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Research article

Enhancing the compost maturation of deer manure and corn straw by supplementation via black liquor

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ABSTRACT

In this paper, the relationship between black liquor and microbial growth, enzymatic secretion and humus formation in composting was studied. The results showed that black liquor inoculation is an effective way to promote fermentation process. After black liquor inoculation, the abundance of *Corynebacterium*, *Aequorivita*, and *Pedobacter*, which have the catalase and oxidase activity, has been significantly increased. The enzymatic activity of alkaline phosphatase, catalase, peroxidase and invertase was 40 mg/(g·24h), 6.5 mg/(g·20 min), 13 100 mg/(g·24h), and 6100 mg/(g·24h) respectively at day 18. Humic acid and fulvic acid concentration was 12 g/kg and 11 g/kg which is higher than that of the treatments of no black liquor inoculation. The results suggested that black liquor inoculation was beneficial to indigenous microorganisms reproduce efficiently, then the secretion of enzymes related to cellulose, hemicellulose, and lipid hydrolysis, and the formation of humic substances.

1. Introduction

Straw biorefinery is a promising value-added technology for the sustainable development and utilization of straw resources. In straw biorefinery process, pretreatment is the critical step to boost the enzymatic hydrolysis efficiency of lignocellulose by disrupting the recalcitrant lignocellulose [1,2]. In various pretreatment methods, sodium hydroxide was employed as the most common approach all over the world [3–5]. In this process, a large quantity of black liquor was produced with the corn straw alkali pretreatment process. Generally, pretreatment of one ton corn straw, about 50–100 tons of black liquor will be produced. It is estimated that 1900 million tons of black liquor were produced each year in China. Black liquor contains the degradation products of hemicellulose and lignin [6] which also contains pollutants of diphenols, dioxins, phenols, and organic halogens [7]. It makes water black or brown, damage the ecosystem, prevent light penetration, inhibit photosynthesis, and reduce dissolved oxygen too [8,9]. Hence, how to use black liquor effectively has become an urgent problem for scientists [10].

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The common solutions include the biological and chemical valorization of black liquor into fuels and chemicals [11]. Cha et al. studied the black liquor recovery and used in Miscanthus pretreatment at a pilot scale. The results indicated that in the subsequent pretreatment with the first and second-generated black liquor, the converted glucose concentrations slightly decreased to 303.0 and 261.1 g/kg biomass respectively [12]. However, because of the higher viscosity of black liquor from ash and lignin, the saccharification yield was low in samples with black liquor pretreatment. Lignin recovery from black liquor is another main utilization direction, but the cost of recycling is too high to industrial production [13]. Several researchers also reported the conversion of black liquor into polyhydroxyalkanoates (PHAs), biodiesel, hydrogen, biogas, and organic acid production [14–17]. Although it may be a promising alternative fermentable substrate in the future, current technology can't resolve the disadvantages from aromatic polymer compounds (guaiacyl, clove and p-coumarin units) and extracts (fatty acids, resinous acids, etc.) to fermentation yet [18].

Composting is an effective means for black liquor valorization that can maintain the sustainable development of the ecology. The composting process can convert black liquor, corn stalks, and livestock manure into nutrient-rich agricultural fertilize under aerobic, water-containing, C/N, and other conditions [10]. In composting, microorganisms play a crucial role. It converts organic matter into humus, and lignin is the key to the formation of humus [19]. Composting process is dominated by the secreted enzymes from the microorganisms in composting [20]. The enzymatic activity with specific functions can reflect the quality of compost products and the types of microbes in the compost. There have been studies on exogenous microbial inoculating to overcome the deficiencies in the species and quantity of microorganisms in compost materials. Xie et al. inoculate bacteria that can oxidize ammonia into nitrite and nitrate to reduce the ammonia nitrogen discharge and achieve the purpose of nitrogen fixation [21]. Kausar et al. found that a carbonaceous *micromonospora* can degrade lignin and cellulose and then inoculate its suspension on the compost, which effectively degrades lignin and cellulose significantly shortens the composting time [22]. These studies showed that microbial inoculation has a beneficial effect and a unique ability higher than of the indigenous microorganisms. The microorganisms worked with the whole composting process by various enzymes they secreted which is closely related to organic-matter degradation, the circulation of nutrients, and the transfer of energy.

Studies showed that lignin degradation products (phenols and quinones, main ingredient of black liquor) could polymerize with nitrogen compounds in the composting process to form humus [23]. This result predicts that the use of black liquor in composting will contribute to the formation of humus. Meanwhile, lignin degradation and humus formation are inseparable from the role of microorganisms in compost. However, there have been no reports on the use of black liquor in composting. The effect of black liquor addition on microbial growth and reproduction, humic acid production is not clear. We hypothesized that the nitrogen source (urea) in the black liquor would promote the growth of indigenous/inoculated microorganisms, then accelerate the composting process and the phenols and quinones in black liquor will facilitate humus formation. To test this hypothesis, the composting experiment inoculated microbial and black liquor was carried out in a laboratory scale. The effects of microbial/black liquor inoculation on bacteria succession, related enzyme activities, lignin, and humus in corn stover/deer manure composting were investigated. This study will achieve black liquor and corn stover valorization by corn stalk and deer manure compost.

