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Original Research Paper

# Effects of *Mucor mucedo* on Corncob Decomposition in Pyr-Contaminated Soil Remediation

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## ABSTRACT

The effect of polycyclic aromatic hydrocarbon and highly effective degradation fungi *Mucor mucedo* (MU) was studied on corncob decomposition in Pyr-contaminated soil for 120 days to identify the impact of a degradable immobilized carrier on the remediation of soil contaminated by persistent organic pollutants. Results showed that the corncob was mainly composed of hemicelluloses, cellulose, and water dissolved (WD) material, which accounted for 85 percent of its total weight. MU addition significantly affected corncob decomposition. Thus, humic acid production and WD and benzene-ethanol dissolved material degradation increased. The peaking of the WD content was delayed for 30 days or more. The extractable pyrene content positively correlated with the WD content in the corncob during the decomposition. These results theoretically support a refined remediation principle of immobilized microorganisms.

## INTRODUCTION

The microbial remediation technique has been widely used because of its low cost, high efficiency and environmental responsibility (Gan et al. 2009, Teng et al. 2010). The immobilized microbial technique (IMT) is a novel microbial remediation technique that favours exogenous degrading bacteria to compete with indigenous bacteria and maintain long-term activity; this approach enhances the efficiency of microbial remediation of organic pollutants in soil (Su et al. 2006, Wang et al. 2008). At present, common immobilized microorganism carriers include clay mineral, corncob, straw, biochar and nano-porous $SiO_2$ synthesized by bio-macromolecules (Wang 2010, Wang et al. 2009, Zhang et al. 2008), among which degradable carriers such as corncob and straw stand out in microorganism immobilization (Ahn et al. 2002, Su 2007a). The degradation rate of B[a]P by immobilized fungi with a corncob as the carrier was previously determined to be 20% higher than that by free fungi (Su et al. 2006, Su et al. 2008). Degradable carriers placed in the soil become decomposed. The decomposition products significantly increase the organic matter content in soil, enhance the aggregate stability, and change the water-soluble organic matter content in soil, soil pH, and microbial activity (Clark et al. 2009, Diehl et al. 2008, Li et al. 2009). Thus, the occurrence state of organic contaminants in soil is affected, and the microbial degradation process and extent are changed. However, how organic contaminant-degrading bacteria work in this process is still unclear. Therefore, the decomposing process of an immobilized carrier (corncob) placed in a farmland was simulated in this study. Immobilized PAH-degrading bacteria were also introduced into the farmland to determine the effects of degrading bacteria on the immobilized carrier decomposition, which can provide a theoretical support to reveal how immobilized degrading bacteria enhance the organic contaminant degradation in soil.

## MATERIALS AND METHODS

### Materials

Corncobs were collected from the National Field Research Station of Shenyang Agroecosystem. Contaminated soil was collected from topsoil (0-20cm) of long-term contaminated farmland in upstream of Shenfu irrigated area. PAHs-degrading fungi *Mucor mucedo* were screened and incubated, which had been successfully applied to *in-situ* remediation of contaminated soil of farmland in Shenfu irrigated area. Pyrene (Pyr) was purchased from Sigma Corporation with purity of 98%.

### Experimental Design

**Preparations:** The treatment of an immobilized carrier followed the procedure of Su (2007). The dried corncobs were crushed to particles with diameter of less than 5 mm and soaked for 24 h after proportionally adding CaO and water (sterilization). Nutrients [i.e., bean flour, sugar, $(NH_4)_2HPO_4$, and $MgSO_4 \cdot 7H_2O$], required by microorganisms, were then proportionally added and stirred evenly. The mixture pH was adjusted to 7 with a $Ca(OH)_2$ solution. The mixture was sterilized at 120°C thrice; each round lasted for 1 h. Distilled water was poured into fresh contaminated soil with a soil-to-water ratio of 1:10. The supernatant was obtained and taken as the soil solution to inoculate indigenous bacteria after 30 min of shacking and 30 min of standing. Previously screened PAH-degrading microorganisms *Mucor mucedo* (MU) were inoculated into a potato liquid medium and cultured in an incubator at 28°C for 3 days. A Pyr stock solution was formulated by acetone to a concentration of 200 mg/L s.

