

A biodegradable rubber by crosslinking poly(hydroxyalkanoate) from *Pseudomonas oleovorans*

G. J. M. de Koning*†, H. M. M. van Bilsen and P. J. Lemstra

Centre for Polymers and Composites (CPC), Eindhoven University of Technology,
PO Box 513, 5600 MB Eindhoven, The Netherlands

and W. Hazenberg†, B. Witholt†, H. Preusting and J. G. van der Galiën

Groningen Biotechnology Centre, Department of Biochemistry, University of Groningen,
Nijenborgh 16, 9747 AG Groningen, The Netherlands

and A. Schirmer and D. Jendrossek

Institut für Mikrobiologie, Georg-August-Universität, Grisebachstrasse 8,
37077 Göttingen, Germany

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Poly(*R*)-3-hydroxyalkanoate)s (PHAs) are bacterial storage polyesters, currently receiving much attention because of their potential application as biodegradable and biocompatible plastics. Among them are the PHAs from *Pseudomonas oleovorans*, which are semicrystalline elastomers. Their applicability is seriously limited by their low melting temperature as well as by their low crystallization rate. Both problems were overcome by crosslinking of unsaturated pendent groups, which were incorporated in the polymer by tailoring the carbon source for biosynthesis. Crosslinking was established by electron-beam irradiation and resulted in a true rubber with constant properties over a large temperature range from -20 to $+170^{\circ}\text{C}$. Even after crosslinking, the material was still biodegradable. To our knowledge this is the first microbially produced biodegradable rubber.

(Keywords: biopolymer; biodegradation; poly(hydroxyalkanoate))

INTRODUCTION

Poly(*R*)-3-hydroxyalkanoate)s (PHAs)¹⁻³ are biopolyesters accumulated by a wide variety of bacteria as a reserve of carbon and energy. The monomer composition of a PHA very much depends on the micro-organism as well as on the substrate used for its accumulation^{4,5}, giving rise to a variety of PHAs. Extensively studied are poly(3-hydroxybutyrate) (PHB) and its copolymers with 3-hydroxyvalerate (HV)¹⁻³, which have been commercialized under the trademark Biopol™ (Zeneca BioProducts). In addition to these relatively rigid materials, recent studies⁶⁻⁸ report the existence of elastomeric PHAs: when grown on *n*-alkane⁹⁻¹¹, *n*-alkanoate^{5,12,13} or *n*-alkanol⁴, *Pseudomonas oleovorans* accumulates a random³ copolymer containing as a major component a 3-hydroxyalkanoate unit with a carbon chain length equivalent to that of the growth substrate. Other monomer units have either one excess or one or more deficit C₂ pairs. Since units smaller than 3-hydroxyhexanoate are hardly detected and units larger than C₁₄ have not been reported, these polymers are referred to

as medium-chain-length PHAs (MCL-PHAs)¹⁴. Interestingly, during growth of *P. oleovorans* on mixtures of *n*-octane and 1-octene, a polymer is synthesized containing unsaturated and saturated pendent groups in a ratio up to 54:46^{10,15}. Recent findings^{3,16,17} demonstrate that several other functionalities can be introduced in the pendent chains as well.

In contrast to many products nowadays, which are touted to be biodegradable, PHAs are genuinely biodegradable¹⁸⁻²⁰, i.e. they can be completely degraded and assimilated into harmless, naturally occurring molecules. The degradation of extracellular PHB has been studied in detail: PHB-degrading bacteria secrete specific PHB-depolymerases^{3,21-30} that hydrolyse the polymer to 3-hydroxybutyrate or oligomeric esters. These water-soluble degradation products are then absorbed by the bacteria and metabolized as nutrients. Recent studies also describe the isolation of bacteria that secrete specific poly(3-hydroxyvalerate) (PHV)³¹ or poly(3-hydroxyoctanoate)³² depolymerases.

