

Co-composting of municipal solid waste with activated biochar: A promising approach to improve the quality of compost

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Abstract

Biochar reduces composting issues and improves compost quality. However, activated biochar (AB) and its size are often overlooked. This research aimed to evaluate the impact of co-composting of municipal solid waste (MSW) with different-sized biochar and AB on the quality of the resulting co-compost. The MSW were mixed thoroughly with different-sized (B_{2-4mm}, B_{1-2mm} and B_{0.5-1mm}) and activated biochars (H₂SO₄-AB_{0.5-1mm} and NaOH-AB_{0.5-1mm}) and co-composted for 90 days until compost maturity. The results revealed that the activation of biochar with NaOH and H₂SO₄ caused the appearance of a mesh structure on the biochar surface, leading to improved stability and maturity, enhanced biodegradation and humification indices. Specifically, NaOH-AB (5%, w/w) showed the highest temperature (71.5°C), germination index (130.9%), and total nitrogen content (1.37%) and the longest thermophilic period (7 days). The highest organic matter content (OM) (37.9%) and the lowest electrical conductivity (7.4 dS/m) were recorded in B_{1-2mm} (10%, w/w). Furthermore, the lowest nitrate concentration (254.4 mg/kg) and the highest C/N ratio (18.1) were achieved in H₂SO₄-AB (10%). Principal component analysis (PCA) highlighted the critical role of the C/N ratio and OM content during the composting. The study recommends the addition of biochar to MSW to achieve an appropriate C/N ratio and prevent nitrogen loss. Overall, incorporating NaOH- and H₂SO₄-activated biochars was found to be a valuable strategy for the composting of municipal solid wastes and the findings provide valuable insights into the potential of biochar in optimizing the composting process.

Keywords: Biochar activation, Co-composting, Humification, Municipal solid wastes.

1 Introduction

The management of municipal solid waste (MSW) is a global concern. According to the United Nations Environment Programme, the global MSW generation will reach 3.8 billion tons by 2050 (UNEP, 2024). In the Middle East, nearly 65% of MSW consists of organic waste, which is higher

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29 than the global average and more than 70% of this MSW is primarily disposed of in unsanitary
30 landfills (UNEP, 2024). Consequently, effective management of MSW has emerged as a pivotal
31 21st-century challenge, which requires innovative technologies to recover resources and facilitate
32 the transition to a sustainable bioeconomy, particularly in developing countries. From both
33 economic and environmental perspectives, composting offers a promising solution for managing
34 the organic fraction of MSW, while also reducing waste disposal costs (Bhattacharjee et al., 2023).
35 Composting involves various stages where diverse microbial communities (bacteria, fungi, and
36 actinomycetes) operate at different temperatures, generating heat as they decompose organic
37 matter. The resulting compost is a nutrient-rich organic fertilizer that improves the
38 physicochemical, and biological properties of soil, enhances soil fertility and reduces the reliance
39 on chemical fertilizers (Babu et al., 2021). Additionally, composting can be associated with
40 challenges such as greenhouse gas emissions, nitrogen loss, and the contamination of soil and
41 water resources (Barthod et al., 2018; Nguyen et al. 2022). To address these challenges, "co-
42 composting," which involves the use of additives at the beginning of the composting, has emerged
43 as a promising approach (Barthod et al., 2018).

44 Recently, biochar has been recognized as a valuable additive and a key component to reduce the
45 adverse effects of MSW composting and improve the quality of the final compost (Feng et al,
46 2024). Biochar possesses a range of beneficial characteristics, including a porous structure, high
47 specific surface area, high ion exchange capacity, active oxygen functional groups, and resistance
48 to decomposition. These properties enhance aeration and accelerate the decomposition of toxic
49 substances through co-metabolism, reducing heavy metal bioavailability, nitrogen loss,
50 greenhouse gas emissions, and composting time (Nguyen et al., 2022). In addition, the
51 physicochemical properties of biochar, including particle size and activation can affect its
52 efficiency in improving compost quality. The particle size affects aeration, moisture; porosity,
53 turning efficiency, and the uniformity of the compost pile (He et al., 2019). However, previous
54 studies have provided limited insights into the effects of biochar particle size on the co-composting
55 process.

56 The main purpose of the activation process is to enhance the oxygen-containing functional groups,
57 surface area, pore volume and diameter, and increase the porosity of the activated biochar (AB)
58 (Panwar and Pawar, 2020). Chemical activation (includes acid and alkaline activation) is the most

widely adopted process for activation, with several advantages over physical methods. Among various chemical activators, NaOH and H₂SO₄ are considered more suitable due to their cost-effectiveness and lower environmental impact (Panwar and Pawar, 2020). Ye et al. (2019) reported that chemical-activated biochar increased available habitats for microorganisms, thereby improving the intensity of microbial respiration. Given the limited studies on AB composting, further investigation is warranted.

The objective of this study was to evaluate the impact of co-composting of MSW with different-sized biochar and AB on the quality of the resulting co-compost. The compost quality characteristics were evaluated, including maturity and stability, enzyme activity, and humification indices.

2 Materials and methods

2-1 Preparation and activation of biochars

MSW was collected from the Municipal Waste Management Organization, Tabriz, Iran. The waste biomass for the production of biochar, was prepared by mixing the pruning branches of plum and pomegranate trees. Biochar was produced by slow pyrolysis at 400 °C with a heating rate of 10 °C per minute and a one-hour holding time at the target temperature. The biochar was separated using 0.5-1 mm (B_{0.5-1 mm}), 1-2 mm (B_{1-2 mm}), and 2-4 mm (B_{2-4 mm}) sieves. For biochar activation, the biochar (B_{0.5-1mm}) was mixed with solutions of 2M NaOH and/or H₂SO₄ at a solid-to-solution ratio of 1:2 (w/v) with gentle stirring for 2 hours under the hood. Then, the suspension was filtered, and the residual solid was washed several times with distilled water until the pH was fixed at approximately 7.0. Finally, the washed biochar was dried in an oven at 65°C for 12 hours (Fan et al., 2010).

