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ORIGINAL ARTICLE

Building a framework of aerobic deer manure/corn stover composting with black liquor/microbial inoculation

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Abstract

Rapid treatment processing for corn stover and black liquor is of the utmost importance with the boom in China's agriculture sector. Aerobic fermentation composting via microbial/black liquor inoculation is superior to traditional composting with enhanced compost maturity and accelerated organic matter degradation. This research aimed to investigate the feasibility of black liquor addition and the effect of microbial/black liquor inoculation on the chemical composition and physiochemical and biological parameters for compost quality assessment. Results indicated that both the microbial/black liquor and the black liquor inoculation improved the heating rate. After composting for 18 days, the C contents and H contents were decreased to $44.29 \pm 0.19\%$ and $5.98 \pm 0.05\%$, while the O contents and N contents were increased to $45.25 \pm 0.15\%$ and $3.48 \pm 0.01\%$, which is consistent with the results of elemental analysis. This result indicated that black liquor and microbial inoculations accelerated the formation of nitrogen structures and recalcitrant nitrogenous oxygenated compounds. The FTIR analysis of four treatments confirmed that the microbial/black liquor inoculation could promote lignocellulose degradation and lignin degradation in subsequent composting. 16S rRNA sequencing revealed *Deinooccola*, *Gemmatimonadota*, and *Chloroflexi* as the predominant bacteria. The study suggested corm stover/black liquor composting as a promising technique for rapid and enhanced quality compost production.

Keywords Corn stover · Compost · Black liquor · Co-composting

1 Introduction

Corn stover (CS) is one of the major agricultural residues which is about 300 million tons per year in China [1]. Dumping CS wastes valuable resources and causes pollutant emissions to the atmosphere and ground water [2]. Methods to utilize crop stover include straw recycling, straw materialization, stockfeed, and straw biorefinery (cellulosic ethanol, cellulosic lactic acid, chemical feedstock, and bio-based materials) [3–5]. Among these methods, straw biorefinery is the most promising and value-added utilization [6, 7]. In the straw biorefinery process, pretreatment technology of corn stover is the key process. Our previous study developed a novel corn stover pretreatment process via NaOH/urea at 60–80 °C [8]. The glucose yield was up to 0.55 t/t pretreated corn stover which showed a promising trend in industrial application. However, it will produce 10–20 times black liquor with the production of glucose. The black liquor contributes 10–15% urea and lignin degradation products (coniferyl alcohol, dibutyl phthalate, 4-hydroxybenzaldehyde, trans-sinapyl alcohol, and acetosyringone). Urea is not only the

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Highlights
• The feasibility of using black liquor in compost was proved for the first time.
• An operational framework of composting with black liquor/microbial inoculation was established.
• The function of black liquor in composting was preliminarily determined.

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most suitable inorganic nitrogen source for the growth of bacteria, but also the source of nitrogen fertilizer for plants. Lignin degraders are important for the formation of humus. Therefore, the addition of black liquor will contribute to the composting process of corn stover and deer manure. Utilization of black liquor from straw pretreatment has attracted widespread attention. Black liquor-based hydrogel (BLH) was prepared and its application as both water retention material and slow release fertilizer was proved [9]. Biochar catalyst was also prepared from black liquor by spray drying and fluidized bed carbonation for biodiesel synthesis [10]. Black liquor played the same role as NaOH during pretreatment at the study of Zhang et al. [11]. Hydrogen production from soda black liquor with V_2O_5 loading amount of 45 wt% was studied by Cao et al. [12]. On the other hand, in agriculture, the continuous and excessive application of chemical fertilizers has led to soil degradation and loss of productivity [13, 14]. The overuse of chemical fertilizer has resulted soil infertility, biodiversity loss, increased salinity, etc. In response, the Chinese government has issued legislation to protect agricultural land and restrict the use of chemical fertilizer. The production and application of organic fertilizer will undoubtedly form part of the solution to these problems. According to studies by scientists and farmers, the effects of organic fertilizer include not only the promotion of plant root growth but also the protection of the environment from manure and agricultural residues pollution. Therefore, composting of corn stover, black liquor, and livestock manure should be an economical, effective, and environment-friendly process for the transformation of corn stover into a safe and stable material for application to the soil. Composting is a promising method for soil reclamation with the help of microorganisms [15, 16]. Composting of livestock manure and agricultural residues is one of the most effective composting techniques [17, 18]. Indeed, many recent studies have suggested composting with agricultural residues and manure as a potential solution for agricultural waste disposal all over the world [19, 20]. Composting is an aerobic, thermophilic, solid-state fermentation process where microorganisms play a major role. Microorganisms are the power system of composting. Inoculation of compound microbial agents can increase enzyme activity and improve the diversity of microbial communities [21]. Indeed, Zhang's study found that *Phanerochaete chrysosporium* inoculation can significantly affect the composting process. The inoculation of lignocellulose-degrading bacteria can effectively reduce arginine in pig manure compost, which affects human health through the food chain [22]. All of these studies indicate the essential role of microorganisms. According to our previous studies, corn stover pretreatment process using NaOH/urea generates black liquor as a by-product and poses a significant effluent problem. However, black liquor can act as a rich source of nitrogen required for microbial activity in fermentation processes [8, 23, 24]. Therefore, the main objectives of this study were to identify dynamic changes of enzyme activity, bacterial succession, and compost quality during microbial and black liquor inoculation in the composting processes. The study attempt to reveal the mechanism of high-efficiency composting based on the correlation of microbial/black liquor inoculation and composting process.

