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Pure Culture Assay with *Streptomyces viridosporus* T7A for Biodegradability Determination of Oxidized Potato Starch/Polyethylene Films

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Abstract

Oxidized potato starch/polyethylene (PE) cast films were prepared with different percentages of linear low density PE (LLDPE), oxidized potato starch and prooxidant. For the determination of biodegradability of the films, lignocellulose-degrading *Streptomyces viridosporus* T7A (ATCC 39115) was used. Films were chemically disinfected and incubated with *S. viridosporus* by shaking at 100 rpm at 37°C for eight weeks. Hydroxyl indices of the films by Fourier-Transform Infrared Spectroscopy, mechanical properties of the films by Instron and film morphology by scanning electron microscope (SEM) were measured. The hydroxyl index of the film containing the oxidized potato starch incubated with *S. viridosporus* T7A was higher than that of the corresponding control. All the films containing 5% and 10% oxidized starch showed a decrease of tensile strength on the films after incubation when the corresponding uninoculated film was compared. In the oxidized starch/PE film incubated with *S. viridosporus* T7A, partial destruction of starch and PE was examined by SEM.

Key words: oxidized potato starch, degradable film, biodegradability, *Streptomyces viridosporus* T7A

INTRODUCTION

Plastic packaging materials are among the newest materials used by the food industry. Plastics are often preferred over metal, wood, paper and glass because of their unique characteristics: inertness to many chemicals and microorganisms, toughness, transparency, elasticity, impermeability by many gases and moisture, ability to be coextruded and design capabilities not available with other packaging materials.

However, the use of degradable plastics has been required in areas with ecological and environmental concerns. In 1985, 13.2 billion pounds of plastics were used for food packaging materials and 22.58 billion pounds of plastics were expected to be used in 2000 (1). In spite of the efforts to increase recycling or incinerating ratios, landfills continue to be the dominant destination for solid waste despite the rapidly decreasing capacity of landfills.

To alleviate environmental pollution from waste plastics, various degradable plastics have been developed. Degradable plastics are degraded by chemical, photo or biological actions. These plastics can be degraded by light, living organisms, oxygen and heat as well as reduced in size by the mechanical effects of wind, rain and so on (2). These mechanisms may operate in a synergistic, concerted or consecutive manner depending on the environmental conditions and the resin.

One kind of biodegradable plastic is a polymer containing a biodegradable filler. Starch is an abundant, low cost and completely biodegradable natural polymer. Griffin (3) developed a process for incorporating starch into blown low-density PE films. Griffin (4) used 15~30% native starches, either directly or blended with lubricants such as ethyl oleate, calcium stearate, iso-octyloleate and paraffin wax, into the polymer.

Starch is inherently water sensitive. When starch is dried, it becomes rigid and brittle from the high degree of molecular branching and hydrogen bonding. The embrittlement imparted to starch/PE plastics by starch can be overcome to various degrees by adding plasticizers and other materials. Otey et al. (5) prepared cast films from aqueous dispersions of starch (gelatinized starch), poly (vinyl alcohol), glycerol, surfactant and formaldehyde. These cast films showed inferior wet strength, but cast films coated with polyvinyl chloride or poly (vinylidine chloride) and acrylonitrile copolymer had improved wet strengths. Otey et al. (6-8) prepared the films by blending starch with poly (ethylene-co-acrylic acid) (EAA). This film was clearer than the film made by dry blending and similar to the appearance of conventional films. Plastics containing starch usually have shown mechanical properties inferior to PE without starch because of the weak interaction between hydrophilic starch and hydrophobic PE. Therefore, studies to improve the mechanical properties of the degradable plastics are required. Griffin (9,10) proposed a process for making films containing polyolefin, fatty acid, fatty acid ester and starches modified by silicone treatment to improve the film properties such as mechanical strength and biodegradation. Swanson et al. (11) evaluated the effects of modified starches on plastic film. The mixture of low density PE and EAA polymers filled with hydroxypropyl or acetyl derivatives of

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starch showed greater elongation and tensile strength than films containing underivatized starch. Evangelista et al. (12) prepared low density PE films with corn starch modified with octenyl succinate groups. These modified, starch-filled PE films, however, exhibited reduced access to amylases compared with native corn starch (13).

