Enhancing rice straw compost with an amino acid-derived ionic liquid as additive

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Abstract

To improve the quality of lignocellulose compost, the effect of a potential new-generation additive—amino acid-derived ionic liquid—on a compost pile comprising 50% rice straw was studied preliminarily. The addition of 1% 1-carboxymethanaminium chloride (glycine hydrochloride [Gly][Cl]) caused observably positive changes in the physical, chemical, and microbiological properties of the compost. After 30 days of composting, the humus and total nitrogen concentrations were 130.85 and 28.8 g/kg, showing an increase of 93.28% and 67.44%, respectively, compared with the concentrations in the beginning of composting; these concentrations were 76.97% and 41.69%, respectively, for the control group (without [Gly][Cl]). Thus, amino acid-derived ionic liquids can be promising additives for enhancing the quality of composts for which straw is used as the primary component.

Keywords: straw compost; ionic liquid; lignocellulose degradation; humus synthesis

1. Introduction

The quality of arable land is generally declining due to the excessive use of chemical fertilizers, thus, organic fertilizers are becoming particularly important in the restoration of arable land. In China, the organic fertilizers production capacity is far below national and international standards. More than 700 million tons of lignocellulose, which primarily comprises cellulose, hemicellulose, and lignin, are produced worldwide every year, thus generating a rich source of biomass feedstock and causing enormous pressure on the environment (Wu et al., 2019). Composts that use straw as the main component have long fermentation time (approximately 2 months) and relatively low quality, because of the low biodegradability due to its complex structure (Dos Santos et al., 2019; Tuomela et al., 2000).

Therefore, several types of exogenous additives are currently being studied to improve the compost quality of lignocellulose. For example, Zhang et al. (2018) demonstrated that the humus (HS) concentration in the compost comprising maize straw, chicken manure, benzoic acid (additive), and soybean residue after oil extraction (additive) increased by 35.46%–45.39% after 62 days compared with that in the initial of composting; this concentration was 35.12% in the treatment without additive (CK). Zheng et al. (2021) reported that the HS concentration of a straw compost reached 16.73% and 18.32% 60 days after the addition of phenylalanine and leucine, respectively (11.82% in CK). Amino acids not only participate in the synthesis of HS directly as precursors but also alter the functions of bacterial communities present in the compost. Meng et al. (2021) added illite/smectite clay in the compost of bagasse pith and cattle manure; the HS concentration increased by 7.16% (3.49% in CK) after 43 days. In addition to HS, the nitrogen concentration can be increased by incorporating additives. For example, a previous study has shown that the total nitrogen (TN) concentration increased by 30.58% with 15% rice husk additive in a two-stage composting of green waste (Wang et al., 2021). In the biosolids composting study conducted by Pan et al. (2019), the TN concentration increased after 52 days by 15.78%–16.62% with the addition of 2%–10% palygorskite (8.51% in CK). Gabhane et al. (2012) have tested five additives on the composts comprising grass cuttings and fallen leaves; the addition of jaggery increased the TN concentration by 13%. However, the HS concentration in several products of lignocellulose composting was <25%, which is the required Chinese standard for high quality products for use as HS biofertilizers (HG/T 5332-2018). This has led to further investigation for identifying additives with higher efficacy.

Ionic liquids (ILs)—ionic compounds whose melting point is below 100°C—have been reported to exhibit several fascinating properties in studies across various domains (Rogers, 2007). ILs may promote lignocellulose degradation by breaking the β-*O*-4 ether (Sun et al., 2016), intra- and intermolecular hydrogen (Hu et al., 2016; Wang et al., 2017), π–π, n–π, and other connecting (De Gregorio et al., 2016) bonds. However, the most studied ILs—imidazolium-based ILs—are associated with toxicity and resistance to biodegradation owing to the presence of the cation (Deng et al., 2011); hence, ILs are rarely applied in composting.

Tao et al. (2005) have reported a new generation of “fully green” ILs—amino acid-derived ILs. These ILs can be easily synthesized by mixing an amino acid (weak base) and a relative strong acid in a suitable molar ratio. The previous exploration of this research has shown that one such IL, carboxymethanaminium chloride (glycine hydrochloride, [Gly][Cl]), produced using glycine and hydrochloric acid is nontoxic toward *Spirulina platensis* at a concentration of 200 mg/L. Moreover, similar to other ILs, it can disrupt the dense structure of the straw surface and exert some dissociative effect on lignocellulose, which is favorable for the composting process. However, the underlying mechanisms behind the altered composition of the compost bacterial community due to the addition of [Gly][Cl] remain unknown. Therefore, in this study, [Gly][Cl] was used as an additive in compost comprising rice straw as the primary raw material, kitchen waste, and cow manure to investigate any changes in physical, chemical, and microbiological properties during the composting process. The enhancement of lignocellulose degradation or humification due to [Gly][Cl] addition may indicate that amino acid-derived ILs improve straw biofertilizer quality as the ILs are compounds that can be "designed" to meet the requirements of people involved in sustainable agriculture.

2. Materials and Methods

2.1. Test materials

Rice straw was obtained from a field in Lianyungang City, Jiangsu Province, China. The kitchen waste was collected from a canteen at Jiangnan University, China. The cow manure was collected from a mountain in Kunming City, Yunnan Province, China. See supplementary material for the basic physicochemical properties of these raw materials.