2. Materials and methods

2.1. Materials

Deer manure and corn straw was collected from Shuangyang District (125.6 °E, 43.5 °N) and the experimental field of Jilin Agricultural University (125.42° E, 43.81° N) respectively. The corn straw was cut into 3 cm after air drying. The properties of raw materials are shown in Table 1. The moisture content, organic carbon, and total nitrogen of corn stalks were 5%, 42.35%, and 0.9% respectively which is consistent with the results of other researchers [24]. The moisture content of deer manure is higher than that of other studies [25]. We think that the corn stalks have a low moisture content and fresh deer manure may retain more sufficient nutrients. Therefore, deer manure we used was not dried. The chemical reagents used in this research were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, AR). Black liquor was prepared according to our previous study and the composition of black liquor was shown in the study [26].

2.2. Preparation of microbial inoculant

The inoculant used in this experiment was from the Education Ministry Key Laboratory of Straw Comprehensive Utilization and Black land conservation (Jilin, China). *Trichoderma reesei, Aspergillus niger, Bacillus subtilis,* and *Bacillus megaterium* which with higher cellulose and lignin degradation property were inoculated at a ratio of 1:1:2:2 in a laboratory scale.

Table 1
Basic properties of corn straw and deer manure.

	Moisture content	Organic carbon	Total nitrogen
Corn stalks	5%	42.35%	0.9%
Deer manure	20%	37.11%	1.8%

2.3. Composting experiment

The composting experiment was carried out in a laboratory-scale reactor (Fig. s1) with forced aeration. The reactor volume was 25 L, with the functions of gas supply, heat preservation, and temperature and humidity monitoring. The bottom of the reactor is provided with a ventilation sieve plate. The composting was adjusted at 25:1 of C/N, 60% of moisture content, 0.6 L/min of air flow rate, and 20 min every 2 h for providing enough oxygen. The composting experiment was applied as follows: corn straw of 3 kg and deer manure of 7 kg per pot named CD. CD with 1% microbial inoculant (m/v) per pot named CDM. CD with 10% black liquor (v/v) per pot named CDB. CD with 1% microbial inoculant (m/v) and 10% black liquor (v/v) per pot named CDMB. Urea was used to adjust the C/N of composting. To ensure the consistency of each experiment, black liquor that contained 17% of urea was used as a nitrogen supplement instead of urea in CDB and CDMB treatment. Compost samples were collected at the 3rd, 9th, and 18th day, and each composting pot is divided into the upper, middle and lower parts for samples selection. Each sample was randomly collected via the three-point sampling method. Mix samples evenly to ensure each sample is about 500g. Each sample was divided into two parts. One part of the sample was for high-throughput 16S rDNA sequencing preserved at −80 °C, the other for analysis and detection of enzyme activity, lignin, and humus concentration air-dried. The supernatant obtained by water extraction from four composting samples were used to test whether the compost product is a non-toxic and harmless to seed germination rate.

2.4. Analytical methods

2.4.1. DNA extraction and 16S rDNA high-throughput sequencing

Total DNA was extracted using a Fast DNA™SPIN Kit for Soil (M.P. Biomedicals, Solon, OH, USA), and stored at −80 °C. The polymerase chain reaction (PCR) was employed to amplify the 16S rDNA for bacterial community analysis. The library was sequenced on an Illumina HiSeq platform III.

2.4.2. Enzymatic activity analysis

Phosphatase activities in soils were assayed as follows. Determination of phenol released by incubation at 37 °C for 24 h of 1 g soil with 4 mL Borate buffer (pH 9.6), and 1 mL 5 mM Disodium phenyl phosphate as substrate [27]. Catalase activity was expressed as the number of milligrams (mg) of 0.1 mol/L KMnO4 released from the added hydrogen peroxide [28]. 2 g dried soil sample and 40 mL of distilled water was placed in a 125 mL conical flask, added 5 mL of 0.3% H_2O_2, then shaking for 20 min, the remaining peroxide was then stabilized by adding 5 mL of 3 mol/L H_2SO_4. The enzyme activity was expressed as the number of milligrams of hydrogen peroxide decomposed per gram of sample within 20 min [29]. The 3, 5-dinitrosalicylic acid colorimetric method was employed for invertase enzymatic activity analysis, and the invertase enzyme activity was explained in milligrams of glucose per gram of sample per 24 h [30]. The activity of peroxidase activity was measured by 2-thio-2,4-dinitrobenzoic acid colorimetry [31]. Take 1 g of soil sample placed in a 50 mL centrifuge tube, and then added 10 mL 1% pyrogallic acid solution and 2 mL 0.5% H_2O_2 solution. After shaking, the culture was placed in a 30 °C incubator for 2 h. The peroxidase activity was expressed as milligram of gallic acid produced in 1g of soil after 2h.

2.4.3. Lignin and humus content analysis

The lignin content of soil samples was analyzed based on a method described by the National Renewable Energy Laboratory [32]. After two steps of acid hydrolysis, the released glucose and xylose concentration were measured using the Glucose RTU kit and D-xylose kit, then the acid-insoluble lignin and acid-soluble lignin were also measured. The humus and fulvic acid (FA) concentration was determined by the method of zhen et al. [33]. All the processes were repeated for triplicate and the mean value was calculated for each case.