Biodegradation is the breakdown of materials by the action of living organisms. Bacteria, fungi and actinomycetes are important for plastic degradation, although larger organisms can play a part (2,14). Plastics may be degraded by microorganisms with a biophysical effect in which cell growth causes mechanical damage. Likewise, there can be biochemical effects in which substances from the microorganisms can degrade polymers. Finally, direct enzymatic action is possible, in which enzymes produced from the microorganisms attack components of the plastic (15).

We prepared degradable PE films containing oxidized potato starch and these films showed improved mechanical strengths compared with the films containing native potato starch in the previous study (16). In this study, we evaluated and compared the biodegradability of PE films containing oxidized potato starch or native potato starch through pure culture assay using *Streptomyces viridosporus* T7A.

MATERIALS AND METHODS

Preparation of oxidized starch/PE films

The films were composed of different percentages of LLDPE, oxidized potato starch and prooxidant (Table 1). Prooxidant (IR1025, Novon International, INC., NY, USA) contained native starch (10%), unsaturates (8.0%), and transition metal compounds (0.2%) in LLDPE. The oxidized potato starches and the cast films were prepared by Lee et al.'s method (16).

Microorganisms

Lignocellulose-degrading *Streptomyces viridosporus* T7A (ATCC 39115) was used for pure-culture biodegradation assay. Microorganisms were maintained on agar slants at 4°C.

Table 1. Composition of the oxidized potato starch/PE films per kilogram

Type of film	Oxidant concentration[1] (%)	Starch content (g)	Prooxidant[2] (g)	PE[3] (g)
5%-Native/PE	0	50	50	900
5%-0.1 OX/PE	0.1	50	50	900
5%-1.0 OX/PE	1.0	50	50	900
5%-1.5 OX/PE	1.5	50	50	900
5%-2.0 OX/PE	2.0	50	50	900
10%-Native/PE	0	100	50	850
10%-0.1 OX/PE	0.1	100	50	850
10%-1.0 OX/PE	1.0	100	50	850
10%-1.5 OX/PE	1.5	100	50	850
10%-2.0 OX/PE	2.0	100	50	850

[1]% active Cl/g starch
[2]Prooxidant contained native starch (10%), unsaturates (8.0%), and transition metal compounds (0.2%) in LLDPE.
[3]polyethylene

Pure-culture biodegradation assay with *S. viridosporus* T7A

Films were cut into strips (2.5×10 cm) in the machine direction and chemically disinfected using the method developed by Kim and Pometto III (17). A chemically disinfected strip was aseptically added to 100 mL of sterile culture medium containing 0.6% (w/v) yeast extract medium plus mineral salts solution (18). The culture flask containing films inoculated with culture spores was incubated by shaking at 100 rpm at 37°C for eight weeks. The control was also prepared by the same procedures except for inoculation with *S. viridosporus* T7A. After incubation, films were washed in 70% ethanol for 30 min and dried at 45°C for 15 hr. The hydroxyl indices by Fourier-Transform Infrared (FT-IR) Spectroscopy, mechanical properties by Instron and film morphology by scanning electron microscope (SEM) were measured for determination of biodegradability of the films.

FT-IR spectroscopy

The hydroxyl index was measured using a FT-IR spectrometer (IFS 120 HR, Bruker, Germany). The hydroxl index is the ratio of the area of the hydroxyl region (871~1190 cm^{-1}) for the area of methylene region (1471~1485 cm^{-1}) (19).

Tensile strength of films

Changes in tensile strength of the films were determined using an Instron (AGS-500A, Shimadzu, Japan). The films were equilibrated to 50% relative humidity for at least 40 h prior to testing (20). Crosshead speed was 100 mm/min and load cell was 50 kg.

Film morphology

Film surface was examined using a SEM (Hitachi S-4200, Japan) with×2,000 after incubation.

