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Biochar

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Composting of municipal solid waste with microbial-inoculated biochar amendment: impact on process and end-product quality

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Abstract

This study investigated the application of biochar and microbial-inoculated biochar in municipal solid waste (MSW) composting to enhance and accelerate the process. Microbial consortium from the active composting phase was utilized for inoculation and biofilm formation on the biochar surface. Five experimental windrow piles were established, including a control pile without biochar, and piles amended with either biochar or microbial-inoculated biochar. The composting process and the quality of the final product were evaluated by analyzing a range of physicochemical and biological parameters. The results demonstrated that piles amended with inoculated biochar exhibited higher levels of FDA hydrolytic activity and organic matter reduction, indicating enhanced microbial activity. Notably, piles 3 and 5, amended with biochar inoculated with a bacterial consortium and a bacterial-fungal consortium, respectively, achieved the highest composting temperatures (65 °C) and produced the highest-quality end products (C/N ratio: 10.1–11.8, Germination index: 100, and fecal coliform levels within acceptable limits) compared to the control piles. These findings provide valuable insights into the practical application of microbial-inoculated biochar in the real field of MSW composting, offering a promising approach to optimize composting efficiency and product quality.

Highlights

- The findings reveal how microbial-inoculated biochar enhances MSW composting efficiency.
- Piles amended with inoculated biochar reached the highest temperature.
- Using biochar containing microbial biofilm improved enzyme activities in MSW composting.
- The best end product was obtained in piles containing microbial-inoculated biochar.
- The FDA hydrolysis is a useful indicator to monitor the microbial activity of composting.

Keywords Composting, Biochar, Amendment, Microbial consortia, Inoculation

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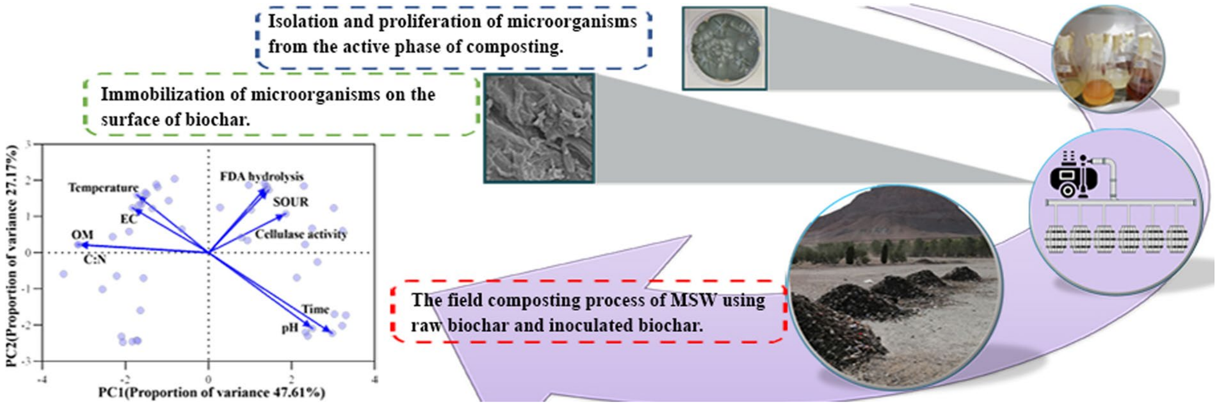
Graphical Abstract



1 Introduction

Municipal solid waste (MSW) management is a crucial aspect of sustainable development. According to estimates by the World Bank Group, global MSW generation currently amounts to 2.01 billion tons annually and is expected to rise to 3.40 billion tons by 2050 (Kaza et al. 2018).

In developing countries, a significant portion of MSW, often exceeding 50% by weight, comprises biodegradable materials, particularly food waste (Xiao et al. 2017). Due to the environmental limitations associated with certain waste management methods like incineration or landfilling, composting emerges as an ideal alternative for managing biodegradable waste (Behera and Samal 2022). However, managing the composting process and producing a stabilized and sanitized product is particularly challenging in developing countries with limited resources and infrastructure due to the rapid increase in MSW production (Behera and Samal 2022; Guo et al. 2020; Awasthi et al. 2017). Therefore, it is necessary to find a technique that can accelerate the composting process as well as improve the quality and maturity of the final product.

The application of biochar as an additive in composting of animal manure and sewage sludge has received increasing global attention over the past decade (Behera and Samal 2022; Guo et al. 2020; Awasthi et al. 2017). Research indicates that incorporating biochar into composting processes can enhance composting efficiency and improve the quality of the final product (Behera and Samal 2022; Guo et al. 2020; Ma et al. 2019). Biochar application enhances the physicochemical properties of the compost mixture, stimulates microbial activity, promotes organic matter decomposition, improves maturity, and reduces phytotoxicity (Behera and Samal 2022; Guo et al. 2020; Ma et al. 2019). Its addition has also been reported to accelerate the composting process and reduce stabilization time by fostering enhanced microbial activity. The porous structure of biochar decreases the bulk density of compost and facilitates aeration within the composting mixture, further contributing to its effectiveness (Behera and Samal 2022; Guo et al. 2020). In addition to improving compost properties, biochar application can mitigate environmental impacts by reducing ammonia volatilization and greenhouse gas emissions during the composting process (Harrison et al. 2022).

Despite the known benefits of using biochar in the composting of animal manure, studies on its application in MSW composting remain limited and the effects of biochar on composting process and quality of the final composted product are not clear. (Parra-Orobio et al. 2023; Malinowski et al. 2019; Awasthi et al. 2017). This knowledge gap highlights the need to investigate the applicability of biochar in real field conditions for MSW composting.

On the other hand, considering the undeniable role of microorganisms in the composting process, modification methods should be effective in improving the conditions to support and promote biological activities. In the environmental biotechnology, augmenting microbial biomass can enhance the levels of active microbes, thereby improving the biodegradation of organic matter. Similar to other microbial processes, the composting process can be enhanced by introducing active microbial communities, as their activities and enzymatic capabilities are pivotal for composting efficiency and acceleration (Wang et al. 2023; Liu et al. 2023; Zainudin et al. 2022; Xu et al. 2019). The direct inoculation of free-living cells or the immobilization of cells using specific carrier substances is a common method for adding microbial biomass in biological processes, with the latter being more prevalent (Tu et al. 2020). Recently, biochar has emerged as an ideal carrier due to its porous structure, high surface area, cation exchange capacity (CEC), and absorption capacity (Behera and Samal 2022; Guo et al. 2020; Xiao et al. 2017).

The use of biochar inoculated with functional bacterial or fungal strains is recognized as an effective and innovative approach for the sustainable remediation of contaminated soils (Qian et al. 2023). Recent studies have also explored the impact of inoculated biochar on the composting performance of animal manure (Wu et al. 2024; Wang et al. 2023; Ji et al. 2023). Wu et al. (2024) demonstrated that inoculating biochar with *Bacillus subtilis* during the composting of chicken manure extended the thermophilic phase, enhanced organic matter biodegradation, and facilitated the humification process of organic waste. Similarly, a study by Ji et al. (2023) on the effects of adding lignocellulose-degrading microbial agents and biochar to pig manure composting revealed that microbial agents significantly reduced the toxicity of compost products and improved compost maturity. While these studies have explored the use of inoculated biochar in the composting of animal manure, to the best of the authors' knowledge, no research has examined the application of microbial-inoculated biochar as an amendment in MSW composting. These investigations primarily focused on the co-composting of inoculated biochar with animal manures, such as chicken, pig, and cattle manure, and were conducted on a small-scale laboratory level rather than in full-scale composting scenarios.

On the other hand, while previous research has examined the effects of introducing specific microbial species into the composting process (Wu et al. 2024; Wang et al. 2023; Ji et al. 2023; Hu et al. 2019), the precise roles of various functional microorganisms involved in MSW composting are still not well understood. Therefore, the potential advantages of using biochar as a carrier for inoculating fungal and bacterial consortia to expedite and enhance MSW composting, especially on a large scale, remain underexplored. To address this gap, this study was designed to explore the practical benefits of incorporating microbial-inoculated biochar in improving the composting process and enhancing the quality of the final product in real field conditions for MSW composting.

2 Material and methods

This study employed a three-phase approach. In phase 1, biochar was prepared, and the suspension of active microorganisms was obtained from samples collected from MSW composting piles. A preliminary experiment in 500-mL containers (detailed in the supplementary file) was conducted to determine optimal conditions for microbial inoculation. In the phase 2, active microbial consortia were introduced into an inoculation reactor containing biochar. This facilitated biofilm formation on the biochar surface (Sect. 2.1.3) and finally in phase 3, the biochar, now enriched with microbial biofilms, was combined with raw MSW according to the procedure outlined in Sect. 2.2. This initiated the composting process. The study protocol diagram is presented in Figure S1.