Owing to their natural origin, PHAs have an exceptional stereochemical regularity. The chains are completely linear and the chiral centres possess only the *R* stereochemical configuration^{9,10}, which implies that these polymers are fully isotactic. This allows MCL-PHAs to achieve some crystallinity¹¹, which can be as

* To whom correspondence should be addressed (at following present address)

† Present address: Institut für Biotechnologie, Eidgenössische Technische Hochschule, Hönggerberg, CH 8093 Zürich, Switzerland

high as 25%⁶. Showing a glass transition temperature well below room temperature¹¹ and with the crystals acting as physical crosslinks, MCL-PHAs consequently are thermoplastic elastomers^{6,7}. If MCL-PHAs are to be applied as elastomers, two major problems will have to be overcome. First, MCL-PHAs exhibit melting temperatures (T_m) varying from 39 to 61°C^{6,7,11,13}. As a result they soften and lose their coherence at a temperature as low as 40°C. The second problem concerns the low rate of crystallization⁷, which gives rise to a crystallization time of several days. This seriously limits the practicability of many processing techniques.

The present study aims to overcome both problems by crosslinking a MCL-PHA. Replacing the crystals (physical crosslinks) by chemical crosslinks would extend the rubber plateau to temperatures far above T_m and eliminate the long processing times due to slow crystallization. Because MCL-PHAs are likely to remain specialty plastics for some time, biodegradable food packaging and biomedical applications are the most attractive applications for now. In this context, crosslinking agents like peroxides and vulcanizers should be avoided. Irradiation is an alternative crosslinking route that does not require the use of additives. Upon irradiation of polymer materials, reactive intermediates are generated, including radicals, which can induce various reactions like crosslinking and chain scission³³. It is well established³³ that the presence of unsaturated bonds enhances crosslinking upon irradiation, pointing to the incorporation of unsaturated pendent groups in a MCL-PHA by adding an alkene to the growth medium. Radiation sources used in polymer modifications are electron beam (e.b.), ultra-violet (u.v.) and γ sources. Since u.v. has a low penetration depth and γ radiation has a low intensity, the use of e.b. was preferred in this study. Of course, it remains to be seen whether a crosslinked MCL-PHA is as biodegradable as it was before crosslinking.

EXPERIMENTAL

Materials

All biopolyesters used were accumulated in *Pseudomonas oleovorans* (ATCC 29347) according to the procedure described previously by Preusting *et al.*¹¹. Different ratios of n-octane and 1-octene were used as the carbon source in order to obtain polymers with different degrees of unsaturation¹⁰. The polymer-containing cells from the several batches were freeze dried and stored separately. The lyophilized cells were ground and suspended in chloroform (10 g l⁻¹). The suspension was refluxed for 4 h to dissolve the MCL-PHA, and subsequently filtered at ambient temperature. The filtrate was reduced to about 100 ml by evaporation and filtered again. Subsequently, MCL-PHA was precipitated by adding the filtrate to a 10-fold volume of ethanol. Further purification was established by analogous reprecipitation. The precipitate was used to prepare a solution-cast film approximately 1 mm thick from chloroform. The resulting transparent film was stored under ambient conditions for 6 days to allow for crystallization. In order to remove any residual solvent, the film was evacuated for 1 day. Each film was subjected to the determination of composition, thermal transitions and molecular-weight distribution.

Subsequently, the films were exposed to electron-beam (e.b.) irradiation using a 3 MeV Van de Graaff accelerator at the Interfaculty Reactor Institute (IRI, Delft, The Netherlands). To prevent degradation, irradiation was performed in nitrogen and at a temperature below 30°C. The radiation doses amounted to 30, 50, 100, 200 or 500 kGy, taking into account the thickness of the sample. Radiation doses were calibrated using polyethylene as a reference. Specimens for mechanical analysis were punched from the irradiated films.

Characterization techniques

Composition. The composition of the non-irradiated polymers was determined by ¹H n.m.r. The spectra were recorded with a 400 MHz (Bruker AM400) spectrometer at 25°C using CDCl₃ as a solvent and locking agent. The spectra were obtained using a spectral width of 6042 Hz, a flip angle of 45° and a pulse delay of 5 s. Spectra were obtained after accumulating 32 scans using a sample concentration of 5% (w/v). The spectra of the unsaturated MCL-PHAs showed additional resonances compared to the saturated MCL-PHA spectrum due to the presence of the terminal unsaturated groups¹⁰. Figure 1 shows a typical spectrum of MCL-PHA from *P. oleovorans* grown on 25 vol% 1-octene and 75 vol% n-octane.