2-2 Chemical analysis of biochars and MSW

For the chemical analysis, the pH and EC were determined in a 1:10 (w/v) compost to water ratio (Singh et al., 2017). The contents of carbon, hydrogen, nitrogen, and sulfur was measured by CHNS analysis (vario ELIII Elementary analyzer, Germany). The biochar CEC was determined by the method of Wang et al. (2013). The ash content was obtained by the Singh et al., (2017) method. The total concentrations of P, K, Na, Fe, Mn, Zn, Cu, Pb, and Ni were determined by ash digestion with a 3:1 mixture of two acids (HNO₃:HCl, 1:3, v/v) (Jones Jr. and Case, 1990). The available P

concentration was determined by the Olsen method (Kuo, 1996), and the NH_4^+ concentration was determined by the indophenol blue method (Li et al., 2015). Water soluble K and Na concentrations were determined at 1:10 ratio (w/v). The concentration of heavy metals was measured by an atomic absorption spectrometer (Shimadzu, AA-6300). Biochar functional groups were determined by the FTIR technique as a general characterization technique (Bruker Tensor 27 FTIR spectrometer); furthermore, the biochar surface morphology was evaluated by SEM (Tuscan FEG-SEM, MIRA3). The characteristics of the produced biochars and the MSW used in this study are presented in Table 1.

Table 1. Basic characteristics of the MSW and biochars.

Properties	MSW	B* _(0.5-1mm)	B* _(1-2mm)	B* _(2-4mm)	NaOH-AB**	H ₂ SO ₄ -AB**
Ash (g/100g)	72.05±3.04	25.57±0.58	19.75±0.35	19.02±0.31	18.80±0.14	17.95±0.35
C (g/100g)	16.03±1.00	54.47±5.67	54.47±5.67	54.47±5.67	63.61±4.28	50.67±2.86
H (g/100g)	0.75±0.01	1.60±0.04	1.60±0.04	1.60±0.04	1.69±0.07	1.54±0.06
N (g/100g)	1.28±0.02	1.01±0.00	0.88±0.00	0.85±0.00	1.22±0.00	0.95±0.00
S (g/100g)	0.74±0.00	0.36±0.01	0.36±0.01	0.36±0.01	0.27±0.00	1.81±0.00
O (g/100g)	8.88±1.59	16.99±6.29	22.94±5.35	23.7±5.4	14.41±4.06	27.08±2.44
O/C	0.42±0.05	0.23±0.15	0.32±0.14	0.44±0.15	0.17±0.08	0.40±0.08
H/C	0.56±0.01	0.35±0.00	0.35±0.00	0.35±0.00	0.32±0.00	0.36±0.00
C/N	12.52±0.98	53.93±6.58	61.9±6.68	64.08±6.6	52.14±4.73	53.34±3.26
CEC (Cmolc/kg)	-	25.04±1.47	14.74±2.88	11.76±1.7	28.34±2.7	25.57±0.74
EC (dS/m)	8.2±0.31	0.55±0.00	0.34±0.00	0.28±0.01	0.47±0.01	1.76±0.01
Total K (g/kg)	10.66±0.20	5.03±0.01	5.03±0.01	5.13±0.01	2.67±0.00	2.47±0.01
Total P (g/kg)	1.41±0.08	1.16±0.01	1.13±0.01	1.12±0.03	0.94±0.01	0.72±0.01
Total Na (g/kg)	2.64±0.06	0.86±0.00	0.78±0.01	0.79±0.01	2.04±0.01	0.35±0.00
Total Fe (g/kg)	1.28±0.33	1.18±0.01	1.07±0.01	0.83±0.03	1.1±0.00	1.01±0.01
Total Mn (mg/kg)	143.34±14.11	110.78±2.86	68.73±2.86	61.20±2.97	98.87±2.85	84.04±1.46
Total Zn (mg/kg)	49.47±1.56	30.1±0.21	28.3±1.41	25.4±0.57	163.71±4.26	138.21±2.86
Total Cu (mg/kg)	58.01±5.67	20.67±0.44	13.85±0.57	14.18±0.45	24.24±0.74	16.77±1.81
Total Pb (mg/kg)	180.52±28.34	161.13±7.11	163.90±4.53	163.9±14.27	172.21±2.98	166.67±11.46
Total Ni (mg/kg)	81.91±12.76	73.12±4.38	71.66±7.79	70.19±14.3	81.18±1.5	74.59±5.69
pH	Min: 7.53 Max: 7.61	Min: 8.58 Max: 8.74	Min: 8.92 Max: 9.20	Min: 9.15 Max: 9.21	Min: 9.06 Max: 9.34	Min: 7.63 Max: 7.77

Values indicate the mean ± standard deviation based on determination with two replications. *B= Biochar **AB= Activated biochar.

2-3 Composting system and experimental design

The experiment was carried out with two factors of biochar type at eleven levels, and time at eight levels of biochar, with two repetitions for three months. For this purpose, biochars of 2-4 mm, 1-2 mm, 0.5-1 mm, activated biochar (AB) with H₂SO₄ and NaOH (H₂SO₄-AB_{0.5-1mm} and NaOH-

107 AB_{0.5-1mm}), were thoroughly mixed with MSW each at two levels of 5 and 10% (w/w). One
108 treatment with no addition of biochar was considered the control. The substrates were
109 accommodated in 100-liter plastic barrels (44 cm diameter and 76 cm height) with an approximate
110 weight of 70 kg. In each barrel, one kg of cow manure was added to increase the microbial activity,
111 and 30 holes were drilled for better ventilation. The temperature of the composting mixture was
112 monitored daily. Once a week, the compost materials were turned and thoroughly mixed. The
113 moisture of the materials was kept in the range of 50-60% by weight until the end of the experiment.
114 Sampling was performed on days 1, 7, 14, 28, 42, 56, 70, and 90, and each time at least five
115 subsamples (approximately 500 g) were taken from different depths of the barrel and mixed
116 thoroughly, and a composite sample was taken (Awasthi et al., 2017a). The composite sample was
117 divided into two subsamples (air-dry and moist). The air-dried samples were used to measure the
118 basic physicochemical properties and elemental concentrations, and the moist samples were used
119 to measure the biological indicators and concentrations of NO₃⁻ and NH₄⁺. Both samples were
120 stored in the refrigerator at 4 °C prior to analysis.