[Gly][Cl] was synthesized following a previously reported protocol (Tao et al., 2005). **Error! Bookmark not defined.**Glycine and hydrochloric acid were mixed in an equimolar ratio in aqueous solution. The solution was then agitated with a magnetic stirrer for 8 hours at 60°C. The reaction is as follows: $[HCl]+[NH_2CH_2COOH]\rightarrow[COOHCH_2NH_3^+][Cl^-]$. Then, the water was removed using a rotary evaporator.

2.2. Experimental design

The rice straw, kitchen waste, and cow manure were mixed in the ratio of 4:3:1 by mass to adjust the initial C/N ratio to approximately 25. Water was then added to adjust the moisture content to approximately 60%. The total mass of each compost group was 7.5 kg. A total of 50 g [Gly][Cl] IL was added to the experimental group (EP), which was 1% of the raw material mass, before water was added. No additives was added to the control group (CP), and 50 g glycine was added to the nutrition group. The experiment was performed in triplicate, and the results were averaged.

The mixture was loaded into a 4.1 × 2.75 × 1.9 L foam box and fermented for 30 days. The piles were turned for aeration. During the first 10 days, the pile was turned once every 2 days. From days 11–30, the piles were turned once every 5 days. Samples were taken on days 0, 2, 5, 10, 15, 20, 25, and 30, according to the five-point sampling method in the upper, central, and lower parts of the box. The samples from the different points were mixed well. The samples were indicated as CP_X and EP_X (X is the number of composting days).

2.3. Measurement of physicochemical properties and chemical constituents

The TN, total phosphorus (TP), and total potassium (TK) concentrations were determined according to the study by Cai et al. (2019). The lignocellulose contents, various HS component concentrations, and 3-dimensional fluorescence spectra of dissolved organic matter were determined following the protocol described in the study of Bai et al. (2020).

2.4 Measurement of enzyme activities

The enzyme activities of cellulose, xylanase, lignin peroxidase, polyphenol oxidase, dehydrogenase, and urease were determined following the protocols described in the studies by Li et al. (2008) and Zeng et al. (2010).

2.5 Determination of microbial communities via high-throughput sequencing

The process used in the sequencing experiment can be summarized as follows: environmental sample DNA extraction → primer design and synthesis → Polymerase Chain Reaction (PCR) amplification and product purification → PCR product quantification and homogenization → Illumina sequencing.

2.6 Statistical analysis

All chemical analyses were repeated thrice in parallel, and the results were reported as means. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$ using the IBM SPSS Statistics 20.0. Redundancy analysis (RDA) was performed to assess the correlation between microorganisms and physicochemical parameters using Canoco 5.0.

3. Results and Discussion

3.1. Physicochemical properties

No significant difference was found in the physicochemical properties measured during the composting process of the nutrition group and CP ($p > 0.05$), except in terms of TN; thus, the results of the nutrition group are not discussed here. The nutrition supplied by the cation of [Gly][]Cl] might not have been the primary reason leading to the differences between the EP and CP.

The trends in the temperature, pH, moisture content (MC), and C/N ratio of the EP and CP were similar throughout the composting process (Fig. 1). All these technical indexes at the end of composting met the technical specifications of organic fertilizer mentioned in NY 525-2012.

The EP and CP reached their thermophilic phase (>50°C) on days 3 and 4, respectively. The duration of the thermophilic phases was 9 and 5 days, with maximum temperatures of 68.3°C and 64.3°C, respectively (Fig. 1a). From day 1 of composting, the temperature in the EP was consistently 1.1°C–8.7°C higher than that of the CP, indicating that the addition of [Gly][Cl] significantly increased the composting temperature. The pH (Fig. 1b) of the EP was consistently lower than that of the CP, probably owing to the acidic nature of the added IL. The final pH between 7 and 8 indicated compost maturity and the absence of alkaline or acidic conditions that inhibit plant growth (Zhang et al., 2013). The change in MC, which is mainly affected by compost temperature, was faster in the EP than that in the CP during the thermophilic phase (Fig. 1c). The C/N ratio (Fig. 1d) in the EP and CP changed from 25.06 and 25.51 to 14.13 and 15.25, respectively, which indicated that both composts were mature. Muscolo et al. (2018) considered final C/N ratio/initial C/N ratio (T) < 0.7 as a proof of good decay. The T values were 0.60 and 0.56 for the CP and EP, suggesting a higher degree of decomposition in the EP.

3.2. Chemical constituents

The primary component of rice straw is lignocellulose, which is the most stable and recalcitrant organic carbon in compost and limits both rapid composting and HS formation (Jurado et al., 2015). From the data shown in Fig. 2a, the degradation percentages of lignocellulose in the EP (66.51%) was 5.11% higher than that in the CP (61.40%). Specifically, the degradation percentages of cellulose, hemicellulose, and lignin were 64.35%, 76.14% and 51.41% in the EP at the end of composting. The IL additive facilitated the degradation of lignocellulose, thus ensuring suitable conditions for the conversion of organic matter and subsequent synthesis of HS. Although only 1% [Gly][Cl] addition can disrupt the structure of straw, it probably has ignorable impact. The more possible reason is that the IL additive changes the physicochemical structures and microenvironment of the compost, and promotes the metabolism and proliferation of lignocellulose-degrading microorganisms.

The concentrations of total nutrients (TN + TP + TK) in the CP and EP were 106.89 and 117.12 g/kg, respectively, after composting, both of which met the Chinese technical index for organic fertilizer. The TN concentration in the CP and EP were 16.8 and 17.2 g/kg in the beginning and 23.8 and 28.8 g/kg at the end of composting, showing an increase of 41.67% and 67.44%, respectively. Zhang and Sun (2019) confirmed that lowering the pH of the compost is beneficial for nitrogen retention. The TP concentration in the CP and EP was 8.77 and 7.99 g/kg, showing an increase of 88.89% and 94.68%, while that of TK was 74.92 and 80.33 g/kg, increased by 32.99% and 42.18%, respectively, which might be because the lignocellulose in the EP degraded more than that in the CP.