2 Materials and methods

2.1 Materials

Corn stover was collected from Changchun Jingyue National High-Tech Industrial Development Zone, Jilin Province, China (125.35° N, 43.88° E). Deer manure was collected from Shuangyang District, Changchun City, Jilin Province, China (125.6° N, 43.5° E). The basic characteristics of the composting materials are listed in Table S1. The chemical reagents used in this study including urea, citric acid, potassium hydroxide, ammonium sulfate, disodium hydrogen phosphate, and 3,5-dinitrosalicylic acid were obtained from Sinopharm (Changchun, China). The preparation of black liquor was referred our previous study [8], and the components are listed in Table S2. All other chemicals were of reagent grade.

2.2 Preparation of microbial inoculation

The microbial inoculation used in this experiment was from microbes preserved in the Education Ministry Key Laboratory of Straw Biology and Utilization (Changchun, China). The inoculation used *Aspergillus niger*, *Trichoderma reesei*, *Bacillus subtilis*, and *Bacillus megaterium* at a ratio of 1:1:2:2 (this ratio in laboratory-scale gave the highest degradation rate of corn stover/deer manure/black liquor and the highest degradation rate of deer manure composting), and the concentration of the liquid inoculant suspended in liquid medium met the requirements for agromicrobial agents at about 1.0×10^9 CFU mL^{-1}.

2.3 Composting experiment design

Composting experiments were carried out in a laboratory-scale reactor (Fig. S1) fitted with a gas supply, thermal insulation, temperature monitoring system, and a leachate collection device. The laboratory was situated in the Education Ministry Key Laboratory of Straw Biology and Utilization. The working volume of the reactor was 25 L (46 cm high, 30-cm base diameter). The optimal composting conditions of oxygen concentration, water content, and initial C/N ratio have been referred by Xie et al. [25]. The C/N ratio of 25:1

is considered optimal for the composting process [26], the moisture content was 40–60% [27], the air flow was 0.6 L/min, and the oxygen supply was used for 20 min every 2 h. The composting process was divided into the four treatments of CD, CDM, CDB, and CDMB, which are defined as the following:

CD: 3 kg of corn straw and 7 kg of deer manure
CDM: 3 kg of corn straw, 7 kg of deer manure, and 1% microbial inoculation
CDB: 3 kg of corn straw, 7 kg of deer manure, and 10% black liquor
CDMB: 3 kg of corn straw, 7 kg of deer manure, 1% microbial inoculation, and 10% black liquor

Compost samples were sampling on days 3, 9, and 18, randomly from three different layers. Each compost treatment was divided into four sections. Approximately 500 g (untreated) of each sample was collected for the following study; one quarter was preserved at − 80 °C for high-throughput 16S rDNA pyrosequencing, and one quarter was dried at room temperature for component and Fourier infrared analysis after crushing and passing a 60-mesh sieve; and one part was crushed, passed through a 60-mesh sieve, and prepared via aqueous extraction for elemental analysis. Urease activities and enzyme activity unit were referred by Yin's study and determined at 578 nm [28].

2.4 Analytical methods

2.4.1 Fourier transform infrared spectroscopy

In order to analyze the changes in chemical bonds during the composting process, the samples were analyzed by Fourier transform infrared spectroscopy (FTIR; Nicolet IS10, Thermo Fisher, MA, USA) according to the published method [29]. The FTIR spectra were recorded within the wavenumber range of 400–4000 cm^{-1}.

