Biodegradability of Scott-Gelead photodegradable polyethylene and polyethylene wax by microorganisms

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SUMMARY: This paper describes the biodegradability of photodegraded polyethylene (PE) and commercial PEwax and molecular size limits of PE for biodegradation. Photodegradation was accelerated by Scott-Gelead System. Photofragmented PE and commercial PEwax were used as a sole carbon and energy source for soil microorganisms, respectively. Biodegradation by microbial consortia was confirmed by the increase of viable cell numbers and GPC analysis of PE added. Significant weigh decrease of PE samples added was also observed. Both photofragmented PE and PEwax were suggested to be biodegradable up to $\overline{Mw}$ 5,000 or more: $\overline{Mw}$ lower than 500 was degraded by more than 40 %. $\overline{Mw}$ 500-2,000 was by more than 10 %. $\overline{Mw}$ higher than 2,000 was only by a few %. Logarithms of biodegradation ratio (%) and $\overline{Mw}$ were correlated to show linearity and to suggest the biodegradability limit close to $\overline{Mw}$ 10,000.

Introduction

Polyethylene (PE) itself is regarded as a chemically "inert" polymer, due to various factors such as its long degradation time. Early stage of studies on the biodegradation of PE by Albertsson et al[1)] indicated that the biodegradation of PE is affected by different factors: preliminary irradiation from a UV source, presence of photodegradative enhancers,

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morphology and surface area, additives, antioxidants, and molecular weight. By measurement of $^{14}CO_2$ generation they showed that the degradation of PE proceeded very slowly. Scott[2] had already concluded in 1975 that an attack to PE by microorganisms is a secondary process. The first process for degradation of PE is an oxidation process, which reduces the molecular weight of the molecule to the degree required for biodegradation to occur. Based on this theory, he developed the so-called Scott-Gilead process[3] to enhance the oxidation of PE molecules. Potts and his collaborators[4] found that the linear paraffin molecules below 500 or so molecular weight were utilized by several microorganisms. The biodegradable range of PE molecular weights is still under discussion. Recently, Otake et al. reported that a remarkable degradation was observed for low density PE thin films buried under soil for over 32 years[5]. They showed whitening, many small holes on the surface of the whitened part and some change in FT-IR spectra, but no datum on molecular sizes was reported.

In this paper, capsules of PE films enhanced through S-G process were successfully used for the controlled-release of fertilizers. After being exposed outdoors for several years, capsules were collected and washed to remove the fertilizers. Washed capsules which were broken into pieces were used as a sole carbon and energy source by soil microorganisms. The biodegradability of capsules and molecular size limits for PE biodegradation is discussed.

Materials and methods

PE sample. Capsules of PE film used for coating controlled-release fertilizers (Nutiricote^{R}, Chisso Asahi Fertilizer Co., Ltd., Tokyo) contained LDPE, 50%, and talc, 50%. Scott-Gelead System (SGS) (PE including photoactivators: iron acetyl acetonate, 2% and nickel dibutyldithiocarbamate, 0. 2%) was added at the level of 3% by weight to PE coat. They

were exposed outdoors for several years. Then capsules were collected, crashed into pieces, washed to remove fertilizers, dried and finally ground into approximately 80 μm particles. These particles (photodegraded PE: PDPE) were used as a sole carbon and energy source for bacterial growth.

Media and screening. The basal medium for screening was composed of PDPE or PEwax, 0.5 %; ammonium sulfate, 0.1 %; sodium nitrate, 0.1 %; potassium diphosphate, 0.1 %; potassium chloride, 0.1 %; magnesium sulfate, 0.02 %; Plysurf A210G (Dai-ichi Kogyo Chem. Co., Japan), 0.03 %; yeast extract, 0.01 %; and a mixture solution of trace metals, 1 ml/100 ml; pH, 7.0-7.2. A mixture solution of trace metals was composed of $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$, each 0.1 g/100 ml. PE samples except PEwax ($\overline{Mw}$=401) were coagulated by autoclaving and could not be dispersed again in a medium. Therefore, a sterile medium was made as follows: The basal medium except PE was autoclaved at 121℃ for 15 min. and cooled down to below 80℃. Then PE samples were added to a medium and sonicated for 15 min and transferred into a sterile shaking flask in a clean bench. PEwax ($\overline{Mw}$=401) was added to a basal medium, sonicated for 15 min, autoclaved at 121℃ for 15 min and cooled down under a flowing of tap water with stirring to disperse PEwax. Microbial consortia which can grow on a PDPE/PEwax medium were screened by enrichment culture techniques. The growth of consortia were confirmed by colony-forming unit (CFU) on nutrient agar plates.

