Study on Biodegradability and Mechanism of Cyanoethylated Waste Paper

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Abstract

With white pollution caused by petroleum plastics becoming an environmental issue of global concern, biodegradable plastics have become a research hotspot, and biodegradable materials made from plants have been attracting attention. Based on previous studies, this paper studied the biodegradability of thermoplastic cyanoethylated waste paper (CWP) by microbial experiment, and preliminarily explored the degradation mechanism. The results showed that: (1) the biodegradability of CWP was better than that of waste paper, and the higher the degree of modification, the better the biodegradability of CWP; under the action of *Trichoderma reesei*, the degradation rate of CWP containing 20.79% nitrogen was about 24% after 144 h (hours) of degradation; (2) The degradation rate of CWP was about 45% after 144 hours degradation by microbial complex bacteria, which was mainly due to the synergistic effect of cellulose degrading enzymes secreted by microbial degrading bacteria, which greatly improved the degradation rate of CWP. (3) The improvement of biodegradability of CWP was mainly due to the

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change of the morphology of the fibers by cyanoethylation reaction, which increased the contact surface and destroyed the crystal structure of the waste paper fibers. The increase of the amorphous region and the distance between the crystal planes was more conducive to the cellulase entering into the CWP, thus improving the degradation rate. (4) Structural analysis of CWP before and after degradation showed that microbial degradation bacteria destroyed C≡N bond and formed hydrocarbon bond, and further destroyed the crystal structure of CWP without changing the crystal form of the fiber.

Keywords: Cyanoethylated waste paper, biodegradation, enzyme activity, degradation rate.

1 Introduction

White pollution caused by traditional petroleum plastics has become a social issue of global concern, so the development of recyclable, renewable, sustainable and biodegradable plastics has received sustained attention. Plastic pollution is a major problem that today's human society is dealing with, and it has the potential to harm our future generations' ability to live comfortably. The phrase "white pollution" refers to our environment's disregard for white plastic bags and single-use plastic cutlery. It's kept in check by limiting the use of throwaway plastic bags. Single-use plastic, in particular, is more damaging than disposable plastic. Many researchers have modified cellulose to make biodegradable plastics [1–7]. The composite microbial system MC1 showed the potential to be used for millet straw degradation, the degradation rates of alkali treated and non-treated millet straw were 61.8% and 60.0%, respectively [8]. The cellulose/PLA composite membrane with good thermal stability and high elongation at break was made by Xia Kunfeng et al. at 70°C [9]. The biodegradability of the composite membrane was good. Biodegradability is the ability of degrading living organisms to biologically organic compounds to their simplest components, such as water, carbon dioxide, methane, basic elements, and biomass. The degradation rate was about 10% after 10 days of degradation in natural soil, and it was decomposed into fragments after 15 days. It can be used as a green environmental-friendly packaging film. Because of the wide source and low cost of plant fiber materials, biodegradable plastics with good properties and low cost can be obtained by plant fiber modification [10]. Some scholars used waste paper fiber and corn straw as raw materials to make a new type of light-weight composite material with straw as the framework and waste paper foaming

material as the matrix [11]. Studies on the biodegradability of plant fibers have been reported at home and abroad [12]. With cellulase activity as an index, fungal degradation of straw was found to be 47% by one of the fungi [13]. Cellulose has good degradation efficiency under the combined degradation of alkaline lignocellulose degrading bacteria *Bacillus* sp. and lactic acid bacteria [14]. The best combination of *Trichoderma* jw-1 and *Trichoderma* jw-2 could degraded straw retreated with 10% NaOH; The degradation rate of straw powder is as high as 76.8% [15]. The strain of *Aspergillus fumigatus* Z5 owns an efficient capacity in lignocelluloses degradation by secreting various extracellular hydrolytic enzymes [16]. In previous studies, it was found that CWP made by cyanoethylation had better thermoplastic properties and mechanical properties [17]. Cyanethylation is the process of nucleophilic addition, in which the acrylonitrile molecule is added to a nucleophile, such as an alcohol, thiol, or amine. It is mostly utilised in the pharmaceutical and dye industries, but it is also employed in chemical polymerization. An anionic polymerization happens when cyanethylation takes place in a medium that cannot release a proton to the product at the conclusion of the process. The carbon atom stays negatively charged due to the absence of protonation, leading the separate cyanethylation products to polymerize. CWP is an abbreviated form of cyanoethylated wastepaper, in which the wastepaper is converted into new thermoplastic material based on the process of cyanoethylating. The major features of CWP are efficient degradation property of cellulose. Meantime, cyanoethylated wastepaper is degraded by means of *Trichoderma reesei* cellulose. On the basis of previous achievements, this paper intends to study the biodegradability and mechanism of CWP.