121

122 2-4 Compost analysis

123 The pH and EC were measured in 1 to 5 (w/v) compost to water ratio (Awasthi et al., 2017a).
124 The organic matter (OM) content was determined by ashing at 550 °C for 6 hours (Haug, 1993).
125 Total N (TN) was determined by the Kjeldahl method (Bremner, 1996). The NH₄⁺ concentration
126 was determined using the methods of indophenol blue (Li et al., 2015). The NO₃⁻ concentration
127 was measured using the sulfosalicylic acid (Cataldo et al., 1975). The germination index (GI), as
128 an indicator of phytotoxicity, was evaluated according to the method proposed by Zucconi et al.
129 (1981). The activity of enzymes, including urease (Tabatabai, 1994), and dehydrogenase (Schinner
130 et al., 2012) and microbial respiration (Anderson, 1983), were also determined in three composting
131 periods of mesophilic, thermophilic, and maturing (1, 7, and 90 days, respectively). The humic acid
132 was extracted and purified from compost according to the method described by Sánchez- Monedero
133 et al. (2002). To determine the E₄/E₆ and E₃/E₅ ratios, the absorbance was measured using a
134 spectrophotometer (SU-6100, Philler Scientific) at wavelengths of 465, 665, 350, and 550 nm
135 (Chen et al., 1977):

136

137 **2-5 Statistical analysis**

138 After the normality test, a repeated-measures ANOVA was performed to evaluate the main and
139 interaction effects of treatments and time on some dynamic response characteristics. The mean
140 comparisons were performed by the Duncan method ($p \leq 0.05$). Principal component analysis
141 (PCA) and cluster analysis were also used to group similar individuals. All statistical analyses and
142 drawing graphs were performed using SPSS 27.0 and Origin software.

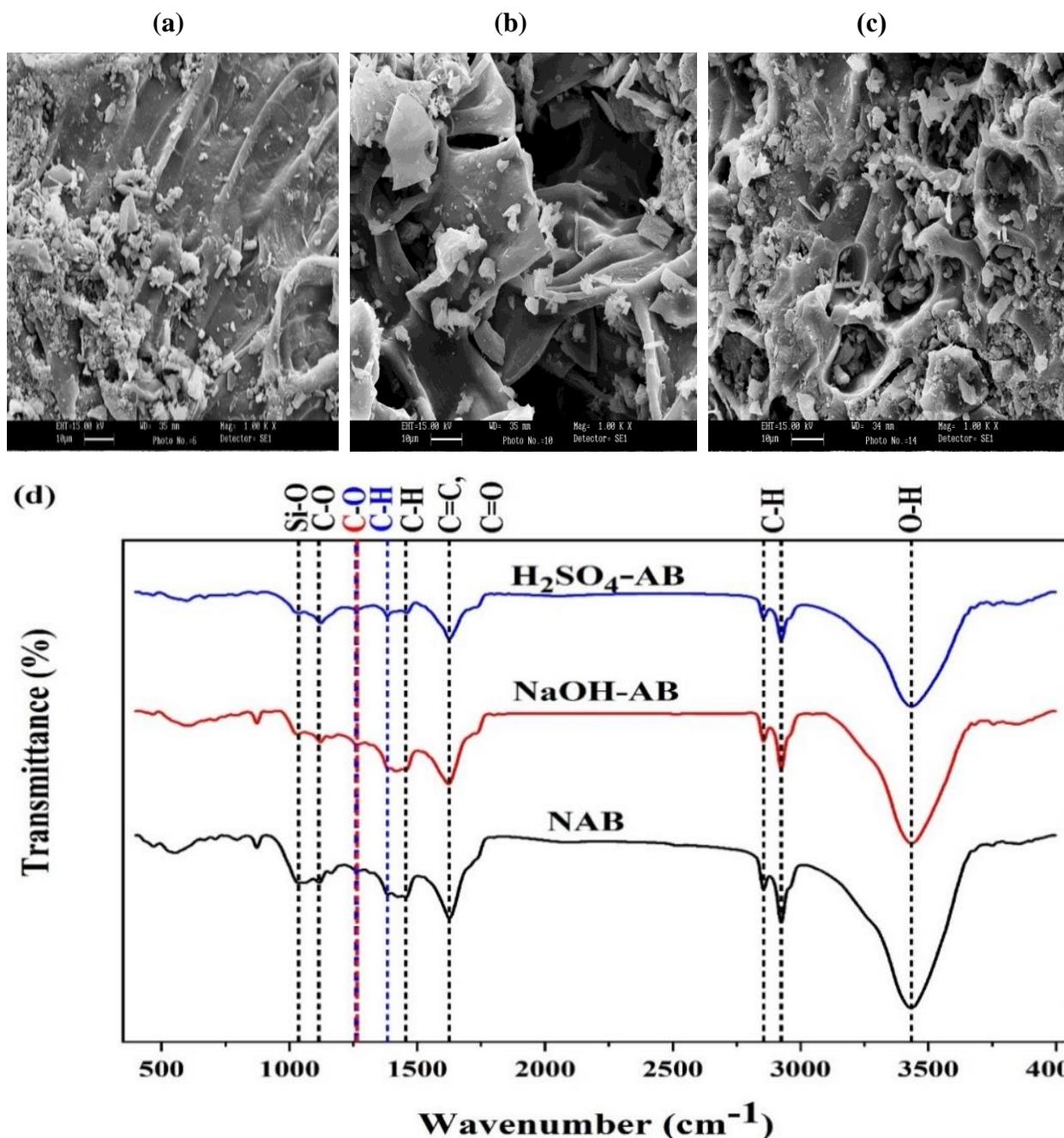
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144 **3 Results and discussion**

145 **3-1 The effect of chemical activation on biochar properties**

146 The chemical activation of biochar caused the appearance of a mesh structure with irregularly
147 sized cavities and deep pores by modifying the biochar surface structure (Fig. 1-a, Fig. 1b, c). An
148 et al. (2020) also reported that the chemical activation of biochar significantly changed the biochar
149 surface structure and increased its specific surface area.

