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An Improved Method for the Measurement of Fungal Degradability of Synthetic Polymers

Rhee, Young-Ha, Jeong-Ae Lee, Pil-Jae Maeng and Chang-Lim Jun*

Department of Microbiology, Chungnam National University, Daejeon 305-764, Korea

**Department of Industrial Chemistry, Hong-Ik University, Jochiwon 339-800, Korea*

진균에 의한 합성고분자재료 분해도 측정법의 개선

이영하 · 이정애 · 맹필재 · 전창림*

충남대학교 자연과학대학 미생물학과

*홍익대학교 공업화학과

ABSTRACT: An improved fungal test method, soft agar overlay method, has been developed for the measurement of biodegradability of synthetic polymers. Advantages of this technique over conventionally used test method of ASTM include better visualization of fungal growth on polymeric materials, shortening of incubation period, and enhanced sensitivity especially to polymeric materials containing highly recalcitrant molecules.

KEY WORDS□Soft agar overlay method, fungal test, biodegradability, synthetic polymers

Studies on biodegradability of synthetic polymers have received a great deal of attention because of the negative impact of discarding synthetic polymers on the natural environment (Reich and Bartha, 1977; Griffin, 1980; Atlas, 1981; Kilbane, 1986). Among the several methods developed for the measurement of biodegradability of synthetic polymers, fungal test method of ASTM(American Society of Testing and Materials; ASTM G21-70) has been widely used. In this method, the degradability of synthetic polymers is determined by the degree of visual growth of fungi on a polymeric material which is over-laid on the surface of nutrient-salt agar medium lacking a carbon source. After a definite time, growth rates are classified according to the fraction of the polymer-surface covered with fungal colonies, *i.e.*, 0: no growth; 1: trace of growth (less than 10%); 2: light growth (10 to 30%); 3: medium growth (30 to 60%); 4: heavy growth (60% to complete coverage).

Although the fungal test method of ASTM is certainly simple and convenient, this method has some problems. Because synthetic polymers are generally hydrophobic and have inert surfaces to water (Guillet, 1973; Kuster, 1979), extremely weak fungal growth appears when fungal spores are inoculated on the surfaces of synthetic polymeric films and sheets according to the method of ASTM. With this reason, the ASTM method is time-consuming (usually more than 4 weeks) and insensitive, especially to polymeric materials which contain highly recalcitrant molecules as their major constituent (Otey *et al.*, 1980; Swanson *et al.*, 1987). Consequently, the ASTM method is considered to be ineffective for accurate measurement of fungal degradability of synthetic polymers.

To avoid these inadequacies, we modified the fungal test method of ASTM. In our modified method, fungal spores were mixed with soft agar containing nutrient-salt medium and were overlaid on the surface of a polymeric material which is placed on a solid medium. In this report, we described the modified method and compared it with the ASTM method in their efficiencies for the measurement of biodegradability of synthetic polymers.

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Table 1. *Fungal degradability of starch-PE films as measured by ASTM and soft agar overlay (SAO) method*

Sample	Formulation(%)		Method	Fungal degradability*								
	Starch	Polyethylene		3	6	9	12	15	18	21	24	(days)
SL-3	10	90	ASTM	1	1	1	1	1	1	1	1	
			SAO	0	1	1	1	1	1	1	1	
SL-4	20	80	ASTM	0	1	1	1	1	1	1	1	
			SAO	0	1	1	1	1	2	2	2	
SL-5	30	70	ASTM	1	1	1	1	1	1	1	1	
			SAO	1	1	1	1	1	2	3	4	
SL-6	40	60	ASTM	1	1	1	1	1	1	1	1	
			SAO	1	1	1	1	2	2	3	3	
SL-7	50	50	ASTM	1	1	1	1	1	2	2	2	
			SAO	1	3	4	4	4	4+	4+	4+	
SL-8	60	40	ASTM	1	1	2	2	2	2	2	2	
			SAO	0	1	2	2	3	3	4	4	
SL-9	70	30	ASTM	1	2	2	2	2	2	2	2	
			SAO	1	3	4	4	4+	4+	4+	4+	

*Fungal degradability was measured by the degree of fungal coverage on each film in accordance with the guidelines of ASTM G21-70, i.e., O (no growth); 1 (less than 10%); 2 (10-30%); 3 (30-60%); 4 (over than 60%).
The symbol "+" means better growth than indicated visual rate.

MATERIALS AND METHODS

Organism and cultivation

Aspergillus niger KCTC 1374 obtained from Genetic Engineering Center of KAIST was used as a testing fungal strain. Subcultures grown on potato dextrose agar medium at 28 to 30°C for 7 to 10 days were used in preparation of conidia suspension.