2 Materials & Methodologies

2.1 Research Materials

(1) Waste paper, office mixed waste paper.
(2) CWP, made in laboratory, cyoethylated waste paper 1#: Nitrogen content 12.85 % (CWP1), cyoethylated waste paper 2#: Nitrogen content 20.79 % (CWP2).
(3) *Trichoderma reesei*, developed by Yunnan Normal University, with an enzyme activity of 500 IU/g.
(4) Microbial degrading bacteria. The existing strains A1 and A2 in the laboratory belong to *Brevibacillus* sp., and A3 belong to *Brevibacillus*

sp., which are inoculated in the degrading medium at the ratio A1:A3:A2 = 1:2:1.

(5) Degradation medium $(NH_4)_2SO_4$2 g, $MgSO_4$ 0.5 g, K_2HPO_4 1 g, NaCl 0.5 g, peptone 1 g, yeast extract 1 g, CWP 1 g, agar 15 g, distilled water 1000 ml, natural pH value, 121°C, high pressure sterilization for 20 min.

(6) Major experimental instruments: 722 visible spectrophotometer, Shanghai Youke Instruments Co., Ltd; Clean bench, SW-CJ-2F, Shanghai Boxun Industrial Co., Ltd; Circulating Water Vacuum Pump, SH2-D (III), Gongyi Yuhua Instruments Co., Ltd; Thermostatic Oscillator, TH2-98A, Shanghai Yiheng Technology Co., Ltd; electronic balance, FA2204C, Shanghai Yueping Scientific Instruments Co., Ltd; Centrifuge, TD6M, Hunan Xiangli Scientific Instruments Co., Ltd; Scanning Electron Microscope, JSM-5600LV, Japan Electronics Company; Fourier Transform Infrared Spectrometer, Brooke Company, Germany; X-ray Diffraction, D/MAX-3B, Japan Mechanics Company.

2.2 Determination of Biodegradability

(1) Determination of cellulase degradation rate

110 ml water solution was added into 116 ml glacial acetic acid to be diluted into acetic acid solution. 164 g sodium acetate was dissolved in 300 ml water to form sodium acetate solution. The above two solutions were mixed to obtain acetic acid-sodium acetate enzyme buffer of pH 5.0. The sample to be degraded was dried, weighed m_0, and added to acetic acid-sodium acetate buffer with cellulase concentration of 0.14 mg/ml. The reaction lasted until the set time in a constant temperature water bath at 50°C. After the reaction, washed with distilled water and dried to constant weight m_1. Enzymatic degradation occurs in two stages: first, enzymes adsorb on the polymer surface, then the bonds are hydro-peroxidized/hydrolyzed. Microorganisms from diverse settings, as well as the digestive gut of some invertebrates, are sources of plastic-degrading enzymes. The degradation rate of CWP before and after enzymatic degradation was calculated according to Formula (1).

$$Y = \frac{m_0 - m_1}{m_0} \times 100\% \tag{1}$$

(2) Determination of degradation rate of microbial degradable materials

Mixed strains were inoculated into the degradation medium and cultured in a shaker with pH 7 at 180 rpm and 35°C. Took centrifugation for 10 min

at 8000 r/min, removed the supernatant. The remaining was degraded CWP. The degraded CWP was dried to constant weight at 85°C, and weighed m_1. The degradation rate was calculated according to Formula (1), in which $m_0 = 1$ g.