2.1 Microbial immobilization on biochar

2.1.1 Preparation of biochar

The biochar used in this study was obtained from a company at Fars Science and Technology Park. It was produced by heating Prunus tree wood waste at a temperature of 550 °C for 6 h in the absence of oxygen. The prepared biochar was then crushed and sieved using a sieve with a mesh size of 6 mm. The physicochemical characteristics of biochar are presented in table S1 (supplementary material).

2.1.2 Isolation of active microorganisms

To establish a microbial biofilm on the biochar surface, bacterial and fungal species present during the active phase of composting were isolated. The objective of this research was to utilize a consortium of active microorganisms for inoculation, without restricting the selection to specific microbial species. It has been reported that the thermophilic phase of composting contains a greater diversity and abundance of bacteria compared to mature compost, with these microorganisms playing a critical role in organic matter biodegradation and humification (Liu et al. 2023). Sampling was carried out from the core of the piles exhibiting temperatures ranging from 45 to 50 °C, which served as the primary indicator of the active phase. Four sampling points were selected in the center of active windrow piles of MSW compost at the Isfahan composting plant in Iran. The samples were mixed, and a homogeneous sample was quickly transferred to the laboratory.

Using a sieve with a mesh size of 2 mm, the sample was sieved, and 5 g of the sieved sample was mixed with 45 mL of a 0.8% w/v NaCl solution. The mixture was shaken at 100 rpm for 30 min. After settling, the supernatant was collected and cultured in three different broths: tryptone soy broth (TSB) contain 40,000 U L^{-1} of nystatin for bacteria, casein starch broth with 40,000 U L^{-1} of nystatin for actinomycetes, and malt extract broth (MEB) containing 50 mg L^{-1} of chloramphenicol for fungi (Nafez et al. 2020). All the cultured media were incubated at 35 °C on an incubator shaker until reaching an OD600 of 1. TSB & MEB culture media were purchased from Merck company and prepared according to the instructions. The casein starch medium was prepared according to the method described in Table S2.

Several aliquots from each broth medium were spread on agar plates. After incubation, macroscopic and microscopic examinations of the microorganisms were performed for confirmation of the presence and identification of bacteria, actinomycetes, and fungi.

2.1.3 Establishment of inoculation reactors

Six containers with a volume of 100 L were selected to establish the inoculation reactor (as presented in supplementary file, Fig. S3). A 10% w/v suspension of biochar was prepared in a minimal nutrient medium with glucose as the carbon source, resulting in 50 L of suspension. Concentrated bacterial (including cultured bacteria and actinomycetes) and fungal cultures prepared from MSW compost (Sect. 2.1.2) were added to containers, with bacterial culture added to three containers and fungal culture added to the other three. Nutrients and glucose were regularly added to the containers every 24–48 h. Aeration was provided through nozzles installed at the bottom of the containers to ensure oxygen supply and mixing. After 15 days, a scanning electron microscope (SEM- Leo 1430, PV, Carl Zeiss Jena, Germany) was used to confirm the formation of a biofilm layer on the surface of the biochar. To calculate the weight of the biofilm, both the inoculated biochar sample and the raw biochar were dried at 105 °C for 24 h to remove moisture. Following this, the biochar samples were heated to 550 °C for 2 h. The biofilm weight was determined by comparing the weights of the biochar before and after the heating process at 550 °C. Additionally, the immobilized microorganisms on biochar were identified based on macroscopic and microscopic characteristics (Xing et al. 2021). For this purpose, biochar samples were immersed in 0.8% w/v NaCl solution, and the suspension was shaken for 30 min to detach microorganisms from the biochar surface. Then duplicate aliquots of the suspension were cultured on tryptone soy agar (TSA), casein starch agar and malt extract agar (MEA) plates. The bacterial and fungal colonies growing on the culture media were isolated, stained and characterized based on the colony and cell morphology as well as molecular analysis (Nafez et al. 2020).

2.2 Composting process

In the outdoor space of the Isfahan composting plant in Isfahan, Iran, five compost piles were created. Each pile consisted of 500 kg of fresh MSW. The main characteristics of municipal solid wastes used in this study are shown in Table S3.

The piles were formed as follows to investigate the effects of adding biochar and biochar inoculated with a microbial consortium:

Pile No. 1: Control pile consisting of 500 kg of MSW.

Pile No. 2: Pile consisting of 500 kg of municipal solid waste + 10 kg of raw biochar (2% w/w) without inoculation.

Pile No. 3: Pile consisting of 500 kg of municipal solid waste + 10 kg of biochar inoculated with a bacterial consortium biofilm.

Pile No. 4: Pile consisting of 500 kg of municipal solid waste + 10 kg of biochar inoculated with a fungal consortium biofilm.

Pile No. 5: Pile consisting of 500 kg of municipal solid waste + 5 kg of biochar inoculated with a bacterial consortium biofilm + 5 kg of biochar inoculated with fungal consortium biofilm.

The rates of biochar addition to different kind of waste materials typically reported in a range from 2 to 20% (w/w). However, because of technical and economic issues of inoculation we used the minimum rate of inoculated biochar in composting piles. Additionally, it was reported that a high biochar application ratio (more than 10%) may interfere with organic waste biodegradation (Xiao et al. 2017).

The piles were formed in a conical shape with final dimensions of 1.1 m in height and 1.8 m in diameter. For the first 14 days, the piles were manually turned every three days. From the 14th day until the end of the maturation phase, the piles were turned at weekly intervals. To maintain optimal moisture levels, humidity measurements were conducted every three days and appropriate amounts of water were added during pile turning to keep the humidity within the optimal range of 40–60%. Sampling and analysis of the piles were performed until the completion of the maturation phase on the 60th day.

2.3 Sampling and analysis of composting process

The temperature at the center of the compost piles was measured daily at 9 a.m. Sampling was conducted on days 0, 5, 10, 14, 21, 28, 45, and 60 after the start of the process to analyze both physicochemical and biological parameters. A composite sampling method was employed in this study. Four random points were selected from each pile during each sampling stage, and samples of appropriate volumes were collected. These four samples were then combined to obtain a homogeneous and representative sample for each pile. The samples were promptly transported to the laboratory and stored at 4 °C for biological analysis. Another portion of the samples was air-dried, crushed using an electric mill, and sieved through a 2 mm mesh sieve for physicochemical analysis.

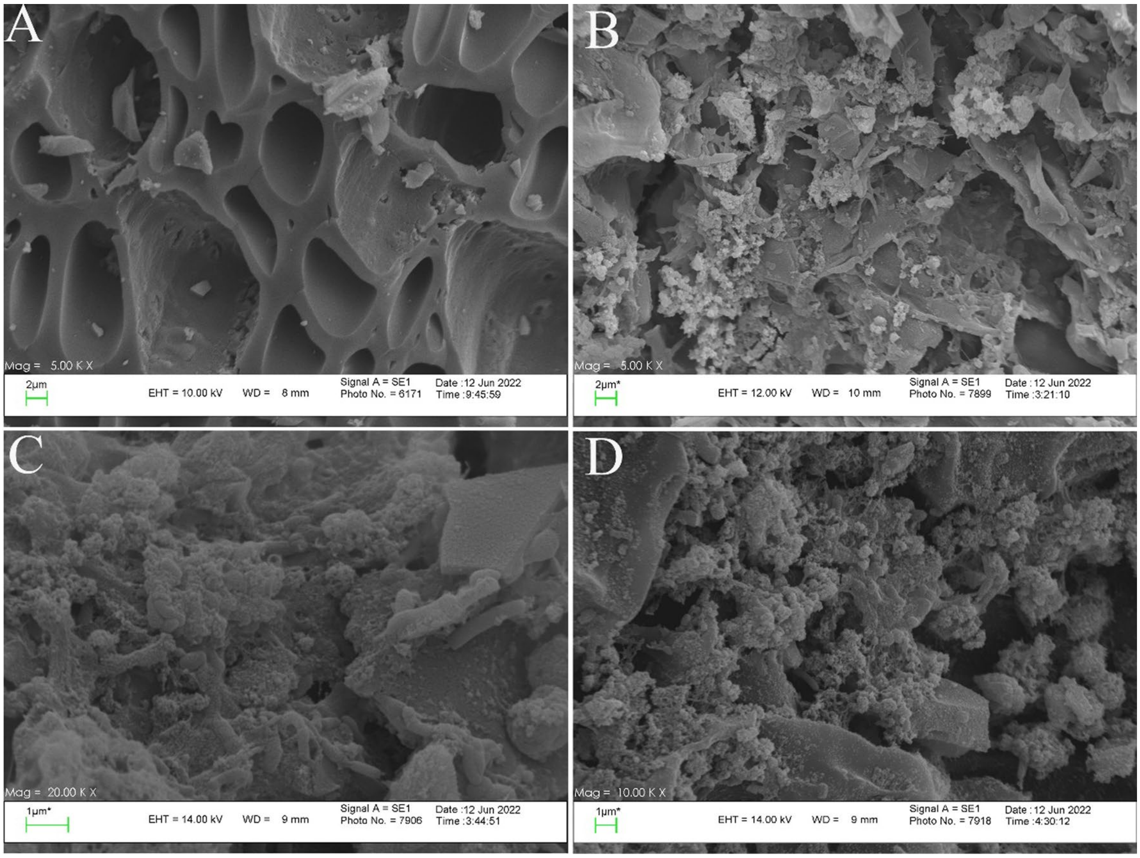


Fig. 1 SEM images of biochar **A** Raw biochar. **B**, **C** & **D** Inoculated biochar

2.3.1 Physicochemical analysis

For the physicochemical analysis, an aqueous extract of the compost was prepared at a ratio of 1:5 (weight to volume) using distilled water. The pH and electrical conductivity (EC) of the extract were measured using a pH meter (AZ-86502, Taiwan) and an EC meter (AZ-86503, Taiwan), respectively (Thompson et al. 2001). Other physicochemical parameters, such as total kjeldahl nitrogen (TKN), organic matter (OM), and carbon content, were analyzed following the *Test Methods for the Examination of Composting and Compost* (TMECC) (Thompson et al. 2001).

