Influence of Biochar on Physico-Chemical, Microbial Community and Maturity during Biogas Residue Aerobic Composting Process

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Abstract: With the rapid development of large and medium-sized biogas projects, the high-value utilization of anaerobic fermentation residues has become a hot spot in recent years. In this study, biogas residue from biogas engineering was used as composting raw material, and 0 (CK), 2.5% (T1), 5.0% (T2), 7.5% (T3), and 10.0% (T4) biochar was added to investigate its effects on physico-chemical properties, microbial populations, and maturity degree during the aerobic composting process. Results show that the addition of biochar shortens the time (3 days) to reach the high-temperature period, increases the composting temperature (63.8 °C) and germination index (GI), decreases the electrical conductivity (EC), reduces the loss of C and N elements, and increases the microbial population during composting. These results suggest that biochar can improve the maturity and fertility of compost products, and significantly regulate the structure and function of microbial communities during the composting process.

Keywords: biogas residue; biochar; aerobic composting; maturity indices; microbial community

1. Introduction

Biogas residue (BS) contains a variety of amino acids, organic acids, vitamins, trace elements, hydrolytic enzymes, phytohormones, and biologically active substances such as humic acids, which commonly serve as the major raw material for high-quality organic fertilizers and soil conditioners [1]. However, due to the presence of irritating gases, high viscosity, phytotoxicity, and residual heavy metals in untreated digestate, as well as the complexity and high cost of the digestate application process, the above factors seriesssly hinder the direct return of digestate to the field for utilization [2]. Therefore, before application to the soil, the digestate needs to be treated accordingly to expand its applicability. Aerobic composting of digestate is a more effective way to solve the comprehensive use and prevent secondary pollution [3]. The efficiency of individual aerobic composting of digestate is poor due to the presence of cellulose-like materials and other hard-to-degrade organic matter (OM). Nevertheless, aerobic composting of digestate can be effectively improved by adding livestock manure, conditioners, and annexing agent to the composting material [4,5].

Biochar reflects a wider diversity in terms of physico-chemical properties, such as pH, structure, particle size distribution, and specific surface area (BET), which makes it...
play different roles in various applications [6]. Currently, the utilization of biochar in agriculture is focused on improving soil to increase agricultural productivity, mitigating climate change, and producing energy [7]. As an exogenous additive, biochar has a certain impact on temperature, electrical conductivity, pH, C/N and nutrients with its, unique physical–chemical properties, thereby changing the micro-environmental conditions of the compost, expediting the composting process, and enhancing the quality of the compost products [8]. Previous studies show that by adding a mixture with a high C/N ratio, a higher amount of NH$_4^+$-N within the pile can be adsorbed and the accumulation of NH$_4^+$-N can be reduced, thus, reducing the loss of N elements [9,10]. In addition, when biochar was added to the compost with livestock manure as raw material, the degradation rate of OM accelerated, the maturity process effectively shortened, and the humic acid content of the compost product was substantially higher than that of the control [11]. Biochar can also significantly improve composting conditions by increasing the number of microorganisms and effectively promoting microbial reproduction [12]. Corn is a common agricultural product, however, a large amount of corn cobs are discarded as waste every year. Studies show that biochar made from corn cobs has a large surface area and pore volume, making it a low-cost biosorbent. In addition, the incineration and disposal of large amounts of corn cobs not only wastes biological resources, but also causes serious environmental pollution. Therefore, the preparation of biochar from corn cobs as an additive to compost is of great significance for waste recycling, as well as environmental protection.

Based on the aforementioned considerations, we are hopeful to study the effect of the biochar addition on composting and its related mechanism when BS is used as the composting material. The present work intends to add different amounts of biochar to the initial stage of BS aerobic composting to explore the dynamic changes in physical–chemical properties, microbial population, and the influence of humification in the composting process, to provide theoretical reference for the utilization of BS composting and the development of biochar-based organic fertilizer.

2. Materials and Methods

2.1. Feedstock

The BS was taken from an anaerobic digester of a company in Heilongjiang Province, China, which was fed with cow manure and corn straw, with an effective volume of 5400 m$^3$ and organic load and hydraulic residence time of 2.5 kg vs. m$^{-3}$ d$^{-1}$ and 30 days, respectively. Pig manure and corn stalk were obtained from the Acheng base of Northeast Agricultural University, and the corn stalk was crushed to small particles (less than 1 cm). The dried corncobs (80 mesh) were heated and carbonized in a tube furnace with N$_2$. The heating rate was 10$^\circ$C/min, and the carbonization temperature was 600$^\circ$C and maintained for 1 h to gain the corncob biochar (0.1–0.5 mm). The initial physico-chemical properties of composting raw materials are shown in Table 1.