GPC analysis. Molecular weights of PDPE and PEwax were measured by high-temperature gel-permeation chromatography using PL-GPC210 (POLYMER LABORATORIES LTD.) equipped with PLgel 10 μm MIXED-B (300 X 7.5 mm) X 2, thermostated at 140℃ with *o*-dichlorobenzene as the mobile phase. The molecular masses [number average ($\overline{Mn}$) and mass average ($\overline{Mw}$)] were analyzed against Universal Calibration curve

using the GPC data for the Polystyrene (PS) standards. Each sample injection was analysed against this calibration to produce PE equivalent molecular weight values.

Results and discussion

Bacterial consortia which biodegrade PDPE and PEwax

PE enhanced with SGS is thought to be fragemented through the pathways similar to Norrish reactions as described previously[6]. PE capsules containing 3 % SGS were photofragmented to average $\overline{Mw}$ lower than 10,000, as described in Materials and methods. PDPE particles and commercial PEwax ($\overline{Mw}$=1,290) were used as a sole carbon and energy

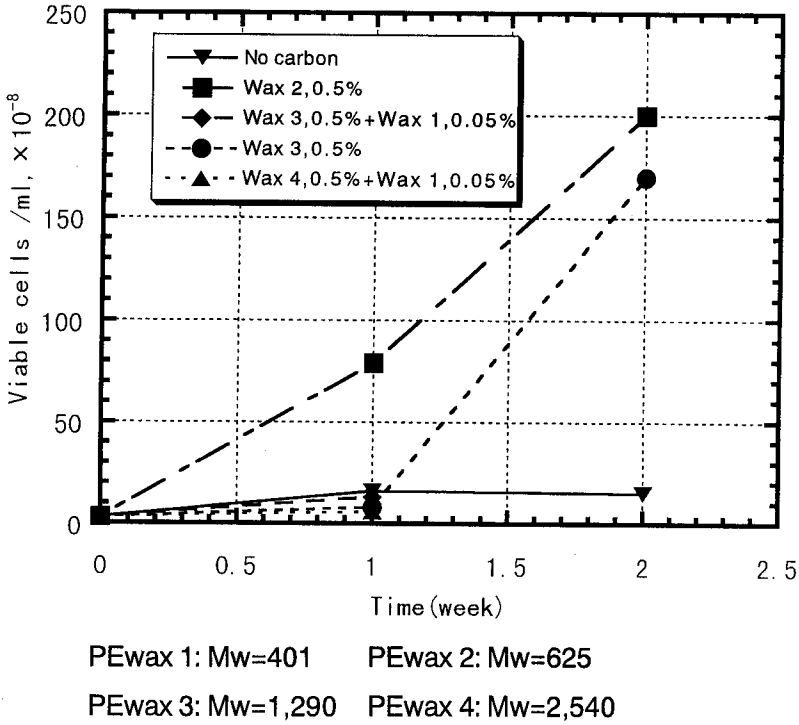
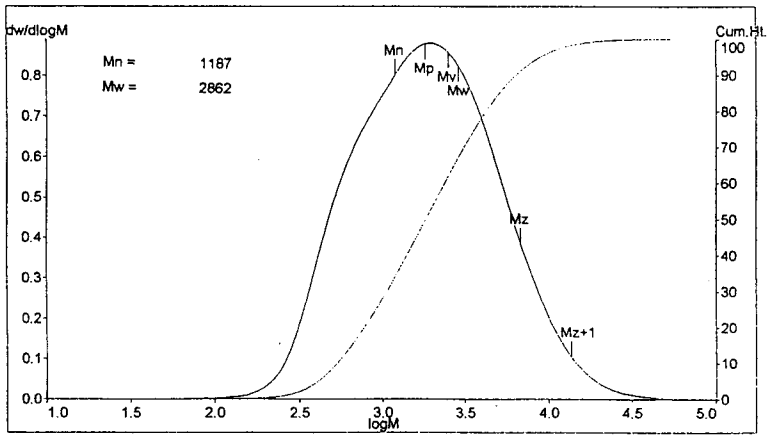
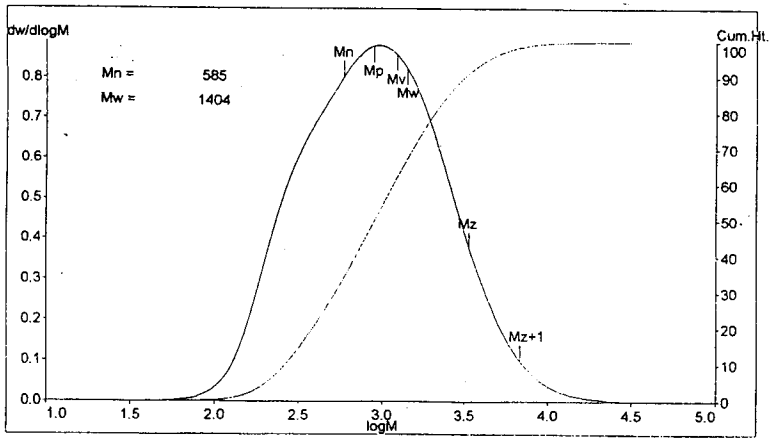