At the end of composting, the HS concentration was 130.85 and 120.25 g/kg in the EP and CP, which increased by 93.28% and 76.97% compared with that in the beginning of composting. During composting, the humic acid (HA) concentration in the EP is consistently higher than that in the CP. The fulvic acid (FA) concentration reached its maximum value 5 days later in the EP, indicating that IL addition accelerated the formation of more mature HS. Zheng et al. (2021) reported an increase in the HS concentration with the addition of phenylalanine and leucine, and they explained that these two amino acids can participate in HS synthesis directly as precursors. However, in the present study, glycine—a small molecule—failed to function as HS precursor.

According to previous studies (Wang et al., 2013; Yu et al., 2018), the excitation-emission matrix (EEM) spectra of dissolved organic matter (DOM) was partitioned into five regions (Fig. 3). Two main substances existed in the initial compost. The highest intensity in the EEM fluorescence spectra was presented in Region IV (Ex < 250 nm; Em > 380 nm), which is associated with soluble microbial byproduct-like substances and lignocellulose. The second highest intensity was in Regions I and II (Ex < 250 nm; Em < 380 nm), which is related to simple aromatic proteins, such as tyrosine and tryptophan, that are primarily from proteins in biological debris or enzymes produced by bacterial decomposition. After composting, the fluorescence intensity in Regions I, II, and IV decreased drastically in the following order: tyrosine-like protein (Region I) > tryptophan-like proteins (Region II) > the lignocellulose (Region IV) the least degraded. The fluorescence intensity of the soluble microbial byproduct-like substances such as simple carbohydrates and fats (Region IV) decreased,, indicating that the microbial activity declined and that the composts showed a tendency to stabilize (Zhang et al., 2016). Simultaneously, the fluorescence in Regions III (Ex < 250 nm; Em > 380 nm) and V (Ex > 250 nm; Em > 380 nm), respectively, indicated that FA-like and HA-like substances were generated. This indicates that the partial aromatic proteins in the initial compost were decomposed by microorganisms, and a large amount of HA-like substances were formed. All changes in the EP were more obvious compared to CP: the simple aromatic proteins were more thoroughly decomposed, and more HA-like organic matters were produced. These results corresponded to the HS concentration outlined above.

3.3. Enzyme activities

The tendency of changes in enzyme activity of both groups were similar (Fig. 4). During the entire composting process, the activities of cellulose (Fig. 4a), lignin peroxidase (Fig. 4b), polyphenol oxidase (Fig. 4c), which are involved in lignocellulose degradation and humification, in the EP were generally higher than those in the CP. The dehydrogenase activity (Fig. 4d) in the EP was higher in the first 20 days but lower than that in the CP in the later composting stage, indicating that the addition of IL induced the redox reactions to occur earlier and faster; this ultimately resulted in a higher degree of decay in the later stage (Castaldi et al., 2008).

The neutral xylanase activity in the EP was always lower than that in the CP, probably because this activity was measured for the enzyme derived from microorganisms with an optimal growth at pH 6–8; EP had a pH that was too low in the initial stage. This low pH might have inhibited the growth and reproduction of neutral xylanase-producing microorganisms, resulting in the lower xylanase activity. In contrast, the hemicellulose degradation rate in the EP was higher than that in the CP at the end of composting, probably owing to the role that acidic xylanase played in degrading xylan and the increased degradation of the other types of polysaccharides, such as glucuronic acid, in hemicellulose

The urease activity reflects the mineralization process of nitrogenous substances during the degradation of organic matter (Bohacz and Korniłłowicz-Kowalska, 2009). The lower urease activity in the EP (Fig. 4f) may be related to the decrease in the number of urease producing microorganisms because of the higher temperature or/and lower pH due to IL addition. The lower urease activity of the compost was beneficial in delaying the release of nitrogen, thus contributing to the higher TN concentration in the EP.

3.4. Microbial community

The addition of [Gly][Cl] significantly modified the microbial community of the compost (Table 1, Fig. 5). During the entire composting period, particularly during the thermophilic phase (day 5), the microbial abundance was significantly higher in the EP than in the CP; however, the microbial diversity was significantly lower in the EP than in the CP (Table 1). This indicated that [Gly][Cl] inhibited certain microorganisms but promoted the proliferation of surviving microorganisms. Conversely, in the study conducted by Zheng et al. (2021), the bacterial diversity was higher in the groups treated with amino acids, which might have been because amino acids provide nutrients for microorganisms and enhance their metabolic activities.

The correlation between physicochemical properties and microflora was evaluated using RDA (Fig. 5a). Among the evaluated physicochemical properties, temperature, C/N, and MC had the most significant influence on *Bacillus* and *Thermoactinomyces*, as well as the microbial community on day 5. HS had significant influence on the other six evaluated genera, as well as the microbial community on days 10 and day 20. In addition, pH did not obviously correlate with the microbial community in the EP on day 5, suggesting that the microorganisms that survived from the initial acidic environment were not easily affected by pH.

