: 20 cm, length: 50 cm and total dry weight: 12-12.6 kg) was adjusted to 60% by addition of sterile water. Fresh air was supplied into piles by mixing for active aeration. The piles was regularly turned to provide sufficient aeration (every 3 days) and maintain the temperature. Temperature of the pin was monitored weekly by a digital thermometer during SS composting. A randomized complete plot design with three replicates per treatment was used. The experiment was performed with the following 8 treatment:

1 (control) - SS (12 kg) and no inoculation.
2- SS (12 kg) and only inoculation of Ilkompost bacterial consortium (1 ml/kg).
3- SS (12 kg) and only inoculation of Micromix bacterial consortium (1 ml/kg).
4- SS (12 kg) and only inoculation of Ilkompost bacterial consortium (2 ml/kg).
5- SS (12 kg) and only inoculation of Micromix bacterial consortium (2 ml/kg).
6- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and inoculation of Ilkompost bacterial consortium (2 ml/kg).
7- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and inoculation of Micromix bacterial consortium (2 ml/kg).
8- SS + WS (12 kg/0.6 kg, 1/0.05, w/w) and no inoculation

The whole time of experiment in this study was 52 days. Every week, Triplicate samples were collected from each pile at a depth of about 10 cm and stored at −4°C immediately until used for determination of microbial population, pile temperature and pH. And, at the end of the composting period, Triplicate samples were collected from each pile at a depth of about 10 cm and stored at −4°C immediately until used for determination of other compost quality parameters such as moisture content, organic matter, N, P, K contents and C/N ratio.

Determination of Microbial Population

Bacteria, fungi and actinomycetes play an important role in the composting. They were determined by using different medium throughout the serial dilution method (Germida, 1993). **MPA** (Meat-peptone agar media: 13 g meat peptone broth and 15 g agar, in 1 L distilled water, pH 7,6), **SAA** (Starch ammonia agar media: 10 g starch, 2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 1 g $MgSO_4$, 3 g $CaCO_3$ and 20 g agar in 1 L distilled water, pH 7,2) and **ASM** (Ashby media: 20 g sucrose, 0.2 g K_2HP0_4, 0.2 g $MgS0_4.7H_20$, 0.2 g NaCl, 0.1 g $FeSO_4$, 5.0 g $CaCO_3$ and 20 g agar in 1 L distilled water, pH 7.3) were used for evaluating bacteria population. **CDA** (Chapek-Dox Agar media: 14 g glucose, 0.7 g $CaCO_3$, 0.7 g KNO_3, 0.35 g $MgSO_4$, 0.35 g NaCl, 0.35 g K_2HPO4, 0.01 g $FeSO_4$ and 20 g agar in 1 L distilled water, pH 6,0) **GAM** (Gause's No.1 media: 20 g soluble starch, 1 g KNO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 0.01 g $FeSO_4$ and and 20 g agar in 1 L distilled water, pH 7,2) and **HUM** (Hutchinson's media: 2.5 g $NaNO_3$, 0.01 g $FeCl_3$, 1 g K_2HPO_4, 0.3 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.1 g $CaCl_2$ 20 g agar in 1 L distilled water, pH 7.2) were used for evaluating fungi and actinomycetes population (Germida and de Freitas, 2008). 1 g of compost sample was diluted in 10 ml sterile water and shaken at 150 rpm for 1 h, then the suspension was diluted with sterile water at 10^{-1}–10^{-6}. 0.1 ml diluted solution (10^{-1}–10^{-6}) was inoculated into 7 ml of MPA, SAA, ASM, CDA, GAM and HUM. Each treatment repeated 3 times. Mediums were incubated at 30 ℃for 7 days. Hutchinson media was incubated 30 ℃for 21 days.

Determination Compost Quality Parameters

The changes in temperature of the compost piles were monitored using mercury thermometer. The pH of the compost sample was determined by pH meter using 1:10 sample water suspension ratios. Compost samples were dried and sieved to less than 0.25 mm. The organic matter content was determined by measuring the loss of dry-solid mass in muffle at 550 ℃for 6 h. Total nitrogen content was determined using the Kjeldahl digestion method. Total phosphorus content was determined using the Vanadomolybdo phosphoric Acid colorimetric method and total potassium content was determined Flame photometric method after wet digestion (Jones et al., 1991; Jones, 2001). All the experiments were performed in triplicate.