2.4.4. Humic acid (HA), analysis and phytotoxicity tests

HA detection was referred to the Dai's study. HA samples of 3 mg was mixed with 0.05 mol/L NaHCO3 of 10 mL and measured at 254 nm using Cary 300 UV–Vis (Agilent, USA). The total organic carbon (TOC), total extractable carbon (TEC), fulvic acid carbon and nitrogen (FAC, FAN), and humic acid carbon and nitrogen (HAC, HAN) were detected according to Zhang et al. [34]. The humification coefficients were calculated as follows: (1) humification index (HI) = (HAC/TOC); (2) percentage of humic acid acids (PHA) = HAC/TEC; ratio of HAN and FAN(HA-N/FA-N) = HAN/FAN. Phytotoxicity tests were conducted according to the National Standard of the People's Republic of China (GB/T 23 486-2009) using cabbage. Composting samples of 8 g mixed with 24 mL distilled water for 1 h at 160 r min^{-1}, then centrifuged and collected the supernatant, stored at 4 °C for further use.

20 seeds were placed in a 7.5 cm culture dish on each layer of filter paper, and 5 mL of the above supernatant was added to the culture dish. Distilled water was used as a blank control. Each experiment was repeated five times, and all culture dishes were incubated at 25 °C for 48 h in the dark. The seed germination index (SGI) was calculated according to Tang's study [35].

2.5. Statistical analyses

The differences in CD, CDB, CDM, and CDMB during the composting process were compared using one-way ANOVA (the significance level of $P < 0.05$). SPSS Statistics 19.0 (IBM, Armonk, New York, USA) was employed for all the analyses with the significance level set at 0.05. Data are showed in means ± standard deviation, n = 3.

3. Results and discussion

3.1. Changes in bacterial community with black liquor and microbial inoculation

3.1.1. Diversity and richness of the bacteria community

The composting process is a solid fermentation process dominated by microbiota and varies with the main microbial species. Changes in the richness of the bacterial community in four treatments are shown in Fig. 1. *Idiomarina*, *Pseudomonas*, and *Moheibacter* are the common microorganisms in CD, CDB, CDB, and CDMB which play an important role in composting. *Ldiomarina* can hydrolyze pectin or lipids and NaCl is necessary for *its* growth [36]. The changes in *ldiomarina* concentration were consistent with the composting period. It was abundant in the heating period and decreased from the high-temperature period according to the 16S rRNA analysis. The result indicated that *ldiomarina* is a temperature-sensitive strain, and the salt content maybe not be enough for *ldiomarina* growth as the composting process progresses. *Pseudomonas* is one of the most diverse and highly adaptable bacteria in water, soil, plants, animals, and humans [37]. It is essential for the supplements of aromatic molecules [38], which is beneficial for composting. The common dominant genus of CD, CDB, and CDMB is *Cellvibrio*. *Cellvibrio* can degrade polysaccharides, and cellulose which is alkalophilic, salt tolerant, not acid resistant [39]. The abundance of *cellvibrio* was higher in black liquor inoculation group (CDB and CDMB), which was related to the characteristics of *cellvibrio*. However, the microbial inoculation group (CDM) which will produce organic acid inhibited the growth of *cellvibrio* and had a lower abundance than other groups. *Cellvibrio* was increased with the composting process and provided nutrients by hydrolyze polysaccharides in the compost to drive the carbon cycle. *Corynebacterium* is the unique genus with a higher abundance only in CDB. It is a Gram-positive, aerobic, non-motile, rod-shapeds, catalase-positive, and oxidase-negative strain. *pseudomonas* and *Aequorivita* are the unique genus with a higher abundance in CDB and CDMB. They are all the kind of halophilic bacterium, oxidase and catalase positive. These three bacteria, under the action of oxidase, degrade lignin and promote humus formation. It also can be shown in Fig. 3B, Figs. 3C and 4B, Fig. 4C. *Fermentimonas* and *Sphingobacterium* were the dominant bacteria in CD and CDB. *Fermentimonas* is a genus of Gram-negative, facultative anaerobic bacilli. The abundance of *Fermentimonas* is higher than other groups which means black liquor inoculation is helpful for the composting. *Sphingobacterium* are gram-negative, rod-shaped, and yellow bacteria, which are strictly aerobic, which can decompose proteins [40], and some of the *Sphingobacterium* are pathogenic bacteria. *Bacillus subtilis* and *Bacillus megaterium* were increased during the high-temperature period and decreased during the cooling period. It was shown that the original *Bacillus* is less in CDMB, and the black liquor has a positive effect on it. In general, microbial inoculation (CDM) with high cellulose degradation capacity should have the greatest impact on the process of composting. However, black liquor inoculation (CDB) has the greatest boost (Fig. 2 and Fig. 4). This is mainly due to the increased abundance of oxidase-producing microorganisms, which degrade lignin and produce large amounts of humus precursor material.