Monomer compositions of the polymers both before and after irradiation were determined via methanolysis and subsequent g.l.c. analysis according to the method of Lageveen *et al.*¹⁰ under the conditions described in detail recently³⁴.

Gel fractions were determined from the weight loss when extracting the irradiated films for 24 h with chloroform using a Soxhlet apparatus. In a triplicate measurement, the deviation of this method appeared negligible.

Molar mass. Molecular weights were obtained by gel permeation chromatography (g.p.c.) at 40°C in tetrahydrofuran using a Waters apparatus (pump model 510; injector WISP 711; DR 410 refractometer) equipped with two Shodex (KF80M; 2 × 30 cm) linear columns. The system was calibrated using polystyrene standards with a low polydispersity. Since Mark-Houwink constants of MCL-PHAs are not known, the required correction to obtain absolute molecular weights could not be performed. To get a notion of the resulting error, the absolute number-average molecular weight (M_n) was determined by means of osmometry using a Hewlett-Packard High Speed Osmometer 501 operated at 37°C with toluene as a solvent. From the minor discrepancy between M_n values from g.p.c. analysis and osmometry, it was concluded that the g.p.c. data can be considered to be reliable.

Thermal transitions. Thermograms were recorded using a Perkin-Elmer DSC-7 differential scanning calorimeter at a heating rate of 10°C min⁻¹. Indium was used for temperature and heat-of-fusion calibration. The peak melting temperatures were taken as the melting point (T_m). The glass transition temperature (T_g) was determined using the point of maximum slope.

Mechanical analysis. Dynamic mechanical measurements were performed with a Polymer Laboratories Dynamic Mechanical Analyzer, operated in the tensile mode. The samples (10 × 10 × 1 mm³) were run at a

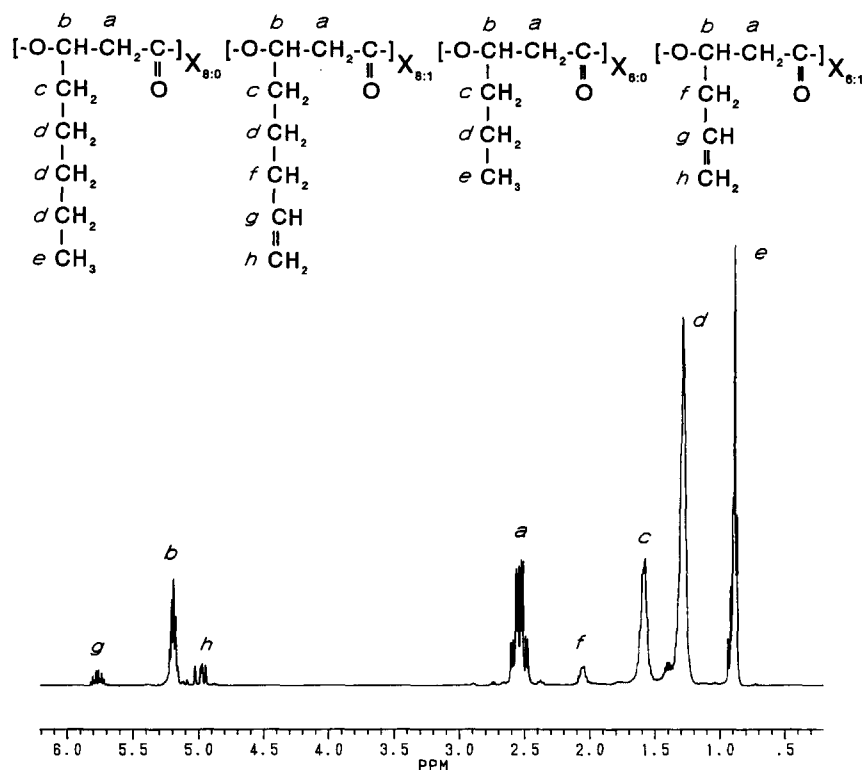


Figure 1 Structural formula and 400 MHz ^1H n.m.r. spectrum of MCL-PHA isolated from *Pseudomonas oleovorans* grown on n-octane/1-octene (75/25)

measuring frequency of 1 Hz, a static force of 0.01 N and a heating rate of $0.5^\circ\text{C min}^{-1}$.