Table 1. Characteristics of the composting raw materials.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Biogas Residue</th>
<th>Pig Manure</th>
<th>Corn Stalk</th>
<th>Biochar</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (%)</td>
<td>29.92 ± 0.58</td>
<td>8.52 ± 0.58</td>
<td>41.53 ± 2.18</td>
<td>46.14 ± 1.76</td>
</tr>
<tr>
<td>TN (%)</td>
<td>1.53 ± 0.03</td>
<td>0.54 ± 0.03</td>
<td>0.78 ± 0.06</td>
<td>0.94 ± 0.03</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>68.20 ± 1.35</td>
<td>69.34 ± 2.86</td>
<td>10.44 ± 0.51</td>
<td>7.42 ± 0.17</td>
</tr>
<tr>
<td>pH</td>
<td>7.46 ± 0.37</td>
<td>7.48 ± 0.32</td>
<td>6.96 ± 0.35</td>
<td>9.90 ± 0.37</td>
</tr>
<tr>
<td>EC (mS · cm$^{-1}$)</td>
<td>12.19 ± 0.48</td>
<td>5.26 ± 0.15</td>
<td>2.58 ± 0.20</td>
<td>12.14 ± 0.48</td>
</tr>
<tr>
<td>OM (%)</td>
<td>59.58 ± 1.26</td>
<td>46.05 ± 1.48</td>
<td>90.82 ± 3.56</td>
<td>64.23 ± 2.87</td>
</tr>
<tr>
<td>BET (m$^2$/g)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>120.24 ± 6.11</td>
</tr>
</tbody>
</table>

The data represent the mean ± standard deviation of three replicates. NA: not analyzed.

2.2. Composting Process

A total of five treatment groups were carried out (Table 2), based on the dry weight of the total compost material, and the addition ratio of biochar was 0–10%. Therefore, the following
treatments were used: CK (control without biochar), T1, T2, T3, and T4. A small amount of crushed corn straw and pig manure was added to adjust the initial C/N ratio to 25 and the moisture content was adjusted to 60–65%. The material was added into the composting reactor with an effective volume of 25 L (outer diameter \( \times \) height \( \times \) wall thickness: 35 \( \times \) 70 \( \times \) 5 cm). The stirring system mixes the pile every 2 days. The aeration device was connected to the bottom of the reactor for ventilation (Figure 1), and the aeration time was set to 5 min and the interval was 40 min. The central temperature of pile was measured per diem. The experiment lasted for 40 days, as the aerobic compost had relatively stable changes in physico-chemical properties at 40 days, and decomposition was basically completed. Approximately 200 g of compost samples were collected from five different areas at 20 cm of the pile on day 1, 2, 4, 8, 16, 24, 32, and 40. Each sample was divided into two equal portions and preserved in a refrigerator at \(-4\) °C for testing of physical–chemical indexes.

Table 1. Amount of composting materials added in each treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biogas Residue (kg)</th>
<th>Corn Stalk (kg)</th>
<th>Pig Manure (kg)</th>
<th>Biochar (kg)</th>
<th>Biochar Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>8.00</td>
<td>1.05</td>
<td>0.85</td>
<td>0.10</td>
<td>2.5</td>
</tr>
<tr>
<td>T2</td>
<td>8.00</td>
<td>0.93</td>
<td>0.87</td>
<td>0.20</td>
<td>5</td>
</tr>
<tr>
<td>T3</td>
<td>8.00</td>
<td>0.83</td>
<td>0.88</td>
<td>0.29</td>
<td>7.5</td>
</tr>
<tr>
<td>T4</td>
<td>8.00</td>
<td>0.74</td>
<td>0.88</td>
<td>0.38</td>
<td>10</td>
</tr>
<tr>
<td>CK</td>
<td>8.00</td>
<td>1.17</td>
<td>0.83</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1. Schematic diagram of composting reactor. 1. Aeration fan, 2. gas flow meter, 3. stirring motor, 4. temperature monitoring, 5. gas collection, 6. sieve plate, 7. leachate outlet, 8. leachate collection.

2.3. Analytical Methods

The moisture content was measured by the drying method (105 °C, 12 h). The pH and EC were determined by taking the supernatant after mechanical shaking for 40 min using deionized water at a ratio of 1:10 (w/v, solid to water mixture). The pH of the supernatant was measured with a digital pH meter (Phsj-3f, Ray magnetic Co., Shenzhen, China) and EC of the supernatant was measured with an EC meter (DDSJ-318, Jingke Co., Shenzhen, China) [13]. The total carbon (TC) and total nitrogen (TN) contents were determined by EA 3000 elemental analyzer (Euro Vector, Italy) to calculate C/N. The microbial enumeration of bacterial, fungal, and actinomycete were measured according to serial dilution and standard plate diffusion techniques by nutrient agar, starch–casein agar, and potato dextrose agar.
as medium, respectively [14]. The dissolved organic carbon (DOC) was determined by total organic carbon analyzer (TOC-VCPH, Japan). The NH\textsubscript{4}\textsuperscript+-N and NO\textsubscript{3}\textsuperscript--N contents were determined using a continuous flow chemistry analyzer (San++ Skalar, Breda, The Netherlands). The germination index was calculated from a plant growth assay by taking 5 mL of the supernatant in a Petri dish with filter paper, placing 25 pak choi seeds evenly and incubating them at a constant temperature of 25 °C for 48 h in an incubator to calculate the GI (%).

