

BIODEGRADATION OF REGENERATED CELLULOSE FILMS BY FUNGI

ZHANG Lina (张俐娜)** and LIU Haiqing (刘海清)

(*Department of Chemistry, Wuhan University, Wuhan 430072*)

ZHENG Lianshuang (郑连爽) ZHANG Jiayao (张甲耀)

DU Yumin (杜予民) and LIU Weili (柳卫莉)

(*Department of Environment Science, Wuhan University, Wuhan 430072*)

ABSTRACT

The biodegradability of *Aspergillus niger* (A. niger), *Mucor* (M-305) and *Trichoderma* (T-311) strains on regenerated cellulose films in media was investigated. The results showed that T-311 strain isolated from soil adhered on the cellulose film fragments has stronger degradation effect on the cellulose film than A. niger strain. The weights, molecular weights and tensile strengths of the cellulose films in both shake culture and solid media decreased with incubation time, accompanied by producing CO₂ and saccharides. HPLC, IR and released CO₂ analysis indicated that the biodegradation products of the regenerated cellulose films mainly contain oligosaccharides, cellobiose, glucose, arabinose, erythrose, glycerose, glycerol, ethanal, formaldehyde and organic acid, the end products were CO₂ and water. After a month, the films were completely decomposed by fungi in the media at 30°C.

INTRODUCTION

Cellulose, a friendly material to environment, is the most abundant organic substance occurring on the earth, and can be decomposed by microorganisms. In our previous work^[1-3], the regenerated cellulose and its blended films prepared from cellulose cuoxam solution were investigated, suggesting that the cellulose films have potential application as substitute for plastic films, where biodegradation is important. The biodegradability of regenerated cellulose films prepared from pulps of linter, cotton stalk and wheat straw in soil was studied, and the type of strains which degrade the cellulose film in soil was identified^[4]. It indicated that the cellulose was

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** To whom correspondence should be addressed.

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degraded by microorganisms in soil more easily than hemicellulose because of cooperative interaction, and their degradation half lives were given to be 30–42 days. The potential of *Aspergillus fumigatus* strains for degrading cellulose or lignocellulosics materials has been investigated^[5], and the strains are able to multiply and sporulate in great profusion in damp grass, straw and hay and appear to be distributed very widely over the earth's continent. This study aimed to understand biodegradation rate, products and process of the regenerated cellulose film by *Aspergillus niger*, *Mucor* and *Trichoderma* strains in culture media.

MATERIALS AND METHOD

Preparation of films

The regenerated cellulose films were prepared from pulps of linter, wheat straw, cotton stalk and linter/hemicellulose, according to our previous method^[2]. The viscosity average molecular weight $\overline{M}_\eta$ and the contents of α -cellulose and hemicellulose of raw materials are summarized in Table 1. The films prepared from cellulose cuoxam were washed in running water, then dried at 110°C for 5 min before used for biodegradation test.

Table 1 Raw materials (pulps) of films

Film No.	Kind	$\overline{M}_\eta \times 10^4$	α -cellulose (%)	Hemicellulose (%)
XCN8	linter	20.0	98	0
JCN8	wheat straw	16.0	78	20
GCN8	cotton stalk	21.0	83	15
XCN-H8	linter/hemicellulose	—	80	20

Strains and culture media

Aspergillus niger (A. niger) was supplied by Chinese Center for Type Culture Collection in Wuhan University, *Mucor* (M-305) and *Trichoderma* (T-311) were isolated from the soil adhered on the cellulose film fragments buried and classified according to the reported method^[4]. Nutrient salt media were prepared by dissolving the designated amount of 0.7g (KH_2PO_4), 1.0g (NH_4NO_3), 0.002g ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 0.002g ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and 0.005g (NaCl) and 1 L of distilled water, then adjusted pH to 6.0–6.5 by addition of 0.01mol/L NaOH aqueous solution. The media were sterilized by autoclaving at 121°C for 20min.

Solid culture test was performed according to ASTM G21-70. The agar media were prepared by adding 15 wt% agar into the above mentioned nutrient salt media, then they were contained in sterilized Petri plate to provide a solidified agar layer.