**Procedures:** Table 1 shows the treatments with pyrene, soil solution and MU, which were added to the immobilized carrier. Pyr was added first, the soil solution and MU were added after 24h to 48h volatilization, and the mixture was then cultured in a 28°C incubator for 120 days in the treatments with Pyr addition. Sterile water was added to the mixture once every 3 days to maintain the moisture content at approximately 45%. Samples were taken on days 0, 7, 14, 28, 42, 63, and 120 after microorganisms were added and all the treatments were performed in a sterile environment.

### Determination Method

For chemical composition determination method of Wen (1984) was followed. The extraction and measurement of Pyr is based on the study of Wang et al. (2012). The reference was determined according to Fourier transform infrared (FTIR) spectroscopy (Wu et al. 2004). The determination instrument was a Nicolet FTIR-6700. The background was scanned with a scan range of 4000-650 $cm^{-1}$, a resolution of 4 $cm^{-1}$, and cumulative scanning times of 64 before determination.

## RESULTS

### Basic Nature of Corncobs

The main chemical components of corncobs include water dissolved material (WD), hemicellulose (HC), and cellulose (CL). The contents of these components were significantly higher than those in straw, Chinese milk vetch, tea, pig manure, and other organic materials (Table 2). However, the contents of benzene-ethanol dissolved material (BD) and lignin (LG) were outstandingly lower in corncobs than in other organic materials (expect for BD in corn stalk and maize root). These components accounted for 8% to 27% and 15% to 45% of the other organic materials, respectively. Similar to the decomposition of other organic materials, the BD and WD of corncobs were easily decomposed by microorganisms to provide an energy source to maintain microbial activities. This condition subsequently affected the decomposition of other corncob components except LG. For instance, the LG content continuously increased, whereas the contents of other components fluctuated with the HC and CL decomposition to BD and WD. The fluctuations of the HC and CL contents were ascribed to the reductions in HC and CL contents with the decomposing process and WD and BD consumption that resulted in relative increase in HC and CL residues (Dai et al. 2004, Chen et al. 2009).

Table 1: Treatments included in the decomposition experiment.

| Treatments | Number | Grouping |
|---|---|---|
| A | 1 | corncob + Soil solution (10%) |
| | 2 | corncob + MU (10%) |
| | 3 | corncob + MU (5%) + Soil solution(5%) |
| B | 1 | corncob + Pyr + Soil solution (10%) |
| | 2 | corncob + Pyr + MU (10%) |
| | 3 | corncob + Pyr + MU (5%) + Soil solution(5%) |

Concentration of Pyr is 20mg/kg. Percentages of soil solution and MU are based on volume ratio.

### Pyr Degradation in Corncobs

Extractable Pyr contents in the three treatments with Pyr addition rapidly decreased in the first 28 days because the immobilized carrier began to decompose. The decrease rate was higher in B2 than in B3 and B1 (Fig. 1). However, the extractable Pyr contents in the three treatments changed differently after day 28. The extractable Pyr content in B3 slightly changed between days 28 and 63 and decreased slowly after day 63, whereas the Pyr contents in B1 and B2 underwent an up-down change (peaking on days 42 and 63, respectively). The extractable Pyr content was generally lower in B2 than in B1 and B3 (except between days 63 and 120). The extractable Pyr contents in B3 and B1 were slightly different, except that the content was higher on day 28 and lower on day 42 in B3 than in B1. These results indicate that MU can significantly promote the degradation of PAHs absorbed by corncobs. Although the inoculation of indigenous microorganisms significantly decreased the Pyr content in corncobs during the early period, the addition of both MU and soil microorganisms did not significantly improve the Pyr degradation probably because of the competition between MU and soil microorganisms for carbon and nitrogen. This process should be validated by further studies on microbial diversity and MU residues.