Fig. 1: Growth of consortium KH12 on commercial PEwaxes.

source for soil microorganisms, respectively. Microbial consortia which can assimilate photofragmented PE or PEwax were screened from 154 field soil samples collected from every parts of Japan. Consequently, several consortia which can grow on PDPF and PEwax were obtained. As they could not grow on an added surfactant, Plysurf A210G, 0.03-0.1 % (data not shown), they were referred to as PE-utilizing consortia. Every consortia were



PS equivalent molecular weight



PE equivalent molecular weight

Fig. 2: Calibration of molecular weights by GPC

composed of three or four major strains. The growth of a consortium referred as KH 12 on commercial PEwax was tested (Fig. 1): The consortium grew on PEwax 1-3 ($\overline{Mw}$=401, 625 and 1,290, respectively), but not on PEwax 4 ($\overline{Mw}$=2,540). Differences seems to be derived from different contents of low $\overline{Mw}$ fractions.

GPC calibration of molecular weights of PDPE and PEwax

As an absolute molecular weight can not be measured for $\overline{Mw}$ lower than approximately 30,000, $\overline{Mw}$ and $\overline{Mn}$ were analysed against Universal Calibration using GPC data for PS standards, as described in Materials and methods. PE equivalent $\overline{Mw}$ and $\overline{Mn}$ values were approximately a half of PS equivalent $\overline{Mw}$ and $\overline{Mn}$ values, respectively (Fig. 2). Thus, biodegradability limit was absolutely dependent on the calibration. We have employed PE equivalent Mw values throughout this study. We should be very careful about the measurement and calibration to compare Mw and $\overline{Mn}$ limits for biodegradation.

Measurement of biodegradation rate of PDPE and PEwax

PE particles were recovered from a medium by filtration with a membrane filter (pore size of 10 μm) (after filtration). PEwax powder was referred to as a before filtration sample. The loss or change of molecular distribution before and after filtration was examined, as shown in Fig. 3. No loss or change was admitted at all, PE seemed to be completely recovered from a medium. Accordingly, the measurement of $\overline{Mw}$ was performed with PE particles recovered by filtration from a medium.

Weight loss of PEwax 3 recovered after cultivation was approximately 30-50% in 18-20 days. These values were beyond error and suggested the consumption of PEwax accompanied