150 Fig. 1-d shows the FTIR spectra of the activated and non-activated biochars (NAB). No distinct
151 differences in the position of the relevant peaks were observed between the FTIR spectra of the
152 non-activated and activated biochars. However, similar to the results of Dehkhoda et al. (2014),
153 the chemical activation of biochar diminished the intensity of the peaks, indicating removal or
154 reduction of some compounds. The activation of biochar each produced a peak of approximately
155 1260 to 1270 cm^{-1} related to the presence of C-O in aryl esters (aromatic structure). On the other
156 hand, H_2SO_4 -AB produced a peak of approximately 1384 cm^{-1} compared to NaOH-AB and NAB
157 (related to the presence of C-H in CH_2 or CH_3 (aliphatic structure)).

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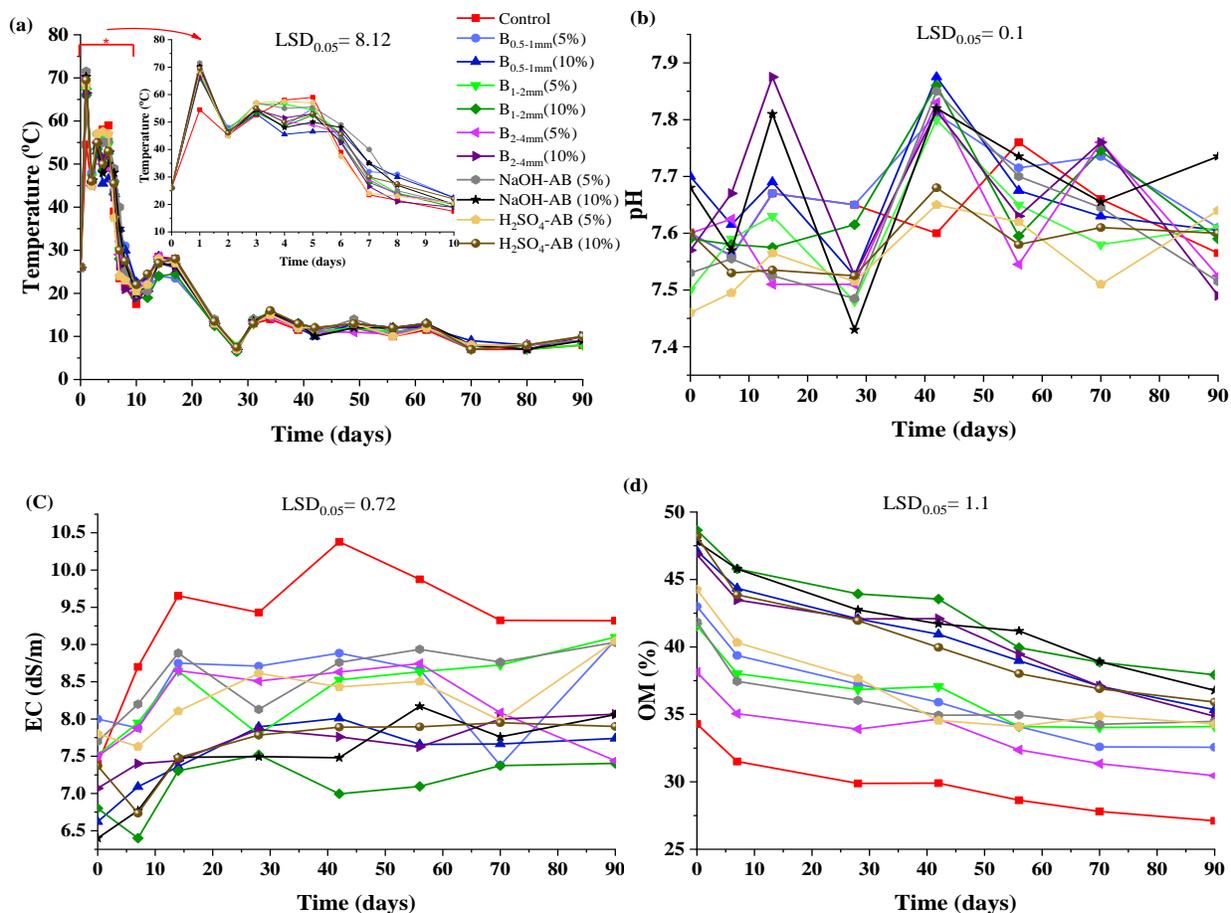


167 **Fig. 1.** Scanning electron microscope (SEM) images of NAB (a), NaOH-AB (b), H₂SO₄-AB (c)
 168 and FTIR spectra of NAB and NaOH-AB and H₂SO₄-AB (d).
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170 3-2 Impact of biochar on some compost maturity and stability indices