### MU Effects on Corncob Decomposition

The content changes in chemical components in corncob

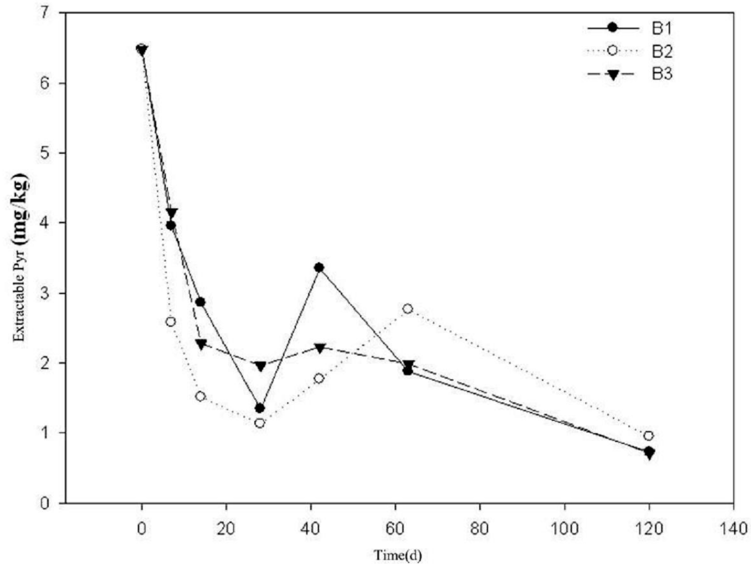


Fig. 1: Change in extractable Pyr content during corncob decomposition.

during decomposition are given in Fig. 2.

**BD**: BD mainly refers to fat, wax and pigments. BD contents in all treatments experienced a down-up-down change despite the differences in time and peak value or BD valley among the different treatments. The BD contents in A1 and A2 in group A had the same change pattern, which decreased rapidly at first and reached a valley on day 7 (9 g/kg and 7 g/kg, respectively). It then increased rapidly and peaked on day 14 (17 g/kg and 25 g/kg, respectively), followed by a continuous decrease. The BD content in A3 had no significant difference with that in A1 and A2 in the first 7 days, but it peaked much later (on day 42) probably because of the interaction of MU and indigenous microorganisms. The BD contents in B1, B2 and B3 also decreased rapidly after the start of decomposition. However, the reduction rate and extent in group B were far smaller than those in group A in the first 7 days, except that the BD content in B1 similarly changed with that in group A. The BD contents reached a valley on day 14 in B2 and B3, peaked on day 42 in B1, B2, and B3, and decreased rapidly afterwards. The BD contents in groups A and B were not significantly different after the 120-day decomposition, except that the BD content in B3 was significantly lower than that in A2. The BD content that remained in each treatment accounted for 43% to 70% of its initial content before decomposition.

**WD:** WD refers to starch, sucrose, oligosaccharides, fructan, and amino acids in corncobs. Similar to the BD content, the WD content also showed a down-up-down trend, but its valley and peak was significantly different from that of the BD content. The WD content reached a valley and peak on days 14 and 42, respectively (valley on day 28 in A1), which were much later than the peak and valley time of the BD content. The WD content that remained in each treatment accounted for approximately 44% to 64% of its initial value after the 120-day decomposition of corncobs. No significant difference in the WD content remained in all treatments ($P > 0.05$), except that the WD content in A2 was significantly higher than that in the other treatments.

**HC:** HC refers to a specific polysaccharide, wherein the polysaccharide is broadly divided into compounds that contain uronic acid and unrecognized special compounds. The former is composed of a polymer of xylose and glucuronic acid, as well as a polymer of galacturonic acid and arabinose. The latter consists of xylan, mannan, and arabinogalactan. The HC contents in group A experienced a down-up-down change. A dramatic difference among A1, A2 and A3 only occurred on day 42. The HC contents in group B largely varied unlike in group A. The HC contents in B1, B2 and B3 first increased and then decreased to a lower point on day 14. It then increased again to a peak on days 28, 63 and 42, respectively, and decreased rapidly afterwards. The HC content that remained in each treatment accounted for 62% to 79% of its initial value after the 120-day decomposition.