\[
\text{GI} (%) = \frac{\text{Treatment germination rate} \times \text{Treatment root length}}{\text{CK germination rate} \times \text{CK root length}}
\] (1)

2.4. Data Analysis

The experimental data were expressed in the form of “mean ± standard error”, and the data were compiled using Excel 2013 software and plotted using Origin 9.0 software. One-way analysis of variance (ANOVA), Duncan’s multiple comparisons, and correlation analysis were analyzed by SPSS 25.0 software, and \( p < 0.05 \) was used as the significant level of difference.

3. Results and Discussion

3.1. Variations of Physico-Chemical Parameters during the Composting Process

3.1.1. Temperature

The temperature variation of the pile is used as one of the most intuitive indicators to characterize whether aerobic composting is proceeding smoothly [15]. The composting temperature of each treatment experiences a trend of a steady rise in the early stage, moderate maintenance of high temperature in the middle stage, and slow decline in the late stage (Figure 2a). The peak temperature of the pile for each treatment ranges from 60.4 to 63.8 °C, with T1, T2, T3, and T4 reaching the highest temperature at day 5, 4, 5, and 3, respectively. The peak temperatures of the treatment groups with the biochar are all higher than those of CK, indicating that the addition of biochar could increase the composting temperature and shorten the time to reach high-temperature period [16]. This is since the porosity of the pile can increase its availability of O\textsubscript{2} by the action of biochar, which is conducive to promoting the metabolic activities of microorganisms and, thus, releasing more heat [11]. At the same time, the heat production by microbial activities and the isolation of the compost pile excellently reduce the heat loss. Each treatment lasted for 7–8 days in the temperature range of 50–65 °C, which could effectively kill pathogenic microorganisms and weed seeds [17]. Compared with CK, the duration of high-temperature period after adding biochar increases by 1–4 days, in which T2 has the longest duration and T4 has the shortest duration. This is due to the microporous structure of biochar, which is conducive to maintaining the temperature of the compost pile [18]. When the percentage of biochar addition is too high, the porosity of the pile also increases and exceeds the optimum range, resulting in rapid loss in moisture during the high-temperature aeration state, which is not favorable for maintaining high temperature.

3.1.2. Moisture Content

As the composting proceeded, the moisture content of each treatment shows a gradually decreasing trend (Figure 2b). The moisture content of each stage basically presented CK > T1 > T2 > T3 > T4, i.e., the moisture content reduces remarkably with the increase in biochar addition, which is because the biochar promotes the reduction in bulk density of the pile, thus, enhancing the aeration performance and causing the evaporation of water [19]. In addition, coupled with the enhanced microbial activity, this process is accompanied by a large amount of moisture being consumed. At the end of composting, the moisture content of T1, T2, T3, and T4 is lower than that of CK. Ravindran et al. (2019) [14] also report the same result that the effect of biochar on the water content is determined by the pore size distribution and connectivity. Biochar could play a role in loosening the structure of the
pile, thus, forming a better ventilation and heat dissipation effect. The improvement in the ventilation environment indirectly promotes the activity of microorganisms in the pile, thereby improving the utilization and absorption of water by microorganisms [20].

3.1.3. pH

With the progress of composting, the pH of each treatment first increases and then decreases, and finally stabilizes (Figure 2c). In the warming stage, the addition of biochar produces NH$_3$ while promoting the decomposition of OM by microorganisms, which leads to a marked increase in pH. In the middle and late stages of the high-temperature phase (4–16 days), some of the OM is converted into organic acids by the bacteria and fungi, causing a decrease in the pH, which, in turn, promotes the breakdown of lignocellulosic. This trend is similar to the results of many studies [21,22]. During the cooling period to the end of composting, the pH gradually tends to be stable. At the end of composting, the pH of each treatment (T1, T2, T3, T4, and CK) is 7.98, 7.84, 7.85, 7.86, and 7.62, respectively, which could meet the optimal pH for composting [23]. Compared with CK, the addition of biochar improves the pH of the pile because of the higher pH of biochar itself. On the other hand, because of the higher ash content of biochar, which contains Na$^+$, K$^+$, Mg$^{2+}$,
Ca\(^{2+}\), and other salt ions that could effectively exchange H\(^+\), thus, increasing the pH [24]. In addition, biochar can retain NH\(_4^+\) while adsorbing NH\(_3\) because of its high porosity and large BET, as shown in Table 1 [25].