Tensile testing was performed under ambient conditions using a Frank 81565. The specimens were dumbbell-shaped according to ASTM-D 1708, their prismatic part measuring $22 \times 5 \times 1 \text{ mm}^3$. A clamp separation of 24 mm and a crosshead speed of 25 mm min^{-1} were used.

Thermal degradation. The degradation behaviour was investigated using a Perkin-Elmer TGA-7 thermogravimetric analyzer. Measurements were conducted at a heating rate of $10^\circ\text{C min}^{-1}$ in air as well as in a nitrogen atmosphere. In addition, 0.5 g of irradiated material was degraded at 250°C in a sealed tube. The degradation products were analysed by means of g.l.c. analysis using a capillary gas chromatograph (Hewlett-Packard 5790A) equipped with a crosslinked methylsilicone column ($l = 25 \text{ m}$, $d = 0.2 \text{ mm}$, film = $0.32 \mu\text{m}$) and a mass spectrometric detector (HP 5970A, electron impact ionization). Samples were injected by $0.5 \mu\text{l}$ split injection and the column temperature program ranged from 40 to 300°C using a heating rate of $10^\circ\text{C min}^{-1}$.

Biodegradation. Materials tested for biodegradability included the octane-based MCL-PHA and irradiated MCL-PHA from *P. oleovorans* grown on 25 vol% 1-octene and 75 vol% n-octane. The 1 mm thick film was cut into pieces measuring maximally $8 \times 8 \text{ mm}^2$. In addition, thin sections of $2 \times 3 \times 0.02 \text{ mm}^3$ were obtained by ultramicrotomy at -196°C using a Reichert Ultracut E Microtome. To minimize bacterial contamination, the samples were incubated in chloroform for 30 min in sterile 100 ml Erlenmeyer flasks. In order to improve the visibility of the thin sections, the swollen polymer was stained with Sudan red. After evaporation of the chloroform, 10 to 20 ml of sterile mineral medium³⁵ were added and the cultures were inoculated with 5 vol% of an

octanoate-grown seed culture of *Pseudomonas fluorescens* GK13 (DSM 7139). The cultures were incubated at 30°C and shaken on a rotary shaker at 150 rev min^{-1} . If necessary, sterile water was added weekly to balance the evaporation of the fluid. At the end of the experiment, the remaining polymer was rinsed, dried and the weight loss was determined.

To assay the enzymatic degradation by the depolymerase, Sudan red-stained thin sections were embedded in a thin layer of agarose in 100 mM Tris-HCl buffer (pH = 8.5), and $10 \mu\text{l}$ of purified depolymerase from *P. fluorescens* GK13³² were added onto the agarose surface. Furthermore, pieces of both irradiated and non-irradiated material were incubated in 1.5 ml Tris-HCl (pH = 8.5) in the presence of 0.25 mg purified depolymerase at 30°C for 4 days. Subsequently, 0.25 mg purified depolymerase were added and the incubation was prolonged for another 3 days. The depolymerase was precipitated with 235 mM trichloroacetic acid and removed by centrifugation. The soluble degradation products were purified by solid-phase chromatography on a Chromabond $\text{C}_{18\text{ec}}$ column and subjected to mass spectroscopy (direct chemical ionization) in the presence of ammonia³⁶ as described previously³².

Polymer samples were fixed in 3% (w/v) glutaraldehyde for 30 min, rinsed six times carefully in double-distilled water, air-dried on paper and stored dust-free at room temperature. The samples were gold-sputtered in an Emscope SC500 and examined using a SEM microscope (Philips 515).

RESULTS AND DISCUSSION

Materials

MCL-PHAs were isolated from *Pseudomonas oleovorans* after growth on mixtures of n-octane and 1-octene. The

Table 1 Characteristics of MCL-PHAs from *Pseudomonas oleovorans* grown on mixtures of n-octane and 1-octene

Carbon source	Unsaturation (mol%)	Monomer composition ^a (mol%)				M_w (kg mol ⁻¹)	M_n (kg mol ⁻¹)	T_m (°C)	ΔH_m (J g ⁻¹)	T_g (°C)	ΔC_p (J °C ⁻¹ g ⁻¹)
		X _{6:0}	X _{6:1}	X _{8:0}	X _{8:1}						
100% octane	0	10	0	90	0	194	126	61	23	-29	0.21
95% octane 5% octene	4±0.3	13	~0	83	4	223	123	55	11	-27	0.33
75% octane 25% octene	15±0.6	9	3	75	12	255	109	-	0	-30	0.42