2.4.2 High-throughput sequencing of four composting process

Use the Fast DNA™ SPIN kit for soil (MP Biomedicals, Solon, OH, USA) to extract the total DNA and store the total DNA at − 80 °C. PCR was used to amplify 16 S rRNA for analysis of bacterial community. Sequencing of the library was analyzed on Illumina HiSeq platform.

2.4.3 Bioinformatics analyses

The original Illumina FASTQ file is demultiplexed, quality filtered, and analyzed using the "Quantitative Analysis of Microbial Ecology (QIIME)" software. The representative sequence was compared with the Silva database, and a confidence level of 0.8 was used.

2.4.4 General analytical methods

Temperature was measured using a soil thermometer. Water content, electrical conductivity, and pH were all monitored by equipment produced by Chinese Jinghe company. Organic carbon was determined using a potassium dichromate wet oxidation method [30]. Total nitrogen was determined by the Kjeldahl digestion method [31].

3 Results and discussion

3.1 Variation of parameters during fermentation

Temperature is one of the main parameters for monitoring the composting process because it reflects the degree of microbial activity while change in temperature can reflect a reduction in the number of pathogenic bacteria. The combination of black liquor and microbial inoculation (CDMB) exhibits a faster heating rate and a higher peak temperature (60.93 °C) than the others, which means that the microbial inoculation played a key role in the whole fermentation phase. Black liquor inoculation alone also showed a faster heating rate than control group (Fig. 1A). This is mainly due to the high concentration of nitrogen source in black liquor, which promoted microbial reproduction. The black liquor also contains sugar, protein, and organic matter hydrolyzed from corn stalk pretreatment [32]. Although the concentrations of these nutrients were low, they can also contribute to microorganism growth in the compost. This is consistent with the study of [33], which showed that black liquor can promote the growth of white-rot fungi.

pH is another key parameter which is related to bacterial diversity and abundance. Under conditions close to neutral pH, bacteria can make good use of the nutrients in compost [34]. The overall trend in pH is an increase from the first day to the end of composting, which may be attributed to the degradation of organic nitrogen during composting to produce a large amount of NH_4^+ (Fig. 1B). The pH values of CD and CDM both changed from acidic to neutral, while those of CDB and CDMB with added black liquor were neutral and rose slowly. These trends indicated that the fermentation maintained a neutral or weakly alkaline environment regardless of the composting process.

Changes in the water content for the four treatments are shown in Fig. 1C. The water content increased in the first 2 days of the fermentations because of the temperature rise and the closed fermentation system. However, in the next few days, the water content began to fall with the increased microbial consumption. If the water content is too

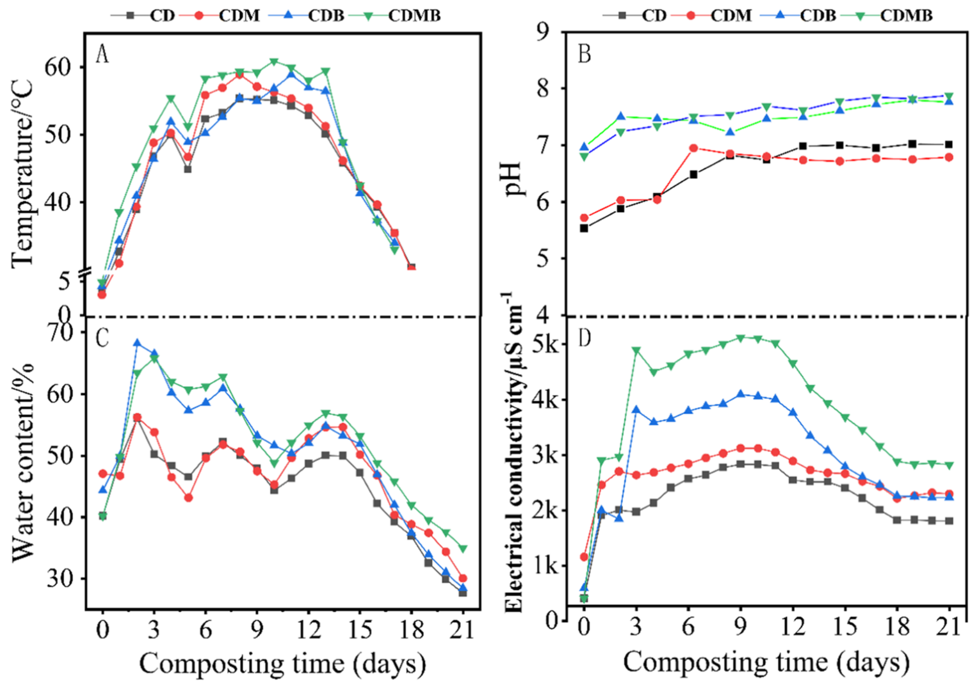

Fig. 1 Parameter data during composting of different treatment groups: **A** temperature, **B** pH, **C** moisture content, **D** electrical conductivity