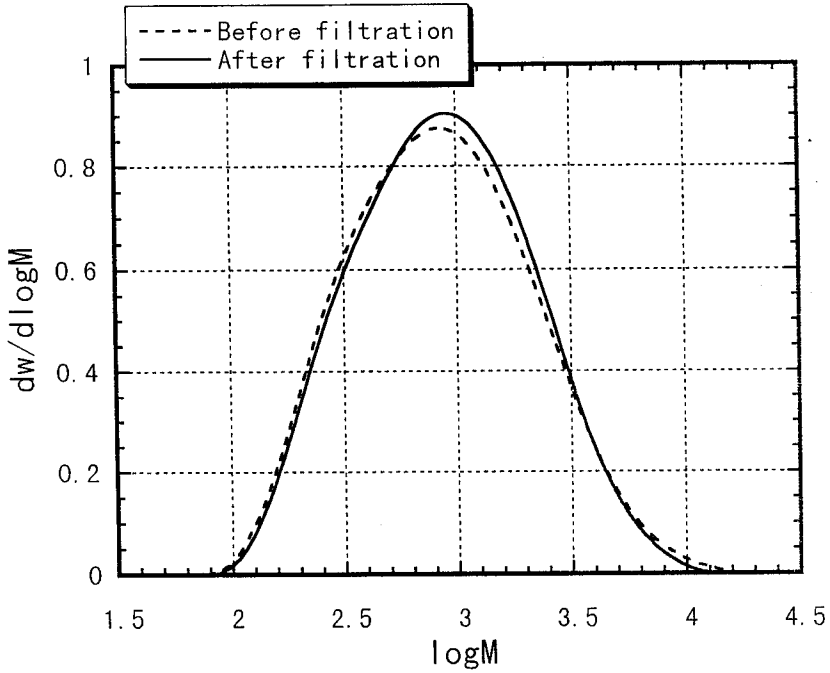
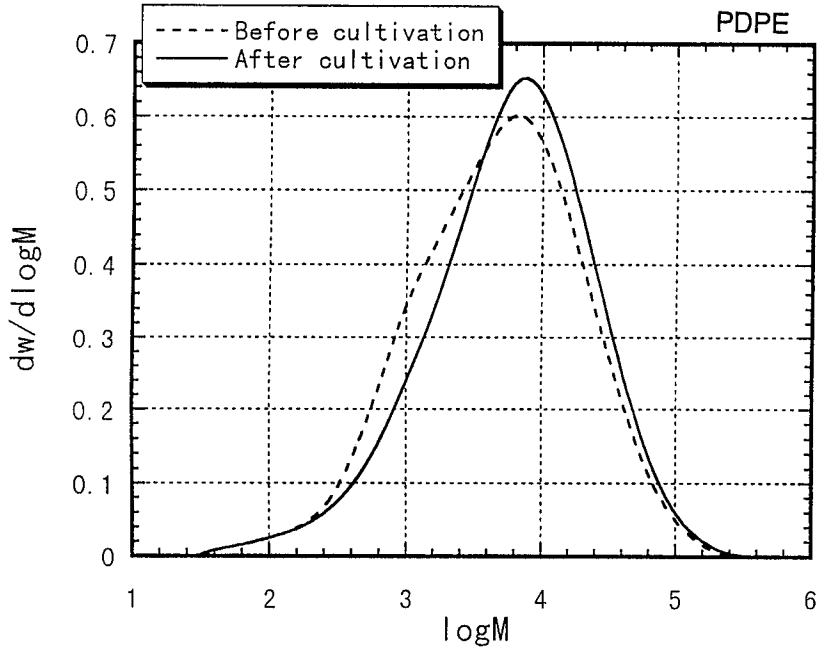


Fig.3: Molecular distribution of PEwax 3 before and after filtration.

with increase of CFU. Recovered particles were dissolved in o-dichlorobenzene at 140℃ and analyzed by GPC as described above. The $\overline{Mws}$ of PDPE and PEwax 3 were compared before and after cultivation, as shown in Figs. 4- a) and 4-b). The $\overline{Mws}$ of PDPE and PEwax were shifted to higher after cultivation, suggesting the fast consumption of the low $\overline{Mws}$. From the crossing points of two GPC patterns before and after cultivation, the biodegradation limit for PDPE was calculated to be more than 3,000 and that for PEwax was suggested to be more than 600.

Furthermore, GPC pattern obtained with PEwax 3 was fractioned and quantity of each

a)