3.1.2. Microbial community succession performed on nonmetric multidimensional scaling analyses

The dominant bacteria were *Psychrobacter, Jeotgalibaca_sp, Pseudoxanthomonas, Sphingobacterium, Fermentimonas, Sphingobacterium, Corynebacterium, Glutamicibacter,* and *Aerococcus* at the family level (Fig. 2A). However, there were differences in bacterial community

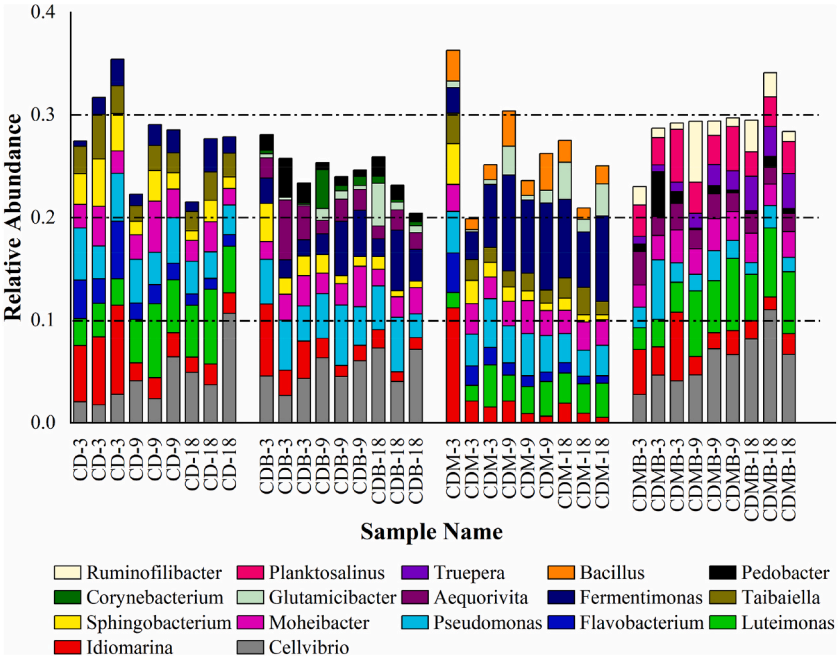

Fig. 1. Diversity and richness of the bacteria community in CD, CDM, CDB, and CDMB at day 3, 9 and 18.

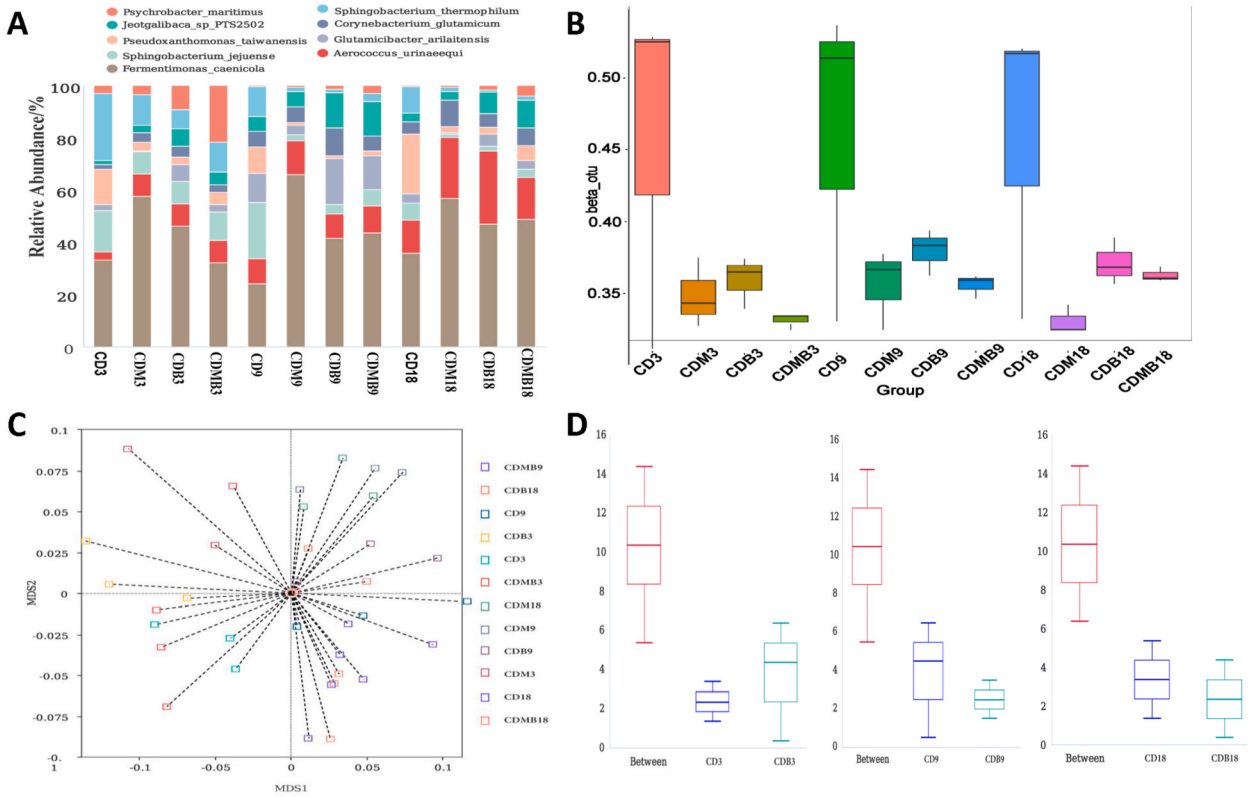

Fig. 2. The relative abundance of bacteria at the family level. (A) among all samples, the bar chart shows the top 10 bacterial community with the largest relative abundance. (B) Beta diversity of CD, CDB, CDM, and CDMB. (C) Microbial community succession performed on nonmetric multi-dimensional scaling (NMDS) analyses. (D) The difference between CD18 and CDMB18 of bacteria.