The autoclaved films ($10 \times 10 \text{ cm}^2$) were placed on the surface of the agar, then the strain pore suspension (0.2ml/dish, 5 million pores/ml) was spread on the surface of the films. They were incubated at 30°C for 6 to 20 days. At intervals of 4–6 days, the degraded fragments were taken out, washed and dried, then the saccharides were extracted from the agar media.

Shake-culture test was performed as follows: 50ml of nutrient salt media were added into 250ml Erlenmeyer flask in which 2wt% film fragments were added as sole carbon source. The strain pore suspensions were inoculated, then incubated at 30°C , 170rpm. At intervals of 2–3 days, the saccharide concentration in the supernatants and $\overline{M}_\eta$ of the degraded film fragments were determined, respectively.

Analysis of degraded products

Both the agar plate and a beaker containing 10ml 0.05mol/L of $\text{Ba}(\text{OH})_2$ solution as CO_2 absorbant were placed in a 1000ml container, the container was sealed and incubated at 28°C . At intervals of 2–5 days, the absorbant was titrated with 0.023mol/L oxalic acid, and an agar media without cellulose film was used as blank control. The experimental value (m_{EXP}) of CO_2 evolution was calculated according to the volume of consumed oxalic acid in titration. the biodegradation rate (R) was given as in the following:

$$R = (m_{\text{EXP}}/m_{\text{THE}}) \times 100\%$$

where m_{THE} is theoretical CO_2 evolution of degraded film, which was calculated from a stoichiometry of aerobic biodegradation^[6].

The water soluble saccharides in degradation products were measured by the colorimetric method^[7]. The saccharide concentration (c_s) was measured on the ultraviolet spectroscopy (UV-160A, Shimadsu, Japan) at $\lambda = 489\text{nm}$ using glucose as standard. The degraded products in the shake culture solution were determined by high pressure liquid chromatograph (HPLC, Shimadsu, LC-6A) with Bondclone 10 NH_2 column (300 $\times$ 3.9 mm, Phenonenex) and a differential refractometer detector. Acetonitrile/water/methanol (65 : 10 : 5) was used as the eluent, and flow rate was 1.6ml/min. The culture solution was purified by centrifugation at 10^4rpm for 30min, and concentrated, then used to the measurement. Infrared (IR) spectra of the degraded film fragments were recorded with a Nicolet FT-IR Spectrometer.

Characterization of degraded films

Viscosity average molecular weights $\overline{M}_\eta$ of the degraded films and raw materials were determined from intrinsic viscosity $[\eta]$ in cadoxen at 25°C by the following equation^[8]:

$$[\eta] = 3.85 \times 10^{-2} \overline{M}_\eta^{0.76}$$

The weight losses $W_{\text{loss}}(\%)$ of the film degraded in culture media were measured at intervals of 3—6 days. Tensile strength the films σ_b were measured by an electronic strength tester XLD-0.1 (the Second Tensile Testing Machine Manufactory of Changchun) according to Chinese standard method (GB4456-84).

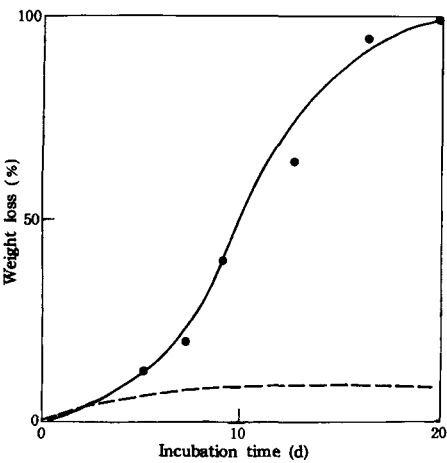


Fig.1 Incubation time dependence of weight loss for XCN8 film (●) in agar media with T-311 strain (—) and A. niger strain (---, see Fig.3)