^aX_{6:0} = 3-hydroxyhexanoate, X_{6:1} = 3-hydroxyhexenoate, X_{8:0} = 3-hydroxyoctanoate, X_{8:1} = 3-hydroxyoctenoate

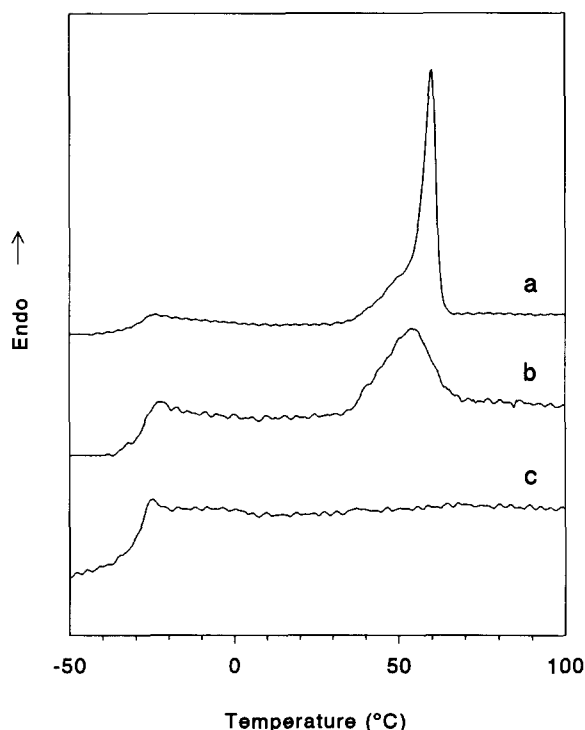


Figure 2 Thermograms of MCL-PHA containing (a) 0 mol%, (b) 4 mol% and (c) 15 mol% unsaturated monomer units

composition (¹H n.m.r.) and characteristics of the products are presented in Table 1. Table 1 and Figure 2 clearly show that the incorporation of unsaturated monomer units gave rise to a decrease in melt endotherm area (ΔH_m) and an increase in relative heat capacity (ΔC_p), which can be attributed to a reduction of the crystallinity¹¹. Obviously, MCL-PHA containing 15 mol% unsaturated monomer does not crystallize at all. At room temperature, this is an amorphous polymer well above its glass transition temperature (T_g) and consequently the material had no strength and is very sticky.

Effects of unsaturation upon irradiation

Upon e.b. irradiation of octane-based MCL-PHA no gel formation was observed. G.p.c. analysis demonstrated a broadening of the molecular-weight distribution at the low-molecular-weight side, which became more pronounced at higher radiation doses (Figure 3). These results demonstrate that, upon e.b. irradiation of saturated MCL-PHA, merely chain scission and no significant crosslinking occurred. However, after irradiation of the amorphous MCL-PHA containing 15 mol% unsaturated monomer, the surface did not feel sticky any more,

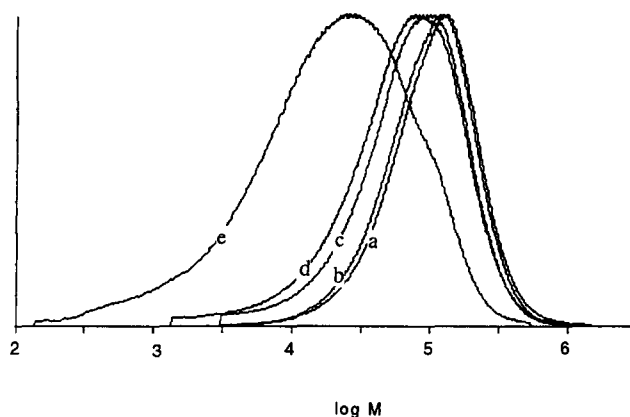


Figure 3 Molecular weight distributions of octane-based MCL-PHA after exposure to an e.b.-radiation dose of (a) 0 kGy, (b) 10 kGy, (c) 50 kGy, (d) 100 kGy and (e) 500 kGy

