Microbiological Community Analysis of the Composting of Poplar Processing Residues

Xiaohui Liang1, Hongyu Si1,*, Yuben Fan2, Bing Wang1, Dongliang Hua1, Zhixin Wang2, Chengwen Dong3

1 Energy Institute, Qilu University of Technology (Shandong Academy of Sciences), Shandong Provincal Key Laboratory of Biomass Gasification Technology, Jinan 250014, China;
2 College of Biological Sciences and Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, China
3 School of Chemistry and Chemical Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, China

*Corresponding author e-mail: sihy@sderi.cn

Abstract. In this study, poplar processing residues was used as the sole substrate for composting, and the microbiological communities such as fungi and bacteria were analysed. Two most favourable fungal phyla Ascomycetes and Basidiomycetes and five kinds of fungal genus such as Aspergillus, Thermomyces, Rhizomucor, Rhizopus, Myceliophthora were mainly distributed (more than 80%) in the high temperature samples. As for the bacteria, Firmicutes, Actinobacteria and Proteobacteria were the three most advantageous phyla in the high temperature samples. At the same time, Geobacillus, Bacillus, Thermobispora and Sphingomonas were the dominant bacteria in the high temperature samples at the genus level.

1. Introduction
Composting is a kind of popular method for organic materials (crop straws, branches and leaves of trees, livestock manures, mushroom substrates, municipal sludge wastes, etc.) utilization, which can achieve high efficiency biotransformation to produce organic fertilizers, nutritional media, soil amendments [1]. In order to obtain high quality organic fertilizers and improve fermentation efficiency, C/N ratio, moisture content, agitation and ventilation should be considered [2]. However, the C/N ratio of some crop straws, branches, leaves and barks of trees are very high, and the output in China could reach nearly 1.4 billion tons per year, and there were not so many nitrogen sources for C/N adjusting for composting [3].

The poultry manure is generally used for reducing C/N ratio to obtain the optimal C/N ratio for composting [4]. However, in some areas, due to actual limited conditions, other supplementary substances cannot be added to adjust C/N ratio during composting. So high C/N ratio materials composting is worth exploring at present. Although compost is widely used for organic wastes treatment, because of the diversity of raw materials, so that there is still a gap in our understanding of microorganisms during composting.
In this study, a kind of high C/N ratio substrate—poplar processing residues was used as the sole substrate for composting, and the microbiological communities including fungus and bacteria were analyzed. The results could apply useful data for further investigation of high C/N ratio substrates composting. And the microorganisms were identified, and these dominant communities could be used on a large scale to improve the composting efficiency and shorten the composting cycle of high C/N ratio substrate in the future.

2. Materials and methods

2.1. Materials

The poplar processing residues was obtained from a local farmland (Jinan, China). Poplar processing residues was randomly crushed, with a length of 5-10 cm and a width of 2–3 cm, and compost adopts natural fermentation. The samples were collected in a poplar processing residues pile (long: 6 m, width:5 m, High: 3 m), and were collected from the bottom to the top of the pile with different temperature such as 55°C (A, surface layer), 65°C (B,30 cm), 70°C (C,60 cm), 7°C (D,80 cm), 37°C (E,150 cm),32°C (F, bottom layer), were obtained. Samples were taken at different places for 3 times at each depth, and then mixed evenly for microbial community analysis, and the temperature was measured three times at different places at the same depth and then averaged. The composting time of this poplar processing residues was 30 days.

2.2. Analysis methods

High-throughput analysis of the microorganisms in the composting samples of poplar processing residues were conducted by Shanghai Personal Biotechnology Co., Ltd., and the data were processed by GenesCloud of Personalbio. All samples used for analyses were performed in triplicates.