### 3.1.4. EC

EC can represent the soluble salt content in the compost, and if its value is higher than a certain level (2.5 mS · cm\(^{-1}\)), it causes toxic effects on crops after application [26]. The variations in EC in each treatment are similar to those of pH (Figure 2d). The study shows that the index of aerobic compost maturity is \(\text{EC} \leq 4.0 \text{ mS} \cdot \text{cm}^{-1}\) [27], and the EC of each treatment (T1, T2, T3, T4, and CK) at the end of composting is 1.11, 1.18, 1.12, 1.20, and 1.23 mS · cm\(^{-1}\), respectively, which are all lower than 4.0 mS · cm\(^{-1}\), indicating that the effect and risk of toxic effect on crops after compost are negligible. These results are less than those reported by Du et al. (2019) [28], who observed \(2 \leq \text{EC} \leq 4.0 \text{ mS} \cdot \text{cm}^{-1}\). The main reason for the difference is that the composting materials used in this study were BS, and most of the soluble salts had already entered the biogas slurry. Compared with CK, the treatments T1–T4 decrease by 9.76%, 4.07%, 8.94%, and 2.43%, respectively, indicating that biochar has a significant effect on reducing EC because of the adsorption of biochar.

### 3.1.5. GI

GI can reflect the changes in plant toxicity by comparing the effects of compost products on seed germination rate. Compared with the treatment group with biochar addition, the GI of the CK group increases more slowly in the early stage (Figure 2e). The reason may be that the NH\(_3\), volatile fatty acids (VFAs), and phenols in the initial pile have a certain inhibitory effect on plant growth, while biochar can bind these toxic compounds [29]. At the end of composting, the GI of each treatment (T1, T2, T3, T4, and CK) is 1.02, 1.08, 0.95, 0.90, and 0.88, respectively, which are all greater than 0.8, showing that the compost reaches maturity and basic non-toxicity level. More importantly, the addition of biochar could provide the OM required for plant growth and has a significant growth-promoting effect [30]. Zhou et al. (2019) [16] also report that biochar is able to combine toxic compounds and additives into the compost, thereby accelerating the release of phytotoxicity.

### 3.1.6. C/N

As one of the most essential factors of total nutrient balance in composting process, C/N can indirectly reflect the process of microbial energy acquisition and synthesis of new cells through metabolism [31]. The overall trend of C/N changes in all treatments is a continuous decline (Figure 2f), because more than 60% of the C elements are converted to CO\(_2\) by microorganisms under the action of inorganicization, and the remaining substances could be used to synthesize new cells with N elements. With the addition of biochar, C/N decreases faster during composting. This is due to the specific surface area of biochar making the particle size of the pile different, and the C that can be used by microorganisms, increasing its decomposition rate [32]. A significant decrease in the C/N of the CK group occurs during 30–40 days, which is because in the later stages of composting, the CK treatment, without the addition of biochar, reduces the porosity and ventilation inside the pile, which increases the anaerobic zone formed and increases the activity of denitrifying and methanogenic bacteria, increasing the loss in total C by increasing the emission of CO\(_2\) and CH\(_4\) [33]. Therefore, it causes a significant reduction in the C/N of CK. As one of the indicators for compost maturity evaluation, the T value reflects the extent to which the composting is proceeding. It represents the ratio of C/N after composting to before composting, and, generally, the T value ranges from 0.53 to 0.72 [34]. In general, when the T value is not more than 0.6, it can basically reflect that the compost has good maturity. According to this criterion, the T values of the products meet the maturity requirements.
3.2. Variations of Physico-Chemical Parameters during the Composting Process