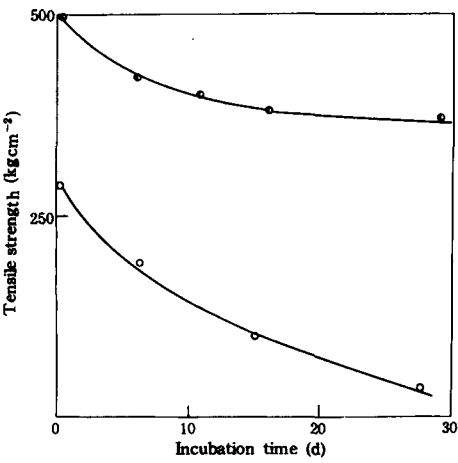


Fig.2 Incubation time dependence of tensile strength for XCN8 film (●) and GCN8 film (○) in agar media with A. niger strain

Table 2 Comparison of biodegradability of the fungi on the XCN8 film

Time (days)	Strain							
	Suspension		A. niger		T-311		M-305	
	of soil							
	c_s $\mu\text{g ml}^{-1}$	$\overline{M}_\eta$ $\times 10^{-4}$	c_s $\mu\text{g ml}^{-1}$	$\overline{M}_\eta$ $\times 10^{-4}$	c_s $\mu\text{g ml}^{-1}$	$\overline{M}_\eta$ $\times 10^{-4}$	c_s $\mu\text{g ml}^{-1}$	$\overline{M}_\eta$ $\times 10^{-4}$
3	1.2	7.87	4.1	7.85	5.58	7.5	5.58	7.42
6	2.44	7.07	7.78	6.2	14.14	5.32	11.04	4.68
8	4.04	6.62	7.80	5.14	34.40	1.97	20.20	2.83

RESULTS AND DISCUSSION

Biodegradation activity of strains

The saccharide concentration produced from the cellulose film in media with fungi and $\overline{M}_\eta$ of the degraded film fragments at the variant incubation time are given in Table 2. The degradability of fungi against the cellulose films can be described by the increase of saccharide concentration and the decrease of $\overline{M}_\eta$ of the degraded

films. The results indicate that the changes of the saccharide concentration are in good agreement with that of $\overline{M}_n$, and the T-311 strain has stronger degradation activity on the XCN8 film than the others. Fig.1 shows incubation time dependence of weight loss of XCN8 film in agar media with T-311 strain and *A. niger* strain. Biodegradability of the XCN8 film in the media with T-311 strain is obviously higher than that with *A. niger*, it is coincident with that in Table 2.

Fig.2 shows that incubation time dependence of tensile strength for two types of cellulose film in agar media with *A. niger* strain. It is clear that the biodegradability of *A. niger* strain against XCN8 film is weaker than against GCN8 film. Incubation time dependence of weight loss for the cellulose films in agar media with *A. niger* strain is shown in Fig.3. After 27 days, the value of weight loss of JCN8 film reached 90%, but that of XCN8 film was lower than 10%. The results imply that the positive effect of hemicellulose on degradation of the cellulose films in the media with *A. niger* strain was enhanced.

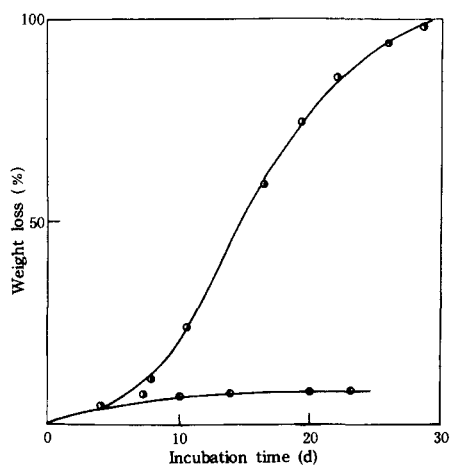


Fig.3 Incubation time dependence of weight loss for JCN8 film (○) and XCN8 film (●) in agar media with *A. niger* strain

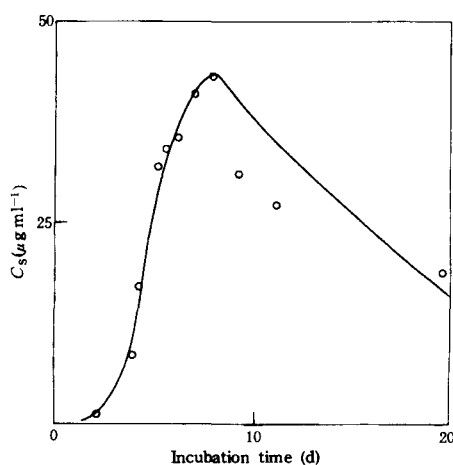


Fig.4 Incubation time dependence of the saccharides concentration (c_s) released from film XCN8 in shake culture media with T-311 strain