The abundance of the three most dominant phyla is summarized in Table 1, and the trend of change occurring in the abundance for the two groups was the same. The abundance of *Firmicutes* decreased less in the EP than in the CP, and it became the dominant phylum on day 20. The abundance of *Actinobacteria* increased more in the CP than in the EP, and it became the dominant phylum on day 20. The abundance of *Proteobacteria* increased less in the EP than in the CP because *Proteobacteria* is less tolerant to high temperature (Wu et al., 2020) and low pH (Shin et al., 2017).

From the perspective of genera (Fig. 5b), on day 5, the two most dominant genera—*Bacillus* and *Thermoactinomyces*—accounted for 54.68% and 3.00% in the CP and 38.31% and 46.46% in the EP, respectively. These two genera are major cellulose and lignin decomposers (Mullings and Parish, 1984). As shown in Fig. 5a, the abundance of *Bacillus* positively correlated with temperature and pH; however, the abundance of *Thermoactinomyces* correlated with only temperature but not pH. The acidic pH in the early stage of composting in the EP exerted a certain inhibitory effect on the growth and reproduction of *Bacillus*, thus reducing interspecific competition with *Thermoactinomyce*, which was more resistant to low pH..

The abundance of *Thermobifida*, *Oceanobacillus*, and *Novibacillus* increased on day 10, and these became the most dominant genera. The primary function of *Thermobifida* and *Novibacillus* is to degrade organic compounds (Chang et al., 2021, Bao et al., 2021) and their abundance in both groups showed no significant difference. The abundance of *Oceanobacillus*, which has been reported to significantly contribute to nitrogen preservation and humification (Qiu et al., 2021), was 15.49% in the CP and 18.29% in the EP. On day 20, *Saccharomonospora* and *Pseudoxanthomonas* became dominant genera in both compost groups. These two genera have a notable effect on lignin degradation (Hua et al., 2014; Wang et al., 2019). T heir abundance in both groups did not differ significantly: only showed a slightly higher abundance in the CP than in the EP.

The abundance of *Gracillibacillus* and *Streptomyces*, which showed significantly positive correlation with HS (Fig. 5a), was 28.79% and 22.00% in the EP, in the CP, these values were 0.64% and 9.44%, respectively. *Gracilibacillus* hydrolyzes macromolecular gelatin and proteins (Waino et al., 1999), whereas *Streptomyces* is involved in cellulose degradation (Mullings and Parish, 1984) and nitrogen fixation (Wu et al., 2020), which is possibly another reason for higher TN concentration in the EP.

Overall, the abundance of *Thermoactinomyces*, which is associated with cellulose and lignin decomposition, along with that of *Gracilibacillus* and *Streptomyces*, which are associated with HS, increased because of the addition of [Gly][Cl]. The microorganisms more sensitive to temperature, pH, and MC were inhibited. Consequently, the lignocellulose degradation percentage and HS concentration were enhanced.

4. Conclusion

In this study, the positive effect of an amino acid-derived ionic liquid on lignocellulose compost was preliminarily established. [Gly][Cl] inhibited the proliferation and/or metabolic activities of certain microorganisms but promoted those of the surviving microorganisms. In the phase when most lignocellulose was degraded, the key factors that influenced the microbial community structures were temperature, C/N and MC. Because [Gly][Cl] showed potential advantages, future studies should focus on the organic matter conversion in lignocellulose compost in presence of other amino acid-derived ILs and more amount of IL additive.

Credit authorship contribution statement

Huanhuan Ma: Investigation and Writing- Original draft preparation. **Ian Beadham**: Writing- Reviewing and Editing. **Wenquan Ruan**: Methodology and Supervision.

Changbo Zhang: Funding acquisition. **Yun Deng**: Conceptualization, Methodology, Writing - Reviewing and Editing, and Supervision.

Declaration of Competing Interest

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

Acknowledgements

This work was supported by the National Key Research and Development Program of China (grant No. 2021YFC2102205, 2017YFD0801100, 2018YFD0800300).

Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

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Figure Captions:

Table 1 Analysis of (a) OTU change, (b) alpha diversity indexes, and (c) the abundance of the three most dominant phyla

Fig. 1 Physicochemical properties of compost: (a) temperature, (b) pH, (c) moisture content, (d) C/N ratio

Fig. 2 (a) Degradation percentage of lignocellulose at the end of composting; (b) total nutrients; (c) changes during composting in (1) humus, (2) fulvic acid, and (3) humic acid

Fig. 3 EEM spectra of DOM: (a) initial compost (b) matured compost in the CP, (c) matured compost in the EP

Fig. 4 Changes in the enzyme activity: (a) cellulase, (b) lignin peroxidase, (c) polyphenol oxidase, (d) dehydrogenase, (e) xylanase, and (f) urease