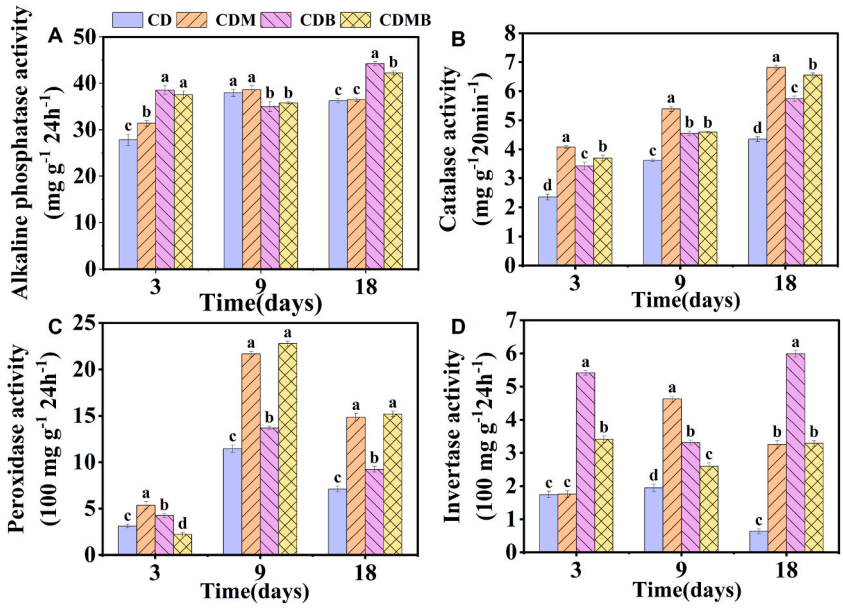

Fig. 3. Enzyme activity changes during warming period (3 days), high temperature period (9 days) and cooling period (18 days), lowercase letters represent distinctiveness ($p < 0.05$) (A. alkaline phosphatase; B. catalase; C. peroxidase activity; D. invertase).

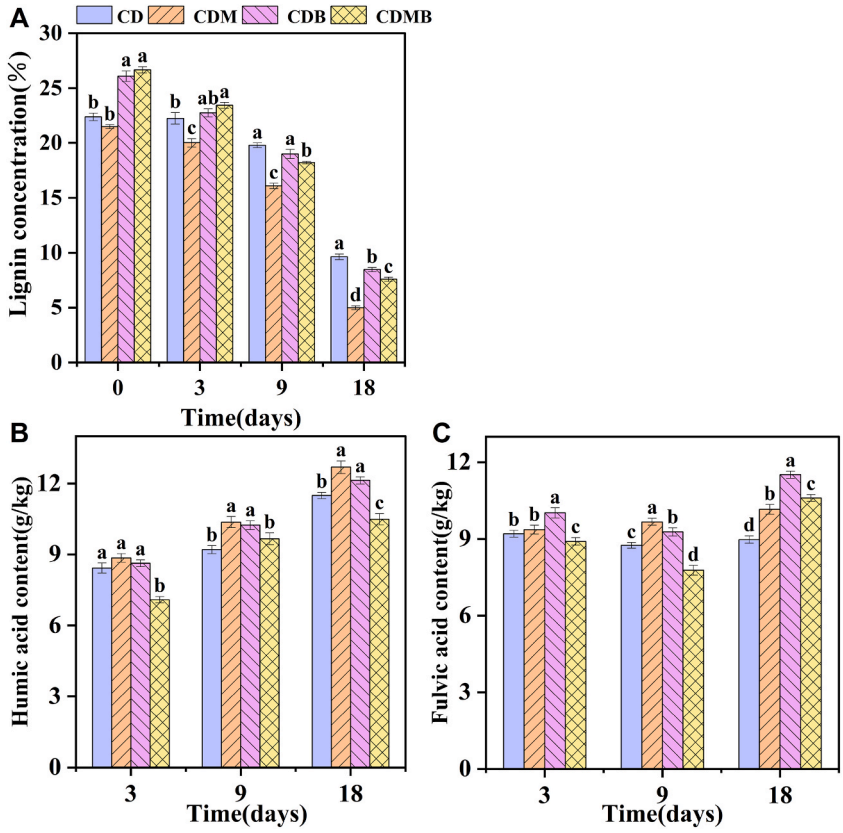

Fig. 4. The lignin, humic acid (HA), and fulvic acid (FA) concentration in CD, CDM, CDB, CDMB, lowercase letters represent distinctiveness ($p < 0.05$). (A) changes of lignin content of four treatments at days 0, 3, 9, and 18. (B) changes of humic acid content of four treatments at days 0, 3, 9, and 18. (B) changes of fulvic acid content of four treatments at days 0, 3, 9, and 18.