Biodegradation process and products

Fig.4 shows that the incubation time dependence of c_s of the cellulose films degraded by T-311 strain. The result indicates that c_s of the degraded cellulose film increases with the increase of incubation time from 1 to 8 days, then decreases with incubation time from 8 to 20 days. The largest saccharide concentration lies in 8th day, and its decrease is related to the consumption of releasing CO_2 . HPLC graphs

of the degradation products of XCN8 and XCN-H8 films in shake culture solution with strain T-311 for 14 days are shown in Fig.5, The XCN-H8 film was prepared from a mixture of linter and hemicellulose (8 : 2). From data shown in Fig.5, it is deduced that the degradation products of the films mainly contain oligosaccharides, cellobiose, glucose, arabinose, erythrose, glycerol, ethanal, and formaldehyde. Organic acid was difficultly detected by the Bondclone NH_2 column, but was shown absorption at $1610\text{cm}^{-1}(\text{COO}^-)$ by IR. The oligosaccharides of the degradation products for XCN8 film are more than that for XCN-H8 in accordance with its compounds added hemicellulose. The biodegradation products of the regenerated cellulose films are similar to natural cellulose, which has been transferred into oligosaccharides, cellobiose, glucose, arabinose, erythritol, erythrose, organic acid and so on^[9-10].

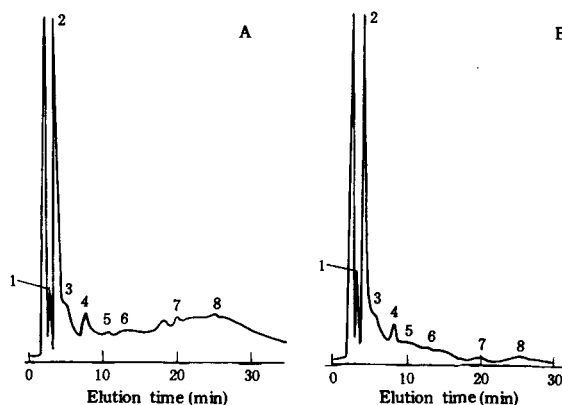


Fig.5 Degradation products of XCN8 (A) and XCN-H8 (B) films in shake culture solution with T-311 strain. 1-glycerol, 2-mixture of erythrose and glycerose, 3-arabinose, 4-glucose, 5-ethanal, 6-formaldehyde, 7-cellobiose, 8-oligosaccharides

Fig.6 shows that incubation time dependence of CO_2 evolution of the XCN8 film and glucose in agar media with strain T-311 at 30°C . CO_2 evolution for both XCN8 film and glucose did not appear until three days of incubation. The glucose, when R value reached 65%, was completely decomposed to CO_2 at 28th day, and the other 35% were resumed as carbon source by the microorganism. CO_2 released from XCN8 film increased with incubation time, and the biodegradation rate reached 55% at 28th day. It suggests that the film was completely decomposed in the solid media at 30°C for a month. The plots of weight loss and the saccharide concentration of the XCN8 film degraded by strain T-311 against incubation time are shown in Fig.7. The weights of the degraded film decreased with incubation time. But the saccharides evolution did not appear until 7th days of incubation, and increased

with the increase of time to reach maximum, then decreased. The change of the saccharides with time is in good agreement with that shown in Fig.4. These results suggested that the saccharide produced from the cellulose film degraded by fungi were continuously consumed as producing CO_2 and water. In view of analysis for Fig.4, 6 and 7, the saccharides were completely decomposed to CO_2 in the shake culture and solid media at 30°C for a month. The end products of the cellulose film degraded by fungi are CO_2 and H_2O .

