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A Study of Biodegradation of Polyethylene and Biodesulfurization of Rubber

Scientific Note

D. RAGHAVAN,[1] R. GUAY,[2] AND A. E. TORMA*,[1]

[1]Department of Materials and Metallurgical Engineering, The John D. Sullivan Center for In-Situ Mining Research, Research and Economic Development, New Mexico Institute of Mining and Technology, Socorro, NM 87801; and [2]On sabbatical leave from Laval University, Quebec City, Canada G1K 7P4

Index Entries: Biodegradation; polyethylene; biodesulfurization; rubber; *Aspergillus niger*.

INTRODUCTION

Polyethylene, which has one of the simplest molecular structures (*1*), possesses a unique property of resisting both chemical and biological (enzymic) degradation. These facts with other attractive features of polyethylene, such as its low price, excellent electrical insulator properties, good processability, toughness, transparency, and flexibility in thin films encouraged the packaging industry to use it as a wrapping material for a variety of applications.

The information regarding the degradability of polyethylene by microorganisms is limited to a few preliminary investigations (*2–5*). The lack of information is related to the fact that synthetic polymers in general are resistant to metabolic reactions, and this discourages many investigators from devoting their efforts to these studies.

Using a radio-respirometric technique, Albertsson (*2,3*) showed that labeled CO_2 was released during growth of white rot fungi on C(14)-polyethylene. Similarly, these authors found that *Fusarium redolens* grew on

*Author to whom all correspondence and reprint requests should be addressed.

low- and high-density polyethylenes. These experiments were carried out for two years (*4,5*) and the kinetics of biodegradation was followed by weight loss measurements (*6*).

It was suggested that the biodegradation of polyethylene may be accelerated by photolytic (*7*) and thermolytic (*8*) pretreatments, as well as by γ ray irradiation, which makes polyethylene packaging films biodegradable (*9*), for example, by *Aspergillus niger.* This microorganism is a fungus that generates oxalic and citric acid when grown on sucrose substrate (*10*). Figure 1 shows a typical optical microscopic characterization of *A. niger,* which is filamentous; these individual filaments are termed *hyphae.* The hyphae grow only at the tip (*11*), and extend their branching regularly behind the tips. They reproduce by sexual and asexual modes; however, the final product of their reproduction system is conidospores.

In 1945, it was reported that the deterioration of rubber hoses was the consequence of microbial oxidation of elemental sulfur present in the rubber (*12*). *Thiobacillus thiooxidans* was identified as the responsible microorganism.

Physiologically and morphologically, *T. thiooxidans* is similar to *Thiobacillus ferrooxidans,* which is able to oxidize all inorganic sulfur compounds (ores) and also was found to contribute in the desulfurization of coal (*13–15*).

All the foregoing studies on biodegradation of polyethylene and desulfurization of rubbers resulted in a limited degree of success. In fact, only a small percentage of the total weight of polyethylene was reported to be degraded and there are no data substantiating the biodesulfurization of rubber. Therefore, there is a need for more systematic investigations in order to assess the efficiency of microorganisms involved in the degradation of commercially available polyethylene samples and the desulfurization of rubber.

MATERIALS AND METHODS

Substrate and Chemicals

Commercially available polyethylene samples of 0.4-mil thickness were used throughout this study. Gamma ray irradiation (preaging) of polyethylene films was carried out at Sandia National Laboratories in Albuquerque, NM, under a continuous flow of air at a dosage rate of approximately 0.5 Mrad/h at 313°K. The total irradiation of the polyethylene samples varied from 1.0 to 39.4 Mrad.

Two pulverized rubber samples (712 and 716), each containing 15% sulfur, that were used in this investigation were supplied by the Goodyear Tire Co., Akron, OH. The sample numbers correspond to unspecified patented chemical composition.