composition of treatment group and CD. On the 9th day and the 18th day of composting, the richness of *Jeotgalibaca* sp in CDB and CDMB was increased, indicating that black liquor/microbial inoculation promoted the reproduction of *Jeotgalibaca_sp.* The richness of *Pseudoxanthomonas* and *Sphingobacterium* in CDB and CDMB were decreased. The beta diversity results showed a significant difference in CD, CDB, CDM and CDMB (Fig. 2B). After inoculation with black liquor or microorganism alone, operational taxonomic units (OTUs) would be enriched, and the enrichment degree was significantly enhanced after inoculation with microorganism and black liquor. Combined with the results of Figs. 4 and 5, we found that after inoculation with microorganisms and black liquor, the species and abundance of microorganisms was adjusted, and the microorganisms with higher straw degradation and humus formation ability were enriched in a large amount. The nonmetric multidimensional scaling (NMDS) analysis was employed to the succession of bacterial communities in composting (Fig. 2C). A significant difference on the 3rd day, the 9th day and the 18th day of composting was observed between CD, CDB, CDM and CDMB. The difference between CDB and CD was the most significant which suggested that black liquor inoculation induced the microbial growth. This could be because the indigenous activated by black liquor in the composting. This result is consistent with the previous study in pig manure and citrus peel composting [41,42]. The bacterial community structure was adjusted by the black liquor and the changes of it were smaller on the 18th day than on the 3rd day and the 9th day. This is consistent with the previous study during the composting [43,44]. However, there was smaller difference between the CDMB and CD than CDB and CD, and the same results were observed between the treatment groups on days 9 and 18. This result indicated that black liquor inoculation had a better regulation ability of microbial species and facilitated the composting process. The same phenomenon of microbial population regulation by black liquor inoculation was observed in Fig. 2D (Fig. 2D).

3.2. Changes in enzymatic activity during composting

3.2.1. Alkaline phosphatase activity

The total phosphorus content in manure varies greatly. Nearly 70% of the entire phosphorus content in manure is unstable [45]. Dangerous phosphorus is difficult to use, while stable organic phosphorus can increase crop yield while being used by plants [46]. The conversion of organic phosphorus or mineral phosphorus to inorganic phosphorus requires the action of phosphatase. Alkaline phosphatase (ALP) comes from soil microorganisms and fauna [47]. Alkaline phosphatase can accelerate the dephosphorization rate of organic phosphorus and catalyze the hydrolysis of phosphoric acid esters and acid anhydrides, and mineralize the soil organic

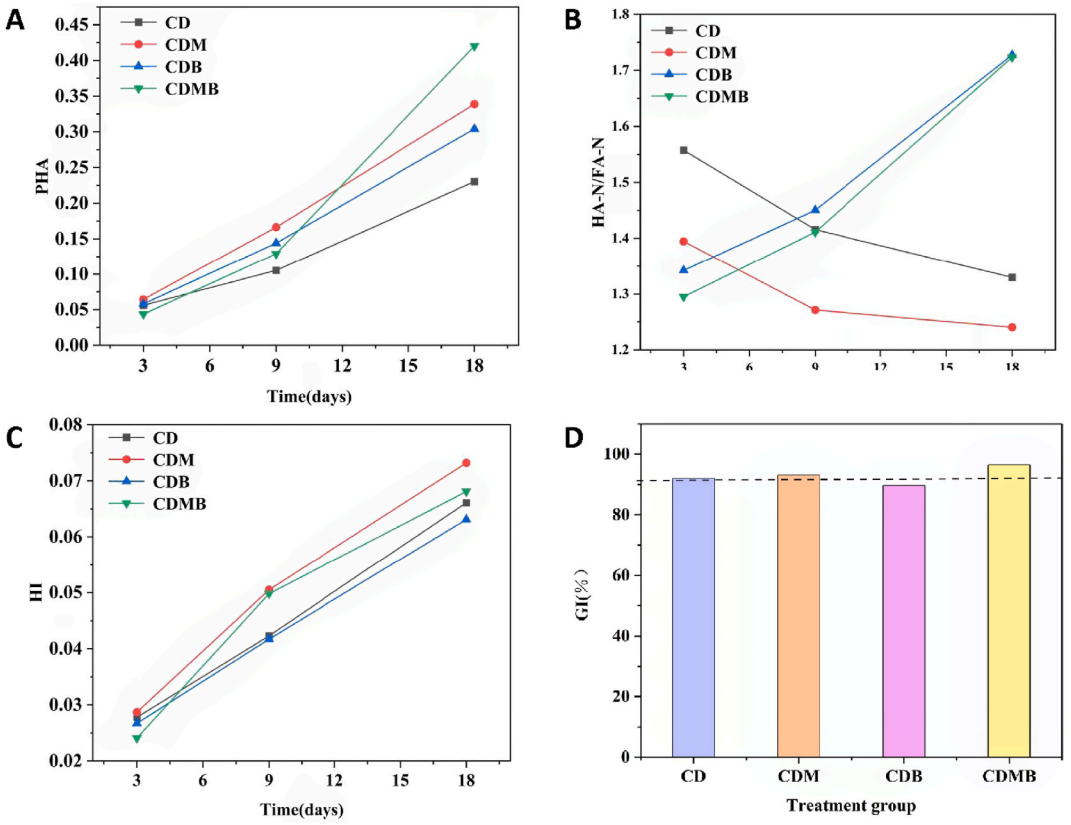

Fig. 5. Quality analysis of compost. (A) Seed germination index (G.I.) of CD, CDM, CDB and CDMB. (B) Change in the percentage of humic acid (PHA) in different treatments. (C) Variation of humification index (HI) in different treatments. (D) Changes of ratio of humic acid nitrogen to fulvic acid nitrogen in different treatments.