3. Results and discussion

3.1. Analysis of fungal communities

![Figure 1. Relative abundance of fungi at phylum level in the composting samples of poplar processing residues](image)

(A: 55°C  B: 65°C  C: 70°C  D: 72°C  E: 37°C  F: 32°C)

According to the fungal phylum level of poplar processing residues compost which was shown in Figure 1, Ascomycota and Mortierellomycota were the two most advantageous phyla, and the highest sample F accounted for 98.37%. At the same time, Ascomycota accounted for the largest proportion among the six samples, with the lowest proportion was 52.13% (E) and the highest proportion was of 94.71% (F). In addition, with respect to sample E, Basidiomycota, Mucoromycota and
Mortierellomycota accounted for only 2.54%. It showed that the community in F was clear, while that in sample E was relatively complex. Ascomycota phylum, from the sample C, D and E, it could be considered that the main fungi population decreased with the increase of sampling depth. Similar results have been reported by Zhang et al. (2016), compared with bacterial community, the structure of fungal community was more stable, and Ascomycota was the only dominant phylum. Except for the surface layer of compost, the abundance of Ascomycota in other samples was usually above 65%, and more than 90% of the sequences were unclassified, which indicated the selective pressure on fungal communities in compost [5]. In addition, the results were also similar to corn straw natural composting, which indicated that Ascomycetes played a dominant role in lignocellulosic composting [6].

![Figure 2. Relative abundance of fungi at genus level in the composting samples of poplar processing residues](image)

In the poplar processing residues composting, it could be found that five kinds of fungal genus such as Aspergillus, Thermomyces, Rhizomucor, Rhizopus and Myceliophthora were mainly distributed (more than 80%) in the high temperature samples (A-D, Figure 2). However, sample F showed higher distribution of Aspergillus than other place, and the reason should be that the temperature was low at the bottom which provided good condition for Aspergillus growing. Meanwhile, for sample E, Aspergillus was the least among the six samples, and Thermomyces was the most. In samples B, C and D, the content of other genera showed an increasing trend, which might be related to the temperature increasing.

Similar experimental results were also obtained in the corn cobs composting performed in a 90 m³ cylindrical fermentor (diameter, 5.4 m; height, 4 m). Corn cobs was crushed and mixed with fresh cow dung to obtain C/N=25-35:1 as compost substrate [5]. It was found that Aspergillus, Mucor and Meyeroyzma were the main fungi in the substrate of 10 cm in the bottom, which might due to the low temperature because of air ventilation. High temperature, aerobic condition and lignocellulose substrate were good for Thermomyces reproduction. In the 1.2 m substrate from the bottom, simple and stable dominant community was constructed by Aspergillus, Bacillus, Thermobifida and Thermomyces, in which Thermomyces and Aspergillus were important in the hemicellulose degradation.

Heuer et al. (2011) reported that with the increase of temperature, the colony number of fungi began to decrease, all thermophilic fungi almost disappeared at 68°C, and were in a state of death or dormancy; when the temperature was lower than 60°C, thermophilic fungi and thermophilic fungi will appear again in composting [7]. Therefore, most of the microorganisms detected in high temperature samples B, C and D were thermophilic fungi. Kang (2019) measured the number of corresponding fungi during cow
dung composting. It was also found that temperature was significantly correlated with fungi abundance. The optimum growth temperature of thermophilic fungi was usually 45-50°C, and the relative humidity was more than 75%, so controlling the appropriate temperature and humidity can effectively improve the degradation rate of organic matter [8].

3.2. Analysis of bacterial communities

![Relative abundance of bacteria at phylum level in the composting samples of poplar processing residues](image)

(A: 55°C  B: 65°C  C: 70°C  D: 72°C  E: 37°C  F: 32°C)