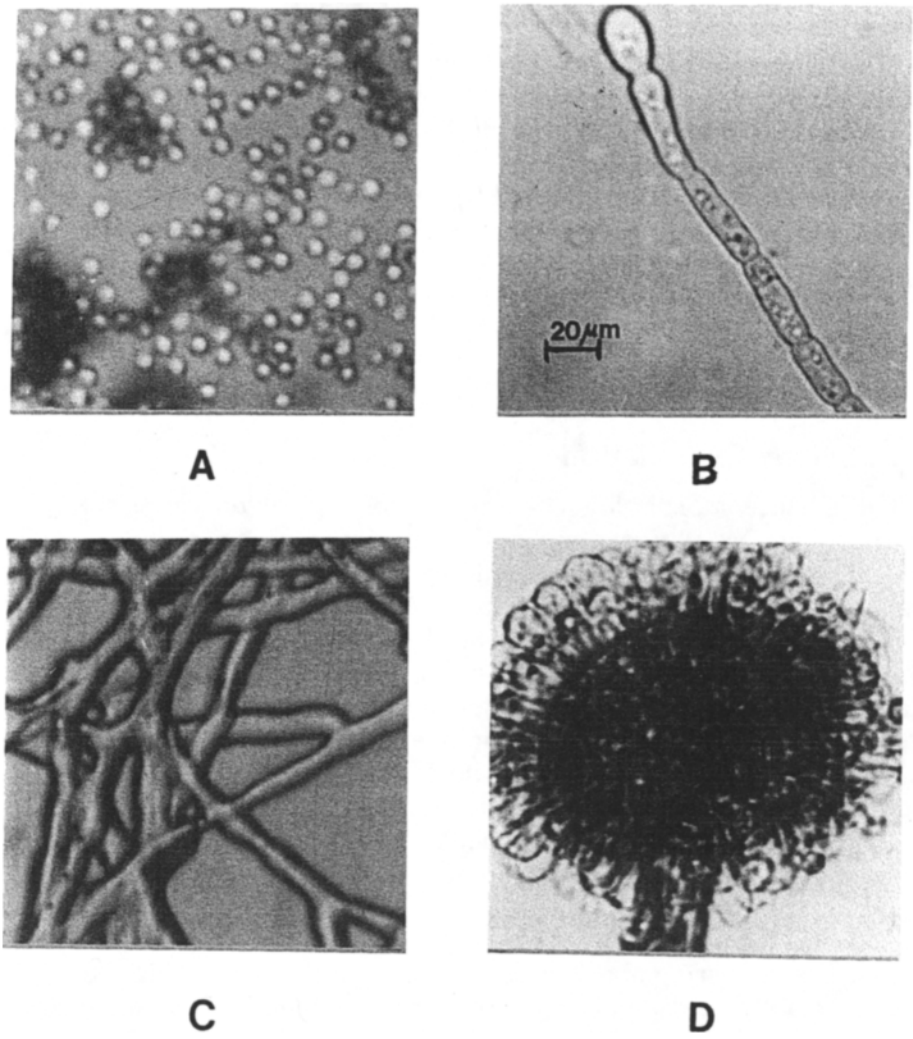

Fig. 1. Morphological characteristics of *Aspergillus niger* during its growth from spores (A) through budding (B), production of hyphae network (C), and finally to fruiting body (conidia) and condiospores (D).

Experimental

Culturing and experimentation using the shake-flask method with *A. niger* has been described in detail elsewhere (*16*).

Elemental sulfur oxidation by *T. ferrooxidans* and *T. thiooxidans* was carried out using a modified 9K medium (*17*) in which sulfur replaced ferrous iron as energy source. Experimental details are described elsewhere for the shake flask-method (*18*), larger scale tank leaching and harvesting of bacteria (*19*), protein determination (*20*), and Warburg respirometric measurements (*21*).