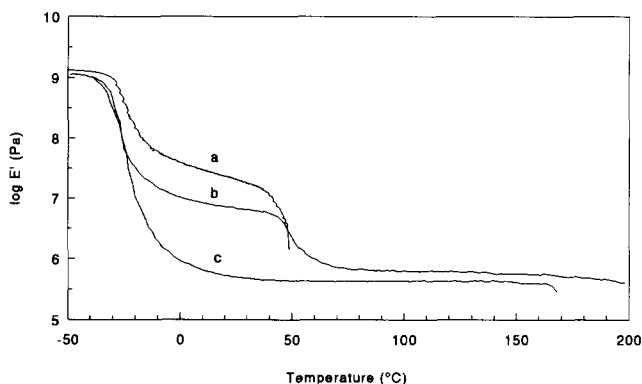


Figure 4 Dynamic modulus versus temperature for (a) octane-based MCL-PHA, (b) MCL-PHA containing 4 mol% unsaturated monomer and e.b.-irradiated with 50 kGy and (c) MCL-PHA containing 15 mol% unsaturated monomer and e.b. irradiated with 50 kGy

indicating that crosslinking had taken place. This was confirmed by Soxhlet extraction. After exposure to a radiation dose of 50 kGy, the MCL-PHAs containing 4 and 15 mol% unsaturated monomer possessed gel fractions of 88 and 93 wt% respectively. Obviously, the presence of double bonds enhanced crosslinking.

Figure 4 presents the results from dynamic mechanical analysis. It clearly shows the drawbacks of octane-based MCL-PHA (curve a). First, this material exhibits a large variation in modulus around ambient temperature. Secondly, it softens already at 40°C due to melting. Since the irradiated MCL-PHA with 4 mol% unsaturated monomer units contains some crystallinity as well, a similar drop in modulus was observed at 40°C, but now

the material retained some strength owing to the presence of chemical crosslinks. As shown above, the introduction of 15% unsaturated monomer appeared to be sufficient to yield a non-crystallizable polymer. Crosslinking of this material resulted in a true rubber possessing chemical crosslinks only and showing constant properties over a large temperature range from T_g to 170°C.

Figure 5 shows the corresponding stress-strain curves at ambient conditions. The relatively small maximum strain of curve b was caused by the presence of defects in the film. Obviously, the modulus decreased with the degree of unsaturation owing to the loss of the ability to crystallize. Notably, only the non-crystalline MCL-PHA returned to its original dimensions after failure in accordance with the behaviour expected for a true rubber.

Effects of radiation dose on crosslink density

Importantly, the non-crystalline crosslinked MCL-PHA showed a constant rubber modulus over a large temperature range (Figure 4). It was anticipated that this plateau modulus, which depends on the crosslink density, could be controlled, as the extent of crosslinking usually increases with the radiation dose³³. Accordingly, Table 2 shows that the gel fraction increased with the radiation dose and T_g rose 15°C, indicative of an increase in crosslink density. The increase in T_g was observed in both calorimetric and dynamic mechanical analyses (Figure 6), and is due to the fact that crosslinking generally tends to reduce the segmental mobility of a polymer.

The increase in crosslink density could also be concluded from dynamic mechanical analysis (Figure 6) and tensile testing (Figure 7). Both techniques demonstrated a similar rise in modulus associated with an increasing radiation dose. The average molecular weight

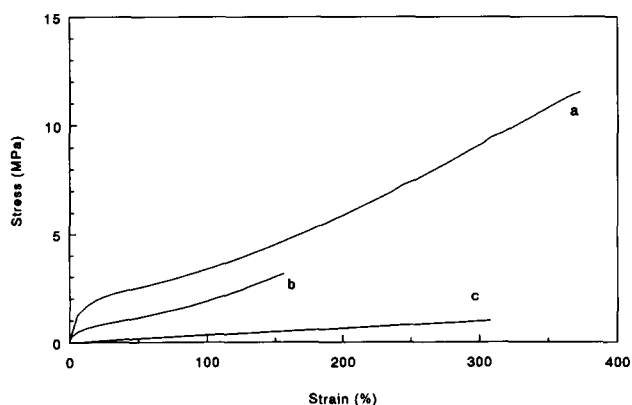


Figure 5 Stress-strain curves under ambient conditions for (a) octane-based MCL-PHA, (b) MCL-PHA containing 4 mol% unsaturated monomer and e.b. irradiated with 50 kGy and (c) MCL-PHA containing 15 mol% unsaturated monomer and e.b. irradiated with 50 kGy