Results and Discussion

Microbial Populations

The change of bacterial population in different media was shown in Figure 1. The MPA (Meat peptone agar media), SSA (Starch ammonia agar media) and ASM (Ashby media) media were used as measures of the number of bacterial community in the piles. The population of bacteria in MPA and ASM media all showed an upward trend over the maturation phases and reached the peak value on the day 50. On the other hand, bacterial population in SAA media showed an upward trend over the mesophilic phase after thermophilic phase and reached the peak value on the day 40. Vargas-Garcia et al (2010) presented the similar trend in population of ammonifying bacteria during the compost of sewage sludge, which was also reported by Meng et al. (2016). In SSA media, at the early mesophilic and thermophilic phases of composting, the population of bacteria had increased due to the presence of readily available organic substrates which was energy for microbial growth. Later, the decrease in the population of bacteria for all treatments from day 40 to the end of composting was probably because the energy sources such as proteins, fats and amino acids were exhausted. The bacterial population in treatments was a little higher than that in control treatments within the initial 10 days and all treatments showed the similar tendency on day 20 to day 50. Therefore, inoculation of enriched Micromix and Ilkompost bacterial consortium was not detrimental to the growth of indigenous bacteria.

The change of fungal population throughout composting was shown in Figure 2. Fungal population in different media had been detected in the piles. The CDA (Chapek-Dox agar media), HUM (Hutchinson's media media) and GAM (Gause's No.1 media) were used as measures of the number of fungal community in the piles. The profile indicated that the fungal populations of inoculation of enriched Micromix and Ilkompost bacterial consortium were much larger than those of control treatment during the whole composting time, with the order as following: Micromix bacterial consortium > Ilkompost bacterial consortium > No inoculation > Control. In treatments, the fungal population increased quickly in the initial mesophilic phase, and later reached at thermophilic and second mesophilic phase. As a comparison, the corresponding peak level of fungal population in treatments was done at fourty days.

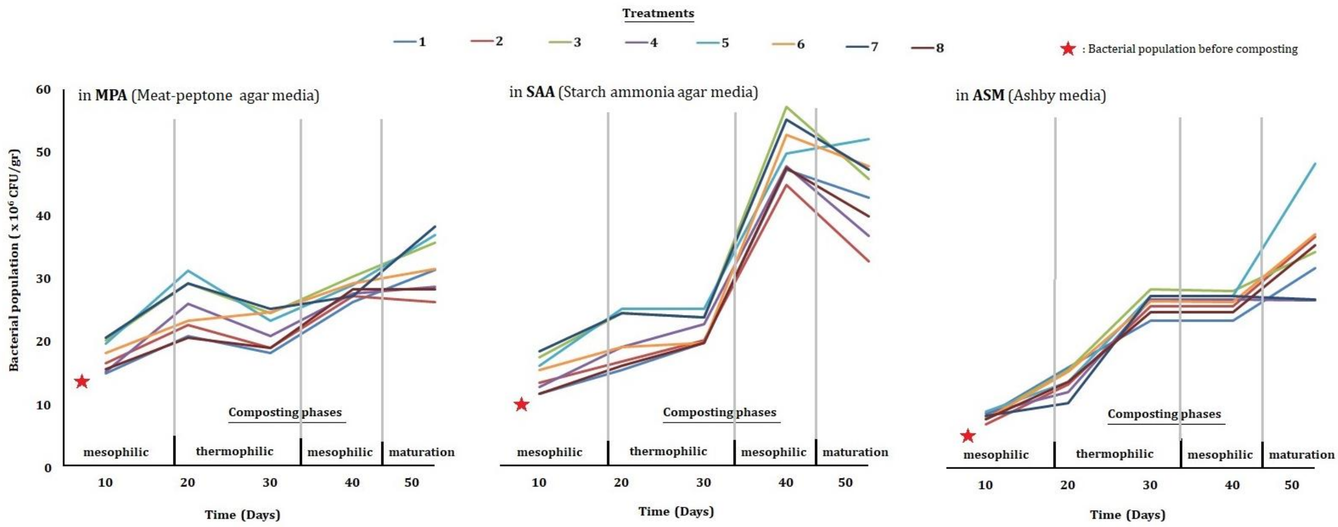

Figure 1. Changes of bacterial population in MPA-Meat peptone agar media (a), SSA-Starch ammonia agar media (b) and ASM-Ashby media (c) during composting