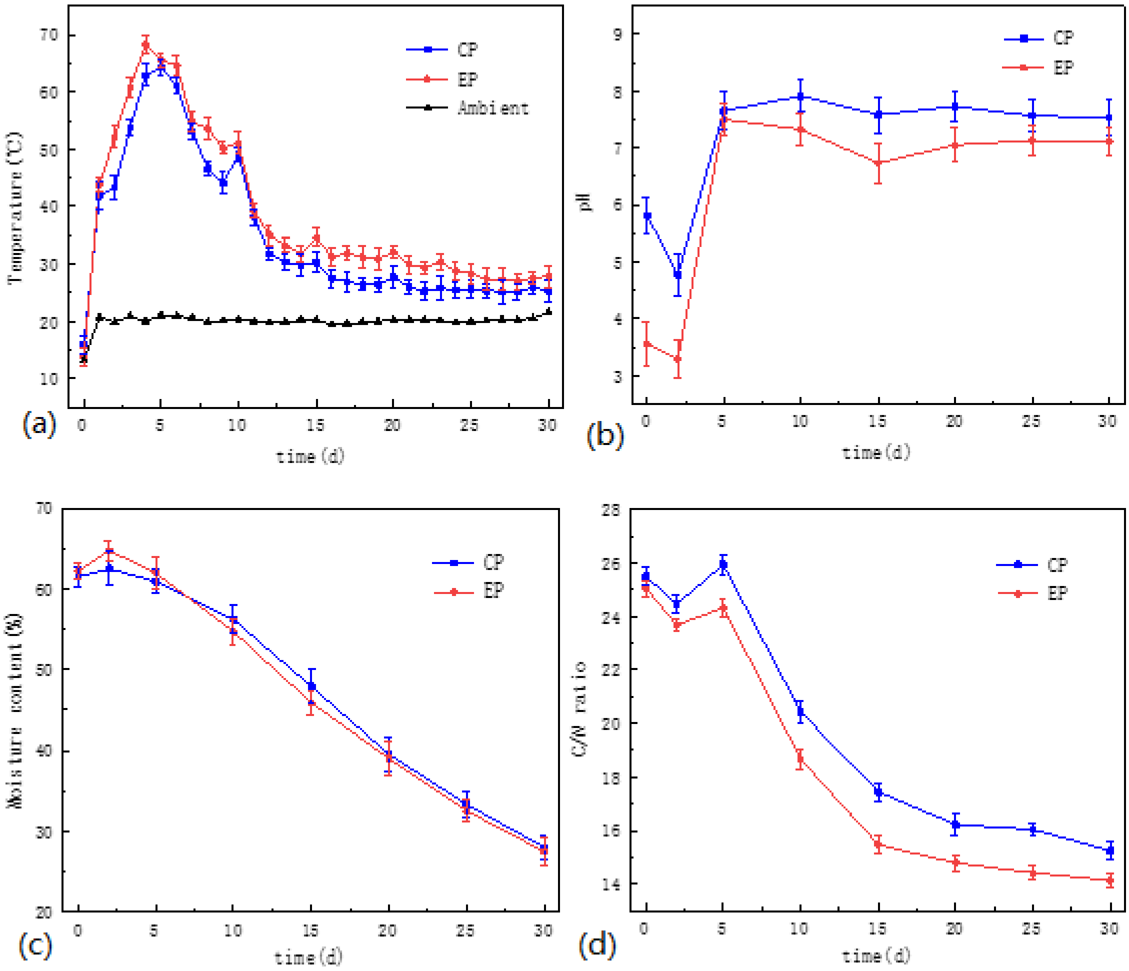
Fig. 5 (a) Redundancy analysis (RDA) of selected environmental parameters and top eight genera in the composting samples, (b) relative abundance of the microbial community at the genus level

Tables & Figures:

Table 1

Group	Sample	OTU number	Number of sequences in OTU	Chao 1	Shannon	*Firmicutes* (%)	*Actinobacteria* (%)	*Proteobacteria* (%)
CP	CP_5	148	41,601	203	2.81	95.69	1.98	2.14
	CP_10	157	37,905	173.07	2.83	58.81	36.28	3.43
	CP_20	205	43,879	212.14	3.26	20.27	50.72	26.12
EP	EP_5	132	50,215	288.52	1.97	94.97	4.92	0.09
	EP_10	154	40,344	188.21	2.83	76.09	22.48	0.17
	EP_20	199	46,237	222.11	3.29	48.29	41.36	8.84

Figure 1



(a)
Temperature (℃)
time (d)
CP
EP
Ambient
10
20
30
40
50
60
70
0
5
10
15
20
25
30
(b)
pH
time (d)
CP
EP
3
4
5
6
7
8
9
0
5
10
15
20
25
30
(c)
Moisture content (%)
time (d)
CP
EP
20
30
40
50
60
70
0
5
10
15
20
25
30
(d)
C/N ratio
time (d)
CP
EP
14
16
18
20
22
24
26
28
0
5
10
15
20
25
30