2.3 Determination of Enzyme Activity

The preparation of crude enzyme medium: the medium was filtered by circulating water vacuum pump, centrifuged at 3500 rpm for 10 minutes, and the supernatant was taken.

CMCase activity (CMCA), Fpase activity (FPA) and β-Glucosidase activity (β-GA) were determined by DNS at light wavelength of 530 nm.

High enzyme activity is caused by higher substrate concentration. It higher enzyme activity increase the reaction rate by colliding the enzymes with substrate molecules.

CMCA determination conditions: added 2 ml CMC-Na solution into 1 ml enzyme solution, water bath at 50°C for 30 minutes.

FPA determination conditions: added 2 ml citric acid buffer and 1 cm^2 filter paper into 1 ml enzyme solution, water bath at 50°C for 60 minutes.

β-GA determination conditions: added 2 ml 1% salicylic acid citric acid buffer into 1 ml enzyme solution, water bath at 50°C for 30 minutes.

Definition of Enzyme Activity Unit: Enzyme activity is a measure of number of micromoles of substrate converted per minute, to the rate of reaction catalyzed by that enzyme. An enzyme unit is the quantity of enzyme required to catalyze the transformation of one mol of substrate per minute under specified pH and temperature conditions. Under the optimum reaction conditions, the amount of enzyme used to produce 1ug glucose with hydrolysis substrate in 1 minute is defined as an enzyme activity unit, expressed in U/ml.

2.4 Analysis of Structures of CWP

(1) Morphological structure

The morphological structure of the fibers was observed by electron microscopy after carbon plating on the washed samples.

(2) Analysis of chemical structure

Fourier transform infrared spectroscopy (FTIR) was used to analyze the chemical structure. FTIR is a technique for obtaining an infrared spectrum of a solid, liquid, or gas's absorption or emission. An FTIR spectrometer obtains

high-resolution spectral data over a large spectral range at the same time. Compounds such as compounded plastics, mixes, fillers, paints, rubbers, coatings, resins, and adhesives are all identified using FTIR. It can be used in all stages of the product lifecycle, from design through manufacturing to failure analysis. The samples were dried and then determined by KBr pressing method and FTIR. The scanning range was 4000–450 cm^{-1}, the resolution was 4 cm^{-1}, and the scanning times were 16.

(3) Analysis of crystal structure

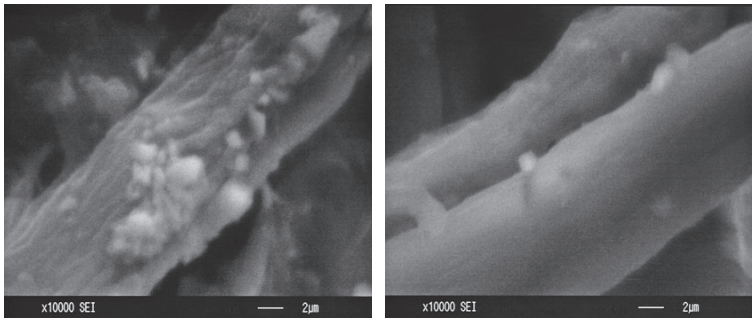
X-ray diffraction (XRD) was used to measure the Cu anode target with a diffraction angle of 5–60°, a scanning speed of 1°/min and a step width of 0.050/step. Constructive interference between monochromatic X-rays and a crystalline sample is the basis of X-ray diffraction. A cathode ray tube produces the X-rays, which are then filtered to produce monochromatic radiation, collimated to concentrate the beam, and aimed onto the sample. The crystallinity of the fibers was calculated by MDI jade 5.0. The scattering of the amorphous region was separated from the diffraction peaks of each crystal plane, and the crystallinity was calculated according to the area of each crystal peak.