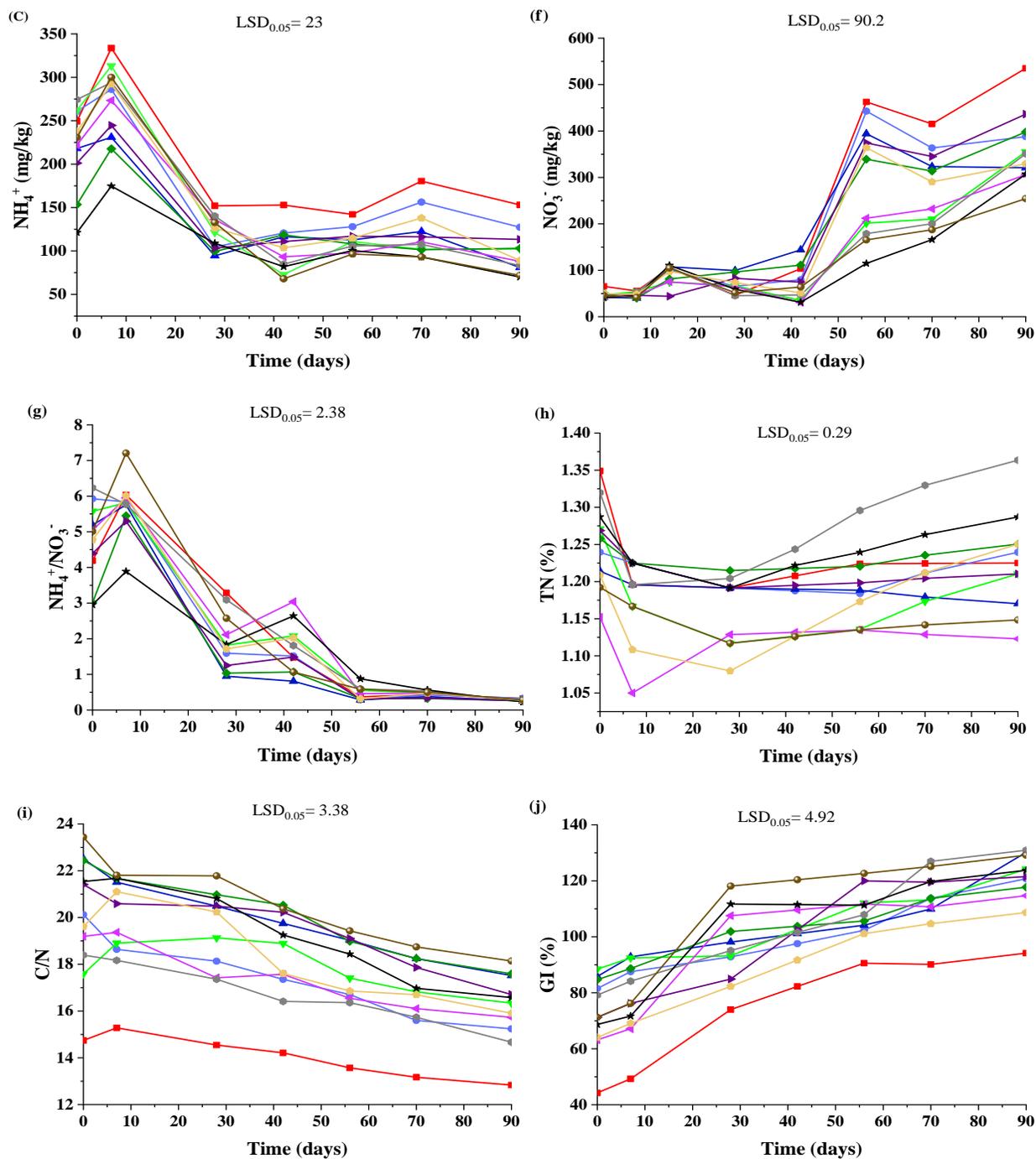
171 The addition of biochar increased the temperature in the thermophilic phase ($P < 0.05$), indicating
 172 the effect of biochar on microbial activity (Fig 2a). Except for the control and H₂SO₄-AB (5%)
 173 treatment, in which the thermophilic period was five days, the rest of the biochar treatments had a

174 longer thermophilic period (6 and 7 days). On the first day of the thermophilic period, the minimum
 175 temperature (54.5 °C) was related to the control and the maximum (71.5 °C) was associated with
 176 the longest thermophilic period (7 days) for the NaOH-AB (5%) treatment. Compared to the
 177 control, biochar treatments led to the early onset of the thermophilic phase, increasing the
 178 temperature and prolonging this phase, which agrees with the results of Manu et al. (2021).



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184 **Fig. 2** Changes in temperature (a), pH (b), EC (c), OM (d), NH₄⁺ (g) and NO₃⁻ concentrations (h),
 185 NH₄⁺/NO₃⁻ ratio (i), TN (j), C/N ratio (k), and GI% (l) during **composting**.

186

187 The pH of the **compost** fluctuated between 7.4 and 7.9. As shown in Fig 2b, at the initial phase
 188 of the **composting**, the pH of the **compost** decreased, probably due to the release of organic acids

189 then decomposition of proteins started and resulted in an increase in pH due to NH_3 production. On
190 days 28 to 42, the pH of the **compost** increased again due to the continued decomposition of organic
191 acids, which was consistent with (Wang et al., 2023). Finally, the pH of the **compost** pile stabilized
192 at values between 7.49 and 7.74 and the **compost** produced in the NaOH-AB ([msocom_110%](#))
193 treatment had the highest pH (7.74) ($p < 0.05$), in response to NaOH solution used in the biochar
194 activation process. **There is confusing literature on biochar effects. Both increasing (Vandecasteele**
195 **et al., 2016) and decreasing (Mao et al., 2018) effects of biochar addition on the final pH of the**
196 **compost piles have been reported. However, some studies, similar to our study, did not observe a**
197 **significant difference in the pH of the final compost (Manu et al., 2021; Janczak et al., 2017). Much**
198 **of the confusion has probably arisen from the different nature of feedstock as well as biochar and**
199 **different conditions of co-composting processes.**

200 According to Fig. 2c, the maximum and minimum values of final EC were recorded in the
201 control (9.32 dS/m) and B_{1-2mm} (10%) treatments (7.41 dS/m), respectively. **The increase in EC was**
202 **more intense in the thermophilic period and can be related to the increase in the activity of**
203 **microorganisms and mineralization of OM.** The most significant decrease was found in the
204 treatments with 10% biochar, which indicated the dilution or absorption effect of added biochar.
205 Qu et al. (2020) also reported the potential of biochar to reduce **compost** EC values through dilution
206 and/or absorption.

207 The content of OM decreased over the time of **composting** process due to the consumption of
208 carbon by microorganisms (Fig 2d). After the thermophilic period, the decrease in OM content
209 slowed. In the final **compost**, the highest and lowest OM contents were observed in the B_{1-2mm}
210 (10%) treatment (37.93%) and the control (27.1% **based on dry weight**), respectively ($p < 0.05$). In
211 general, the level of OM in the 10% biochar treatments was almost higher than that in the 5%
212 biochar treatments. Due to the chemical recalcitrance of biochar aromatic structure, it does not go
213 through severe degradation during **composting**. This is consistent with the results of Manu et al.
214 (2021). The reduction in OM content was the highest in the H₂SO₄-AB (10%) treatment (12.26%)
215 and the lowest in the control (7.18%).