Figure 2

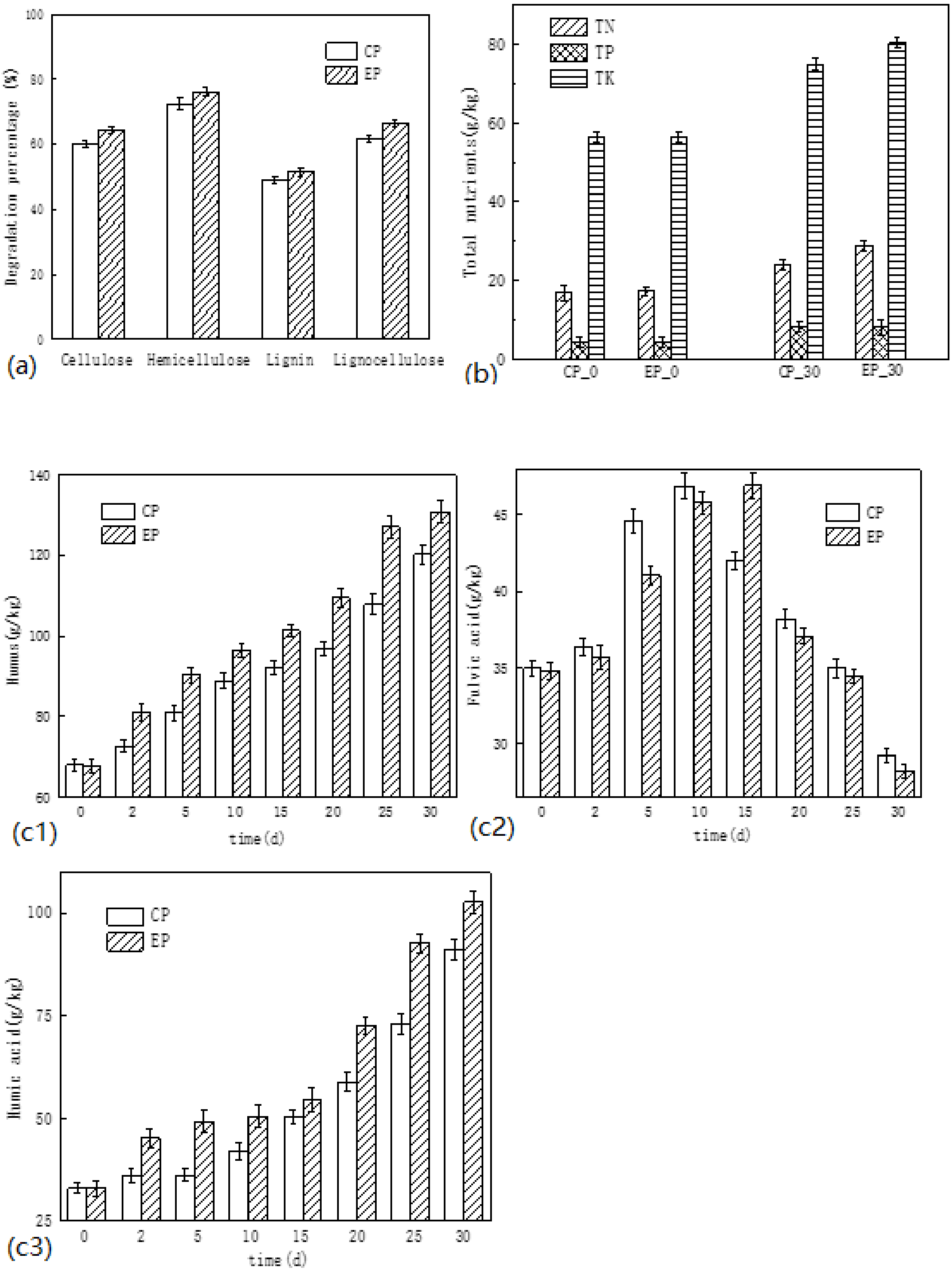

(a)
Degradation percentage (%)
0
20
40
60
80
100
CP
EP
Cellulose
Hemicellulose
Lignin
Lignocellulose
(b)
Total nutrients(g/kg)
0
20
40
60
80
TN
TP
TK
CP_0
EP_0
CP_30
EP_30
(c1)
Humus(g/kg)
60
80
100
120
140
CP
EP
0
2
5
10
15
20
25
30
time(d)
(c2)
Fulvic acid(g/kg)
30
35
40
45
CP
EP
0
2
5
10
15
20
25
30
time(d)
(c3)
Humic acid(g/kg)
25
50
75
100
CP
EP
0
2
5
10
15
20
25
30
time(d)