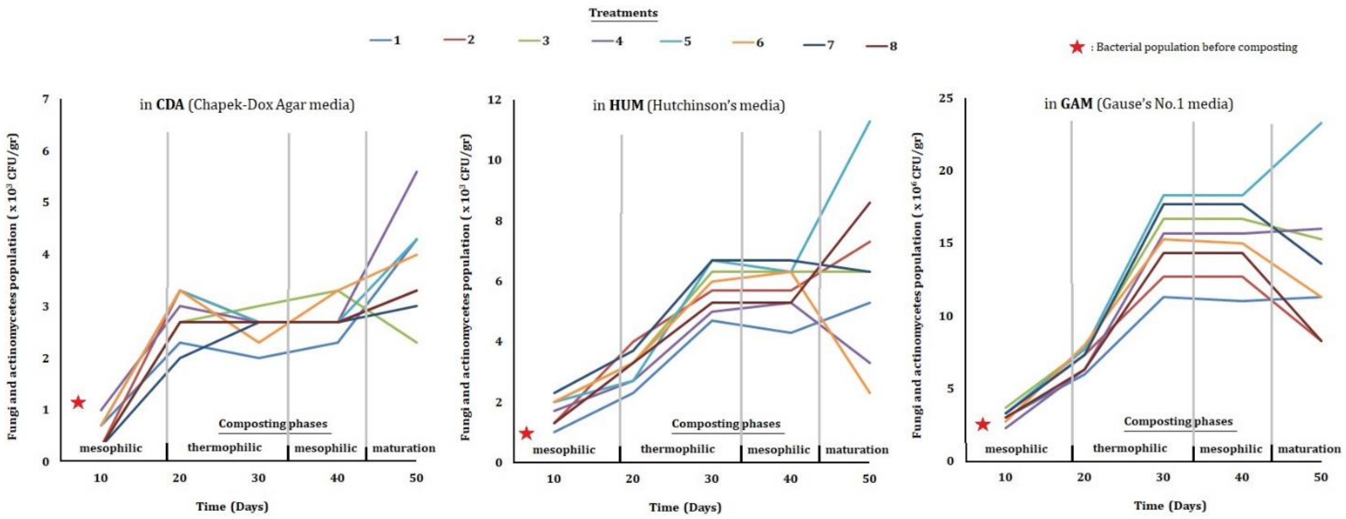

Figure 2. Changes of fungal population in CDA-Chapek-Dox agar media (a), HUM-Hutchinson's media media (b) and GAM-Gause's No.1 media (c) during composting

Compost Quality Parameters

Temperature has been widely recognized as one of the most important parameters in the composting process (Sullivan and Miller, 2001; Zhou, 2017), which can directly reflect the composting efficiency and microbial activity (Manu et al., 2017; Wang et al., 2019b). The variation of temperature during composting process was shown in Figure 3a. The temperature during composting went through three distinct phases, including mesophilic phase (0–2,5 weeks), thermophilic phase (2,5-5 week) and cooling phase (5-7 week), which were similarly found by Li et al. (2019) and Wang et al. (2019c). Compared to control (treatment 1), the temperature of treatments rapidly increased after inoculated Micromix and Ilkompost bacterial consortium at the beginning of compost. As shown in Figure 1a, Treatment 3, 5 and 7 reached the highest temperatures on 3 weeks at 42.5°C, 42.8°C and 43.1°C, respectively. The peak temperature, or heating rate, of Micromix bacterial consortium inoculated treatments (3, 5 and 7) was clearly higher than that of Ilkompost bacterial consortium inoculated treatments (2, 4 and 6). This may be due to the inoculation of the Micromix bacterial consortium in piles, which would result in a more affective microorganism in this consortium. In addition, Micromix bacterial consortium inoculated treatments had a long high thermophilic duration of 3 weeks and 5 weeks (>40°C), which could meet the hygienic index of composting, while effectively killing pathogenic microorganisms and weed seeds in the compost (Wang et al., 2019b), respectively. It could be the result of inoculation of enriched Micromix and Ilkompost bacterial consortium, which could accelerate the decomposition rate of organic matters and contribute more heat from microorganisms, thereby promoting a large temperature fluctuation. As the reaction progressed, when the temperature of composting decreased to 40 °C on 4 weeks for Micromix bacterial consortium, the process had mostly finished (Bertoldi et al., 1983). Inoculation with enriched Ilkompost bacterial consortium had no significant impact on shortening composting time in comparison with control ratment. However, inoculation with Ilkompost bacterial consortium could accelerated the rise of pile temperature and had a longer duration of high temperature, which could kill many kill many pathogenic microorganisms and weed seeds (Wan et al., 2020).