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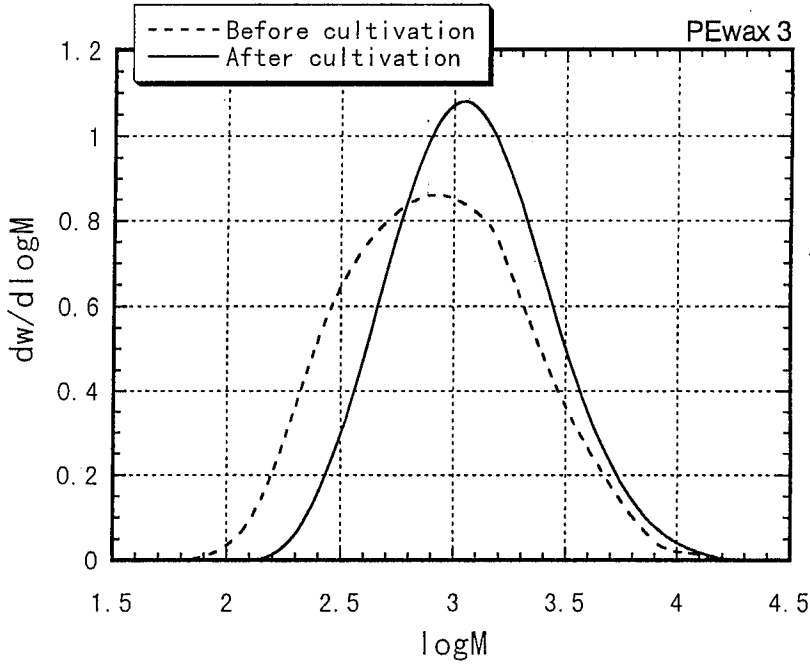

Fig. 4: GPC of PDPE and PEwax 3 before and after cultivation by a consortium KH-12.

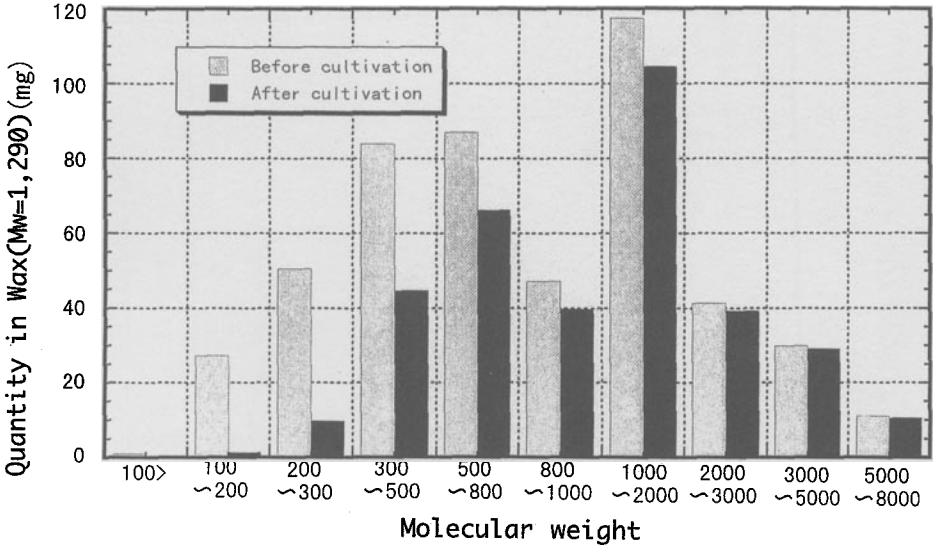

Fig. 5: Comparison of each molecular size fraction before and after cultivation.

fraction was compared before and after cultivation , as shown in Fig 5. Throughout all fractions, some biodegradability was suggested. Correlation between $\overline{Mw}$ and biodegradation ratio was figured, as shown in Fig. 6. Fractions ranging to 500 were consumed approximately by more than 40% and fractions ranging from 500 to 2,000 were suggested to be degraded by more than 10 %. From Figs 6 and 7, $\overline{Mw}$ more than 5,000 seemed to be biodegraded to some extent and appreciable biodegradation rate was expected for $\overline{Mw}$ lower than 2,000. In addition, the logarithm of the biodegradation ratio (%) was plotted against the logarithm of $\overline{Mw}$, as shown in Fig 7. Linearity was observed. From Fig. 7, biodegradation is expected up to very close to $\overline{Mw}$ 10,000. As PDPE is expected to be biodegraded with $\overline{Mw}$ more than 3,000 and PEwax was biodegraded ranging up to $\overline{Mw}$ more than 5,000, we can conclude