3 Results and Discussions

3.1 Morphological and Crystal Structures of CWP

(1) Morphological structure

The study of individual fibers present inside the material is called fiber morphology, also it delivers substance potential performance indicator by variety of conditions. The form of individual fiber is important, because it represents how the material retain its shape in maximum pressure. It is used to measure the dimension of the individual fiber to predict how the product get degraded after use. According to Figure 1(a), the fiber morphology of waste paper was: flat, complete, no obvious breakage, rough surface, impurities and fine fibers on the surface, less long fibers exposed out of the surface, and the connection between the long fibers had few cracks due to the filling of fine fibers in between. According to Figure 1(b), the fiber morphology of waste paper had significant changes after cyanoethylation reaction: a large number of impurities on the surface of the fibers had been removed, the fibers were separated (a), the surface of the CWP was smooth, and the edge swelled (b).



a× 10000 b× 10000

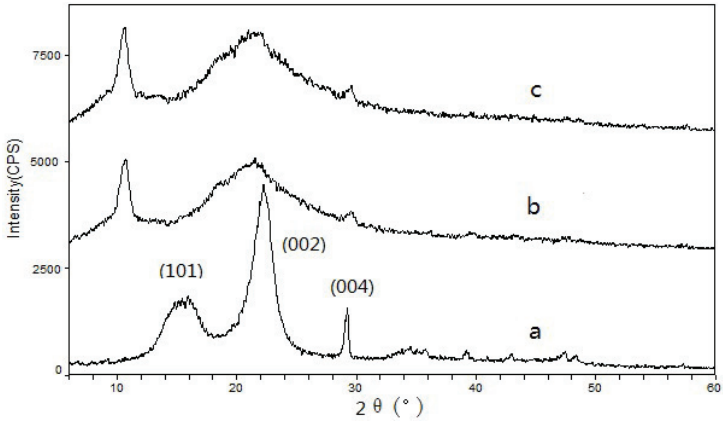
a: waste paper; b : CWP

Figure 1 Scanning electron microscope photographs of waste paper before and after cyanoethylation.

This morphological change increased the contact area of CWP, which was conducive to cellulase entering the fiber and degrading CWP.

(2) Crystal structure

There were a lot of hydroxyl groups in the cellulose molecular chain, which formed intramolecular hydrogen bonds and intermolecular hydrogen bonds. The hydrogen bonding is the process of interacting two atoms between a pair of other atoms with maximum affinity electron. But, the bonding process of hydrogen is classified into two types such as intermolecular bonding and intramolecular bonding. In which, intramolecular bonding is the process of bonding two hydrogen atoms by using two functional groups. The strength and change of location of these hydrogen bonds made cellulose present four crystal structures, and at the same time made the structure of amorphous region more complex. The micro-structure of early cellulose can be divided into crystalline and amorphous regions: the part that produces characteristic crystalline diffraction peaks is called crystalline cellulose by XRD test, which is used to determine the structure of crystalline materials. X-ray diffraction test is also known as non-destructive test method. It is considered as one of the important way of determining crystal structure by analyzing the phases present in the material to establish the chemical composition information in the crystal and the part that cannot produce characteristic crystalline diffraction peaks but only steamed bun peaks is called amorphous cellulose [18]. An amorphous cellulose is determined from different types of cellulose, in order to determine the amorphous cellulose by regerating ethanol from



a: waste paper; b : CWP1; c: CWP2.

Figure 2 XRD pattern of wastepaper.

SO_2-diethylamine- dimethyl sulfoxide (SO_2-DEA-DMSO) solvent system but the crystalline cellulose is used to describe the amount of crystal materials present in the cellulose. XRD analysis of waste paper fibers showed that the main characteristic peaks of cellulose I were (101), (002) crystal plane [19], $2\theta = 15.9^\circ$, 22.2°, and the crystallinity Xc of waste paper was 67.07%. According to the analysis of data in Table 1, it was found that the diffraction peaks of waste paper fibers near $2\theta = 15.9^\circ$, 22.2° showed typical cellulose crystallographic patterns, while the crystallographic patterns of CWP had changed significantly. The strength of peaks of CWP at $2\theta = 22.2^\circ$ had decreased, shape widened and crystallinity decreased. The (101) diffraction peak shifted to $2\theta = 10.8^\circ$, and such shift was the result of the increase of the structure space of CWP. The crystallinity of CWP decreased with the increase of nitrogen content, and the crystallinity of CWP was 13.83%. From the data in Table 2, it was found that the distance between (101) and (002) crystal planes increased with the increase of nitrogen content, and the maximum diffraction intensity of (101) and (002) crystal planes shifted to the direction of less than 2θ [20]. The distance between crystal planes of d101 and d002 increased correspondingly, and the shape of crystal plane (004) was widened [21]. This indicated that cyanoethylation of waste paper changed the crystal structure of waste paper cellulose and had de-crystallization effect. This was mainly due to the substitution of hydrogen group of cellulose by $-CH_2CH_2CN$ and the substitution reaction of acrylonitrile into the crystalline region [22]. The substituent groups had been embedded between the