216 The NH_4^+ concentration increased at the beginning of the thermophilic period and then decreased
217 (Fig. 2e). The initial increase could be due to ammonification reactions. In the final **compost**, the
218 NaOH-AB (10%) treatment had the lowest NH_4^+ concentration (70 mg/kg), and the control had the

219 highest NH_4^+ concentration (153 mg/kg), with significant differences from the rest of the treatments
220 ($p < 0.05$) and indicating the positive effect of biochar on the reduction in NH_4^+ concentration. The
221 activated biochars, especially NaOH-AB (5%), had the highest impact on reducing NH_4^+
222 concentration, while the control had the lowest. This observation can be explained by combined
223 roles of adsorption (the high absorption capacity of the activated biochars for NH_4^+) and microbial
224 immobilization in reducing nitrogen loss. On the other hand, the carboxylic and phenolic functional
225 groups attached to the surface of biochar, as a result of the aging process (Nguyen et al., 2017) or
226 activation with NaOH, have negative charges to adsorb NH_4^+

227 During composting, the concentration of NO_3^- , in contrast to NH_4^+ , increased (Fig 2f). In the
228 first few weeks of the composting process, the NO_3^- concentration was low, and there were no
229 significant changes in its concentration because the temperature, pH, or NH_4^+ concentration was
230 high enough to prevent the activity and growth of nitrate-producing bacteria (Ren et al., 2019;
231 Wang et al., 2023). After 42 days, the NO_3^- concentration rapidly started to increase and finally
232 reached a relatively constant level. The control and the H_2SO_4 -AB (10%) treatment had the highest
233 and lowest NO_3^- concentrations, respectively ($p < 0.05$). These results were consistent with the
234 results of Manu et al. (2021). The higher concentration of NO_3^- in the control was due to its lower
235 temperature, which favored the nitrification process.

236 The $\text{NH}_4^+/\text{NO}_3^-$ ratio is used as a nitrification index to check the compost maturity and stability.
237 Ratios less than 0.5 are considered fully mature compost. All the composts produced in this
238 research were fully mature regarding the $\text{NH}_4^+/\text{NO}_3^-$ ratio (Fig. 2g). The highest and lowest ratios
239 in the final compost were detected in the control (0.33) and the NaOH-AB (10%) treatment (0.23),
240 respectively. It was observed that the treatments with 10% biochar had lower $\text{NH}_4^+/\text{NO}_3^-$ molar
241 ratios and reached the mature phase earlier. Furthermore, in the NaOH-AB treatments, the
242 $\text{NH}_4^+/\text{NO}_3^-$ ratio decreased with decreasing biochar particle size ($B_{0.5-1\text{mm}} < B_{1-2\text{mm}} < B_{2-4\text{mm}}$).

243 In this study, the TN content first decreased sharply and then gradually increased during the
244 composting process ($p > 0.05$) (Fig 2h). The most significant decrease in TN content coincided with
245 a sharp increase in NH_4^+ concentration. These results were consistent with the reports of Wang et
246 al. (2023). The reason for the reduction in TN content in the thermophilic phase can be associated
247 with denitrification and/or ammonia volatilization. The TN content decreased in all treatments from
248 the first to the seventh day (thermophilic period). However, the highest initial TN content (1.35%)

249 and the maximum of its subsequent decrease from the first day to the seventh day (11.35%) were
250 both observed in the control ($p < 0.05$). This sharp decrease was accompanied by a significant
251 increase in NH_4^+ concentration (Fig. 4d). Manu et al. (2021), reported that N losses during the
252 composting averaged 31.4% TN, 17.2% NH_3 , and 1.4% N_2O .

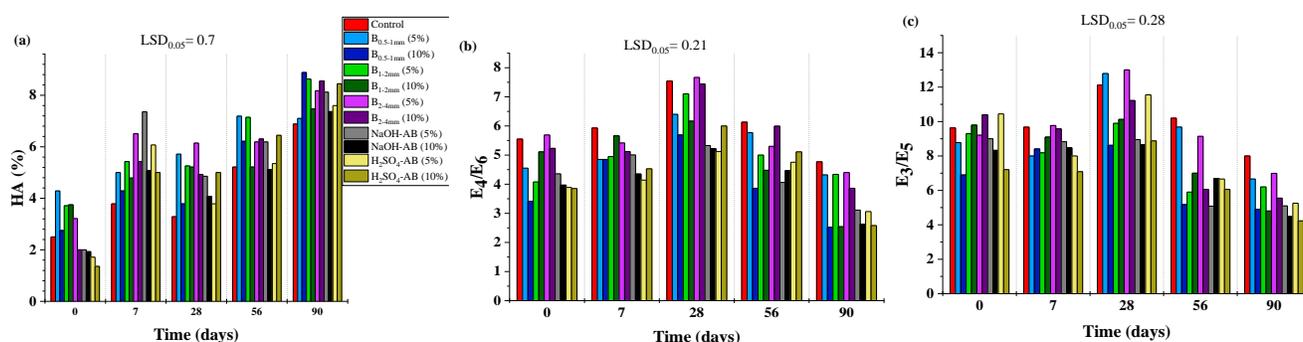
253 The decline in the C/N ratio in the control was less than that in the biochar-amended treatments
254 (Fig. 2i). The lowest C/N ratio at both the beginning and end of the composting process was
255 detected in the control (14.74 and 12.83, respectively), which indicated the loss of nitrogen through
256 ammonia volatilization and/or NO_3^- leaching from the compost pile. At low C/N ratios, carbon is
257 consumed before nitrogen fixation, and an unpleasant smell is generated due to ammonia
258 volatilization. In the conditions of this research, the decline in the C/N ratio in the composts that
259 received biochar was 28-46% higher than the decline in the C/N ratio in the control. The average
260 C/N ratio in the composts receiving 10% biochar was higher than those receiving 5% biochar.
261 Wang et al. (2023) recommended the use of biochar to decrease the C/N ratio of final compost,
262 likely due to enhanced nitrogen conservation and OM degradation in the compost pile. However,
263 other researchers, such as Vandecasteele et al. (2016), found the addition of biochar to be
264 inappropriate, likely due to the high doses used. Some studies also reported no significant
265 difference in the C/N ratio between composts with and without biochar incorporation (Malińska et
266 al., 2014). One of the reasons for these conflicting results is the difference in the C/N ratio of the
267 raw materials. Considering the carbon content in the biochar used in this research (51-64%), it is
268 recommended to add biochar to MSW in order to increase the C/N ratio to the appropriate range
269 of 20-30 and prevent nitrogen loss.