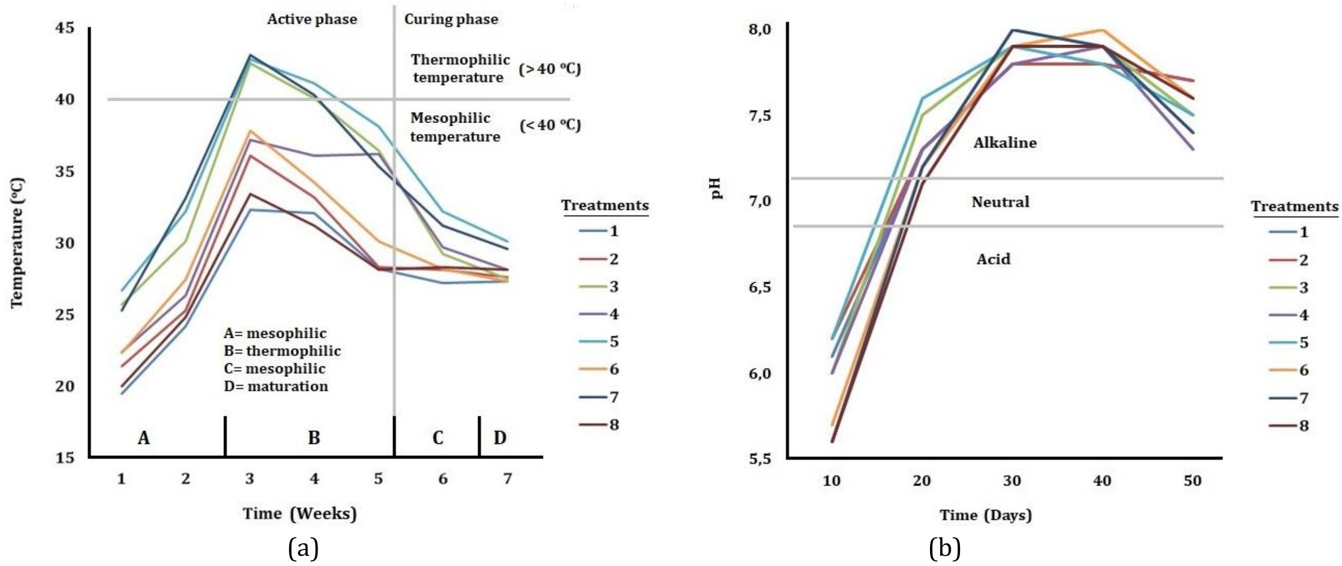

Figure 3. Changes of temperature (a) and pH values (b) during composting

The pH also strongly affects microbial activity during composting (Bareither et al., 2013), and the degradation process can be enhanced by appropriate pH control (Gajalakshmi and Abbasi, 2008). The change of pH in treatments was shown in Figure 3b. As shown in Figure 1b, the trend of the eight piles was similar, with an overall trend of a gradual increase in the pH with ultimate stabilization at an alkaline pH, which is consistent with previous results (Chang et al., 2019). During the mesophilic phase, the pH in all piles increased slightly. The increase in pH might be attribute to consumption of short-chain organic acids and the formation of ammonium (NH_4^+) ions (Tong et al., 2019). Moreover, the addition of matured compost significantly reduced the pH in the mesophilic phase, and significantly increased the pH of thermophilic phase. However, there was no significant difference in the pH among the piles during the cooling phase of composting. Afterward, the pH of treatments slowly decreased and kept approximately 7.5 until the end of compost because of the ammonia emission and ammonium oxidation by nitrobacteria (Rihani et al., 2010). Wang et al. (2017) pointed out that the optimal pH value of final composting production ranged from 6.9 to 8.3. In this study, the final pH value of composting production of all treatments reached the standard (pH 7.29–7.67).