2.3.2 Biological activity

The specific oxygen uptake rate (SOUR) and cellulase enzyme activity were analyzed following the TMECC (Thompson et al. 2001). Additionally, the fluorescence diacetate hydrolysis (FDA) rate was determined using the previously described method (Nikaeen et al. 2015).

2.4 Quality of the final product

To assess the quality of the final compost product and its compliance with compost standards, chemical and microbial indicators were analyzed. Microbial analysis included the determination of total and fecal coliforms, *E. coli*, and *Salmonella* using the multi-tube fermentation technique as described earlier (Sadeghi et al. 2022) to ensure the microbial safety of the final compost.

Furthermore, the chemical quality of the final compost product was evaluated by analyzing the concentrations of total and soluble heavy metals (cadmium, copper, lead, zinc, and nickel) and the ammonium to nitrate ratio (Thompson et al. 2001). The analysis of heavy metals was performed using an atomic absorption spectrophotometer (Perkin-Elmer, model 3030, USA).

In addition, the germination index (GI), which serves as an indicator of maturity and phytotoxicity, was determined by using watery extracts of the compost placed on filter paper in a Petri dish with cress seeds (Nikaeen et al. 2015).

2.5 Principal component analysis

The principal component analysis (PCA) was performed using the ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) and ggfortify (https://cran.r-project.org/web/packages/ggfortify/index.html)

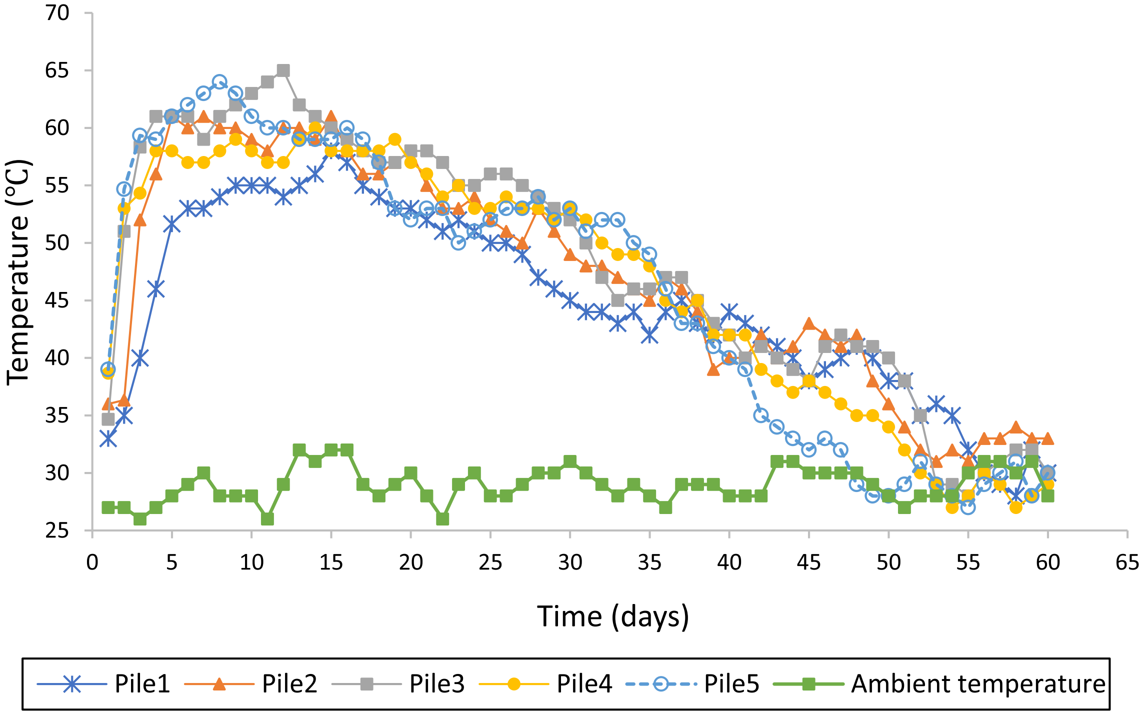


Fig. 2 Temperature variation trends during the composting process in control and amended piles

packages to evaluate the relationship between analyzed parameters.

3 Results and discussion

3.1 Immobilization process

As mentioned in Sect. 2.1, the isolated microorganisms were immobilized on biochar to form a biofilm. Figure 1 displays the SEM images of the biochar surface before and after microbial inoculation.

The SEM results demonstrated that the designed inoculation reactors (Sect. 2.1.3) provided suitable conditions for the formation of microbial biofilms of different types of microorganisms studied. Previous studies have acknowledged biochar as an excellent carrier and habitat for microorganisms (Liu et al. 2020; Tomczyk et al. 2020). The large surface area and porous structure of biochar make it an ideal medium for microbial immobilization (Feng et al. 2020). The functional groups generated during the pyrolysis process of biochar production create polarity on the biochar surface, facilitating the adsorption of microorganisms (Feng et al. 2020). The ability of biochar to retain water and nutrients on its large surface area also provides an ideal habitat for microorganisms, particularly bacteria, actinomycetes and fungi (Behera and Samal 2022; Guo et al. 2020). Additionally, biofilm-forming microorganisms produce extracellular polymeric substances (EPS) that bind them to each other and to the biochar surface. EPS not only mitigates the harmful effects of toxic substances but also enhances bacterial resistance to adverse conditions (Feng et al. 2020; Tu et al. 2020). Moreover, the mineral elements present in biochar can promote the growth of microbial populations. Therefore, immobilizing microorganisms on biochar can enhance the survival and activity of microbial communities (Liu et al. 2020). Studies on wastewater treatment or polluted soil bioremediation have shown that using immobilized cells on a specific carrier substance can enhance and improve microbial activity. In our study, we inoculated biochar with a consortium of dominant microorganisms from the active phase of compost. This is expected to kick start the active phase faster in the amended piles and accelerate the composting process.

Figure 1 demonstrates that the cultured microorganisms were effectively colonized, resulting in the formation of biofilms. The weight of the stabilized biofilm on the biochar surface was calculated, and the average ratios were determined to be 19.8% (w/w) for the fungal biofilm and 25.3% (w/w) for the bacterial biofilm. In line with our findings, Tu et al. (2020) reported the ability of *Pseudomonas* sp. NT2 to immobilize on the biochar surface and produce EPS.

Analysis of immobilized microorganisms on biochar indicated that yeasts and *Aspergillus* were the prevalent fungi, while the bacteria primarily consisted of

Gram-negative and Gram-positive bacilli. Additionally, filamentous structures resembling the morphology of actinomycetes were noted in the colonies isolated from the culture medium.

3.2 Temperature trends

Temperature is a crucial indicator of microorganism activity, humification degree, and the succession of microbial communities during composting (Wang et al. 2022; Zhou et al. 2019). The temperature changes over time are presented in Fig. 2. The results show that the highest temperature level (65 °C) was reached in pile 3 on the 12th day. Pile 5 achieved the second highest temperature, reaching 64 °C on the 8th day. These peaks in temperature can be attributed to the application of biochar inoculated with bacterial biofilm in pile 3 and biochar inoculated with both fungal and bacterial biofilms in pile 5, indicating high microbial activity. This behavior is consistent with findings from other studies, which reported that the inoculation of microbial agents combined with biochar contributed to a faster rise in temperature, an increased peak temperature, and a prolonged thermophilic phase during composting (Wu et al. 2024; Wang et al. 2023; Ji et al. 2023). In Wang et al. (2023) study on composting sheep manure mixed with corn straw, the application of biochar carried microbial agent was used in composting process, which increased the peak temperature and prolonged the thermophilic phase, and it was observed in comparison of control piles. In the control pile (pile 1), the maximum temperature reached was 58 °C on the 15th day, which was lower than the maximum temperature observed in the other piles. Additionally, pile 1 maintained the thermophilic phase (>50 °C) for 18 days, while the thermophilic phase was longer in the other piles. Pile 5 exhibited the highest stability in the thermophilic phase, lasting for 26 days. It has been reported that the porous nature of biochar reduces the bulk density of compost piles, thereby improving aeration and enhancing microbial activity (Parra-Orobio et al. 2023; Behera and Samal 2022). Furthermore, the micro- and macro-pores, comparable in size to bacteria, provide a protective environment that allows bacteria to proliferate while minimizing exposure to inhibitory compounds (e.g., NH_3, H_2S, and heavy metals), competitors such as pathogens, and environmental stressors like pH fluctuations, leaching, and desiccation (Behera and Samal 2022; Guo et al. 2020). As a result, biochar-amended compost piles exhibit enhanced microbial activity, leading to elevated temperatures, with even greater improvements observed in piles amended with inoculated biochar (Wu et al. 2024; Wang et al. 2023; Ji et al. 2023).