low, the growth and metabolism of microorganisms in the composting process are affected [35]. Therefore, we regularly replenished water and strictly controlled the moisture content of the four treatments to 40–60%. Changes in the electrical conductivity of the fermentations are shown in Fig. 1D. Electrical conductivity is one of the main factors to affect the composition of the bacterial community and is related to the salt content in the material [36]. In addition, the transfer capacity of surface electrons in compost is related to the redox activity of microorganisms [37]. In the four compost treatments, the electrical conductivity reached a peak on the ninth day. At this time, the fermentations had reached or were close to peak temperature. With the addition of black liquor in CDB and CDMB, the initial conductivity, upward trend, and peak value were all higher than those without added black liquor. Studies have shown that the electrical conductivity of compost should be lower than 5500 μS/cm; otherwise, the high salt content responsible for the high conductivity reading will damage the organisms [38]. The observed results suggest that adding black liquor can supplement salt in the compost and increase the activity of microorganisms as long as the electrical conductivity of fermentation does not exceed 5500 μS/cm.

3.2 Characteristics of organic carbon, total nitrogen, and C/N ratio

Changes in organic carbon, total nitrogen, and C/N ratio of compost samples reflect the composting process. During the entire composting period, microbial and black liquor inoculation (CDMB) showed the largest decrease of organic carbon (Fig. 2). Organic carbon content of 130 g/kg and 140.3 g/kg was observed on days 3 and 21 at CDMB and CDB, respectively. Nitrogen is also important as the main nutrient element for microbial activities. The nitrogen content was observed to decline in each of the four composting treatments. The nitrogen content was higher in black liquor inoculation alone (CDB) than control group (CD) in the whole composting phase which means that the nitrogen cannot be quickly utilized by the natural microorganisms in deer dung. However, the nitrogen source was well utilized in the microbial and black liquor inoculation (CDMB). This result means that the inoculation of microorganisms improved the fermentation efficiency. It produced a lot of biological heat,

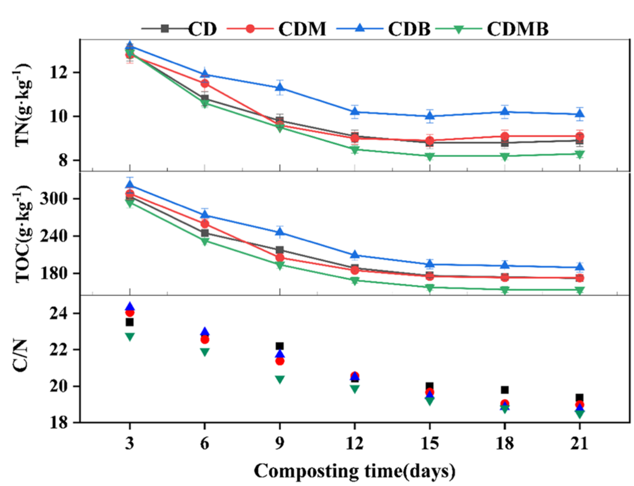

Fig. 2 Changes in organic carbon, total nitrogen, and C/N ratio

which also can be proved in the result of Fig. 1A. The C/N ratio in four treatments also declined. It reflects the relative accumulation of nitrogen during substrate utilization and because of loss of organic carbon to carbon dioxide [39].