Data analysis

The data obtained from the experiments was analyzed by PC-SAS program (version 6.12) using analysis of variance (21). Values with $p<0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Hydroxyl index of films

There are various methods for the measurement of biodegradability of polymers. The most widely applicable methods are the measurement of changes in mechanical properties and chemical compositions of polymers during biodegradation (22-26). Hydroxyl, carbonyl and carboxyl groups were detected by FT-IR during the PE degradation (24-26). The amount of these functional groups, therefore, can be used to determine oxidative degradation degree. Changes of the hydroxyl indices on the films are presented in Fig. 1 and Fig. 2. The hydroxyl index of the films containing 5% oxi-

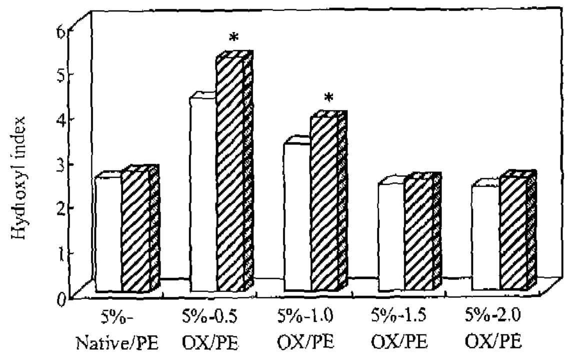

Fig. 1. Hydroxyl index of oxidized potato starch (5%)/PE films incubated with *Streptomyces viridosporus* T7A for eight weeks. □, Control ; ▨, Incubated with *S. viridosporus* T7A. Each value represents the mean for four replicates. *Film inoculated with *S. viridosporus* T7A is significantly different from the control film at $p<0.05$.

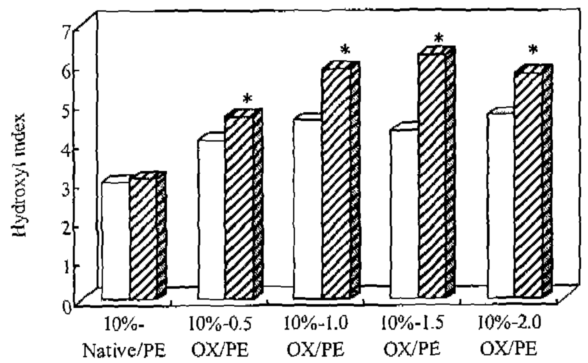

Fig. 2. Hydroxyl index of oxidized potato starch (10%)/PE films incubated with *Streptomyces viridosporus* T7A for eight weeks. □, Control ; ▨, Incubated with *S. viridosporus* T7A. Each value represents the mean for four replicates. *Film inoculated with *S. viridosporus* T7A is significantly different from the control film at $p<0.05$.

dized potato starch (5%-0.5 OX/PE, 5%-1.0 OX/PE, 5%-1.5 OX/PE, and 5%-2.0 OX/PE) incubated with *S. viridosporus* T7A was higher than that of the corresponding control (Fig. 1). In particular, hydroxyl indices of 5%-0.5 OX/PE and 5%-1.0 OX/PE inoculated with *S. viridosporus* T7A remarkably increased when compared with the each control film. However, there was not a significant difference between the hydroxyl index of the inoculated 5%-Native/PE film and that of the uninoculated control 5%-Native/PE film. The hydroxyl index difference between the inoculated 10%-Native/PE films and uninoculated 10%-Native/PE films was not significant, whereas increases of hydroxyl indices were observed on films containing 10% oxidized potato starch after incubation with *S. viridosporus* T7A compared to their corresponding control films (Fig. 2). These results indicate that the oxidized starch/PE films biodegraded faster than native starch/PE films. Oxidized starch has many more hydroxyl and carbonyl groups than native starch. These hydrophilic groups can help enzymatic access to films. Moreover, hydroxyl and carbonyl groups could take part in the oxidative degradation of the films (26), which could synergistically increase the biodegradation of the films.