Scanning Electron Microscopy (SEM)

The as-received, preaged, biologically and abiologically treated polyethylene specimens were initially washed with distilled water and allowed to air dry. The samples were then mounted on aluminum stubs (2.5 cm in diameter) using tungsten/carbon glue, and dried at 295°K. Each specimen was coated with Au-Pd in a sputtering unit and finally examined by SEM (Hitachi-Perkin Elmer HHS-S2 electron microscope). Irradiated samples were further characterized by Fourrier transform infrared spectroscopy (FTIR) using Perkin Elmer equipment, model 1700 GC-IR.

RESULTS AND DISCUSSION

Polyethylene Degradation

The surface morphology of an as-received polyethylene sample is shown in Fig. 2A. Observation of this specimen suggests that there is a uniform microstructural surface pattern throughout the polymeric film. Figure 2B and 2C illustrate the surface textures of the polyethylene samples removed from a synthetic metabolite solution and from the sterile growth medium, respectively. The synthetic metabolite solution consisted of a mixture of equal volumes of 0.1*N* oxalic acid and 0.1*N* citric acid, and represents the combination of end products of sucrose metabolism by *A. niger* (*10*). Even after 4 mo of treatment with both the sterile medium and the synthetic metabolite solution, marginal effects on polyethylene surface may be seen, as depicted in Fig. 2B and 2C.

Following inoculation with fungal spores of *A. niger,* the growth started in liquid medium and spread to the polymer surface, as shown in Fig. 2D. A week later (day 17), colonies have spread and the hyphae could be seen covering about 50% of the examined surface (Fig. 2E). After 160 d of treatment, the polyethylene surface exhibited dramatic changes from its initial appearance: it lost its original homogeneity and preexisting uniformity. The SEM micrographs suggest that the surface transformation of the polyethylene samples is attributed to fungal metabolic activity (growth), since experiments carried out with the synthetic metabolite solution did not result in similar surface alterations. However, further characterizations are in progress, such as the use of filter-sterilized spent growth medium to see whether other metabolites take part in the degradation process. These data will be published elsewhere. Other investigators (22) were unable to demonstrate the mechanism of biodegradation of polyethylene.

Radiated Polyethylene Degradation

The influence of irradiation on the chemical modification of polyethylene is presented in Fig. 3A in the form of a modified Fourrier transform infrared spectrum. The preaging effect of irradiation on polyethylene can