**CL:** CL is often combined with hemicellulose, pectin and lignin. The CL contents in A1, B2 and B3 largely varied during the first 14 days. The CL content in B2 remained steady in the first 7 days and then decreased rapidly. The CL

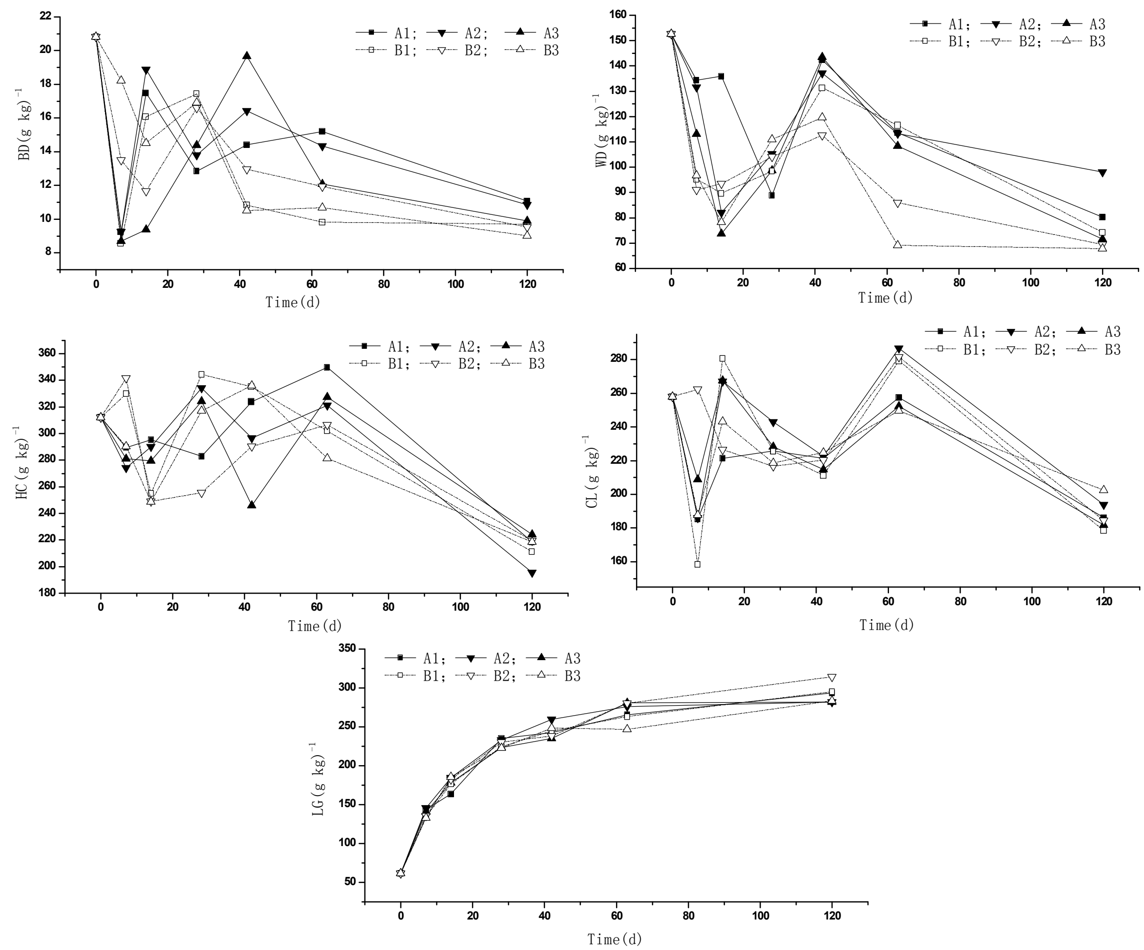


Fig. 2: Content changes in chemical components in corncob during decomposition.

contents in A1 and B3 decreased rapidly since the beginning of decomposition, reached a valley on day 7, and then increased gradually. The CL contents in A1, B2 and B3 remained relatively stable from days 14 to 42, increased rapidly to a peak on day 63, and then decreased rapidly. The CL contents in A2, A3 and B1 underwent a down-up-down-up-down change, with valleys on days 7 and 42 and peaks on days 14 and 63. The CL content that remained in each treatment accounted for 70% to 83% of its initial value after the 120-day decomposition. In addition, the CL content in each treatment slightly differs from each other, except that the CL content was significantly higher in B3 than in A3, B1 and B2.

**LG:** LG is an aromatic compound and polymer composed of a derivative unit of phenylpropane with a chemical composition between $C_{10}H_{10}O_3$ and $C_{41}H_{40}O_{10}$. The LG content in each treatment increased significantly and continuously, which reached 282 g/kg to 315 g/kg or as much as 4.6 times to 5.1 times of the initial value during the 120-day corncob decomposition.