Table 1 XRD analysis results of CWP

Sample	Crystal Plane	2θ (°)	Crystal Plane Distance (A)	Crystallinity (%)
Waste paper	101	15.92	5.56	67.07
	002	22.24	3.99	
CWP1	101	10.56	8.37	16.46
	002	21.12	4.20	
CWP2	101	10.53	8.39	13.83
	002	21.14	4.20	

Table 2 Degradation rate of CWP by cellulase

Degradation Time (h)	Waste Paper (%)	CWP1(%)	CWP2(%)
24	8.6	14.1	15.6
48	11.4	16.9	18.8
72	15.5	18.8	21.2
96	17.1	20.4	22.2
120	18.9	21.1.	22.5
144	19.8	22.9	24.0
168	19.9	23.1	23.1
192	22.4	23.9	23.8

crystal planes, and the lattice structure can't return to its original state, which led to the increase of distance between the crystal planes.

3.2 Degradability of Cellulase

From the experimental data of the degradation rate of CWP in Table 2, it could be seen that CWP had biodegradability. The degradation rate of CWP was higher at the beginning of the degradation reaction, which indicated that the degradation efficiency of cellulase was high; the degradation rate slowed down after 72 hours of the degradation reaction, and remained unchanged after 144 hours. This may be the result of difference in the cellulase reaction and the reaction of other enzymes. Cellulase was a multi-component enzyme system with extremely complex structure of the substrate. Because of the water insolubility of the substrate, cellulase must be adsorbed specifically on the substrate cellulose and then decomposed the substrate cellulose into glucose under the synergistic action of several components. Therefore, at the beginning of degradation, cellulase entered the amorphous zones of CWP at a relatively rapid rate and broke the cellulose chain and decomposed it. After a period of reaction, the volume of amorphous zones of CWP decreased, making it difficult for cellulase to enter the crystal zone with regular structure, so

the degradation rate of CWP slowed down and basically remained unchanged at later stages.

Comparing waste paper with CWP, the degradation rate of CWP was significantly higher than that of waste paper at the same degradation time. This indicated that Cyanoethylation of waste paper would not reduce the degradation rate of cellulase, but enlarge the space structure of waste paper and reduce the crystal zone (Table 1), reduce the difficulty of cellulase entering the cellulose and improve the enzymatic degradability of CWP. Therefore, the CWP with 20.79% nitrogen content was selected for the subsequent microbial degradation experiments.

3.3 Microbial Degradability of CWP

From the experimental data of microbial degradation of CWP in Table 3, it could be found that the degradation rate of CWP was low at 48 h after degradation, and then increased until the peak at 72 h, and then basically remained unchanged. This was because the number of microorganisms was small at the initial stage of inoculation and the growth was delayed. The main physiological process was to regulate the expression of related genes in cells to adapt to the new environment. When microorganisms entered the logarithmic growth stage, they grew and multiplied vigorously, and the concentration of substrate was high. Metabolic waste was very scarce. At this time, the degradation efficiency was the highest at the highest rate. After a period of degradation, metabolic wastes accumulated gradually, substrates were consumed gradually, and microorganisms entered the plateau stage. At this time, the number of microorganisms in the culture system was relatively stable. The final stage of cultivation was to enter the decline phase, when metabolic waste accumulated in large quantities, nutrient was exhausted, and the efficiency of degrading CWP decreased gradually.