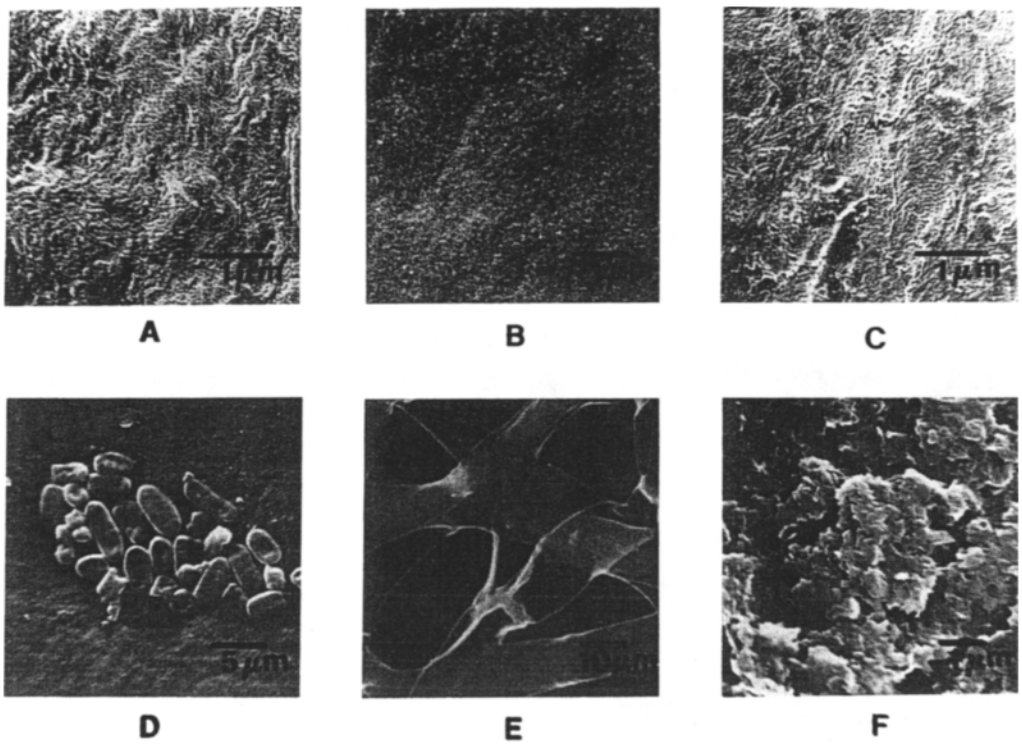

Fig. 2. Scanning electron micrographs of 0.4 mil polyethylene samples. A) As-received sample; B) Sample suspended in sterile medium for 160 d, sterile control; C) Polyethylene sample treated with a mixture of oxalic and citric acids; D) *A. niger* colony on a polyethylene sample after 10 d of treatment; E) *A. niger* hyphae network on polyethylene sheet after 17 d of growth; F) Sample recovered after 160 d of treatment.

be attributed to molecular oxygen reaction with the polymer, leading to the formation of carbonyl groups. This functional group shows absorption at 1760–1650 cm^{-1} on FTIR spectrum.

Figure 3B is a SEM micrograph of an irradiated polyethylene specimen recovered from the uninoculated culture medium. It can be compared with the micrograph appearing in Fig. 2C, where practically no effect from the medium can be observed on the sample surface. Here irradiation did not seem to increase any visible morphological change, even at large doses (39.5 Mrads). However, when the irradiated samples were placed in the inoculated flasks, *A. niger* colonized the polymeric material as shown in Fig. 3C after 10 d of culture. Striking similarities can be observed between Fig. 2F and Fig. 3D when considering the overall flaky appearance of the polyethylene surface, but this result was obtained after 30 d of treatment with the irradiated specimens, whereas it took 160 d to achieve approximately the same effect with the untreated polyethylene samples. The fungi seemed to have caused quite extensive damage to the irradiated