**FTIR Spectroscopy Analysis**

The functional corncob groups were mainly composed of carbohydrate, followed by aliphatic and aromatic compounds; this observation was consistent with the results of component analysis based on the IR spectrogram of the samples (Fig. 3) and the absorption bands of the IR spectrum characterized by many investigations (Wu et al. 2004, Wu & Lv et al. 2004, Xu et al. 2004, Peng et al. 2013). The variations in the main functional groups in the different treatments were similar. The vibrations in each treatment underwent fluctuations during the decomposing process, except that the peaks were severely weakened and new peaks appeared in some treatments. For instance, the peaks at 2850 $cm^{-1}$ to 2920 $cm^{-1}$ were weakened and even disappeared at some point, which indicates that the lipid in corncobs decomposed rapidly. The C=O deformation vibrations of hydrogen bond and C=O stretching vibrations centred at approximately 1640 $cm^{-1}$. The emergence of new peaks at 1620$cm^{-1}$ to 1650$cm^{-1}$, 1460$cm^{-1}$ to 1440$cm^{-1}$, and 1370$cm^{-1}$

Table 2. Main chemical components of the corncob and other organic materials (g/kg).

| | Ash | Benzene-ethanol dissolved material | Water dissolved material | Hemicellulose | Cellulose | Lignin | Data sources |
|---|---|---|---|---|---|---|---|
| Maize leaf | 176.1 | 90.9 | 286.8 | 231.2 | 316.3 | 74.9 | (Wu et al., 2000) |
| Corn stalk | 27.8 | 11.3 | 197.1 | 280.1 | 418.3 | 93.3 | |
| Maize root | 64.5 | 18.0 | 193.3 | 355.6 | 302.9 | 130.1 | |
| Corn straw | 96.1 | 47.9 | 238.4 | 257.6 | 371.3 | 84.8 | |
| Straw | | 51.2 | 69.9 | 228.4 | 283.4 | 128.5 | (Zhu et al., 2001) |
| Sawdust | | 102.5 | 27.8 | 165.5 | 118.0 | 433.0 | |
| Pig manure | | 232.4 | 75.2 | 127.3 | 132.0 | 140.8 | |
| Chinese milk vetch | | 332.8 | 203.2 | 58.1 | 115.0 | 147.0 | (Wu et al., 2004) |
| Tea | | 305.4 | 133.4 | 68.3 | 104.7 | 245.1 | |
| Corncob | 37.6 | 27.6 | 153.0 | 315.8 | 258.9 | 63.2 | This study |

Table 3: Relationship between the extractable Pyr and corncob components during the decomposition.

| Extractable Pyr | Benzene-ethanol dissolved material | Water dissolved material | Hemicelluloses | Cellulose | Lignin |
|---|---|---|---|---|---|
| B1 | | * | | | **- |
| B2 | * | * | | | **- |
| B3 | * | * | | | **- |

(Note: "-" means negative correlation; "*" means P < 0.05 level; and "**" means P < 0.01 level.)

to 1350cm$^{-1}$ suggests the generation and continuous accumulation of humic acid, $NH_4^+$-containing compounds, and aromatic substances during the decomposing process.

The MU addition significantly improved the humic acid generation without the pyrene addition. The peaks at 1620 cm$^{-1}$ to 1650cm$^{-1}$ occurred rapidly on day 7, continued, and were more prominent after the pyrene addition. The peaks at 1620cm$^{-1}$ to 1650cm$^{-1}$ appeared on day 7 in B2. The pyrene addition enhanced the peaks at 1735cm$^{-1}$ to 1690cm$^{-1}$, which indicates the presence of acid lipid and ketone compounds during the decomposing process.

### Relation Between the Extractable Pyr Content and Chemical Components of the Corncob

Table 3 shows that Pyr degradation was significantly positively correlated with the WD content, but significantly negatively correlated with the LG content. This observation indicates that WD in corncobs was a key factor that affects the degradation of contaminants absorbed in corncobs. Unlike the treatments with indigenous bacteria, the relation between the chemical components of corncobs and extractable Pyr was not changed by the MU addition. The extractable Pyr content was not only significantly related to WD and LG contents but also significantly related to BD content in the treatment with only the added MU. This condition indicates that MU itself can strengthen the corncob decomposition. However, the competition between MU and indigenous bacteria weakens the MU impact on the corncob decomposition.