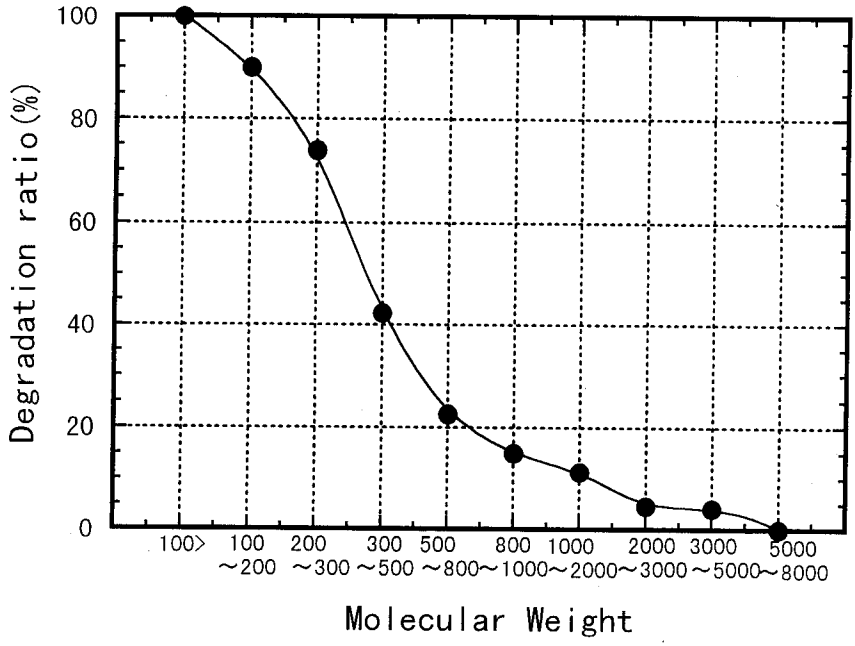

Fig. 6: Biodegradation ratio of each molecular size fraction

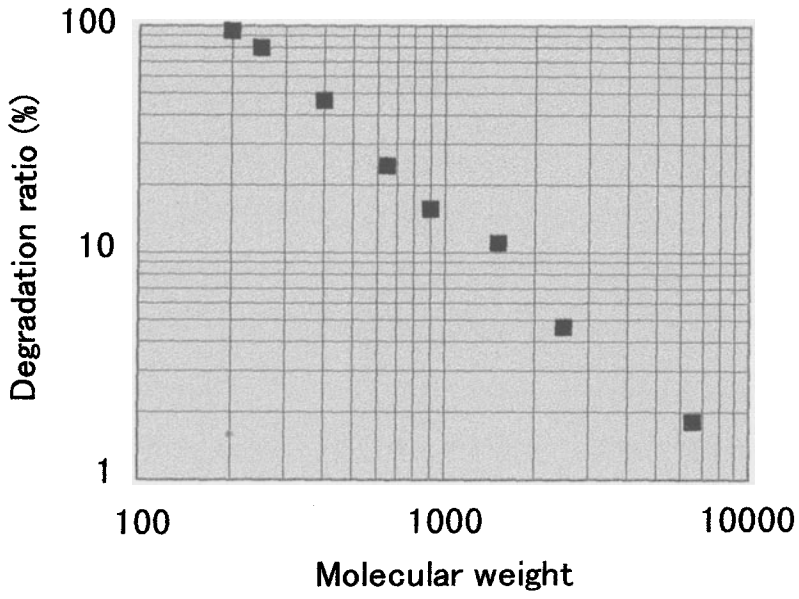

Fig. 7: Correlation between logarithms of $\overline{Mw}$ and biodegradation ratio

that PE is more biodegradable than expected so far.

Conclusion

PDPE-degradative microbial consortia were obtained from normal field soils in Japan. Each consortium was composed of three to four bacterial species with other minor contaminants. They could not grow on 0.03-0.1 % of a surfactant, but grew on PDPE or PEwax. They could show good growth on PEwax ranging from $\overline{Mw}$ 401 to $\overline{Mw}$ 1,290, but showed very poor growth on PEwax of $\overline{Mw}$ 2,540. Growth seemed to be dependent on the content of degradable low $\overline{Mw}$ PE in each samples. The weight loss of PEwax after cultivation reached approximately 30-50%. Standard conditions for calibration of PE with $\overline{Mw}$ lower than 10,000 were settled by GPC with o-dichlorobenzene as solvent at 140℃ using Universal Calibration method. PE equivalent $\overline{Mw}$ values were approximately a half of PS equivalent $\overline{Mw}$ values, indicating that biodegradability can be evaluated with its measurement. PE value was employed throughout this study. By PE equivalent $\overline{Mw}$ measurement, biodegradability of PE with $\overline{Mw}$ higher than 5,000 was suggested by consortia, $\overline{Mw}$ below 2,000 being the optimal biodegradation limit.

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