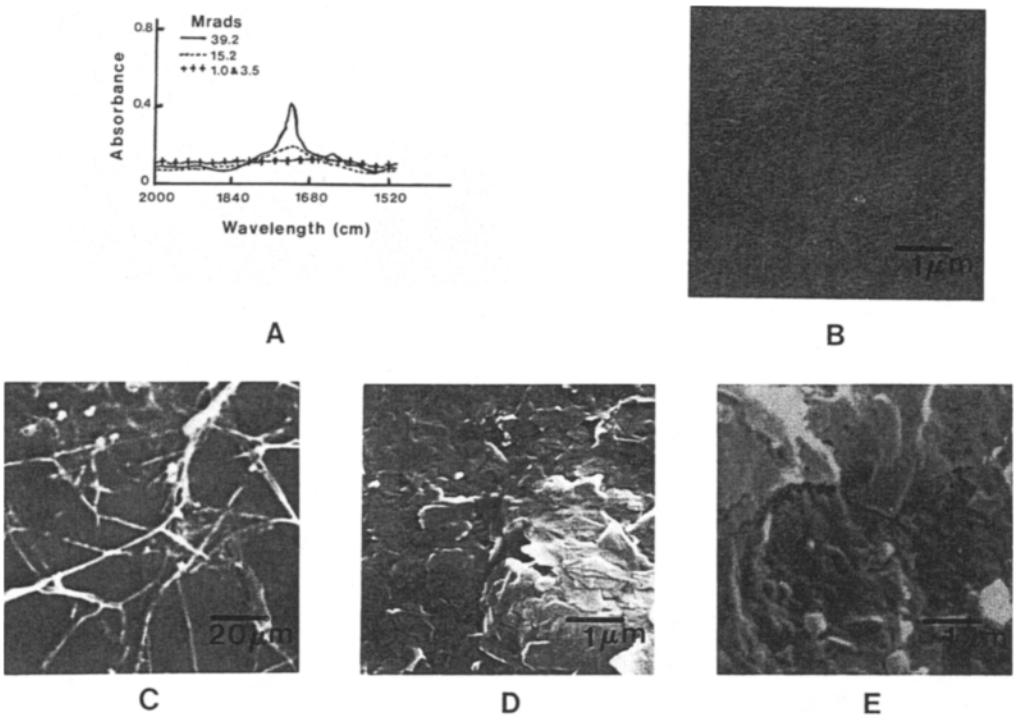

Fig. 3. A) Fourrier-transform infrared spectra of polyethylene 0.4 mil sheets irradiated with various doses of γ rays; B) SEM of an irradiated polyethylene sample from a sterile control experiment after 10 d of treatment; C) *A. niger* growth on a sample; D) Partial degradation of the surface sample by molds after 30 d; E) Degraded polyethylene sample after 90 d of fungal treatment.

polyethylene sample after 90 d of culture at room temperature, as shown in Fig. 3E.

Growth of Thiobacilli on Elemental Sulfur

Oxidation of elemental sulfur by pure and mixed cultures of *T. ferrooxidans* and *T. thiooxidans* are depicted in Fig. 4. In pure cultures, *T. ferrooxidans* and *T. thiooxidans* seem to have similar sulfur-oxidizing ability, but in mixed cultures their activity increased because of a synergistic growth effect. However, we can see that the acid production by these shake-flask cultures is maximal after 7 d of growth, with an approximate 35% transformation of the substrate. After 7 d of treatment, the pH of the culture dropped to 1.50. At this low pH, the bacterial activity was reduced because of the accumulation of metabolic sulfuric acid. It is known that the optimum pH for growth of *T. ferrooxidans* and *T. thiooxidans* is 2.30 (*18,23*).

Since the mixed culture of *T. ferroxidans* and *T. thiooxidans* resulted in an improvement in the oxidation of elemental sulfur, larger scale experi-

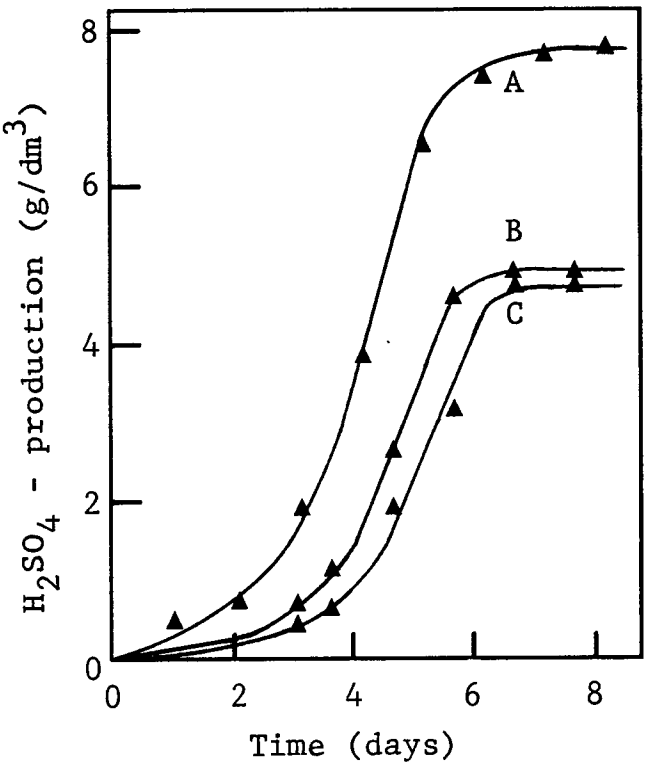

Fig. 4. Oxidation of elemental sulfur at 294°K and 200 rpm, where A = mixed culture of *T. ferroxidans* and *T. thiooxidans*, B = pure strain of *T. thiooxidans*, and C = pure strain of *T. ferroxidans*.

ments with 20 dm^{-3} nutrient solutions were used for mass-culturing these mixed bacterial cells for Warburg respirometer studies.