## DISCUSSION

The immobilization technique can avoid physical and chemical damages of the cell, which is suitable for microbial remediation (Hum et al. 2008). Li et al. (2005) investigated the phenanthrene and pyrene degradation in soil based on the IMT. The said researchers believed that IMT can help to enhance the competitiveness of introduced microorganisms in soil and is an effective method for PAH degradation in soil. After analysing the degradation dynamics of benzo[a]pyrene in soil, Wang et al. (2011) found that immobilized mixed bacteria made by adsorption with vermiculite as a carrier exhibited effective mass transfer performance; its B[a]P degradation rate was 20% higher than that of free fungi. Su et al. (2007) also showed that the degradation rates of B[a]P and Pyr in soil by MU were relatively high, and MU can be used for the remediation of PAH-contaminated soil. However, how immobilized carriers, especially biodegradable carriers or decomposed products, affect the bio-availability of PAHs in soil by activation and absorption during the remediation of contaminated soil has not been reported.

The present study determined that adding MU can significantly affect the decomposing process of corncobs, which was reinforced by the Pyr presence. Taking A1 as the control, adding MU (in A3) resulted in the rapid decrease in WD content in corncobs at the beginning of the experiment. The WD content only accounted for 54% of the control on day 14. The BD accumulation also slowed, and its peak oc-