Table 3 Different cellulase activities and degradation of CWP

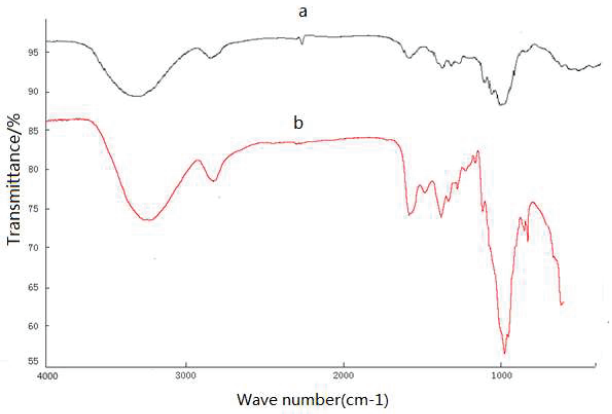
Degradation Time (h)	CMCA (U/ml)	FPA (U/ml)	β-GA (U/ml)	Degradation Rate (%)
48	68.80	435.73	160.27	28.11
72	73.33	231.33	190.13	41.96
96	135.73	153.47	221.60	44.34
120	157.87	124.27	114.53	44.60
144	114.53	95.07	85.33	45.00
168	–	7.47	–	45.76

The experimental results of cellulase activity in the degradation process also validated the change of degradation rate. A cellulose derivative having carboxymethyl groups bonded to some of the hydroxyl groups of the glucopyranose monomers to make up the cellulose backbone is known as carboxymethyl cellulose or cellulose gum. It is often utilized some sodium counterpart, sodium carboxymethyl cellulose. Meantime, β-glucosidase is the major component present in the cellulose, with help of this component cellobiose to glucose is converted in the final step of cellulose hydrolysis process. The major benefits of this reaction are constantly under control since its product glucose inhibits it. The activities of carboxymethylcellulase and β-glucosidase were low at the beginning of incubation, and increased gradually along with time, reaching the maximum at 96 hours, and the two enzymes could hardly be detected at 192 hours. Different from these two enzymes, the activity of filter paper enzymes reached its maximum at the beginning of incubation, and gradually decreased along with time. It was speculated that the degrading bacteria needed to secrete a large number of filter paper enzymes to cut off the long carbon chain molecules of CWP at the beginning of incubation, so the activity of filter paper enzymes was the strongest and then the breaking of long carbon chains resulted in the emergence of substrates corresponding to carboxymethylcellulase and β-glucosidase, and their activity gradually increased, while filter paper enzymes gradually decreased due to the reduction of reaction substrates. After 192 hours of degradation, the growth of degrading bacteria entered the decline phase, and the activity of enzymes gradually decreased. At last, the activity of enzymes could hardly be detected.

3.4 Structure Analysis of Degraded CWP

(1) FITR analysis

The degraded CWP was tested by FTIR. FTIR results (Figure 3, Table 4) before and after degradation showed that there was an absorption peak at 2250 cm^{-1}, where the peak was the stretching vibration peak of C≡N, which was the characteristic peak after cyanoethylation substitution reaction. However, the absorption peak of the biodegraded paper material appeared at around 2889 cm^{-1}, which was the characteristic peak of -C-H group, while the stretching vibration peak of C≡N disappeared. It was preliminarily speculated that the cellulase secreted by the composite bacteria destroyed the C≡N to form hydrocarbon bonds during the degradation process.



a Undegraded CWP; b CWP Degraded by Microorganisms.

Figure 3 FTIR test results.