As shown in Fig. 2, the maximum temperature in piles 2 and 4 was almost the same and was reached on days 14 and 15, respectively. However, it was observed that the peak temperature in pile 4 was lower than that in piles 3 and 5. This suggests that fungal biofilm inoculation may not have a significant effect on increasing the temperature in the initial phases of composting.

During the thermophilic phase, the maximum breakdown of recalcitrant materials occurs (Sahu et al. 2019; Tchobanoglous and Kreith 2002) and high temperatures in this phase are effective in controlling weed seeds and pathogens, as they cannot survive under such conditions (Wang et al. 2021; Tchobanoglous and Kreith 2002). Therefore, piles 3 and 5, with their higher temperatures, are expected to exhibit more efficient composting process, leading to greater organic matter reduction and improved hygiene. After approximately 35 days, the temperature in all treatments dropped below 45 °C, marking the beginning of the maturation phase. During this stage, the proportion of resistant materials increases, while microbial activity decreases, resulting in a gradual decrease in temperature towards ambient levels (Tchobanoglous and Kreith 2002).

Since temperature change is a rapid and easy indicator to determine the activity of the microbial population in the composting process (Onwosi et al. 2017), a higher maximum temperature or a faster-reaching temperature peak in piles 3 and 5 indicates that a high microbial population immobilized on the surface of biochar. Therefore, more efficient reduction of OM and better hygiene quality are expected in these piles.

3.3 pH evolution trend

The pH evolution trend is a significant factor in microbial succession during composting. pH plays a crucial role in the activity and growth of bacteria and fungi. The optimal pH range for bacteria is 6.0–7.5, while for fungi, it is 5.5–8.0 (Tchobanoglous and Kreith 2002). However, the initial level of pH in the composting process depends on the characteristics of the feedstock. In this study, the initial pH value was approximately 7.8–7.9 (Fig. 3). Although a slight increase in pH was observed on the 5th day in some piles, it was followed by a gradual decrease in all compost piles. The pH changes observed in this study were relatively small, with the minimum pH value ranging between 7.6 and 7.8. Previous research has indicated that during the initial stage of composting, labile organic materials are rapidly decomposed, resulting in the accumulation of ammonium nitrogen and an increase in pH (Wang et al. 2023). However, as the composting process progresses (around day 10), the pH typically decreases due to the activity of acid-forming bacteria and the breakdown of complex carbon materials into organic acids (Wang et al. 2023; Tchobanoglous and Kreith 2002). Therefore, the faster pH decreases in pile 3 could be

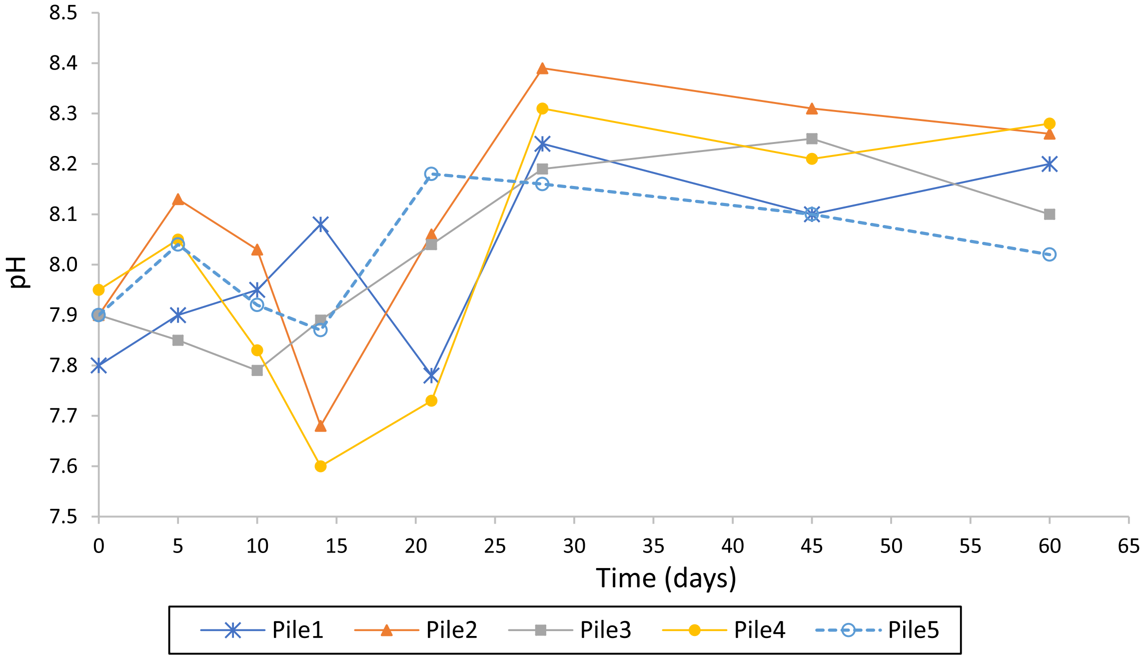


Fig. 3 pH variation trends during the composting process in control and amended piles

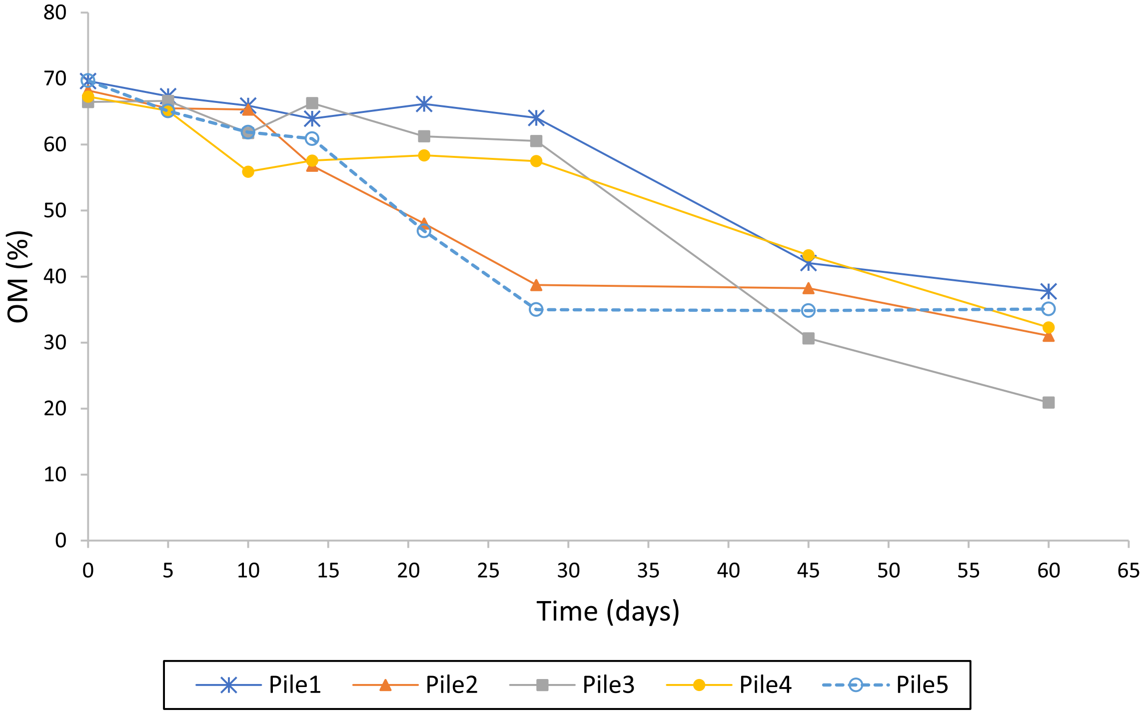


Fig. 4 Organic matter degradation trends during the composting process in control and amended piles

attributed to the more activity of microorganisms in this pile which is comparable to temperature trend (Figs. 2, 3). It was also observed that pile 1 (without biochar) had a delayed attainment of the minimum pH value in comparison to other piles, which may be attributed to the need for microbial acclimation. Consistent with our findings,