270 The most important tests to evaluate compost quality characteristics is the GI. The standard
271 value of the GI is reported to be $\geq 80\%$ for compost maturity (Zucconi et al., 1981). The GI had an
272 increasing trend during composting (Fig. 2j). However, in the final compost, the control had the
273 lowest GI (94%), and the NaOH-AB (5%) treatment had the highest GI (131%) ($p < 0.05$). Similar
274 to the results of this research, some researchers also reported that the GI in composts with biochar
275 was higher than that in composts without biochar (Manu et al., 2021; Wang et al., 2023). The higher
276 GI in the biochar treatments compared to the control in this study, can be attributed to their higher
277 temperature and longer thermophilic period, which helped remove pathogenic bacteria and
278 improve compost quality.

279 **3-3 Humic acid yield, E₄/E₆ and E₃/E₅ ratios**

280 The HA yield increased during composting, mainly in the thermophilic phase (Fig 3a). The
 281 highest and lowest HA% were related to the B_{0.5-1mm} (10%) treatment (8.9%) and control (6.9%),
 282 respectively. By adding 0 to 10% biochar to the compost, the HA% of the final compost increased,
 283 on average, from 0 to 22.54%, which is consistent with the results Jindo et al. (2016). The enhanced
 284 humic acid production induced by biochar addition during composting may be due to the release
 285 of aromatic precursors from biochar or the adsorption of soluble organic compounds on the active
 286 surfaces of biochar (Jindo et al., 2016).

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289 **Fig. 3.** Time dependence of humification indices during the co-composting process: Humic acid
 290 yield (a), E₄/E₆ ratio (b), and E₃/E₅ ratio (c).

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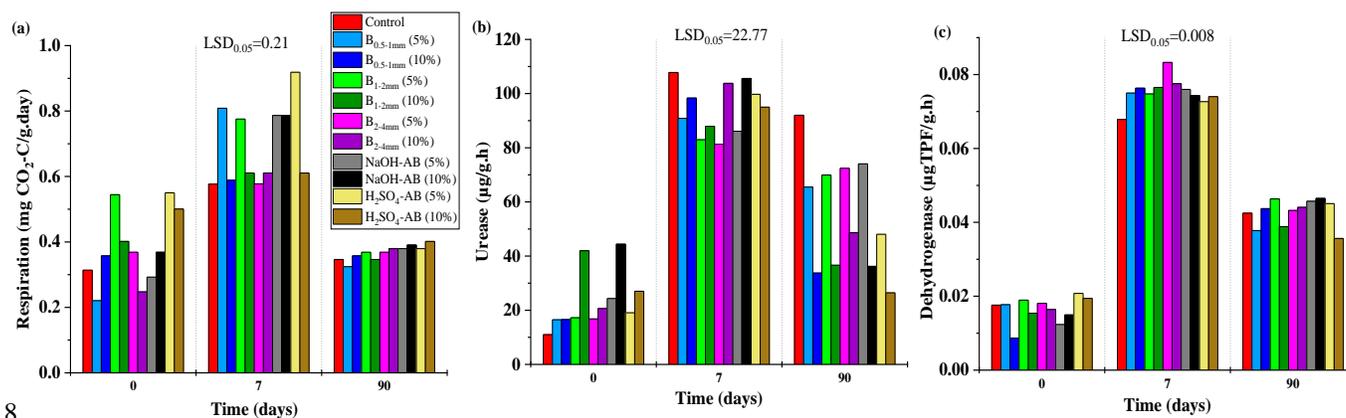
292 The E₄/E₆ ratio is inversely related to the degree of aromatic condensation of the humic
 293 substances (Ren et al., 2019). The E₃/E₅ ratio also denotes a more or less similar concept. In this
 294 research, the trends of both the E₄/E₆ and E₃/E₅ ratios over time first increased and then decreased
 295 (Fig. 3b, c). For composts with low degrees of humification due to the presence of proteins and
 296 carbohydrates, the ratios of E₄/E₆ and E₃/E₅ were high (Chen et al., 1977). As the degree of
 297 humification increased, large molecules were formed, and the E₄/E₆ and E₃/E₅ ratios decreased.
 298 The E₄/E₆ ratios of the extracted HA varied between 2 and 10. The ratios near 2 are considered to
 299 be mature compost. In this research, the control had the highest E₄/E₆ and E₃/E₅ ratios (4.77 and 8,
 300 respectively), and the B_{0.5-1mm} (10%), B_{1-2mm} (10%), NaOH-AB (10%), and H₂SO₄-AB (10%)
 301 treatments had the lowest ratios of E₄/E₆ (2.52 to 2.63) and E₃/E₅ (4.23 to 4.9) (P < 0.05). Wang et
 302 al. (2023) also reported E₄/E₆ ratios of 2.84 to 3.47 for biochar-treated composts. The control in
 303 the compost production process, showed decreases in the E₄/E₆ and E₃/E₅ ratios, indicating the

304 lower production of humic substances without biochar application. These results were consistent
305 with those of Wang et al. (2023) and Manu et al. (2021).

306

307 3-4 Microbial respiration, activity of urease, and dehydrogenase

308 The biochar treatments, compared to the control, caused a significant increase in microbial
309 respiration for the thermophilic period only ($p < 0.05$). Microbial respiration increased in the
310 thermophilic period due to the presence of easily degradable compounds, whereas it decreased in
311 the final days of composting (Fig. 4a). In the thermophilic period, the control and the AB treatments
312 had the lowest and highest microbial respiration, respectively. Biochars with smaller sizes ($B_{0.5-1mm}$
313 $_{1mm}$ and B_{1-2mm}) had greater rates of microbial respiration than biochars with larger sizes (B_{2-4mm}).
314 This is probably because of their higher specific surface area supporting a large proportion of the
315 total microbial community in the compost pile. The biochar treatments had higher levels of
316 microbial respiration (17.5%, on average) than the control. This finding is consistent with the
317 results of Steiner et al. (2011).