3.3 Changes of urease activity

Urease is an important indicator to characterize the conversion of urea to ammonia and carbon dioxide, and its activity is closely related to nitrogen metabolism in compost [40]. Urease initially showed a trend of increased activity and then decreased during the remainder of the composting process (Fig. 3). For days 3–18, the highest urease were measured at 1.25-, 1.75-, 1.5-, and 2.25-g amino acid nitrogen $(g·24\ h)^{-1}$ on the ninth day for CD, CDM, CDB, and CDMB, respectively. By comparing the urease levels of these four treatments, inoculation of microorganisms will increase the metabolism of microorganisms, thereby resisting high temperature, accelerating mineralization and decomposing nitrogenous organic matter, and increasing urease activity. In comparing the data for CDB and CDMB, it appears that black liquor also promoted urease production during the whole composting process. At the end of the thermophilic stage period and when the temperature began to drop, the urease activity of the four treatments was decreasing. Continuous decrease in urease activity can be attributed to the exhaustion of easily biodegradable organic matter [41]. The urease activity of CD, CDB, CDM, and CDMB at day 21 was similar, although the nitrogen content and microbial biomass were different. This result means the manure of composting.

3.4 Fourier transform infrared spectroscopy

The addition of black liquor and lignocellulose-degrading bacteria promotes the degradation of cellulose and promotes the humus process (Fig. 4). Samples collected from four different treatments had similar peaks: 3430–3410 cm^{-1} for the wide peak stretching vibration of -OH in carbohydrates and water molecules, and the absorption of N–H stretching vibration in protein and amide compounds. A band at 2850 cm^{-1} was attributed to the $-CH_3$ and $-CH_2$- groups in aliphatic compounds and lignin, while the band at 1650–1630 cm^{-1} was used to characterize C-O stretching vibration connected with aromatic groups in lignin. Observation of an absorption at 1506 cm^{-1} was considered the characteristic of lignin degradation [42, 43]. Samples from the four compost treatments were analyzed by FTIR spectroscopy in the wavenumber range of 400–4000 cm^{-1}. Six major peaks were detected at 3402, 2930, 1506, 1030, 875, and 670 cm^{-1}, which was consistent with other studies [44–46]. These results indicate that the content of protein, cellulose, and polysaccharides will change and degrade at the same time during composting which is the same as Ouaqoudi's study [47]. The transmittance at 1506 cm^{-1} was enhanced in CDM, CDB, and CDMB, and the results were suggestive of the partial removal of lignin. This evidence suggests