phosphorus [48]. Some researchers have obtained alkaline phosphatase from *Bacillus licheniformis*, and researchers have obtained alkaline phosphatase from *Bacillus subtilis*. The alkaline phosphatase activity of CD and CDM increased significantly during the high-temperature period and reached a peak of 37.92 mg g^{-1} 24 h^{-1} and 38.59 mg g^{-1} 24 h^{-1} at days nine, respectively (Fig. 3A). However, the alkaline phosphatase activity of CDB and CDMB was decreased to 34.97 mg g^{-1} 24 h^{-1} and 35.78 mg g^{-1} 24 h^{-1} during the cooling period at day 18. The highest abundance of *Enterococcus* and *Bacillus* was observed during the high-temperature period (Fig. 1). This result was consistent with the levels of the alkaline phosphatase activity in CD and CDM. The *enterococcus* and *Bacillus* produced a large amount of alkaline phosphatase in a high-temperature period. During the cooling period, the *Enterococcus* and *Bacillus* stopped growing or dying, which led to the decline of available phosphorus (Fig. 1). The alkaline phosphatase activity of CDM was significantly higher than that of CD. This may be because of the lack of relevant composting microorganisms in CD. The alkaline phosphatase activity in CDB and CDMB was ultimately different from the previous two groups. Black liquor inoculation will increase nitrogen and cause an increase in phosphorus. Microbes in compost produce a large amount of alkaline phosphatase to stabilize nitrogen during the warming period. So, black liquor and microbial inoculation will accelerate the conversion of unstable phosphorus in livestock and poultry manure and maintain higher activity to increase crop production.

3.2.2. Catalase activity

Catalase is also a crucial enzyme in soil. It can quickly capture hydrogen peroxide (H_2O_2) and degrade it into oxygen and water. It can provide nutrients to plants and reduce the loss of H and O [49,50]. Catalase can be expressed in particular members of the Pseudomonas genus, such as *Pseudomonas aeruginosa* [51]. Some researchers have also detected higher catalase activity in *Pseudomonas stutzeri.*, *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus licheniformis* [52]. The catalase activity of CD, CDM, CDB, and CDMB decreased with the increase of *pseudomonas* (Fig. 3B). It was shown that the catalase activity in CDM and CDMB is higher than that of CD and CDB, which means that *Bacillus* significantly promotes the production of catalase during the composting process. The catalase activity of CDB is higher than that of CD, which indicated that the black liquor was also had a positive effect on the growth of microorganisms associated with catalase secretion. The substances composed of H and O elements are decomposed into hydrogen peroxide, which becomes a robust substrate for conversion into catalase.

3.2.3. Peroxidase activity

Peroxidase is also an essential endogenous active oxygen scavenger in cells, closely related to plant disease resistance. With the participation of dehydrogenase, peroxidase can catalyze the oxidation of many essential phenols. Moreover, peroxidase can participate in photorespiration, oxidizing the by-product of photosynthesis glycolic acid to glyoxylic acid and hydrogen peroxide. In composting process, peroxidase is thought to be closely involved in the synthesis of humus. The peroxidase activity of CDM and CDMB with *Aspergillus niger* inoculation reached the highest enzyme activity of 2165.2 mg g^{-1} 24 h^{-1} and 2276.65 mg g^{-1} 24 h^{-1} in a high-temperature period (Fig. 3C). The final peroxidase activity of CDM and CDMB was 1486.75 mg g^{-1} 24 h^{-1} and 1520.65 mg g^{-1} 24 h^{-1}, higher than that in CD and CDB. The results indicated that the peroxidase activity of CDB is always higher than that in CD.

3.2.4. Invertase activity

Invertase activity is an important indicator of soil biological activity representation. It can characterize the conversion intensity of soil organic matter and promote the increase of soluble nutrients in the soil. It also plays an important role in the carbon cycle. The invertase activity of CD and CDM reached the highest of 194.7 mg g^{-1} 24 h^{-1} and 463.05 mg g^{-1} 24 h^{-1} at day nine and decreased at day 18 (Fig. 3D). It means that the microbial growth activity was enhanced in compost via microbial inoculation and secreted more invertase. The invertase activity of CDB and CDMB was up to 541.65 mg g^{-1} 24 h^{-1} and 340.95 mg g^{-1} 24 h^{-1} and decreased to 598.8 mg g^{-1} 24 h^{-1} and 328.38 mg g^{-1} 24 h^{-1} until days 18 (Fig. 3D). *Bacillus subtilis* is one of the main strains of producing invertase [53]. The result is consistent with the growth characteristics of microorganisms in black liquor inoculation (refer to Fig. 1). This may be because black liquor inoculation promotes the growth of *Bacillus*, which own high invertase production ability. Black liquor contains C, N, and other elements, which is also conducive to the reproduction of microorganisms. In conclusion, microbial and black liquor inoculation will increase invertase activity.