The change of some compost quality parameters in different media was shown in Table 2. The initial organic matter and organic carbon contents in SS and WS were higher than in all treatments. The total carbon and organic matter content of the compost showed a decreasing trend with the advancement of composting. The release of CO_2 leads to a reduction in C/N ratio. The composting is an aerobic process, in which diverse microbes are involved. By changing the microbial diversity, the composting process can be altered. Most of the organic material consists of macromolecules, which cannot be penetrated easily. Therefore, microorganisms secrete enzymes, which degrade the polymers to small organic materials. According to Golueke (1992) and Sivapalan et al. (1994), low colony forming unit's value must be taken as an indicator of matured compost. Death cells of microbes in turn, increase the nitrogen content.

Table 2. Changes in some compost quality parameters at the end of the composting period

Treatments	Organic matter,%	Organic C, %	Total N, %	Total P, %	Total K, %	C/N
1	34.25	19.87	1.77	1.87	0.21	11.22
2	34.10	19.78	1.94	1.62	0.31	10.19
3	35.29	20.47	1.97	1.87	0.23	10.39
4	36.74	21.31	2.04	1.89	0.41	10.44
5	35.52	20.60	2.07	1.75	0.24	9.95
6	42.52	24.66	4.37	1.91	0.30	5.64
7	43.86	25.44	4.02	1.80	0.27	6.32
8	40.52	23.50	3.94	1.72	0.26	5.96

Composting materials with a low C/N ratio results in greater N losses than waste with a high C/N ratio (Zhou, 2007). The C/N ratio is an important quality parameter in composting because it gives a characterization of the decomposition of organic matter in the compost. Microorganisms use about 30 parts of carbon for every part of N. Thus, an initial C/N ratio of 20–35 will be most favorable for quickly converting organic waste into compost. Sewage sludge typically has a C/N ratio of less than 15. Although decomposition will be rapid at this ratio, nitrogen can be lost in large amounts as ammonia (Jiang et al., 2011). In the present study, the C/N ratio was 5.4 in the treatment 1, 2, 3, 4 and 5 and 8.82 in the treatment 6, 7 and 8 with WS added at the beginning of composting. The C/N ratio of compost pile provides an indication of the kind of compost and how it can be managed while mixed to the soil. Initially the C/N ratio of SS and WS were 5.4 and 77.3, respectively. At the end of composting period, it was determined that the C/N ratios of the treatments varied between 5.64 and 11.22. Asija et al. (1984) also recorded a decrease in C/N ratio with the increase in the period of decomposition (Table 2).

In this study, results showed that the level of P in all piles was high compared to the initial contents of SS and WS. Similar results have been determined Wei et al. (2015) and Du et al (2018). On the contrary, there was also a significant decrease in the N concentration in the final product compared to the initial contents of SS and WS. Regarding the loss of nitrogen during composting, Wang et al. (2016) determined that nitrogen is reduced as a result of ammonia volatilization at the initial stage of composting. Similarly, Hua et al (2009) determined that the decrease in total N content in the early stages of composting was due to loss of N in the form of ammonia, which in turn depends on the type of material and its C / N ratio.

Conclusion

In this study, Ilkompost and Micromix bacterial consortium were inoculated in composting of SS. Compared to control, the temperature of piles rapidly increased after inoculated Micromix and Ilkompost bacterial consortium at the beginning of compost. Moreover, inoculation increased the population of bacteria and fungi in the composting phases. During aerobic composting of SS, addition of WS enhanced the population of bacteria and fungi during the composting phases, but reduced the peak temperature. WS addition in aerobic SS composting as a bulking agent is not necessary. But, the inoculation of Micromix is more active bacterial consortium than Ilkompost might be a useful strategy to improve compost quality parameters and increase microbial population in aerobic SS composting.

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