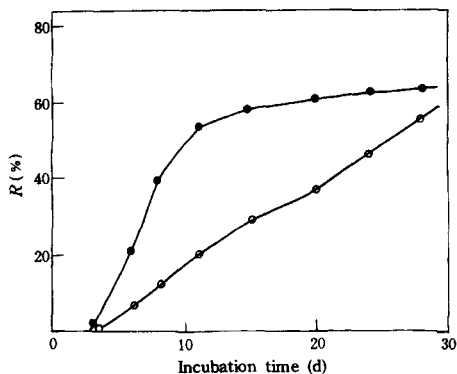


Fig.6 Incubation time dependence of CO_2 evolution for film XCN8 ($\ominus$) and glucose ($\bullet$) in agar medium with T-311 strain at 30°C

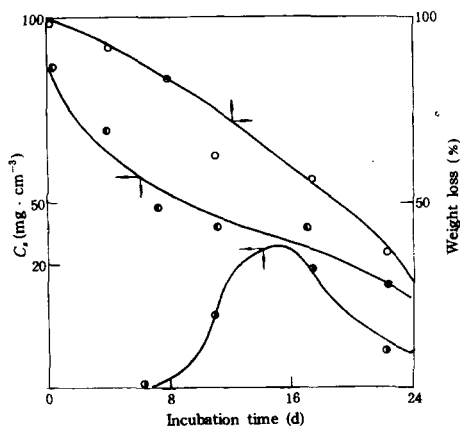


Fig.7 Plots of weight loss ($\circ$) and saccharide concentration c_s ($\bullet$) of the XCN8 film degraded by T-311 strain at 30°C against incubation time; ($\bullet$) represents glucose

CONCLUSION

The T-311 strain isolated from soil has obviously stronger degradation activity on the regenerated cellulose film than *A. niger* strain. The biodegradation process of the films in the agar media or in shake nutrient salt media with T-311 strain includes breakdown of cellulose chains, production of the saccharides from the cellulose macromolecules, and decomposition of the saccharides. Hence, the degradation products with very different molecular weights and different structures exist in the culture solution, and mainly contain cellulose with low molecular weight, oligosaccharides, cellobiose, glucose, arabinose, erythrose, glycerose, glycerol, ethanal, formaldehyde and organic acid, and the end products are CO_2 and water. The polydispersed constituent of these products coincides with the conclusion from ^{13}C NMR^[4]. The cellulose film was completely decomposed to CO_2 and water in the media with T-311 strain at 30°C after a month.

Keywords:

Biodegradation; Regenerated cellulose film; Fungi; Medium.

REFERENCES

- [1] Zhang, L.N.; Yang, G. and Xiao, L., *J. Membrane Sci.*, **1995**, 103: 65.
- [2] Zhang, L.N.; Yang, G. and Yan, S. et al., *Chinese Patent* C08J 5/18 CN1091144A, **1994**, 8.
- [3] Zhang, L.N. and Yan, S., *Chinese J. Polymer Sci.*, **1995**, 13(1): 78.
- [4] Zhang, L.N.; Liu, H. and Zheng, L. et al., Biodegradability of regenerated cellulose films in soil, *Ind. Eng. Chem. Res.* (in press).
- [5] Wase, D.A. John; "Cellulosics: Pulps, Fibre and Environmental Aspects", by Kennedy, J.F.; Phillips G.O. and Williams P.A., Ed., Ellis Horwood, **1993**, p.409.
- [6] Larry, R. Krupp and William, J. Jewell, *Environment Sci. & Technol.*, **1992**, 26: 193.
- [7] Stoffer, J.O., "Methods of Enzymatic Analysis" Vol. VI, **1985**, p.21.
- [8] Brown, W. and Wirskstom, R., *Euro. Polym. J.*, **1965**, 1: 1.
- [9] Blazej, A. and Kosik, M., "Cellulosics; Pulp, Fibre and Environmental Aspects", by Kennedy J.F. Ed., Ellis Horwood, **1993**, p.397.
- [10] Veness, R.G. and Evans, C.S., "Cellulosics; Pulps, Fibre and Enviromental Aspects", by Kennedy J.F. Ed., Ellis Horwood, **1993**, p.451.