Tensile strength of films

Tensile strength means the stress at a fracture of the specimen. Tensile strength is weakened when the film structure is destroyed by biodegradation. Changes in the tensile strength of the films after incubation for 8 weeks are shown in Fig. 3 and Fig. 4. There is no significant change in comparison of tensile strengths between the inoculated native film and the uninoculated native film. However, all films containing 5% and 10% oxidized starch showed a decrease in tensile strength of the films after incubation with *S. viridosporus* T7A culture when the corresponding uninoculated film was compared with it. Significant reductions in tensile strength were represented in 5%-0.5 OX/PE and 5%-1.0 OX/PE. This result is consistent with the change in hydroxyl index of films after incubation. *S. viridosporus* T7A is a lignin-degrading

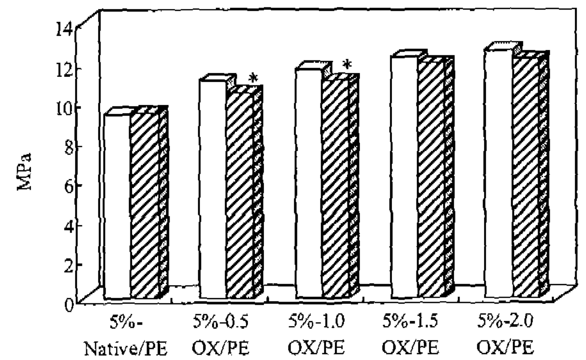

Fig. 3. Tensile strength of oxidized potato starch (5%)/PE films incubated with *Streptomyces viridosporus* T7A for eight weeks. □, Control ; ▨, Incubated with *S. viridosporus* T7A. Each value represents the mean for five replicates. *Film inoculated with *S. viridosporus* T7A is significantly different from the control film at $p<0.05$.

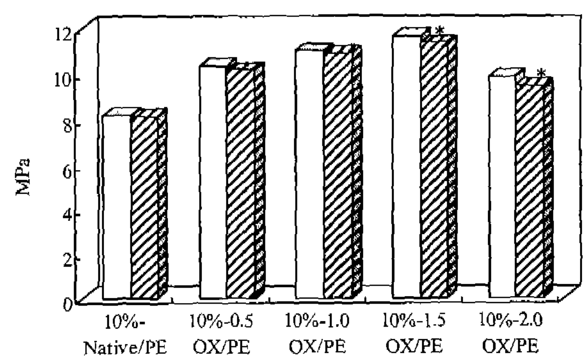

Fig. 4. Tensile strength of oxidized potato starch (10%)/PE films incubated with *Streptomyces viridosporus* T7A for eight weeks. □, Control ; ▨, Incubated with *S. viridosporus* T7A. Each value represents the mean for five replicates. *Film inoculated with *S. viridosporus* T7A is significantly different from the control film at $p<0.05$.

Streptomyces and can also degrade starch by producing amylase. Therefore, it is suggested that *S. viridosporus* T7A would degrade starch in the films and the matrix of the films would be destroyed, which could cause the reduction of the mechanical strength of the films. Additionally, the ability of microorganisms to adhere to the surface of insoluble substrates is critical for their survival in the environment because of intense competition for limited resources (14). Therefore, the degree of biodegradation depends on the easiness of microbial attack to the plastics. The hydrophilic groups of the films are susceptible to microbial access. Because the oxidized starch has more hydrophilic groups such as hydroxyl, carbonyl and carboxyl groups than native starch (16), degradation of films containing oxidized starch could be accelerated.

Sometimes a high level of bacterial attachment to the films was attributed to an inhibition factor of the biodegradation (24). Large amounts of cell mass accumulated on the film surface could lead to the reduction in oxygen tension, which causes a corresponding reduction in chemical oxidative degradation as well as biodegradation. In this study, because the flasks containing the films inoculated with *S. viridosporus* T7A were incubated by shaking in the incubator, cell mass accumulation on the films was not extensive.

Film morphology

Film surface examined using a SEM is presented in Fig. 5. Biodegradation of the films can be visualized on SEM micrographs. (A) is the control film and (B) is the film cultured with *S. viridosporus* T7A. In the film incubated with *S. viridosporus* T7A, partial destruction of starch and PE was examined. Destruction or removal of starch granules from the films could cause the weakness of the film strength. It supports the results obtained from the FT-IR spectroscopy and Instron test.

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(A) (B)

Fig. 5. Scanning electron micrograph (×2,000) of oxidized potato starch/PE films (5%-2.0 OX/PE) incubated with *S. viridosporus* T7A for eight weeks. (A), Control ; (B), Inoculation with *S. viridosporus* T7A.

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