3.2.1. Nitrogen Variations

In composting, the changes in the forms and contents of N have a great impact on the application of compost products. The effects of biochar on NH$_4^+$-N, NO$_3^-$-N, and TN during the composting are shown in Table 3. At the beginning of composting, the TN content of each treatment decreases, indicating that easily degradable materials in the raw material are relatively copious, and the physiological metabolic activity of microorganisms is vigorous. Some organic N is converted into NH$_4^+$-N, which causes a rapid increase in NH$_4^+$-N and pH [35]. The NH$_4^+$-N content increases with the increase in biochar addition ratio, indicating that the functional groups (-COOH, -CH=O, and -CR=O, etc.) and rich pore structure of biochar can effectively adsorb NH$_3$, which makes the NH$_4^+$-N content significantly higher than that of CK [36, 37]. As the compost enters the high-temperature phase, the organic materials rapidly decompose and the volatilization of NH$_4^+$-N is vigorous. Some organic N is converted into NH$_4^+$-N, which causes a rapid increase in NH$_4^+$-N, NO$_3^-$-N, and pH [35]. The NH$_4^+$-N content increases with the increase in biochar addition ratio, indicating that the functional groups (-COOH, -CH=O, and -CR=O, etc.) and rich pore structure of biochar can effectively adsorb NH$_3$, which makes the NH$_4^+$-N content significantly higher than that of CK [36, 37]. As the compost enters the high-temperature phase, the organic materials rapidly decompose and the volatilization of NH$_3$ gradually increases, thus, causing the NH$_4^+$-N content to decrease. However, the N loss in the T1–T4 treatments is not obvious, indicating that biochar helps reduce the N loss. During the cooling period, the TN content of all treatments shows a slow increase. At the end of the composting, the TN content of T1–T4 increases by 18.41%, 17.96%, 11.62%, and 19.30%, respectively, compared with that of CK. This is because the biochar promotes the degradation of cellulose by cellulase, providing nutrients for the composting system. Biochar also plays a role in nitrogen fixation and provides N sources for the growth and reproduction of microorganisms [38, 39].

Table 3. Effect of biochar on the change of ammonia nitrogen, nitrate nitrogen, and total nitrogen during composting.

<table>
<thead>
<tr>
<th>Nitrogen Variations</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$-N (g kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>2.11 ± 0.09 b</td>
<td>2.48 ± 0.12 b</td>
<td>3.09 ± 0.14 b</td>
<td>3.27 ± 0.16 c</td>
<td>2.58 ± 0.11 c</td>
<td>1.86 ± 0.06 b</td>
<td>1.01 ± 0.04 a</td>
</tr>
<tr>
<td>T2</td>
<td>2.33 ± 0.11 a</td>
<td>2.44 ± 0.08 b</td>
<td>3.11 ± 0.17 b</td>
<td>4.76 ± 0.22 b</td>
<td>3.49 ± 0.18 ab</td>
<td>2.12 ± 0.09 a</td>
<td>1.15 ± 0.06 a</td>
</tr>
<tr>
<td>T3</td>
<td>2.46 ± 0.08 a</td>
<td>2.43 ± 0.10 b</td>
<td>3.11 ± 0.18 b</td>
<td>5.04 ± 0.24 a</td>
<td>3.54 ± 0.09 b</td>
<td>2.14 ± 0.10 a</td>
<td>1.07 ± 0.08 a</td>
</tr>
<tr>
<td>T4</td>
<td>2.44 ± 0.14 a</td>
<td>2.83 ± 0.11 a</td>
<td>3.61 ± 0.13 a</td>
<td>5.29 ± 0.23 a</td>
<td>4.01 ± 0.19 a</td>
<td>2.35 ± 0.07 a</td>
<td>1.17 ± 0.03 a</td>
</tr>
<tr>
<td>NO$_3^-$-N (mg kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>2.12 ± 0.07 b</td>
<td>2.47 ± 0.13 b</td>
<td>3.13 ± 0.15 b</td>
<td>3.32 ± 0.17 c</td>
<td>2.42 ± 0.13 c</td>
<td>1.76 ± 0.08 b</td>
<td>0.96 ± 0.03 a</td>
</tr>
<tr>
<td>T2</td>
<td>16.32 ± 0.87 c</td>
<td>18.06 ± 0.90 c</td>
<td>11.38 ± 0.44 d</td>
<td>11.79 ± 0.55 c</td>
<td>12.21 ± 0.74 a</td>
<td>237.80 ± 10.58 c</td>
<td>264.77 ± 10.28 b</td>
</tr>
<tr>
<td>T3</td>
<td>17.14 ± 0.64 b</td>
<td>19.88 ± 0.81 b</td>
<td>14.26 ± 0.59 b</td>
<td>16.70 ± 0.62 b</td>
<td>89.55 ± 3.64 c</td>
<td>265.32 ± 17.62 b</td>
<td>263.49 ± 13.74 b</td>
</tr>
<tr>
<td>T4</td>
<td>17.26 ± 0.95 ab</td>
<td>20.17 ± 1.21 a</td>
<td>17.40 ± 0.86 a</td>
<td>9.02 ± 0.47 d</td>
<td>10.16 ± 0.52 d</td>
<td>286.79 ± 14.37 a</td>
<td>296.87 ± 15.93 ab</td>
</tr>
<tr>
<td>TN(%)</td>
<td>0.06 a</td>
<td>0.03 a</td>
<td>0.06 a</td>
<td>0.04 a</td>
<td>0.07 a</td>
<td>0.08 a</td>
<td>0.11 ± 0.06 a</td>
</tr>
</tbody>
</table>

The different letters denote significant differences (p > 0.05), while the same letters show no significant difference (p < 0.05). The data represent the mean ± standard deviation of three replicates.