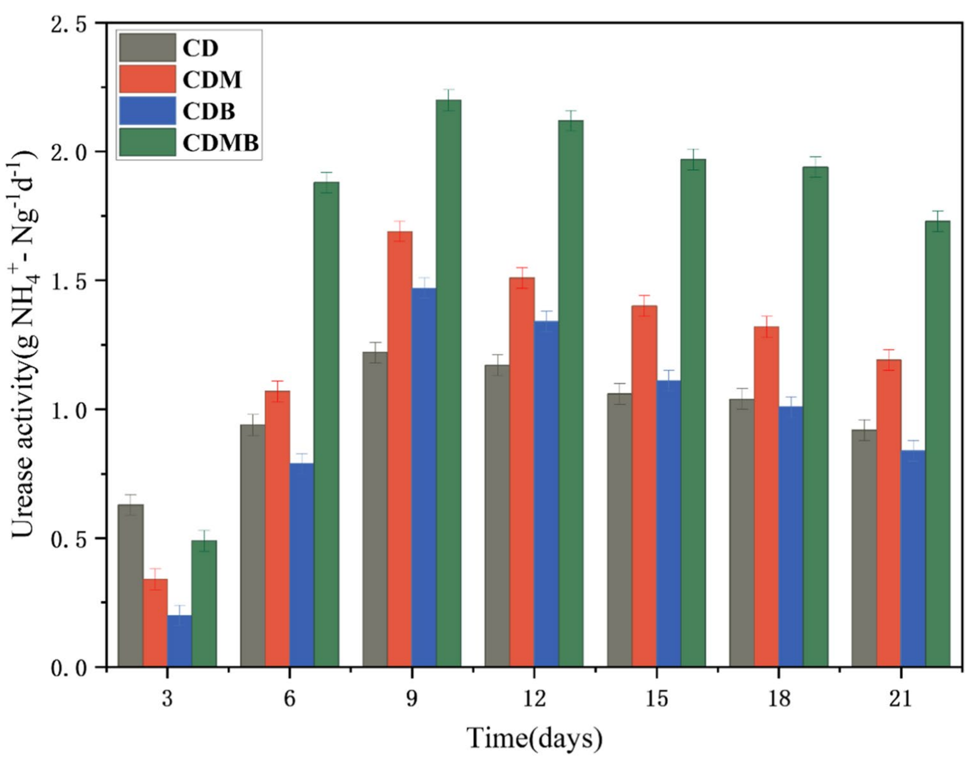

Fig. 3 Urease activities during composting for different treatment groups

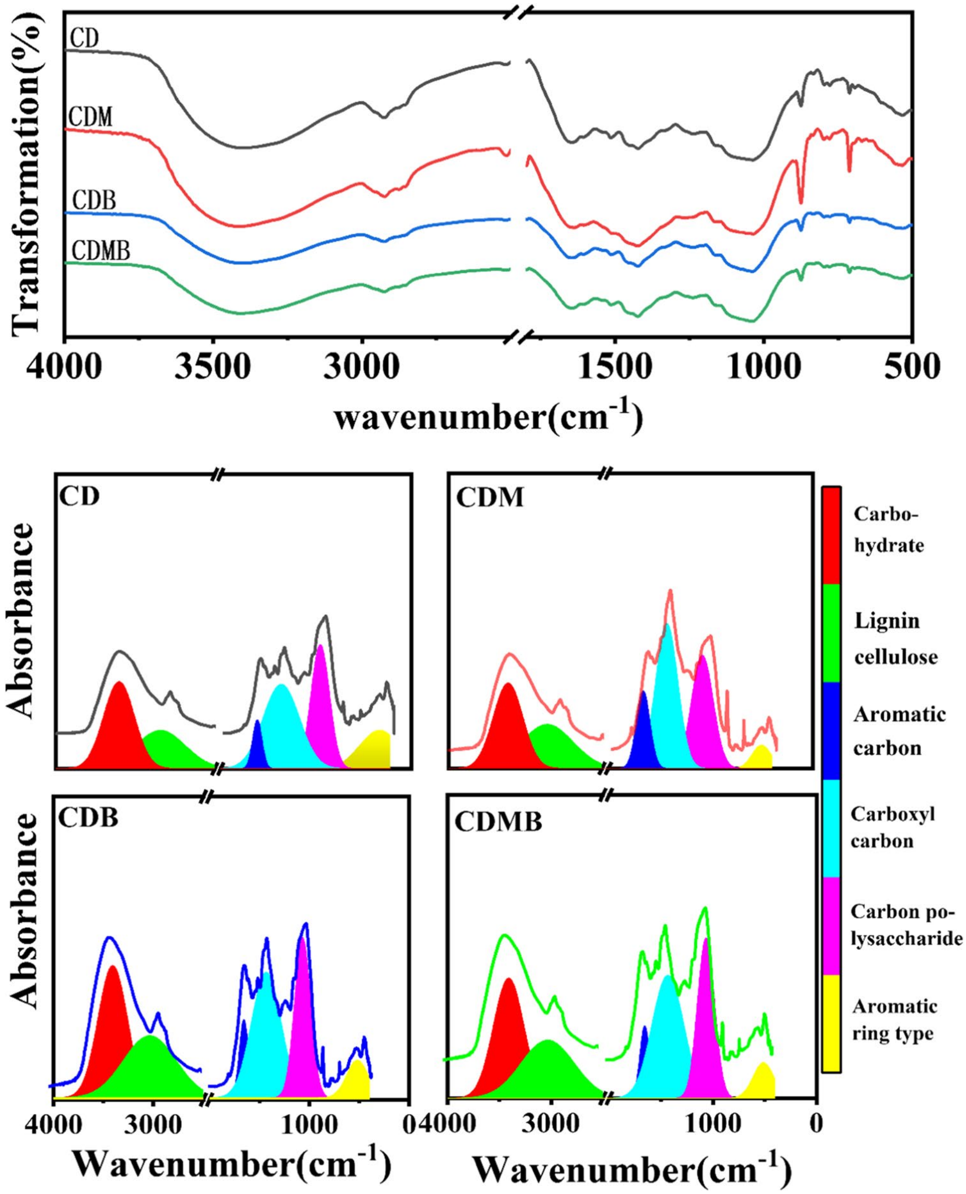

Fig. 4 Fourier transform infrared spectroscopy of different composting treatments at day 18

that thermophilic lignocellulose-degrading strains seem to play a key role. The spectral changes are similar to previous studies, in which the lignin content changes are quantitatively analyzed by FTIR spectroscopy [48]. After adding lignocellulose-degrading bacteria, the transmittance of CDM and CDMB at 873–875 cm^{-1} and 670 cm^{-1} was all increased which indicated that the degree of cellulose penetration increased during the composting process. Furthermore, the transmittance of CDM compost was higher than CDMB compost, which indicated that black liquor had a negative effect on the composting process. Transmittance at 1030 cm^{-1} for the four treatments was also increased, indicating the destruction of cellulose and lignin structures.