318
319 **Fig. 4.** Changes in biological indices during **composting**: Respiration (a), urease (b), and
320 dehydrogenase (c).

321
322 Urease is an enzyme that its activity is determined by measuring the concentration of NH_4^+
323 produced. In the final compost, the highest and lowest urease activities were observed in the control
324 and the H_2SO_4 -AB (10%) treatment, respectively. Similar results were obtained for the NH_4^+
325 concentration. As expected, urease activity increased from the beginning of **composting** to the
326 thermophilic phase and then decreased toward the maturing phase (Fig. 4b).

327 The activity of dehydrogenases enzyme often matches the microbial activity. In this study, the
328 changes in dehydrogenase activity were similar to those observed for microbial respiration and

329 urease activity, with a maximum in the thermophilic period (Fig. 4c). Zhang and Sun, (2014) also
330 reported that the incorporation of biochar can increase the activity of dehydrogenase in the
331 thermophilic phase. The possible reason for this is the supply of nutrients from the biochar.

332

333 3-5 Principal component analysis (PCA)

334 Based on the results obtained, 61% of the cumulative variance is explained by the first and
335 second components (Fig 5). Among the quality parameters of the produced **composts**, those with
336 the most impact on the first component were C/N> OM> urease activity> temperature> NO₃⁻>
337 EC> E₃/E₅ ratio> E₄/E₆ ratio> NH₄⁺ concentration, and those with the most influence on the second
338 component were HA> dehydrogenase> GI. In addition, PCA enabled the grouping of different
339 **composts**. As shown in Fig 5 (red points), there was a significant distance between the control and
340 the biochar-treated composts, indicating the significance of biochar incorporation in the
341 composting process. **To elaborate on specific interactions between variables, it can be noted that**
342 **the reduction in the C/N ratio was associated with an increase in OM, indicating improved**
343 **decomposition of OM in the presence of AB. The NaOH/H₂SO₄-activated biochars enhanced**
344 **microbial activity and surface interactions, which contributed to a faster reduction in the C/N ratio.**
345 **Additionally, a positive correlation between OM content and temperature during the thermophilic**
346 **phase highlighted biochar's role in enhancing microbial activity and enhancing compost quality.**
347 **Similar to our findings, Awasthi et al. (2017b) reported that the PCA showed the strongest**
348 **correlation with OM degradation and the C/N ratio.**

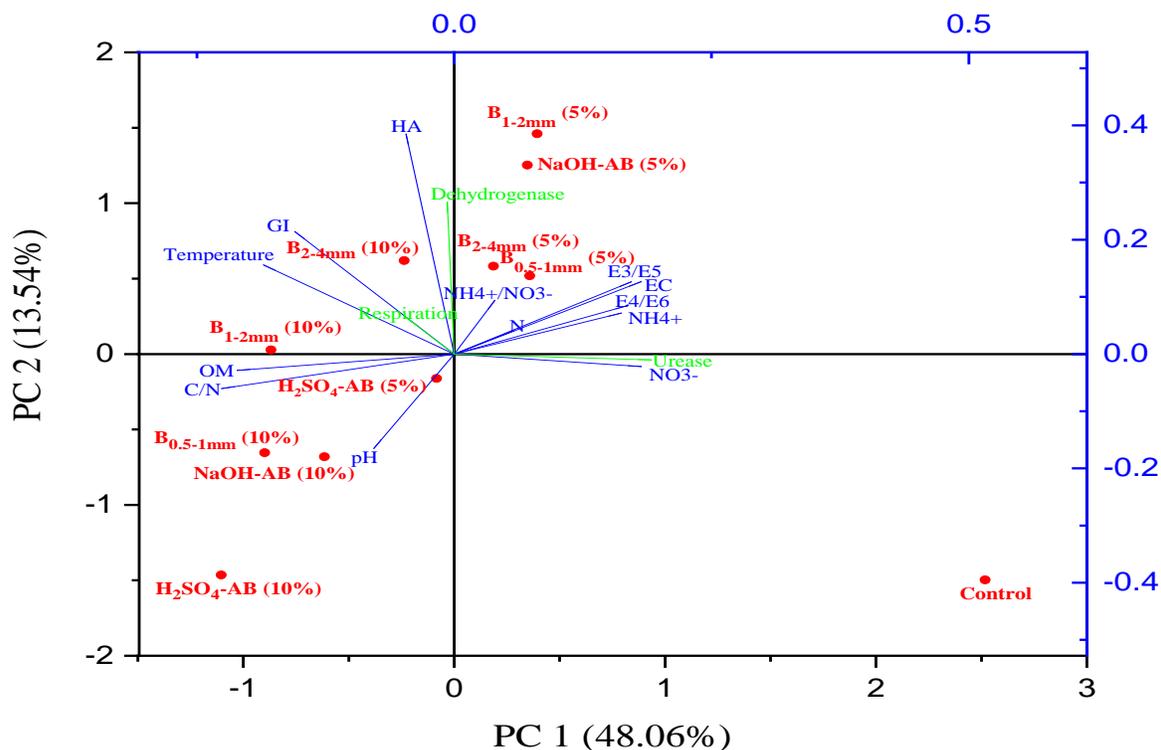


Fig. 5. PCA biplot during composting.

4 Conclusions

The results indicated that chemically AB (NaOH-AB and H₂SO₄-AB), particularly with small-sized biochar (0.5-2 mm) and when incorporating 10% biochar compared to 5% (w/w), enhanced stability and maturity indices, promoted OM biodegradation, and improved humification indices. This presents an effective strategy for improving the quality of MSW co-compost. PCA further highlighted the critical role of the C/N ratio and OM content in the co-composting. We recommend that future studies compare various activation methods, optimize biochar activation conditions to maximize composting efficiency, and conduct cost-benefit and carbon footprint analyses.

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