In the heating stage of composting, the NO$_3^-$-N content increases at a higher rate because the most suitable temperature for nitrifying bacteria is about 30 °C, and activities of nitrifying bacteria are inhibited under high temperatures [40]. During the cooling stage, the activity of microorganisms involved in nitrification improves, which promotes the conversion of NH$_4^+$-N to NO$_3^-$-N. At the end of composting, the NO$_3^-$-N content of T1–T4 increases from 56.41% to 87.69% compared with CK. Compared to NH$_4^+$-N, NO$_3^-$-N is more easily absorbed and utilized by plants, so high-quality organic fertilizer based on compost must have abundant amounts of NO$_3^-$-N [26].

3.2.2. DOC Variations

During the early stage of composting, except for T4, the DOC of each treatment first gradually decreases (Figure 3b), because of the growth and reproduction of microorganisms, and the rate of DOC utilization is higher than the rate of degradation of macromolecular OM, as shown in Figure 3. This is consistent with the study of Chan et al. (2016) [41]. When the compost enters the high-temperature stage, the DOC content tends to rise because the physiological activities of microorganisms are limited by the temperature and...
microorganisms cannot utilize DOC directly, but hydrolyze OM by secreting extracellular enzymes \[42\]. During the cooling period, the DOC content gradually decreases because microbial activity gradually recovers and the OM degradation rate slows down. At the same time, the amount of easily decomposed OM is less, and the oxidative decomposition rate of DOC is higher than the degradation rate of OM. Ko et al. (2005) \[43\] think that the accumulation of refractory compounds leads to the slow utilization of DOC. At the end of composting, the DOC content does not decrease compared with that at the beginning. It shows that biochar can reduce the loss in C and retain more C.

Figure 3. Variations of OM (a) and DOC (b) of different treatments during the composting process.

3.3. Microbiological Analysis

3.3.1. Bacteria

In the middle-temperature stage of aerobic composting, bacteria of all kinds of flora have a key role in the enhancement of pile temperature. The effect of biochar on bacteria in the composting process is shown in Figure 4a. The number of bacteria reaches the peak in the warming and cooling periods, and decreases in the high-temperature period. In the first 8 days of composting, the bacterial population of each treatment is \( T4 > T3 > T2 > T1 > CK \), and the highest bacterial population is \( 4.98 \times 10^{11} \text{ cfu/g} \) at first day in the T4, manifesting that the microporous structure and microporous environment possessed by biochar confer favorable conditions for bacterial growth and reproduction \[44\]. During the warming stage, the number of mesophilic bacteria rises on account of them using easily degradable OM, such as crude protein and soluble monosaccharides, for their own physiological activities. In the high-temperature stage, due to the high temperature and the gradual reduction in nutrients, mesophilic bacteria enter into an inactive or dormant state, resulting in a decrease in the number of bacteria. Subsequently, in the cooling stage, the compost temperature once again provides a suitable environment for the growth of microorganisms, and the rebound of the number and activity of mesophilic bacteria has a catalytic effect on the improvement
in the degree of humification. From the late stage to the end of composting, the rate of OM degradation gradually slows down and cannot reach the needs of bacterial growth, leading to a decrease in the number of bacteria [45].

Figure 4. Microbial population of different treatments during the composting process. Bacteria (a), actinomycetes (b), and fungi (c).
3.3.2. Actinomycete

Actinomycetes can decompose cellulose and dissolve lignin. Meanwhile, actinomycetes can withstand higher temperature and pH than fungi [46]. The effect of biochar on actinomycetes in the composting process is displayed in Figure 4b. Overall, the number of actinomycetes in each treatment shows a decreasing–increasing trend and reaches the lowest value during the high-temperature period (4–8 days) of composting, which is because most actinomycetes survive inside the pile mainly in the form of spores and became the advantage flora for the decomposition of lignocellulose. Moreover, some actinomycetes that are not suited to high temperatures are dormant [47], so the number of actinomycetes is significantly reduced. Fang et al. (2019) [48] show that thermophilic actinomycetes can survive in the high temperature, cooling, and decaying stages of composting. In the whole composting process, the number of actinomycetes is noteworthy higher than that of CK when biochar is added at 7.5% and 10.0%, probably because biochar protects and provides a favorable living environment for microorganisms, but also influences the number of C sources and has a certain effect on the survival of microorganisms [49].