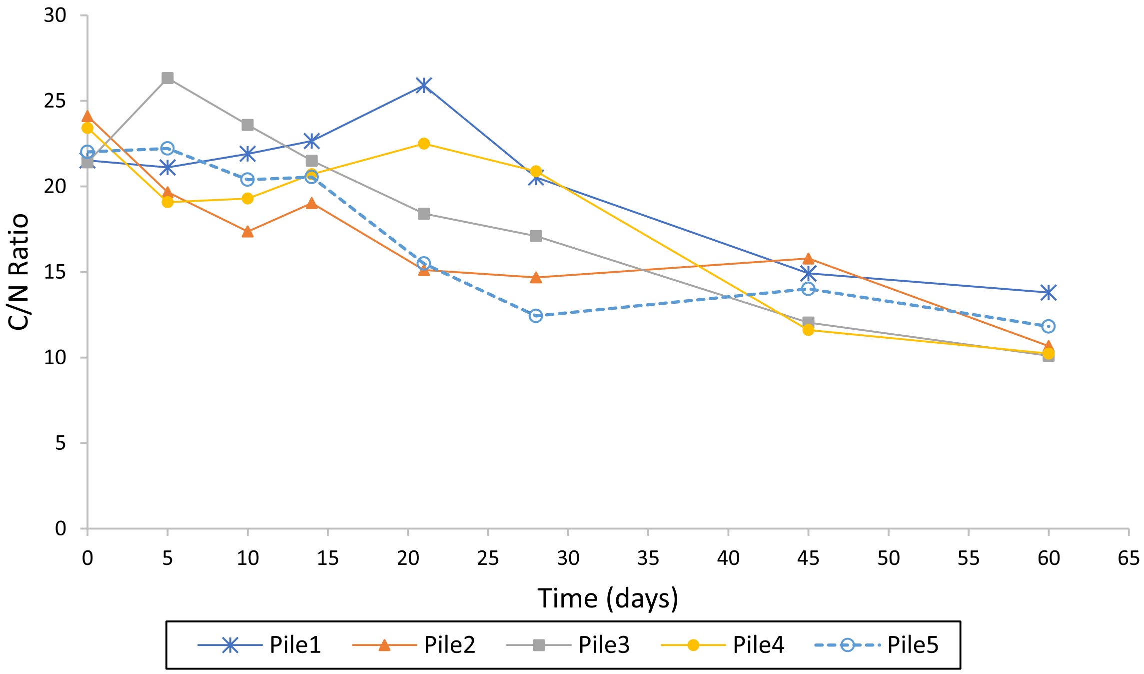


Fig. 5 C/N ratio variations during the composting process in control and amended piles

it has been reported that compost piles with biochar exhibit a more rapid decrease in pH compared to those without biochar. This effect is attributed to the enhanced production of organic and inorganic acids resulting from the degradation of organic matter driven by increased microbial activity (Guo et al. 2020).

3.4 Organic matter trend

The degradation of OM during MSW composting is depicted in Fig. 4. During composting, microorganisms break down organic compounds to obtain energy and proliferate. The initial OM contents ranged from 67 to 69%. The results indicate a gradual downward trend in OM degradation. The concentrations of OM decreased by 45%, 54%, 66%, 52%, and 50% in piles 1–5, respectively. Notably, pile 1 had the lowest final reduction of organic matter, while pile 3 exhibited the highest reduction. This suggest that the presence of biochar inoculated with bacterial biofilm can accelerate the decomposition of organic matter, leading to more efficient and cost-effective composting processes by reducing the required time and space. Biochar accelerates the degradation of organic matter by improving aeration within the compost material and stimulating both microbial and enzymatic activity (Behera and Samal 2022; Guo et al. 2020). Piles 3 and 1, with the lowest and highest OM reduction rates, respectively, exhibited a strong relation with temperature variations and therefore microbial activity. In contrast, piles 2 and 5 achieved faster OM reduction (43% and 47% by day 28). The slower initial OM reduction of pile 3 compared to pile 5 suggests a lack of adapted fungal populations in the early stages. However, once appropriate fungal populations were established, pile 3 experienced increased degradation efficiency. As shown in Fig. 4, compost piles amended with biochar exhibited lower OM content on the final day, indicating higher stability compared to pile 1. This observation aligns with findings from other studies (Wang et al. 2023; Antonangelo et al. 2021). It has been reported that the addition of biochar, particularly microbial-inoculated biochar, enhances microbial activity and the humification process, thereby promoting the formation of stabilized humic substances during composting (Guo et al. 2020).

3.5 Carbon to nitrogen ratio

The ratio of carbon to nitrogen (C/N) is a crucial factor in composting, as other nutrients are usually present in sufficient quantities and proportions in most organic wastes (Tchobanoglous and Kreith 2002). As shown in Fig. 5, the initial C/N ratios ranged from 21 to 24 and gradually decreased over time. The final C/N values ranged from 10 to 14, with the highest value observed in pile 1. The C/N ratio below 12 is indicative of advanced OM stabilization and compost maturity (Tchobanoglous and Kreith 2002). During composting process, carbonaceous materials are continuously decomposed, leading to a decrease in carbon concentration (Raut et al. 2008). On the other hand, a part of

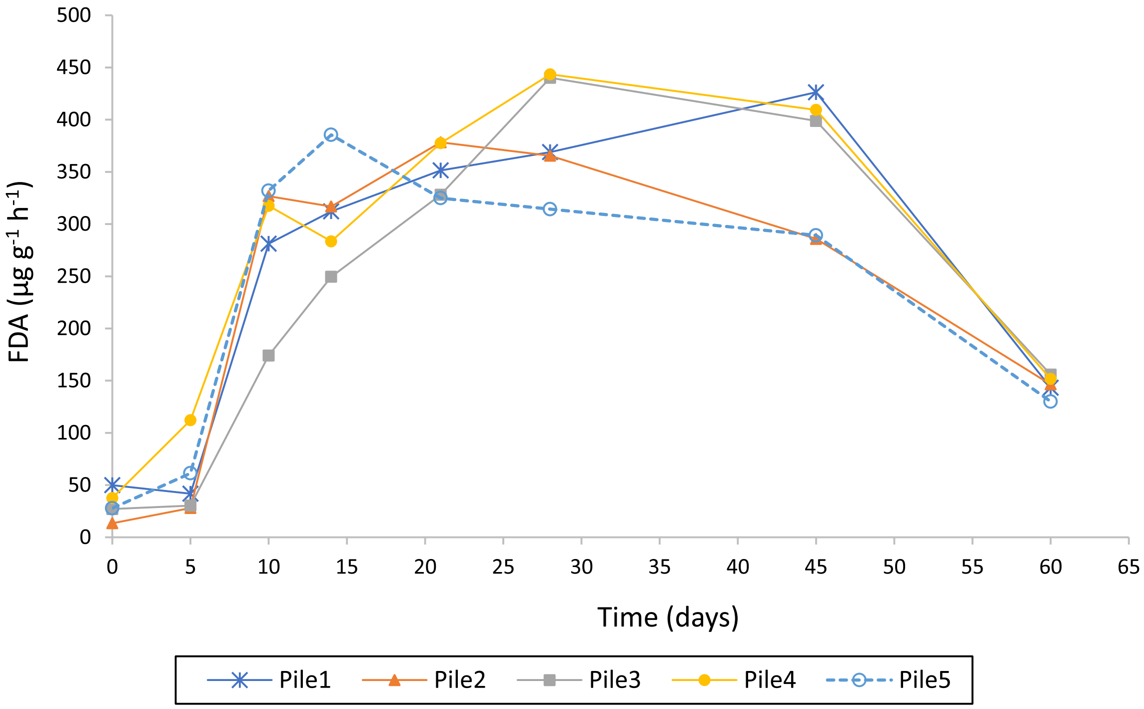


Fig. 6 FDA hydrolysis variations during the composting process in control and amended piles

organic nitrogen may be lost as NH_3 through evaporation or converted to NO_3^-. As a result, the C/N ratio of mature compost tend to decrease (Wang et al. 2023; Sahu et al. 2019).

The C/N trends observed during the composting process indicate a slight increase in the early stage (Fig. 5). During composting, the organic nitrogen converted firstly into ammonium and therefore, this increase could be attributed to the loss of nitrogen through NH_3 emission, as discussed by Wang et al. (2023). This increase occurred on the 5th day for piles 3 and 5, with a greater increase in pile 3, potentially due to higher microbial activity and nitrogen volatilization as indicated by temperature and OM profiles (Parra-Orobio et al. 2023).

Additionally, the C/N ratios of 14.6, 17.3, and 12.42 in piles 2, 3, and 5, respectively, on the 28th day of composting indicate an accelerated mineralization process in these piles compared to piles 1 and 4, which had C/N ratios of approximately 21, which can be attributed to higher biological activities.