3.5 Elemental analysis

Changes of C, H, O, and N content indicate that dehydrogenation, oxidation, and incorporation of nitrogen occur throughout the composting process [49]. Throughout the composting treatments of 18 days, both the C and H contents were decreased, while the O and N contents were increased. The O contents and N contents at the end of composting in CDMB were $45.25 \pm 0.25\%$ and $44.29 \pm 0.19\%$, and in CD were $44.42 \pm 0.07\%$ and $44.97 \pm 0.03\%$, respectively (Table 1). This suggests that black liquor plus microbial inoculation could promote the formation of stable nitrogen structures and recalcitrant oxygenated compounds. These results also suggested that organic carbon compounds in CDM and CDMB degraded faster than in CD and CDB at

Table 1 Elemental composition of four composting treatments at different stages of composting

Composts	Elapsed time (d)	C	N	H	O	Atomic ratios		
						C/N	C/H	C/O
CD	3	48.95 ± 0.01^{a}	3.22 ± 0.04^{d}	6.3 ± 0.12^{a}	40.55 ± 0.09^{a}	15.20	7.77	1.21
	9	47.87 ± 0.01^{b}	3.56 ± 0.01^{b}	6.35 ± 0.07^{a}	41.23 ± 0.09^{b}	13.45	7.54	1.16
	18	44.97 ± 0.03^{h}	3.57 ± 0.05^{b}	6.06 ± 0.14^{a}	44.42 ± 0.07^{ef}	12.60	7.42	1.01
CDM	3	45.74 ± 0.05^{e}	2.96 ± 0.04^{e}	5.99 ± 0.15^{a}	44.32 ± 0.07^{f}	15.45	7.64	1.03
	9	45.34 ± 0.09^{g}	3.3 ± 0.02^{c}	5.72 ± 0.04^{b}	44.64 ± 0.07^{e}	13.74	7.93	1.02
	18	40.44 ± 0.03^{j}	2.85 ± 0.01^{f}	4.85 ± 0.05^{c}	50.87 ± 0.09^{c}	14.19	8.34	0.79
CDB	3	46.73 ± 0.01^{d}	3.3 ± 0.03^{c}	6.5 ± 0.01^{a}	42.48 ± 0.04^{i}	14.16	7.19	1.10
	9	45.86 ± 0.22^{e}	3.53 ± 0.01^{b}	6.27 ± 0.04^{a}	43.34 ± 0.17^{g}	12.99	7.31	1.06
	18	46.39 ± 0.22^{d}	3.61 ± 0.05^{b}	6.19 ± 0.13^{a}	42.82 ± 0.03^{h}	12.85	7.49	1.08
CDMB	3	47.86 ± 0.18^{c}	3.46 ± 0.01^{b}	6.4 ± 0.03^{a}	41.29 ± 0.14^{j}	13.83	7.48	1.16
	9	46.16 ± 0.06^{d}	3.75 ± 0.04^{a}	6.09 ± 0.05	43.01 ± 0.15^{h}	12.31	7.58	1.07
	18	44.29 ± 0.19^{i}	3.48 ± 0.01^{b}	5.98 ± 0.05	45.25 ± 0.25^{d}	12.73	7.41	0.98

Means with the different superscript letters in a column differ significantly ($P < 0.05$)

the beginning of the composting phase (day 3). This was likely caused by the microbial inoculation, which accelerated the composting process. Table 1 also shows the changes of C/H, C/N, and C/O element ratios. The C/N ratio in CDB and CDMB was significantly reduced, indicating that the compost can concentrate the nitrogen-rich structure as the compost proceeds. This result was attributed to the decomposition of biodegradable materials, such as protein decomposition products, formed by condensation of nitrogen-rich compounds. In addition, during days 3–18, the decreases in the C/N ratio for CD, CDM, and CDB were higher than that in CDMB. From these results, the C/N ratio declined with the temperature rising. These observations were supported by the fact that microbial inoculation enhanced humification, which was apparent in CDB and CDMB treatments. Based on the above analysis, we conclude that microbial inoculation attributed the aromatization reactions during composting process, while black liquor inoculation playing a supporting role on the composting process.