Table 2 Characteristics of MCL-PHAs containing 15% unsaturated monomer as a function of the e.b. radiation dose

E.b. dose (kGy)	T_g (°C)	Gel fraction (%)	E_{pl} (MPa)	M_c (kg mol ⁻¹)
0	-30	0		
30	-27	90	0.15	60
50	-27	93	0.43	20
100	-26	98	1.2	8
200	-22	99	2.5	3
500	-15	99	12.2	0.7

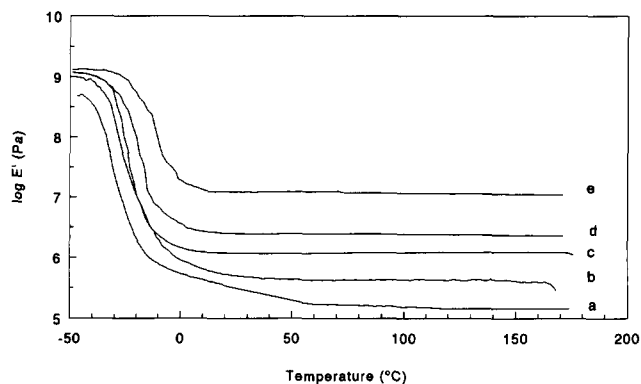


Figure 6 Dynamic modulus versus temperature for MCL-PHA containing 15 mol% unsaturated monomer after an e.b. radiation dose of (a) 30 kGy, (b) 50 kGy, (c) 100 kGy, (d) 200 kGy and (e) 500 kGy

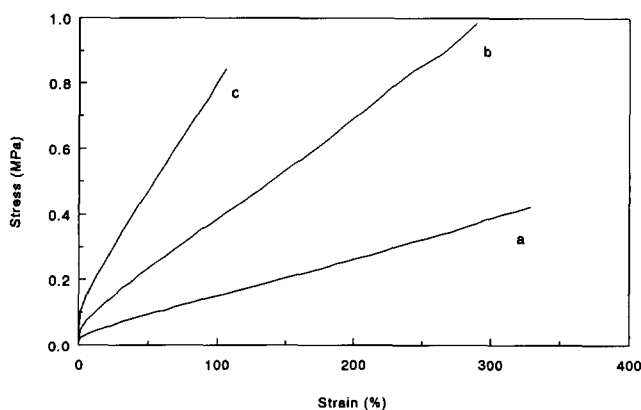


Figure 7 Stress-strain curves under ambient conditions for MCL-PHA containing 15 mol% unsaturations after an e.b. radiation dose of (a) 30 kGy, (b) 50 kGy and (c) 100 kGy

between two crosslinks (M_c) was estimated from the plateau modulus (E_{pl}) according to the following equation:

$$M_c = \frac{3\rho RT}{E_{pl}}$$

where $\rho = 10^3 \text{ kg m}^{-3}$, $R = 8.31 \text{ J mol}^{-1} \text{ K}^{-1}$ and $T =$ temperature (K).

Upon increasing the dose to 500 kGy, M_c decreased to approximately 700 g mol^{-1} (Table 2), which corresponds with one crosslink every five monomer units. Notably, this is somewhat higher than the original concentration of double bonds. As is common behaviour for rubbers, tensile testing (Figure 7) showed a considerable reduction of the tear resistance concomitant with an increasing crosslink density. At high radiation doses, tensile testing could not even be performed owing to failure of the material in the clamp.