Figure 3

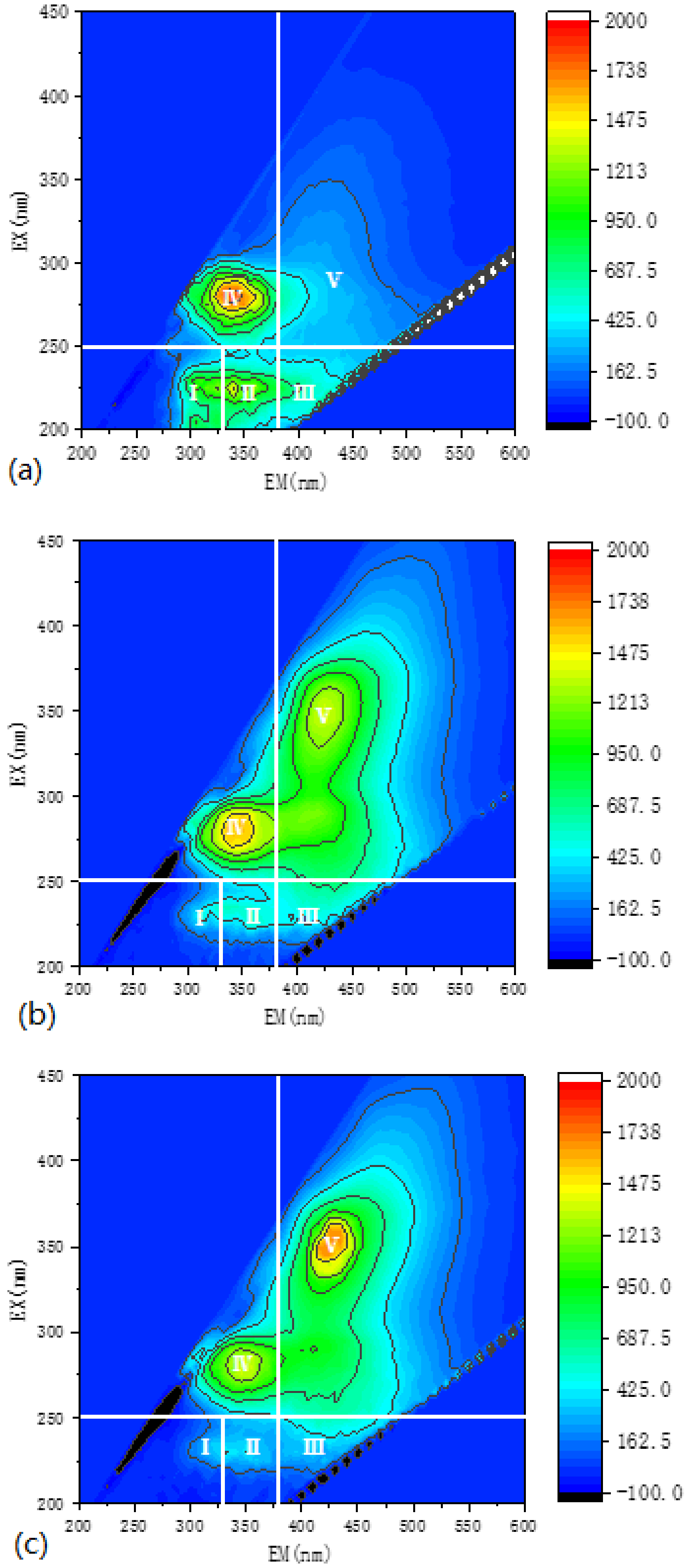

(a)
(b)
(c)
EX (nm)
EM (nm)
200
250
300
350
400
450
500
550
600
I
II
III
IV
V
2000
1738
1475
1213
950. 0
687. 5
425. 0
162. 5
−100. 0

Figure 4

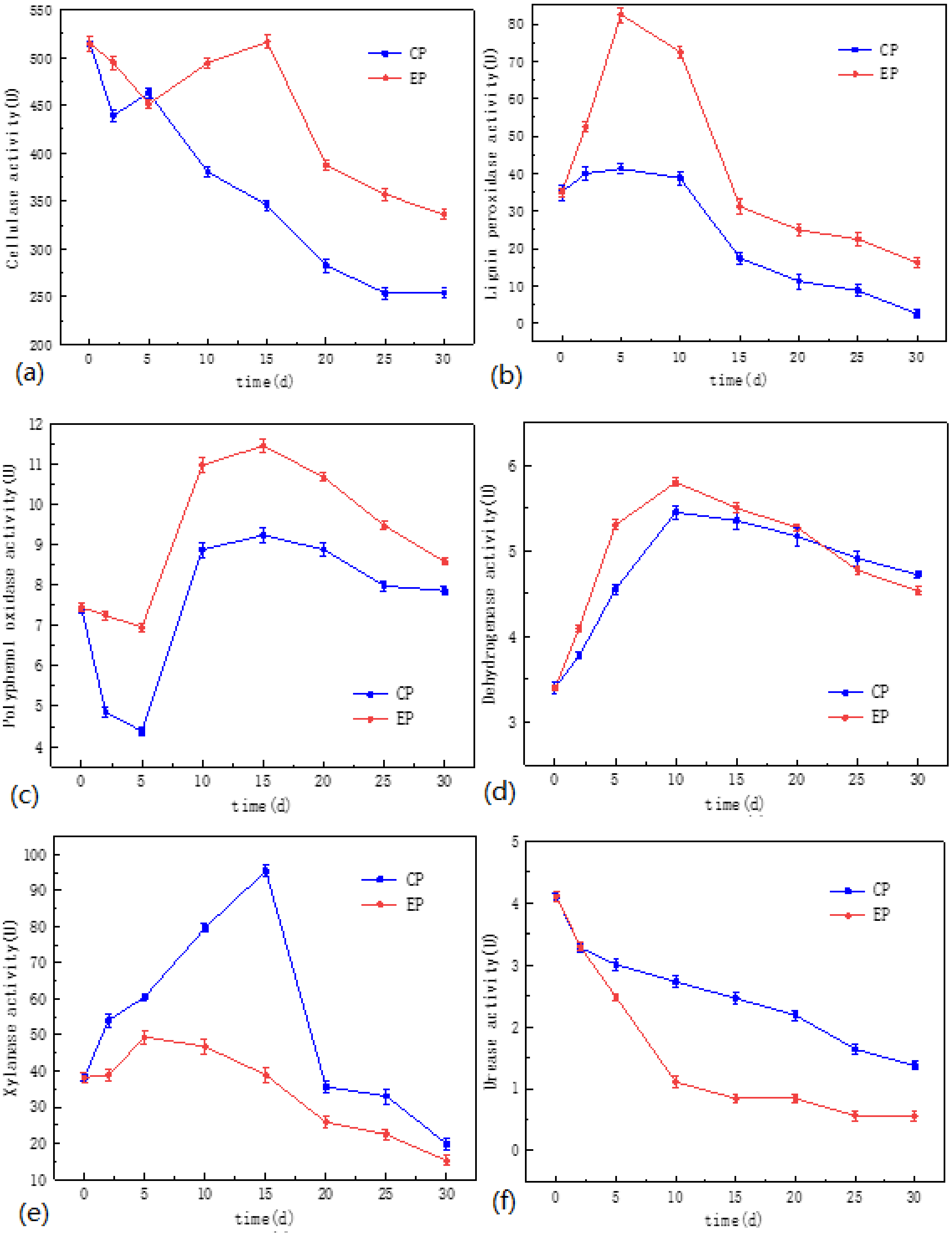
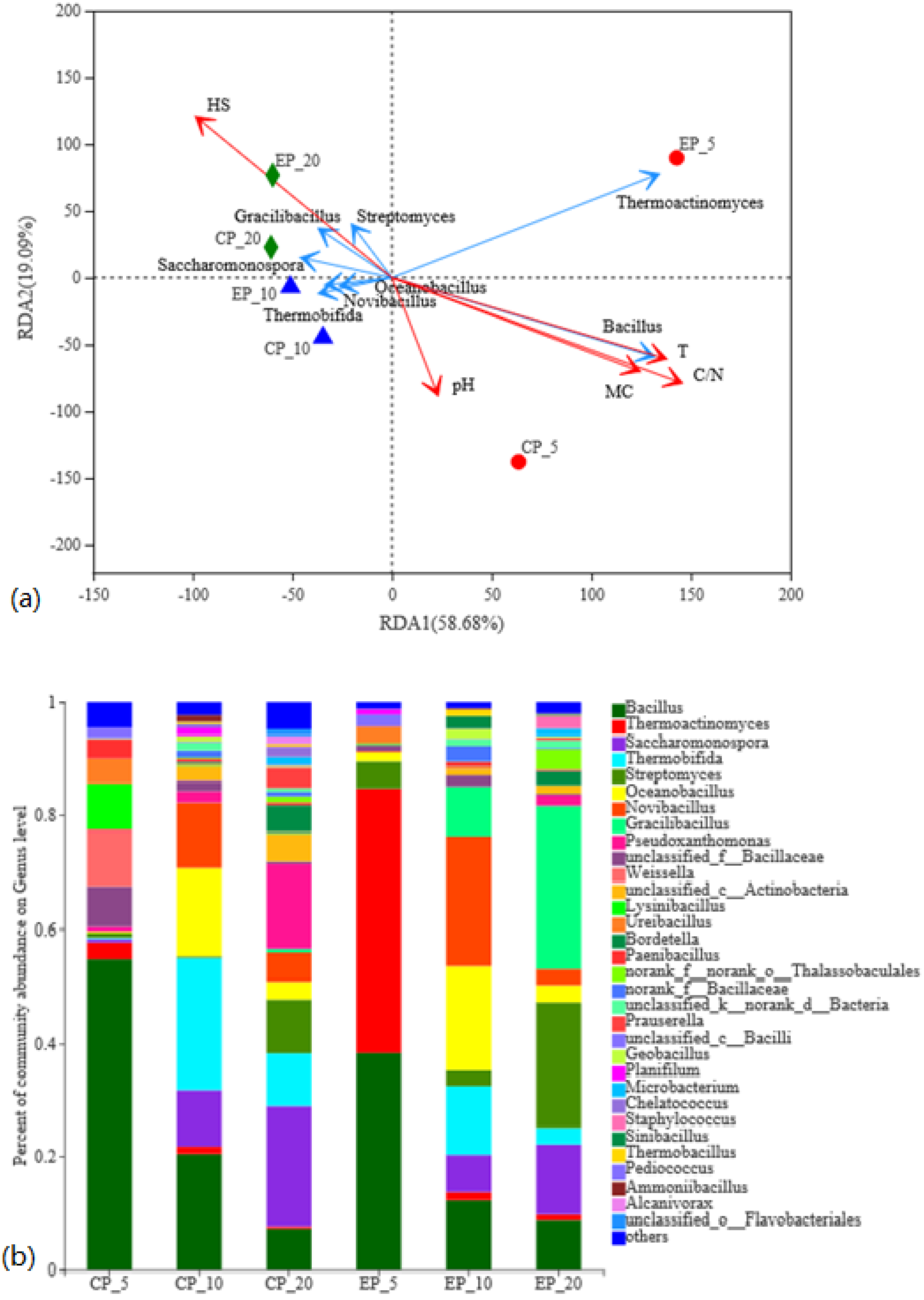