3.3.3. Fungi

In the aerobic composting process, fungi can hydrolyze the organic substances and secrete extracellular enzymes, which can destroy the physical structure of the pile through the mechanical interpenetration of internal mycelium and promote the biochemical reaction and the degradation of the pile. The number of fungi is much smaller than that of bacteria and actinomycetes (Figure 4c), and the fungi are sensitive to environmental changes, so the fluctuation of fungi in the composting process is more obvious. The number of fungi decreases evidently as the temperature increases to the peak. Research shows that temperature is one of the most considerable factors affecting the growth of fungi. Most of the fungi belong to the mesophilic fungi, and its optimal growth temperature is 25–30 °C. When the temperature of the pile reaches 60 °C, most of the fungi stop physiological activities [50]. When the temperature drops below 60 °C, the number of fungi begins to increase. The extracellular enzymes secreted by fungi could hydrolyze organic substances, which is conducive to promoting the maturation of pile. Overall, the addition of biochar has a certain protective effect on the fungi and provides a favorable microenvironment for the growth, reproduction, and activities of fungi.

3.4. Correlation Analysis

The maturity represents the degree of stabilization of OM in aerobic compost after the mineralization and humification processes. Compost products with complete maturation do not have adverse effects on crops and the environment when applied to the soil. If compost products with low levels of decay are applied directly to the soil, some intermediate products (NH$_4^+$, butyric and valeric acid, etc.) may be generated through microbial degradation, which may seriously affect the normal growth of crops [51]. In this study, nine indicators indexes were selected to evaluate the maturity of compost (Table 4). The NH$_4^+$-N and NH$_4^+$-N/NO$_3^-$-N are the common indicators for the evaluation of compost maturity [52]. The TN has an extremely significant negative correlation with NH$_4^+$-N/NO$_3^-$-N and a significant negative correlation with NH$_4^+$-N, respectively. The NH$_4^+$-N and GI are significantly negatively correlated. The NH$_4^+$-N/NO$_3^-$-N is significantly negatively correlated with DOC and GI. It is known that the above parameters can be used as a reference for whether the compost is fully rotten or not, but cannot be used as an absolute index, because the N content changes with the change in pile temperature, microbial metabolism, and pH. Considering the practical significance of compost decay, a plant growth experiment is a more convincing parameter to evaluate the decay degree. Table 5 shows the GI in different studies with and without the addition of biochar at the end of composting. It can be found from Table 5 that the GI of the experimental group with biochar addition is higher than that of those without, indicating that, with the addition of biochar, the maturation of the compost is promoted. However, the maturation-promoting
effect of biochar is different due to the different composting raw materials, biochar ratio, and composting time. The comparison of GI also demonstrates that the addition of biochar to the aerobic compost of BS has better decay promotion, suitability, and economic advantages compared to other composting raw materials.

### Table 4. Correlation analysis of compost maturity evaluation indexes.

<table>
<thead>
<tr>
<th></th>
<th>EC</th>
<th>TN</th>
<th>NH₄⁺-N</th>
<th>NH₄⁺-N/NO₃⁻-N</th>
<th>DOC</th>
<th>DOC/TN</th>
<th>C/N</th>
<th>T</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TN</td>
<td>−0.656</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺-N</td>
<td>0.909 *</td>
<td>−0.903 *</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₄⁺-N/NO₃⁻-N</td>
<td>0.686</td>
<td>−0.981 **</td>
<td>0.892 *</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>−0.261</td>
<td>0.905 *</td>
<td>−0.625</td>
<td>−0.884 *</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC/TN</td>
<td>0.012</td>
<td>0.554</td>
<td>−0.417</td>
<td>−0.606</td>
<td>0.940 *</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/N</td>
<td>0.700</td>
<td>−0.600</td>
<td>0.325</td>
<td>0.503</td>
<td>−0.255</td>
<td>−0.009</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0.763</td>
<td>−0.549</td>
<td>0.343</td>
<td>0.458</td>
<td>−0.121</td>
<td>0.149</td>
<td>0.982 **</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>−0.989 **</td>
<td>0.807 *</td>
<td>−0.882 *</td>
<td>−0.961 **</td>
<td>0.907 *</td>
<td>−0.408</td>
<td>0.929 **</td>
<td>−0.993 **</td>
<td>1</td>
</tr>
</tbody>
</table>