During composting, the mesophilic microorganisms are responsible for converting ammonium to nitrate through the nitrification process. This transition affects the nitrogen forms present in the compost, with ammonium and NO_3^- becoming the main forms in matured compost. Therefore, the ratio of ammonium to nitrate (NH_4^+/NO_3^-) is a valuable parameter for evaluating the degree of compost maturity. Brinton (2000) suggests that a nitrification index (NH_4^+/NO_3^- ratio) ranging from 0.5 to 3 indicates mature compost. In this study, the NH_4^+/NO_3^- ratios in composted products of piles 1 to 5 were found to be 3.3, 1.9, 3.0, 3, and 2.9, respectively, indicating compost maturity in piles 2–5, whereas in pile 1 as control pile without biochar amendment the ratio was higher than the recommended value. However, the lowest ratio was observed in pile 2 with biochar amendment. This finding is consistent with previous studies that have shown the impact of biochar amendment on the nitrification index (Xiao et al. 2017). The addition of biochar enhances the nitrification process by stimulating nitrifying bacteria while suppressing the ammonification process. Moreover, it has been reported that incorporating 5% (w/w) biochar can increase the total nitrogen content of the final compost product by approximately 45% compared to the control (Fuchs et al. 2021; Guo et al. 2020). Overall, the use of biochar in composting processes contributes to the reduction of NH_3 emission and accelerates the mineralization process. The biochar amendment positively influences the nitrification index, leading to the production of mature compost. These findings highlight the potential benefits of incorporating biochar in composting practices for improved nutrient cycling and environmental sustainability.

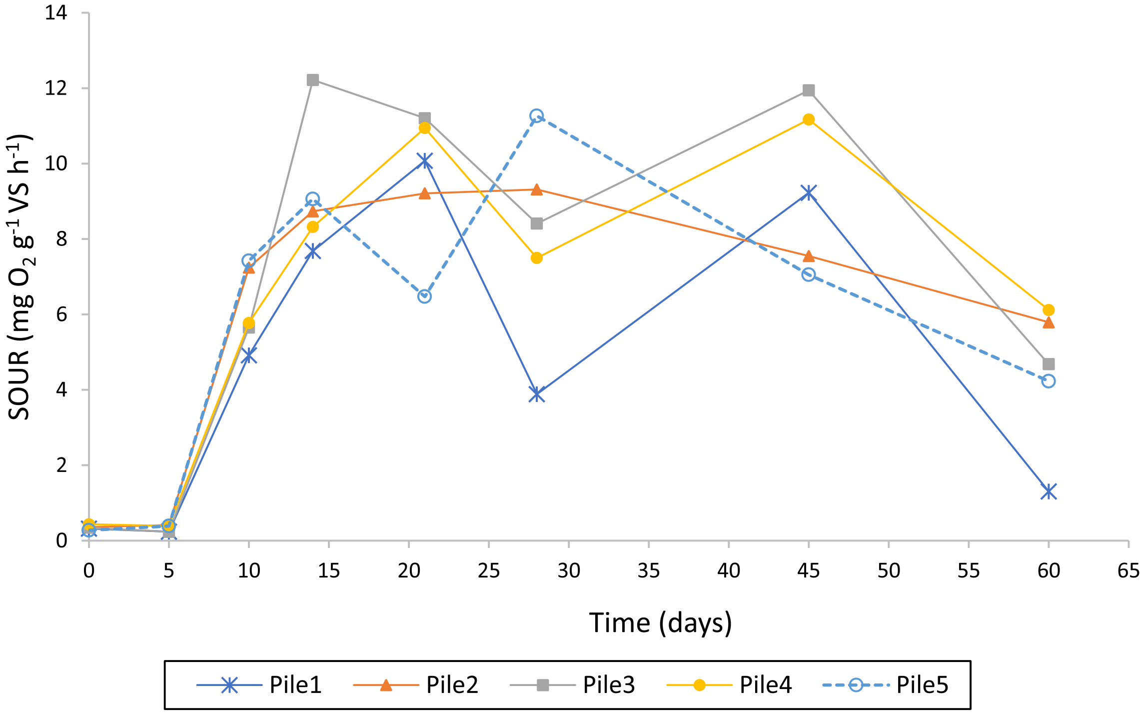


Fig. 7 Specific oxygen uptake rate variations during the composting process in control and amended piles

3.6 FDA hydrolysis

Fluorescein diacetate hydrolysis is attributed to enzymes such as proteases, lipases, and esterases (Zhang et al. 2023), making it a reliable and rapid indicator of microbial activity (Nikaeen et al. 2015).

In the first samples, the FDA hydrolytic activity ranged from 49 to 13 μg g^{-1} h^{-1}, remaining relatively constant in the second sample on the fifth day. However, on the 10th day, the FDA hydrolytic activity increased significantly in all piles and continued to increase until reaching maximum values. Pile 3 and pile 4 both reached a maximum FDA hydrolytic activity of 443 μg g^{-1} h^{-1} on the 30th day, while pile 1 reached its maximum activity on the 45th day. Piles 5 and 2 exhibited peak FDA hydrolytic activity on the 15th and 21st days, respectively, maintaining high levels until the 45th day (Fig. 6). Notably, pile 1, without any amendment, exhibited a delayed maximum FDA hydrolysis compared to the other piles. In contrast, pile 5 (inoculated with a bacterial and fungal consortium) achieved the fastest maximum FDA hydrolysis, likely due to the presence of active fungi in the early stages of composting and their synergistic interaction with bacteria for biodegradation. This observation is consistent with the degradation chart of organic matter (Fig. 4). Previous studies have shown that microbial inoculation or biochar application can increase FDA hydrolytic activity during composting (Sahu et al. 2019). The availability of sufficient substrate for microbial metabolism, biomass, and enzyme secretion contributes to the high FDA hydrolytic activity (Jiang et al. 2021).

Piles amended with inoculated biochar exhibited higher levels of FDA hydrolysis, indicating higher microbial activity (Fig. 6). Similar to the findings of Zambrano et al. (2023), this study noted that the FDA hydrolysis increased during the thermophilic phase and remained relatively constant across all piles. Studies have shown that the FDA release rate correlates with all compost stability indicators. Higher enzyme activity is expected to lead to an increased FDA release rate, oxygen consumption rate, and mineralization rate (Komilis et al. 2011). Therefore, this parameter can be used to compare the level of microbial activities in compost piles which was in the highest value in pile 3 as other microbial indicators in this pile. Finally, the FDA hydrolytic activity decreased in the last stage of the process due to nutrient deficiency and low organic matter content (Zambrano Riquelme et al. 2023). The final FDA hydrolytic activity values ranged from 130 to 151 μg g^{-1} h^{-1} (Fig. 6).

3.7 Specific oxygen uptake rate

Oxygen uptake is a valuable and reliable indicator as it directly reflects microbial oxygen consumption through

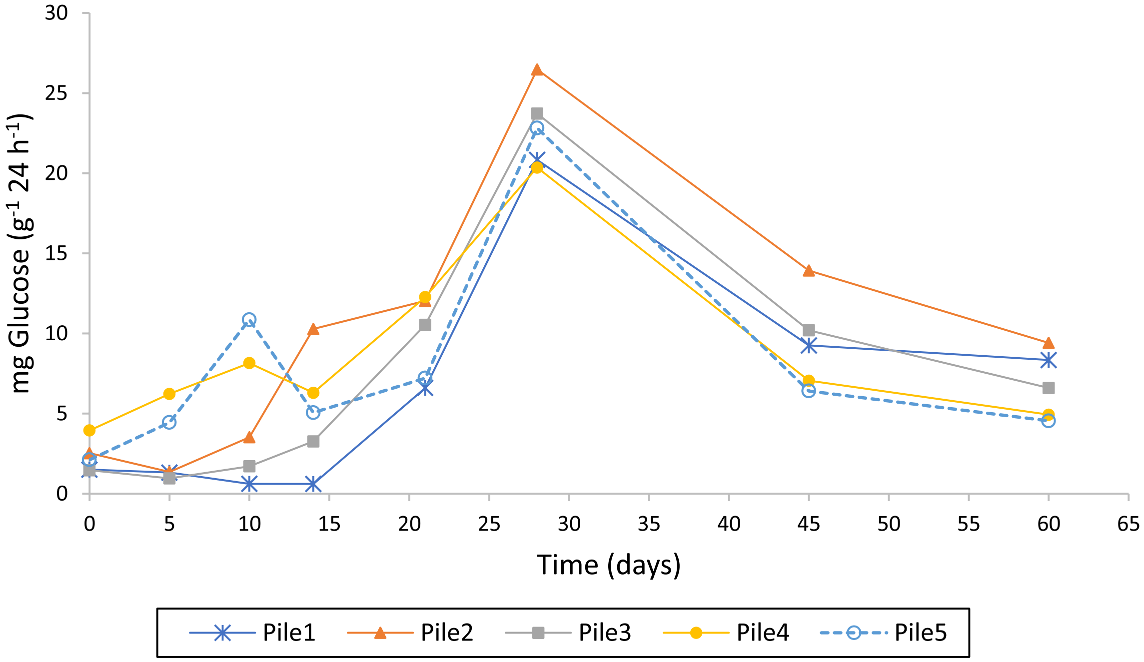


Fig. 8 Cellulase activity variations during the composting process in control and amended piles