(a)
Cellulase activity(U)
550
500
450
400
350
300
250
200
0
5
10
15
20
25
30
time(d)
CP
EP
(b)
Lignin peroxidase activity(U)
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
time(d)
CP
EP
(c)
Polyphenol oxidase activity(U)
12
11
10
9
8
7
6
5
4
0
5
10
15
20
25
30
time(d)
CP
EP
(d)
Dehydrogenase activity(U)
6
5
4
3
0
5
10
15
20
25
30
time(d)
CP
EP
(e)
Xylanase activity(U)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
time(d)
CP
EP
(f)
Urease activity(U)
5
4
3
2
1
0
0
5
10
15
20
25
30
time(d)
CP
EP

Figure 5


(a)
RDA1(58.68%)
RDA2(19.09%)
-150
-100
-50
0
50
100
150
200
-200
HS
EP_20
EP_5
Thermoactinomyces
Gracilibacillus
Streptomyces
CP_20
Saccharomonospora
EP_10
Oceanobacillus
Novibacillus
Thermobifida
CP_10
Bacillus
T
C/N
MC
pH
CP_5
(b)
Percent of community abundance on Genus level
0
0.2
0.4
0.6
0.8
1
CP_5
CP_10
CP_20
EP_5
EP_10
EP_20
Bacillus
Thermoactinomyces
Saccharomonospora
Thermobifida
Streptomyces
Oceanobacillus
Novibacillus
Gracilibacillus
Pseudoxanthomonas
unclassified_f__Bacillaceae
Weissella
unclassified_c__Actinobacteria
Lysinibacillus
Ureibacillus
Bordetella
Paenibacillus
norank_f__norank_o__Thalassobaculales
norank_f__Bacillaceae
unclassified_k__norank_d__Bacteria
Prauserella
unclassified_c__Bacilli
Geobacillus
Planifilum
Microbacterium
Chelatococcus
Staphylococcus
Sinibacillus
Thermobacillus
Pediococcus
Ammoniibacillus
Alcanivorax
unclassified_o__Flavobacteriales
others