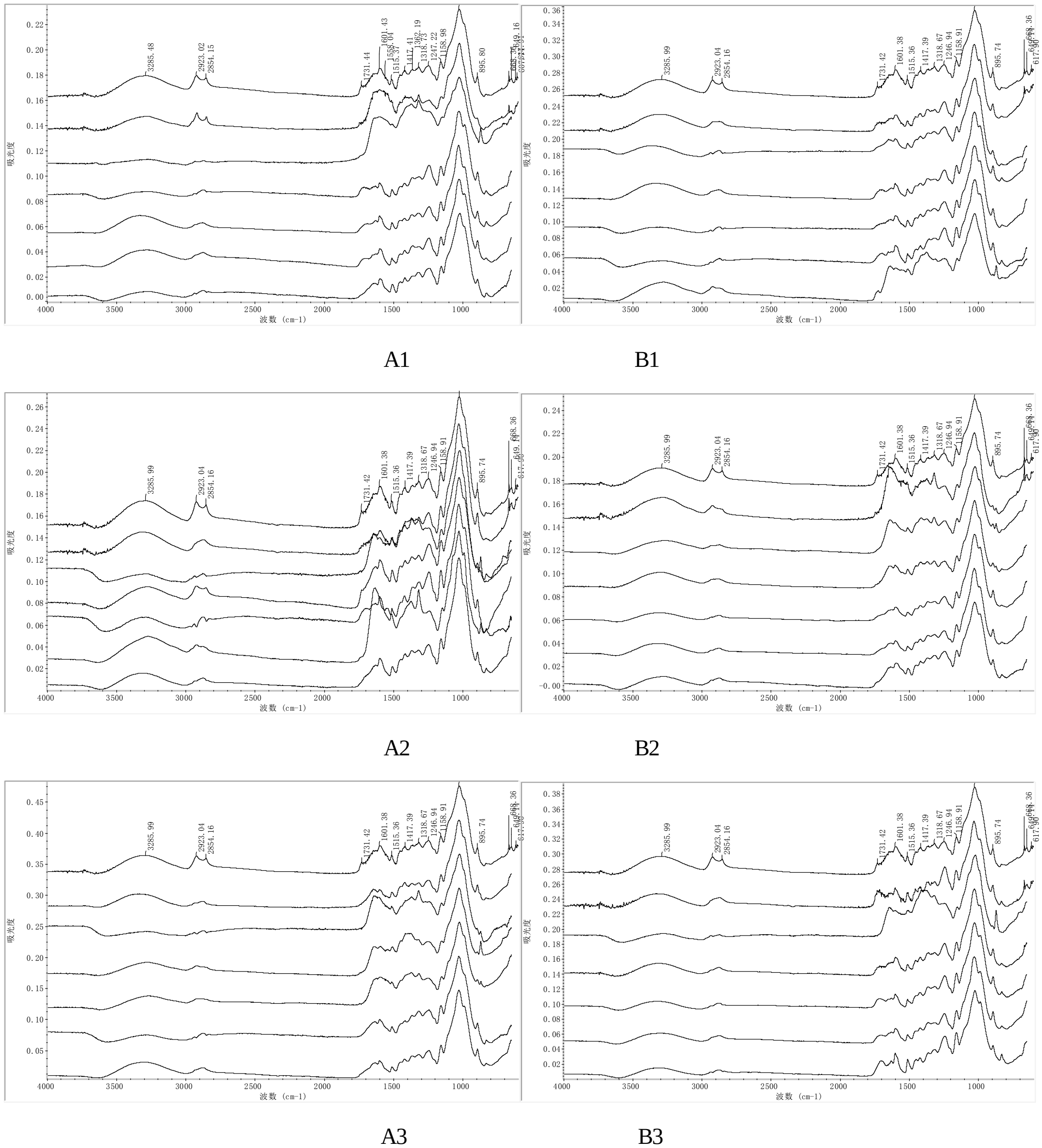


Fig. 3. Change in FTIR spectra during decomposition.

curred nearly 30 days later than that in the control. The CL residue was higher than that in the control in the first 14 days, which was probably due to the growth competition between indigenous microorganisms and the added MU. However, no significant difference between the control and A3 was observed, which indicates that the decomposing effect enhanced by MU addition disappeared. Adding Pyr probably stimulated the growth of indigenous microorganisms.

The BD content in B1 was significantly lower than that in the control after day 28, which accounted for 65% to 75% of that in the control. The WD content accounted for only 66% to 71% of that in the control before day 14, whereas the HL content peaked at approximately a month ahead of that in the control. A joint action of MU and Pyr (in B3) significantly lowered the WD content unlike the control during the decomposing process (except on day 28). The BD and

HC contents were far higher than those in the control from days 0 to 40 and significantly lower after day 60. The Pyr addition provided accessible and degradable carbon for MU (with respect to HC) in the early days of experiment, which resulted in more degradable materials for microorganisms in the same period and significant reduction in the HC, WD and BD contents in B3 than in the control in the late period.

The results of this study are different from the findings of Wu et al. (2010). These researchers found that extraneous bacteria should compete to work. The effects of the three types of inoculated microorganisms on straw decomposing were unclear, which indicates that the complete straw decomposition mainly relied on its own microorganisms. Their results were probably related to the differences in microorganisms added or in conditions under which microorganisms were added. Indigenous microorganisms and PAHs were added to sterilized corncobs in the present study. However, Wu et al. (2010) did not process straw and its own microorganisms should play an important role in the decomposing process of straw.

## CONCLUSION

This study focuses on the effects of PAH-degrading bacteria and PAHs on the decomposing process of corncobs added into soil. The following conclusions were derived:

1 MU significantly promoted the degradation of PAHs absorbed by corncobs, whereas the addition of both MU and soil microorganisms cannot significantly improve the Pyr degradation probably because of the competition between MU and soil microorganisms for carbon and nitrogen.

2 Adding MU can significantly affect the decomposing process of corncobs, and the Pyr presence reinforced this effect. Adding MU resulted in the rapid decrease of the WD content in corncobs and slower BD accumulation. Adding Pyr stimulated the growth of indigenous microorganisms.

3 Adding MU significantly improved the generation efficiency of humic acid, and the effect was more prominent after adding pyrene.

4 Pyr degradation was significantly positively correlated with the WD content, whereas it is significantly negatively correlated with LG content. This condition indicates that WD in corncobs was a key factor that affects the degradation of contaminants absorbed in corncobs. MU itself can strengthen corncob decomposition, whereas the competition between MU and indigenous bacteria weakened the effects of MU on corncob decomposition.

The results of this study are preliminary. Further in-depth studies combined with microbial population changes, binding characteristics of microorganisms, and immobilized carriers, as well as the long-term dynamics of both the structure and composition of immobilized carriers, can be conducted in the future.

## ACKNOWLEDGEMENTS

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