Test polymers

Two kinds of plastic films, starch-polyethylene (PE) and starch-polyethylene-vinylacetate (EVA) copolymer films, were used as test materials in this study. Starch-PE or starch-EVA films were prepared by blending various concentration (10 to 70%) of corn starch with low density polyethylene or ethylene vinyl acetate copolymer (vinyl acetate content 15%), respectively, by using Brabender internal mixer at 150°C with running speed of 50 rpm for 40-50 min. A thin film was made with these blended mixture by simple press method (Jun *et al.*, 1989).

Measurement of biodegradability

Conidia suspension of *A. niger* KCTC 1374 and nutrient-salt agar medium were prepared in accord with the ASTM guidelines for fungal test (ASTM G21-70, 1985). The plastic films (5 × 6 cm in size) were sterilized with 70% ethylalcohol and placed aseptically on hardened nutrient-salt agar medium. One milliliter of conidia suspension (1.5×10^6 conidia/ml) was added to 7 ml of soft agar medium (same as nutrient-salt medium except 0.8% of agar), and the mixture was overlaid on test material. The samples were incubated for 24 days at 30°C with more than 85% of a relative humidity. Biodegradability was measured by the degree of fungal coverage on each film after 3 to 24 days and the visual rate of fungal growth was by the method of ASTM G21-70. The above stated test method was designated as soft agar overlay method in this study.

RESULTS AND DISCUSSION

Incorporation of biologically labile polymers, such as starch, into normally stable polymer has been one approach to the preparation of biodegradable plastics. The rate of biodegradation of plastics has been reported to be increased by inclusion of a highly biodegradable polymer in the plastic matrix (Otey *et al.*, 1980; Griffin and Tarverdi, 1983; Pitt *et al.*, 1987). In this respect, starch-PE and starch-EVA films with various concentration of starch were considered to be appropriate test materials for the comparison of the efficiency of soft agar overlay method with that of ASTM method.

The biodegradabilities of starch-PE films meas-

Table 2. *Fungal degradability of starch-EVA films as measured by ASTM and soft agar overlay (SAO)*

Sample	Formulation(%)		Method	Fungal degradability*								
	Starch	EVA		3	6	9	12	15	18	21	24	(days)
SV-31	10	90	ASTM	1	1	1	1	1	1	1	1	
			SAO	0	1	1	1	1	1	1	1	
SV-32	20	80	ASTM	1	1	1	1	1	1	1	1	
			SAO	0	1	1	1	1	1	1	1	
SV-33	30	70	ASTM	1	1	1	1	1	1	1	1	
			SAO	1	1	1	1	1	2	2	3	
SV-34	40	60	ASTM	1	1	1	1	2	2	2	2	
			SAO	0	1	1	1	3	4	4	4	
SV-35	50	50	ASTM	1	2	2	2	2	3	3	3	
			SAO	0	2	4	4	4+	4+	4+	4+	
SV-36	60	40	ASTM	1	1	1	1	1	2	2	2	
			SAO	1	1	2	2	3	3	4	4	
SV-37	70	30	ASTM	1	2	2	2	2	3	3	3+	
			SAO	1	3	4	4+	4+	4+	4+	4+	

*Fungal degradability was measured as described in Table 1.

ured by ASTM and soft agar overlay method are shown in Table 1. As the starch level increased, the film resistancy to fungal attack decreased significantly. However, the fungal degradabilities measured by ASTM method were not significant at or below 40% of starch level. The lack of fungal attack at or below 30% of starch level in starch-poly(ethylene-*co*-acrylic acid) has been also informed by other investigators who used ASTM method (Otey *et al.*, 1980; Swanson *et al.*, 1987). In contrast, soft agar overlay method showed a marked increase of fungal growth rate in accordance with the increase of starch levels. These results indicate that soft agar overlay method is more effective than ASTM method for the measurement of biodegradabilities of highly recalcitrant polymeric materials.

Moreover, much more rapid growth of *A. niger* on starch-PE films was observed when the soft agar overlay method was used. For example, more than 60% of the surface area of SL-7 (50% starch level) was covered by fungal colonies after 9 day-incubation as measured by soft agar overlay method, whereas less than 30% covered even after 24 day-incubation as measured by method. This result obtained by soft agar overlay method was undoubtedly due to more successful supply of fungal spores and nutrients on polymeric films by overlaying the mixture of spores and soft agar medium on polymeric film which was highly hydrophobic and water-imper-