Bacterial Desulfurization of Rubber

The results obtained in the respirometric experiments are presented in Fig. 5. The mixed culture of thiobacilli used in these experiments was found to be effective in the oxidation of sulfur from pulverized rubber samples, as shown by the large difference between the oxygen uptake of active bacterial cells and that of the control experiments. Similarly, Table 1 shows the kinetic evaluation of oxygen uptake data. As shown, no endogenous respiration (bacteria without substrate) was observed, although in the sterile controls (substrate with killed bacteria or without bacteria), substrates exhibited limited degree of autooxidation.

Conclusions

The present study provides preliminary data on the involvement of such microorganisms as *Aspergillus niger* in the biodegradation of commercially available polyethylene packaging thin films and *T. ferroxodans*

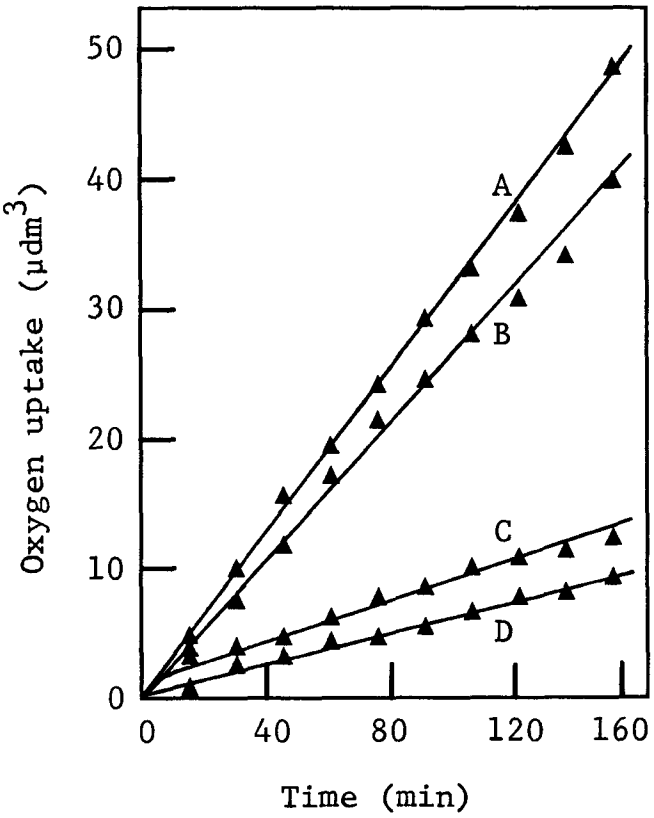

Fig. 5. Warburg respirometric data; at 308°K, 120 strokes/min where A=rubber sample 712 inoculated run, B=rubber sample 716 inoculated run, C=712 sterile control, and D=716 sterile control.

Table 1
Comparative Kinetics of Oxidation of Sulfur
in Rubber Samples by a Mixed Culture of *T. ferroxidans* and *T. thiooxidans*

Experimental Conditions	Specific rate of oxygen uptake μdm^3O_2/(h mg protein)	
	Rubber "712"	Rubber "716"
Inoculated	4.44	3.55
Sterile killed bacteria + rubber substrate	1.47	0.59
Sterile no bacteria + rubber substrate	1.28[a]	0.59[a]
Control bacteria without rubber substrate	0.04	0.01

[a] μdm^3/h units (no protein)

and *T. thiooxidans* in pure and mixed cultures in the desulfurization of rubber samples. Further studies are in progress, and it is expected that the generated results will contribute to the solution of certain problems associated with the disposal and management of polymeric waste materials.

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