3.6 Variation of microbial community composition during composting

16S rRNA clone libraries obtained from the four composting samples at day 18 were analyzed after quality filtering. Comparing the number of 16S rRNA OTUs between different treatments, it was found that there were differences in the diversity of bacterial communities between the samples (Fig. 5). The common OTU data between CD, CDM, CDB, and CDMB amounted to 35.94%, 55.28%, 57.78%, and

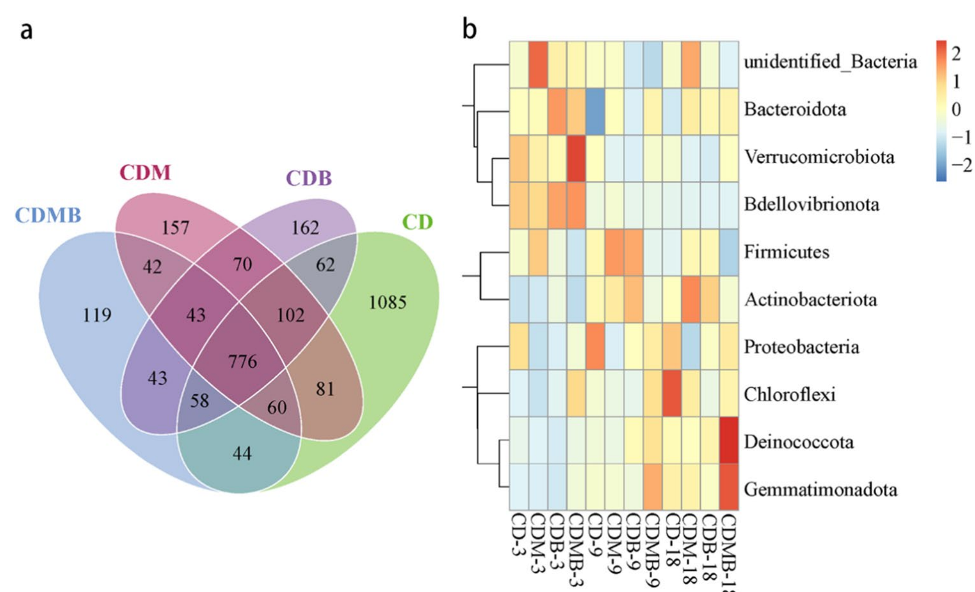

Fig. 5 Venn diagram of shared 16S rRNA OTUs (**a**) and heatmap of the log relative abundance of top genera from CD, CDM, CDB, and CDMB samples (**b**) during the composting process

62.94%, respectively, which means that most of the bacterial community was different (Fig. 5A). The CDMB process contains the largest number of unique OTUs. The diversity of functional bacteria in four composting treatments was determined by 16S rRNA sequencing. Figure 5B shows a heatmap analysis of the 36 identified bacterial species. The results were same as the previous studies [50]. The relative abundances of *Deinooccola*, *Gemmatimonadota*, and *Chloroflexi* were high during the fermentation. These observations are consistent with Green's study [51]. The microbial community composition analysis was not enough to explain the mechanism of carbon and nitrogen cycle. Black liquor and microbial inoculation helped the composting process. The bacterial and fungal community should be analyzed in detail in future study.

4 Conclusions

This study demonstrated the feasibility of composting from corn stover and deer manure with the addition of black liquor and microbial inoculation. The results showed that fermentation processes were improved with the addition of bacteria and black liquor. Changes in physicochemical factors after black liquor addition were similar with those observed without black liquor, although a higher temperature peak was observed. FTIR spectroscopy proved the effect of black liquor on composting process. Elemental analysis, microbial analysis, and monitoring of urease activity showed that CDMB treatment had higher dissolved N concentration, which was more favorable for the conversion of corn stover into fertilizer. Such technology should be useful for the resource utilization of both black liquor and corn stover. It may be also helpful for developing a straw biorefinery industry without pollution or emissions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13399-021-01792-4.

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Author contribution Shijun Pan: conceptualization, methodology, software, investigation, writing original draft.

Huan Chen and Futai Ni: writing (review and editing).

Yanli Li and Mingzhu Guo: validation, formal analysis, visualization.

Sitong Zhang: Writing (review and editing).

Guang Chen: Resources, writing (review and editing), supervision, data curation.

Mingzhu Guo: Investigation, writing original draft.

Futai Ni: Review and editing.

Gang Wang: conceptualization, resources, writing (review and editing), supervision, data curation.

Declarations

Conflict of interest The authors declare no competing interests.

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