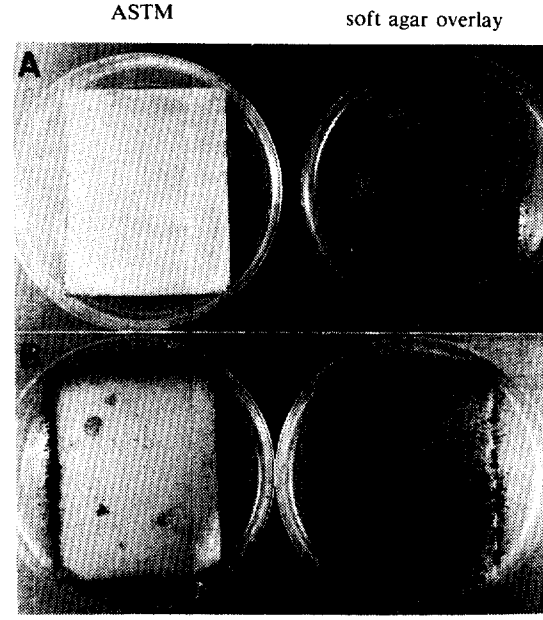

Fig. 1. *Comparison of fungal growth on the surface of polymeric films tested by ASTM and soft agar overlay method.*
A: fungal growth on starch-PE(SL-7) after 10 days incubation.
B: fungal growth on starch-EVA (SV-35) after 18 days incubation.

meable.

Similar results were also observed from the biodegradability tests for starch-EVA films (Table 2). There was little variation of the extent of fungal growth when the samples with 20% of starch content or less were tested using both methods. On the other hand, more remarkable difference among the fungal degradabilities of the starch-EVA films containing more than 20% of starch level was found in the case of soft agar overlay method, which indicates that soft agar overlay method can reflect the biodegradability of the polymeric films more accurately than ASTM method. Furthermore, soft agar overlay method allowed more rapid growth of *A. niger* on starch-EVA films, so that this test was able to shorten the incubation period.

More critical problem in an attempt to measure fungal degradability of synthetic polymers by ASTM method was that the fungal spores did not easily attach on the hydrophobic surface of polymers and that the fungal growth on the surface of polymers was restricted by the difficulty of nutrient supply through water-impermeable polymers. Actually, the fungal growth was largely localized at the marginal regions of the polymeric materials when ASTM method was applied (Fig. 1), and hence accurate measurement of visual rate of fungal growth was not possible. However, fungal colonies were easily formed and distributed evenly on the surfaces of test materials when the soft agar overlay method was applied.

From these results, we conclude that soft agar overlay method is more efficient than the conventional ASTM method for the measurement of fungal degradability of plastic materials. By using soft agar overlay method, incubation time for the measurement of biodegradability is reduced approximately to half, and more accurate determination of visual rate of fungal growth, especially on the polymers of low susceptibility to fungal attack, is practicable. In addition, the method is believed to be applicable to a wide variety of synthetic polymers of particular interest in industries.

적 요

진균에 의한 합성 고분자재료 분해도의 측정을 보다 효율적으로 수행하기 위한 soft agar overlay법을 고안하였다. 이 방법은 고분자재료에서의 균 생장도를 쉽게 파악할 수 있도록 하고, 생분해도 측정기간을 크게 단축시킬 수 있을 뿐만 아니라, 난분해성 고분자재료에서의 분해도 차이를 정확하게 나타내 줌으로써 기존의 측정방법에 비하여 그 효율성이 높았다.

REFERENCES

1. **ASTM G21-70,** 1985. Standard practice for determining resistance of synthetic polymeric materials to fungi. Annual Book of ASTM Standards.
2. **Atlas, R.M.,** 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **45,** 180-209
3. **Griffin, G.J.L.,** 1980. Synthetic polymers and the living environment. *Pure & Appl. Chem.* **52,** 399-407.
4. **Griffin, G.J.L. and K. Tarverdi,** 1983. Microbiodeterioration of thermoplastics: starch filter as an attractant for woodlice. In *Biodeterioration* (ed. T.A. Oxley and S. Barry), John Wiley & Sons Ltd.
5. **Guillet, J.E.,** 1973. Polymers with controlled lifetimes. *Polymer Sci. Technol.* **3,** 1-25.
6. **Jun, C.L., H.B. Lee and Y.H. Rhee,** 1989. Characteristics of blended polyethylene film: mechanical properties and biodegradability. *Proc. IUPAC International Symposium* (Korea), pp. 148-149.
7. **Kilbane, J.J.** 1986. Genetic aspects of toxic chemical degradation. *Microbial Ecology* **12,** 135-146.
8. **Kuster, E.,** 1979. Biological degradation of synthetic polymers. *J. Appl. Polym. Sci.* **35,** 395-404.
9. **Otey, F.H., R.P. Westhoff and W.M. Doane,** 1980. Starch-based blown films. *Ind. Eng. Chem. Res.* **19,** 592-595.
10. **Pitt, C.G., Z.W. Ingram and R.W. Hendren,** 1987. The synthesis of biodegradable polymers with functional side chains. *J. Polym. Sic.* **25,** 955-966.
11. **Reich, M. and R. Bartha,** 1977. Degradation and mineralization of a polybutene film-mulch by the synergistic action of sunlight and soil microbes. *Soil Sci.* **124,** 177-180.
12. **Swanson, C.L., R.P. Westhoff, W.M. Doane and F.H. Otey,** 1987. Starch-based blown films for agricultural mulch. *Polymer Preprints* **28,** 105-106.

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