biological activities and is strongly correlated with the metabolism of microorganisms (Nikaeen et al. 2015; Tchobanoglous and Kreith 2002). In the process of biological decomposition, microorganisms utilize oxygen and generate carbon dioxide and water. As a result, assessing the SOUR rate can help evaluate the stability of compost and the pace at which the process is advancing (Thompson et al. 2001). The results (Fig. 7) indicate that the SOUR was at its lowest value in the initial stage and remained stable until the second sampling on the 5th day. This delay in the SOUR trend may be attributed to the need for microbial acclimatization (Nikaeen et al. 2015). A significant increase in SOUR value was observed during the thermophilic phase. The simultaneous increase in SOUR and temperature can be interpreted as the temperature rise caused by microbial metabolism (Molina-Peñate et al. 2023). Similar results have been reported in previous studies (Molina-Peñate et al. 2023; Ballardo et al. 2017). As shown in Fig. 7, the first peak of SOUR was observed in piles 3 and 5 on the 15th day, while it occurred later in other piles. Additionally, the highest values of SOUR were observed in pile 3 followed by pile 5. This fact indicates higher microbial activity in piles 3 and 5, possibly due to amended biochar inoculated with microbial biofilms. The addition of microbial-inoculated biochar to compost accelerates organic matter degradation, reduces the time required to reach the thermophilic phase, and results in higher SOUR values, as observed in Fig. 2 and Fig. 7 for piles 3 and 5. Consequently, this improves the overall composting efficiency and enhances the humification process (Wu et al. 2024; Wang et al. 2023). Between the 15th and the 45th days, some fluctuation in SOUR variation has been recorded. Previous studies attributed these fluctuations to excessive temperature above the optimal range or changes in microbial succession (Ming et al. 2008; Miyatake and Iwabuchi 2006). Additionally, a high percentage of organic matter and high oxygen demand of the process may have created a small anaerobic zone, which could have had a temporary adverse effect on SOUR values. Pile 2, amended with biochar, exhibited lower fluctuations and a relatively stable SOUR value from day 14 to 28. This stability might be attributed to the large surface area of biochar, which absorbs organic matter and gradually releases it for microbial degradation. Additionally, the porous structure of biochar has been reported to increase pile porosity, thereby enhancing oxygen supply (Guo et al. 2020). The final SOUR in piles 1 to 5 reached 1.3, 5.79, 4.68, 6.11, and 4.22 mg O_2 g^{-1} VS h^{-1}, respectively. According to the US Department of Agriculture and the Composting Council Research and Education Foundation, a SOUR < 3 is interpreted as very stable, and a SOUR of 3–10 as stable (Thompson et al. 2001). However, in pile 1 because of the higher concentration of organic matters (Fig. 4), a low SOUR value (less than 3 mg O_2 g^{-1} VS h^{-1}) cannot be interpreted as indicating more stability of produced compost compared to other experimental piles. Hence,

Table 1 Chemical and biological characteristics of the final compost

		Pile 1	Pile 2	Pile 3	Pile 4	Pile 5	Standard value	Reference
Total heavy metals mg kg_{DW}^{-1}	Cd	4.9	3.8	4.8	4.9	4.8	39	(EPA 2023)
	Cu	232	103	391	78	103	1500	(EPA 2023)
	Pb	229	157	151	133	72	300	(EPA 2023)
	Zn	1879	2014	813	489	1251	2800	(EPA 2023)
	Ni	40	35	51	65	61	420	(EPA 2023)
Soluble heavy metals mg kg_{DW}^{-1}	Cd	0.1	0.1	0.1	0.1	0.1	Unregulated	
	Cu	4.6	3.5	3.7	3.2	3.8	Unregulated	
	Pb	5.7	4.5	5.7	9	6.4	Unregulated	
	Zn	2	3.9	1.1	3.3	1.2	Unregulated	
	Ni	1.9	1.9	1.9	2.4	1.5	Unregulated	
pH		8.2	8.26	8.1	8.28	8.02	5- 8.5	(Thompson et al. 2001)
EC (dS m^{-1})		2.56	2.41	2.55	3.05	2.47	<5	(Thompson et al. 2001)
SOUR (mg O_2 g^{-1} VS h^{-1})		1.30	5.79	4.68	6.12	4.23	<10 is stable <3 is very stable	(Thompson et al. 2001)
Cellulase activity (mg glucose g^{-1} 24 h^{-1})		8.34	9.41	6.60	4.93	4.54	Unregulated	
FDA activity (μg g^{-1} h^{-1})		143.29	146.42	155.88	151.72	130.03	Unregulated	
C/N ratio		13.80	10.67	10.10	10.23	11.82	<15 (Grade A) <20 (Grade B)	(Tchobanoglous & Kreith 2002)
GI		80	100	100	90	100	>80	(Thompson et al. 2001)
NH_4^+/NO_3^-		3.27	1.95	3.04	3.06	2.87	0.5–3	(Brinton 2000)
Total coliform (MPN)/g_{DW}		46000	46000	2700	1400	720	Unregulated	
Fecal coliform (MPN)/g_{DW}		15000	4300	430	1400	110	<1000	(Brinton 2000)
E. coli (MPN)/g_{DW}		1400	1500	91	430	ND	<1000	(Brinton 2000)
Salmonella (MPN)/g_{DW}		ND	ND	ND	ND	ND	ND in 25 gDW	(Brinton 2000)
Viable weed seeds		ND	ND	ND	ND	ND	None	(Thompson et al. 2001)

ND Not detected, *DW* Dry weight

the lower microbial activity may have reduced the final SOUR value in pile 1 (control).

3.8 Cellulase activity

Cellulose is known as a relatively resistant material against biological activity, and it is known to limit the rate of compost production more than any other compounds (Ma et al. 2019; Raut et al. 2008). Cellulose hydrolysis, catalyzed by cellulase, produces D-glucose (Ma et al. 2019). Cellulase enzymes are produced by several fungi such as *Aspergillus*, *Trichoderma Penicillium*, and aerobic and anaerobic bacteria including *Pseudomonas*, *Bacillus*, and *actinomycetes* (Ramos and Malcata 2011). As shown in Fig. 8, the initial values of cellulase activity were negligible. Although an unbalanced trend of cellulase activity can be observed up to the 15th day, a significant increase in cellulase activity was observed in all piles from the 15th day onwards. The peak values of cellulase activity in piles 1 to 5 reached 20.8, 26.5, 23.7, 20.3, and 22.8 mg glucose g^{-1} 24 h^{-1}, respectively. Previous literature suggests that the optimal temperature for the degradation of cellulose materials is about 55–65 °C, with the maximum activity of the cellulase enzyme corresponding to the thermophilic phase (Zhu et al. 2021; Ma et al. 2019). Additionally, the optimal pH for lignocellulosic material is around 7–8. The optimum pH for some cellulase-producing fungi is between 4.5 and 5.2, while the optimum pH value for actinomycetes is 7 to 8, and most cellulase is produced at pH 8 (Raut et al. 2008). Therefore, the maximum cellulase activity was observed on day 30 when the temperature and pH were optimal. Maximum cellulase activity was obtained in pile 2, while the minimum was obtained in pile 1. Studies have suggested that biochar can promote cellulase production and activity in both thermophilic and cooling phases (Yin et al. 2023). Addition of biochar in the co-composting of green waste and food waste resulted in greater lignocellulose degradation compared to treatments without biochar (Parra-Orobio et al. 2023). Eventually, cellulase activity decreased along with decreasing temperature in the cooling phase (Qiao et al. 2019). Cellulase activity is influenced by the concentration of nutrients, amounts of cellulose and hemicellulose, and growth of cellulase-producing organisms (Jiang et al. 2021; Qiao et al. 2019; Sahu et al. 2019). An initial peak of cellulase activity was observed in piles 4 and 5, which could be attributed to the degradation activity of fungal biofilms in these piles as cellulase-producing organisms. Therefore, the lower maximum cellulase activity in piles 4 and 5 on the 30th day compared to other amended piles is due to less degradable cellulose residue.

3.9 Chemical and biological characteristics of the final product

The chemical and biological characteristics of the final compost samples compared to the standard values are presented in Table 1. One of the main concerns with applying composting products, especially those derived from MSW, is the potential to increase heavy metal concentrations in soils. Heavy metals may accumulate in the soil and damage the food chain through biomagnification (Xiao et al. 2017; Reyes Pinto et al. 2020). Therefore, it is important that MSW compost contains low levels of heavy metals, as recommended in the guidelines. Qian et al. (2023) stated that while both biochar and compost can immobilize heavy metals in the soil, co-application of biochar with compost can immobilize heavy metals more efficiently and promote the reduction of bioavailability and mobility of heavy metals in soil. It has been proposed that a higher proportion of biochar results in greater stabilization of heavy metals (Qian et al. 2023). According to our results, analysis of heavy metals in the final products and comparison of the heavy metal concentrations with the standard values showed that the final concentration of heavy metals, including Cd, Cu, Pb, Zn, and Ni, in all piles is several times lower than the standards. As shown in Table 1, unlike Zn, the concentrations of other investigated soluble heavy metals were approximately lower in pile 2 compared to both the control pile and the piles amended with inoculated biochar. Biochar exhibits an affinity for certain heavy metals and can influence their mobility and bioavailability through mechanisms such as adsorption, complexation, and precipitation (Guo et al. 2020). Biochar chelates and stabilizes heavy metals relying on its porous structure and functional groups on its surface (Wang et al. 2022). However, in piles 3 to 5, biofilms covering the biochar surface may have reduced the ability of biochar to adsorb heavy metals (Xing et al. 2021).