Table 4 FTIR spectra data of CWP

Wave Number (cm^{-1})	Peak Assignment
3397	Oscillation of hydroxyl O-H bond
2903	Expansion Vibration of -CH_2-, -CH-
2889	Characteristic peaks of -CH group
2253	Shrinkage vibration peak of C≡N
1641	Shrinkage vibration peak of C=O
1429	Shear vibration of cellulose's -CH_2- or Bending vibration of lignin's -CH_2-
1373	Bending Deformation Vibration of -CH-
1162	Telescopic vibration of C-O-C
1029	Telescopic vibration of C=O
897	Vibration of the β-D glycoside bond

(2) XRD analysis

X-ray diffraction analysis (XRD) is a materials science technique, which is used for determining material's crystallographic structure. It is a technique that involves irradiating a material with incoming X-rays and then measuring the intensities and scattering angles of the X-rays that exit the substance and the XRD patterns are used to provide information about defects and size of the particles, meantime the atomic distribution of the unit cell is delivered during peak relative intensities. But, the major present in the process of XRD patterns is good peak-to-peak background ratio and powder diffractogram

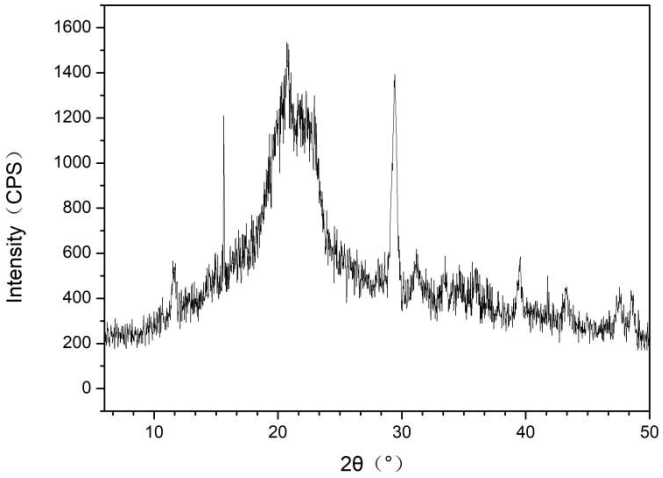


Figure 4 XRD patterns of CWP.

interpretation. From the XRD pattern and analysis results of degraded CWP in Figure 4, it could be seen that the crystal form of degraded CWP remained unchanged as cellulose type I crystal, maintaining the coexistence of crystal and amorphous regions. The main characteristics of XRD was discussed as follows: represent information of crystal structure, crystal texture and structural parameters like strain, crystal defects, size and average gain, etc... The (101) crystal plane of CWP disappeared basically at $2\theta = 10.5°$, while the strength of the peak of the (002) crystal plane decreased at $2\theta = 22.2°$ (002) and the shape widened, which was the result of the decrease of the crystallinity of CWP, while the strength of the (004) crystal plane increased, but the crystallinity of degraded CWP was 10.58%. This was mainly due to the synergistic effect of cellulase decomposed during the degradation process of composite bacteria, which destroyed the crystal structure of cellulose, increased the amorphous region, and increased distance between crystal planes, which was more conducive to cellulase entering the cellulose for degradation. These results indicated that cyanoethylation of waste paper improved the biodegradability.

4 Conclusions

(1) Compared with waste paper, CWP has better biodegradability. Under the action of *Trichoderma reesei*, the degradation rate of CWP containing

20.79% nitrogen is about 24% after 144 hours of degradation, which is mainly due to the swelling of the fibers, the increase of the contact area and the destruction of crystal structure in cellulose caused by cyanoethylation reaction. The increase of the amorphous regions and the distance between crystal planes of cellulose is more conducive to the cellulase entering into the CWP.

(2) The degradation rate of CWP is about 45% after 144 hours degradation by complex bacteria. The degradation rate has such obvious increase due to the synergistic effect of carboxymethyl cellulase, β-glucosidase and filter paper enzyme, which was consistent with the conclusions of Zhang Li et al. [23].

(3) In the process of degradation of CWP by microorganisms, cellulose degrading enzymes produced by microorganisms destroy the carbon-nitrogen triple bonds of the substituted groups in the cellulose chain and convert them into hydrocarbon bonds; after degradation, the crystalline form of CWP remains unchanged, but degradation destroys the crystal structure of CWP and increases the volume of amorphous zones.

Combining the above research results with the previous research results of the project team, CWP not only has good thermoplastic properties, but also has good biodegradability, which preliminarily shows that CWP has the potential of being biodegradable plastics.

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