**: p < 0.01, *: p < 0.05.

### Table 5. Comparison of GI of composting with and without biochar by different researchers.

<table>
<thead>
<tr>
<th>GI</th>
<th>Biochar Content</th>
<th>Composting Raw Materials</th>
<th>Periods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.08</td>
<td>5%</td>
<td>BS + CS + pig manure</td>
<td>40 days</td>
<td>NA</td>
</tr>
<tr>
<td>0.89</td>
<td>5%</td>
<td>Rice straw + pig manure</td>
<td>42 days</td>
<td>(Zhou et al., 2019) [16]</td>
</tr>
<tr>
<td>1.05</td>
<td>12%</td>
<td>Cow manure + wheat straw + bacterial consortium</td>
<td>42 days</td>
<td>(Awasthi et al., 2020) [53]</td>
</tr>
<tr>
<td>1.1</td>
<td>10%</td>
<td>Swine manure + sawdust</td>
<td>50 days</td>
<td>(Ravindran et al., 2019) [14]</td>
</tr>
<tr>
<td>0.85</td>
<td>0</td>
<td>Biogas residues</td>
<td>80 days</td>
<td>(Meng et al., 2020) [26]</td>
</tr>
<tr>
<td>0.65</td>
<td>0</td>
<td>Green waste</td>
<td>30 days</td>
<td>(Zhang et al., 2014) [54]</td>
</tr>
<tr>
<td>0.86</td>
<td>0</td>
<td>Rice straw + BS</td>
<td>84 days</td>
<td>(Du et al., 2019) [55]</td>
</tr>
<tr>
<td>0.86</td>
<td>0</td>
<td>Food waste</td>
<td>56 days</td>
<td>(Chan et al., 2016) [41]</td>
</tr>
</tbody>
</table>

NA: T2 group in this study.

Many scholars used GI as an evaluation criterion for the screening of relevant decay indicators and established the corresponding aerobic compost decay evaluation system based on the selected indicators [29]. The correlation coefficients between EC, TN, NH₄⁺-N, NH₄⁺-N/NO₃⁻-N, DOC, DOC/TN, C/N, T, and GI show that, except for DOC/TN (−0.408), the other seven indexes could be considered as more reliable evaluation indicators of the decay of BS aerobic composting. Meng et al. [26] studied the full-scale of the composting process of biogas residues from corn stover anaerobic digestion and they used the Spearman correlation analysis to evaluate the relationships between physical and chemical composting measures and between physical and chemical measures and GI. They discover that GI is highly significantly correlated with NH₄⁺-N, C/N ratio, and TOC (p < 0.01) and significantly correlated with NO₃⁻-N (p < 0.05), which is consistent with the reliable maturity index obtained in this study. Although plant growth is the most reliable method for assessing compost maturity, plant growth takes a lot of time. Therefore, it is important to find quick and easily measurable physical and chemical indicators to assess compost maturity [23]. Meng et al. [23] found a strong correlation between composting time and TOC, C/N ratio, NH₄⁺-N, NO₃⁻-N, TP, TK, available potassium, and available phosphorus in the co-composting of the biogas residues and spent mushroom substrate. Therefore, they chose the above indicators as compost maturity indicators. In summary, the selection of compost maturity indexes is related to compost raw materials, compost additives, composting time, etc. It fundamentally reflects the changes in the physical and chemical properties of compost, and how to select the appropriate maturity index is the key to future research.
4. Conclusions

The present study demonstrates that biochar significantly influences physico-chemical properties and improves the stability and maturity of BS aerobic composting. The addition of biochar prolongs the high-temperature period (8 days) and peak temperature (63.8 ºC), promotes the degradation of lignocellulose, increases the number of microbial populations, and improves the humification of compost. Combined with the actual effect and cost, the best biochar addition of biogas residue aerobic composting is 7.5%. Additionally, taking GI as the evaluation standard, a reliable evaluation index of BS aerobic composting maturity was found. Finally, adding biochar to compost substrate is a potentially sustainable method of managing agricultural wastes. However, its effect on soil remediation as a biochar-based organic fertilizer needs to be studied.

Author Contributions: Conceptualization, W.Y. (Wencong Yan) and Y.S.; methodology, Y.Q., Y.S., and J.Q.; validation, Y.Q., Y.S. and W.Y. (Weiming Yi); formal analysis, Y.Q. and T.Y.; investigation, Y.Q. and J.Q.; resources, Q.Z., W.Y. (Weiming Yi), and X.L.; data curation, Y.Q., Y.S. and J.Q.; writing—original draft preparation, Y.Q., Y.S. and J.Q.; writing—review and editing, W.Y. (Wencong Yan), T.Y., and X.L.; supervision, Y.S. and J.Q.; project administration, Y.S.; funding acquisition, Y.S., J.Q., Q.Z., W.Y. (Weiming Yi), and X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key R&D Program of China, grant number 2019YFD1100603, and Special project of Heilongjiang Provincial Academy of science and technology cooperation, China, grant number YS20B01.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to thank the academic backbone project of Northeast Agricultural University.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

GI Germination index
EC Electrical conductivity
BS Biogas residue
HAs Humic acids
OM Organic matter
LM Livestock manure
PS Particle size
BET Specific surface area
CS Corn straw
MC Moisture content
TC Total carbon
TN Total nitrogen
DOC Dissolved organic carbon
VFAs Volatile fatty acids

References

1. Arthurson, V. Closing the global energy and nutrient cycles through application of biogas residue to agricultural land—potential benefits and drawbacks. Energies 2009, 2, 226–242. [CrossRef]