Environmental mobility and complexes of heavy metals with soil organic matter are directly correlated with the water-soluble fraction (Reyes Pinto et al. 2020). Even though biochar application cannot decrease the total heavy metal concentration, their availability and solubility would decrease (Xiao et al. 2017). In this study, bioavailable concentrations of heavy metals were found to be negligible and several times lower than the total heavy metal concentrations.

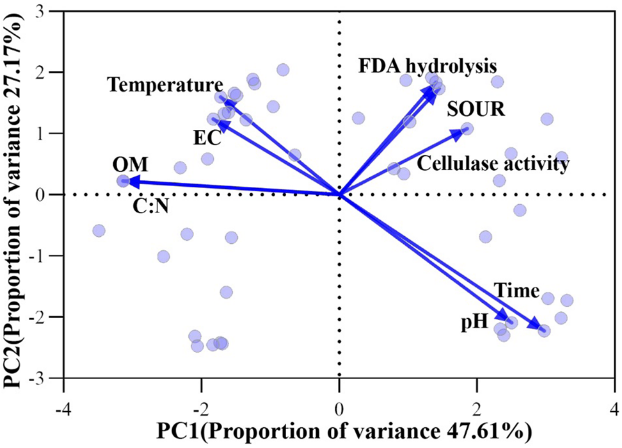


Fig. 9 Principal component analysis of various operational parameters of composting

Electrical conductivity is a measure of soluble salts and ions in the soil. A value higher than 4 dS m^{-1} can have adverse effects on plant growth. High salinity content in the soil can lead to low osmotic ability, ion toxicity, lack of nutrients, and consequently, reduced soil productivity (Cao et al. 2020; Lin 2008). The EC levels are presented in Table 1. The final EC value in the control group (pile 1) and experimental groups (piles 2–5) was within the standard range.

Compost sanitation requirements are proposed to ensure that pathogens are reduced or eliminated (Qian et al. 2023). For this purpose, the Environmental Protection Agency (EPA) recommends a minimum temperature of 55 °C or greater for at least 15 days during the composting process and at least 5 turnings in the thermophilic phase (EPA 2019). The results indicate that in piles 3 and 5, the fecal coliform concentration is consistent with microbial standards. However, in pile 5, no *E. coli* was detected, while relatively low numbers of *E. coli* were detected in piles 3 and 4. Additionally, *Salmonella* was not detected in any of the samples. The temperature investigation results showed that piles 1, 2, and 4 did not reach a temperature above 60 °C, which likely prevented further reduction of fecal coliforms. In a study on the microbial characteristics of municipal solid waste compost, Sadeghi et al. (2022) reported that 27% of the samples were found to have fecal coliform and 16% with *Salmonella* above the standard.

The GI indicates the maturity of compost. The maturity of compost is typically linked to the presence or absence of harmful compounds within it. Immature compost often harbors phytotoxic substances. Hence, assessing the GI parameter is essential for gauging the quality of compost (Núñez et al. 2022; Ma et al. 2019). The germination index in all piles was within the standard (> 80%). However, piles 2, 3, and 5 had a GI of 100, confirming the low phytotoxicity and high maturity of the produced compost (Wang et al. 2023). The GI index in pile 4, while meeting the standard at 90%, is lower than in other inoculated piles. This could be attributed to lower microbial activity, as evidenced by the lower temperature peak observed in pile 4. Several studies have reported an increasing GI with biochar addition to compost, attributed to improved conditions through the adsorption of toxins on biochar (Xiao et al. 2017). Wang et al. (2023) reported that the addition of biochar carrying microbial agents accelerated compost stabilization and reduced its phytotoxicity.

3.9.1 Principal component analysis

Principal component analysis (PCA) was conducted to assess the clustering and relationships among various parameters investigated during the composting process (Fig. 9), including temperature, FDA hydrolysis, OM, C/N ratio, SOUR, cellulase activity, time, and pH. The parameters are linked to the origin, with blue lines indicating their contribution to the principal components. PC1 and PC2 values accounted for 47.61% and 27.17% of the variance, respectively, highlighting their dominance in explaining the data structure. Combined, they accounted for about 74.8% of the total variance, underscoring the significance of these components in capturing the primary trends and relationships in the composting parameters. The graph illustrates that pH and time were aligned, with pH increasing over time. Conversely, temperature moved in the opposite direction to time, decreasing as time progressed. OM and C/N ratio were closely aligned and exhibited an angle of approximately 45 degrees with temperature, showing changes that corresponded closely with temperature variations. Additionally, the parameters of FDA hydrolysis, SOUR, and cellulase activity were closely aligned with each other and demonstrated simultaneous increases or decreases. However, these parameters formed an angle of about 90 degrees with temperature and time. This suggests that the fluctuations in these parameters did not entirely align with changes in temperature and time due to ongoing microbial activities despite a decrease in their intensity. Additionally, the results of PCA indicated that FDA hydrolytic activity and SOUR were closely related, implying that one of these parameters could be analyzed when economic considerations are important or when infrastructure for SOUR analysis is limited. Clustering and relationships among various parameters presented by PCA (Fig. 9) highlighting the importance of analyzing different parameters including temperature, C/N, SOUR and enzymatic activities in evaluation of performance a modified composting process.

3.9.2 Implications and future research

Our findings demonstrate that microbial-inoculated biochar can enhance the composting process and improve the quality of composted products. This acceleration of the active composting phase has the potential to increase facility throughput and add economic value. However, further research is needed to assess the economic feasibility of applying inoculated biochar, particularly in comparison to the additional costs associated with biochar production or procurement.

The physicochemical properties of biochar, influenced by feedstock and pyrolysis conditions, significantly impact its suitability as a biofilm carrier and its performance in MSW composting. At present, no standardized method exists for producing biochar used as a composting amendment (Fuchs et al. 2021; Guo et al. 2020). Therefore, it is essential to compare the efficiency of different types of biochar. Additionally, the ideal dose of biochar should be determined to optimize composting efficiency while accounting for cost-effectiveness.

Future studies could explore the use of coarse biochar particles, sieved from the final compost product, to evaluate their effectiveness in composting. Moreover, research is required to monitor the effects of biochar and inoculated biochar on the microbial profiles of MSW composting processes. Field-scale investigations are also necessary to assess the use of co-composted biochar in soil applications.

To gain deeper insights, additional parameters should be incorporated into studies to evaluate the impact of biochar and inoculated biochar on nitrogen dynamics during co-composting, as well as their role in immobilizing or eliminating toxic materials and xenobiotics.

An emerging concern related to compost application is the release of antibiotic resistance genes into the environment (Wu et al. 2024). Therefore, it is crucial to examine the effects of biochar and inoculated biochar on the elimination of antibiotic-resistant bacteria and resistance genes during the MSW composting process.

As highlighted in the introduction, most studies on biochar and inoculated biochar in MSW composting have been conducted at a pilot scale. Consequently, field-relevant findings are needed to provide valuable information that will support the design and practical application of biochar-based strategies in large-scale MSW composting operations.

4 Conclusion

This study evaluated the impacts of microbial-inoculated biochar on the MSW composting process and the quality of the resulting product. The addition of biochar inoculated with bacterial and bacterial/fungal consortia significantly enhanced biological activity, as evidenced by higher temperatures and an extended thermophilic phase. The findings also demonstrated improved organic matter degradation and enzymatic activity with the use of microbial-inoculated biochar. Our results indicate that biochar inoculated with bacterial and bacterial/fungal consortia can effectively enhance the composting process under real-world conditions. This improvement was reflected in a higher GI and better hygienic quality of the final product, facilitating its use as a soil improver. Furthermore, the study identified FDA hydrolysis as a reliable indicator of microbial activity during the composting process, providing valuable insights into process monitoring. These findings open new possibilities for using inoculated biochar as an additive in large-scale MSW composting processes. However, while microbial-inoculated biochar reduces the time and space needed to produce stable and mature compost, further research is required to assess the cost–benefit of its application in MSW composting facilities.

Supplementary Information

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Additional file 1.

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Author contributions

Mohammad Javad Tahsini: Investigation, Methodology, Formal analysis, Writing; Mahnaz nikaeen: Conceptualization, Supervision, Funding aquistion, Writing, Reviewing, Editing; Farzaneh Mohammadi: Software, Formal analysis; Ahmadreza Taghipour: Methodology; Meghdad Tahmasebi: Methodology; Amir Hossein Nafez: Conceptualization. All listed authors have approved the manuscript before submission.

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Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors reviewed and approved the final manuscript.

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