3.3. Lignin and humus

Lignin is a complex aromatic polymer. Its biodegradation proceeds, including two stages: the depolymerization of natural lignin and the mineralization of heteroaromatic hydrocarbons [54]. Microorganisms and enzymes were essential in lignin biodegradation. Oxidoreductases with peroxidases depolymerize lignin by forming lignin phenoxy radicals. Some bacteria can secret decomposing lignin enzymes to break down lignin. The mineralization of aromatic hydrocarbons produced by lignin depolymerization is believed to be mainly controlled by bacteria. Researchers have reported that the catabolism of lignin-derived aromatic hydrocarbons is applicable to various bacteria, such as *Pseudomonas putida* [55]. The lignin degradation was increased with the increase of bacteria abundance and enzyme activity (Fig. 4A). Among the four treatments, CDMB has the highest degradation rate. The lignin degradation rate of CDM and CDB was higher than that of CD. The humic acid concentration in CDM and CDB increased faster than CD and CDMB, reaching 13 g/kg and 12 g/kg at day 18 (Fig. 4B). The fulvic acid concentration in CDM increased fastest, reaching 11.5 g/kg on day 18 (Fig. 4C). The results mean that microbial inoculation can contribute to the formation of humus and black liquor inoculation has no inhibition on humus formation. The construction of the humus was consistent with the change of lignin concentration. Black liquor may assist *fungi* that degrade lignin, such as *Aspergillus niger* and *white-rot fungi* to degrade lignin but cannot promote humus formation.

3.4. Quality analysis of compost

Humification index (HI) is generally expressed as the ratio of carbon content of humic acid (HA-C) to total organic carbon (TOC). The HI coefficient of the four pile rot samples increased with the prolongation of composting time, and the highest increase was observed in the CDB group, indicating that black liquor played an extremely important role in the process of soil humification (Fig. 5A). The result was consistent with the cultivation experiment of Chinese cabbage in greenhouse (Fig. s6). The percentage of humic acid (PHA) was expressed as the ratio of carbon content of humic acid (HA-C) to carbon content of humus (HS–C) (Fig. 5B). Compared with the beginning of composting, the overall trend of the four treatment groups showed an upward trend, among which the microbial inoculation group (CDM) increased fastly, and the HI coefficient was the highest after the cooling period on the 18th day. The increasing trend of PHA coefficient in the process of composting indicates that high temperature composting is conducive to the formation of humic acid and the quality of humus, which can promote the formation of soil aggregate structure after being applied to the soil. The HA-N/FA-N of the CDB and CDMB groups inoculated with black liquor showed an upward trend, while the CD and CDM groups showed a downward trend. The results showed that the inoculation of black liquor not only added nitrogen in the material, but also added nitrogen in humic acid, which made the nitrogen structure in humus more stable (Fig. 5C). The seed germination experiment is considered the most effective method for evaluating the harmless maturity of composting products. Seed Germination Index (G.I.) comprehensively reflects the phytotoxicity of compost. It was considered the most sensitive and reliable evaluation. The Germination Index (G.I.) should be greater than or equal to 70% according to NY/T 525–2021. Chinese cabbage seeds were chosen to be cultured in a flat plate containing a compost product aqueous solution for three days at a constant temperature of 25 °C for seed germination experiments. It was shown that the seed germination of the four treatment groups CD, CDM, CDB, and CDMB reached 91.96%, 93.06%, 89.73%, and 96.50%, respectively (Fig. 5D), which were all higher than the organic fertilizer standard. The results were consistent with the nitrogen content of humus, the nitrogen content in humic acid of CD, CDM, CDB, and CDMB, and the nitrogen content in fulvic acid of CD, CDM, CDB, and CDMB (Fig. s2, Fig. s3, and Fig. s4). This proves that the compost product is mature and can germinate the seeds.

4. Conclusion

Black liquor promotes composting by activating indigenous microorganisms that are closely related to the fermentation process of composting. After black liquor inoculation, the abundance of *corynebacterium*, *aequorivita*, and *pedobacter* has been significantly increased and the humic acid and fulvic acid concentration was up to 12 g/kg and 11 g/kg. These microorganisms secrete large number of composting-related enzymes (alkaline phosphatase, catalase, peroxidase, and invertase) which accelerate the degradation of lignin, promote the composting process. This study proved that black liquor inoculation was an effective solution for humus formation, soil fertility improvement, and environmental protection in composting.

Author contributions

Shijun Pan: Conceptualization, Methodology, Software, Investigation, Writing Original Draft.
Huan Chen & Yide Fan: Writing: Review & Editing.
Juan Liu & Mingzhu Guo: Validation, Formal analysis, Visualization
Guang Chen: Resources, Writing - Review & Editing, Supervision, Data Curation.
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Conflict of interest statement

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13246.

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