**Figure 3.** Relative abundance of bacteria at phylum level in the composting samples of poplar processing residues

According to the relative abundance analysis at the bacterial phylum level which was shown in Figure 3, *Firmicutes, Actinobacteria* and *Proteobacteria* were the three most advantageous except for sample A accounting for at least 92.25% of all 16S rDNA sequences. Mao et al. and Li et al. found that the dominant bacteria were *Firmicutes, Proteobacteria* and *Actinobacteria* in the swine manure composting at the phylum level [9,10]. However, the relative abundance varied in different periods of composting. Except the above three phyla, *Bacteroidetes* was also one of the larger proportion, up to 12.53% in sample A, which might be its surface place, so the diversity of bacteria was high. Among the six samples, *Firmicutes* accounted for the largest proportion in samples B, C and D, reached 67.90% to 75.70%, and was the main bacteria that played a role, which might be related to high temperature; *Actinobacteria* in samples E and F were the most dominant phyla, up to 51.00%-67.61%, which should be related to the low temperature. The same results were also obtained during the corncob and fresh cow dung composting in cylindrical fermenter reported by Zhang et al.(2016) [5]. As for sample A, however, *Proteobacteria* was the largest phylum, accounting for 39.87%, the second was *Firmicutes*, was 23.22%, and *Bacteroidetes* and *Actinobacteria* were similar. Nine bacterial phyla were detected in the experiments, among which *Bacteroidetes, Firmicutes, Actinobacteria* and *Proteobacteria* were considered as dominant phyla in the corncob and fresh cow dung composting [5]. At the same time, these four phyla were also reported as the dominant phyla in the composting of other lignocelluloses (corn stalk and dry cow dung/urea fertilizer, or pure corn stalk composting) [6].
Figure 4. Relative abundance of bacteria at genus level in the composting samples of poplar processing residues

According to the relative abundance analysis of the top 11 dominant bacteria at the bacterial genus level in Figure 4. The main dominant bacteria in samples B, C and D were Sphingomonas, Geobacillus, Bacillus and Thermobispora, which could account for 64.67% to 69.78% of the total. In addition, the proportion of other genera was similar, which might be related to their temperature above 65°C. The strains of samples E and F were scattered, and there were no obvious dominant strains, which showed that there were more kinds of microorganisms suitable for growth at low temperature; For sample E, Geobacillus, Thermobispora and Thermopolyspora had similar proportions (within 5% difference); While in sample F, Geobacillus and Streptomyces accounted for 15.17% and 11.22%, and others were lower than 8%. At the same time, unknown categories accounted for 49.68% of the total, meaning that the microbial communities compositions of this component were complex. In case of sample A, the unknown bacteria reached 62.02%, the next dominant strain was Pseudoxanthomonas with 11.31%, and others were all below 7%, which might be related to the complex microbial communities on the surface of composting.

Berg et al. (1972) found that with the decreased of compost height, the dominant genera changed from Cellvibrio to those genera belonging to Firmicutes and Actinobacteria, such as Bacillus, Planifilum and Thermobifida [11]. Besides, Gannes et al.(2013) found that the composting time was also an important factor affecting the community composition: at the initial stage of composting, the community was dominated by Bacteroides and Proteus, including Petrimonas, Pseudomonas and ProteiniPhilum, and it was replaced by Bacillus and Bifidobacterium thermophilus at the end of composting [12] Bacillus was widely distributed in lignocellulosic composting system because of its heated resistance. The advantages of these genera indicate that at the end of composting process, selected communities with thermal stability and lignocellulose degradation ability should be conducted.

4. Conclusion

In this study, poplar processing residues was used as the sole substrate for composting, and the microbiological communities such as fungi and bacteria were analysed. Two most favorable fungal phyla Ascomycetes and Basidiomycetes and five kinds of fungal genus such as Aspergillus, Thermomyces, Rhizomucor, Rhizopus, Myceliophthora were mainly distributed (more than 80%) in the high temperature samples. As for the bacteria, Firmicutes, Actinobacteria and Proteobacteria were the three most advantageous phyla in the high temperature samples. At the same time, Geobacillus, Bacillus,
Thermobispora and Sphingomonas were the dominant bacteria in the high temperature samples at the genus level.

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