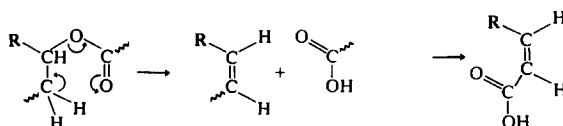
Thermal degradation

Figures 4 and 6 show that the rubber plateau of the crosslinked materials ranges up to 170°C, which temperature is associated with decomposition. Similar results were obtained in the presence of air and of nitrogen, which indicates that thermal degradation is non-oxidative. As main degradation products, 2-hexenoic acid and 2-octenoic acid were detected by g.l.c. analysis, in a ratio that corresponded with the composition of the polymer. Other degradation products include dimer, 2-decenoic acid and 7-octenoic acid from the obvious

Table 3 Relative weight loss (wt%) of PHA in cultures incubated with *Pseudomonas fluorescens* GK13 at 30°C for 25 days

Initial polymer concentration (wt%)	Relative weight loss (wt%)			
	PHA (saturated)	PHA (15 mol% unsaturated), 30 kGy e.b.	PHA (15 mol% unsaturated), 100 kGy e.b.	PHA ^a (15 mol% unsaturated), 500 kGy e.b.
0.03	>98	>98	82	6.3
0.06	>98	91	66	4.5
0.10	80	88	64	5.4
0.14	76	97	60	3.6
0.20	86	85	58	3.7
0.30	62	86	45	2.2
0.50	73	74	41	2.1

^a Twenty-five days after inoculating the culture for the second time

**Scheme 1** Thermal degradation

reorganization reaction. The observed degradation behaviour is analogous to that of poly(hydroxybutyrate), which is known to depolymerize into 2-butenic acid³⁷⁻³⁹. The reaction responsible is a β elimination, the significance of which increases with temperature.

The main chain folds to the conformation depicted in *Scheme 1* to enable chain scission. The chain ends are more flexible and, consequently, at high enough temperatures rapid unzipping occurs. Apparently, a temperature of 170°C is sufficient to cause a rapid fall of the molecular weight, giving rise to the loss of coherence.

Biodegradation

Crosslinked MCL-PHA (15 mol% unsaturated monomer units) was exposed to *Pseudomonas fluorescens* GK13, which is known to degrade octane-based MCL-PHA³². Liquid cultures with the crosslinked material as the sole source of carbon and energy were inoculated with *P. fluorescens* and incubated at 30°C for various periods. Initially, the turbidity of the fluid increased and the polymer surface became dim. After a lag period, the rate of degradation was almost constant (*Figure 8*). An increasing crosslink density significantly lengthened the lag period and reduced the rate of degradation, possibly due to a decreasing accessibility of the material. Degradation proceeded to completion within experimental error. Only the most densely crosslinked polymer had such a long lag period that the culture lost viability. When adding some fresh culture after 20 days, biodegradation commenced. This material might well be degraded when being co-fed with a readily accessible carbon source. Hydrolysis of the octane-based MCL-PHA, possessing physical crosslinks only, occurred at a similar rate as for sparsely (chemically) crosslinked material. Control experiments without bacteria demonstrated no weight loss of the materials tested, even after 71 days of incubation.

Raising the initial polymer concentration by incubating a bigger piece of polymer somewhat increased the absolute weight loss, but the relative weight loss decreased (*Table 3*). However, the lower surface/volume

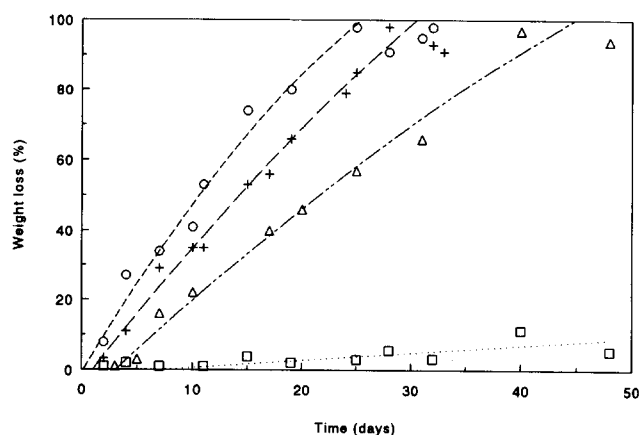


Figure 8 Relative weight loss versus time when incubated with *Pseudomonas fluorescens* GK13 at 30°C (initial polymer concentration 0.10 ± 0.01 wt%) for octane-based MCL-PHA (+) and for MCL-PHA containing 15 mol% unsaturated monomer e.b. irradiated with 30 kGy (O), 100 kGy (Δ) or 500 kGy (□)

ratio of a bigger piece suggests that the amount of degradation attained is largely dependent on the surface area presented to the bacteria. Indeed, when the experiments were performed with Sudan red-stained thin sections, the polymer seemed to hydrolyse rapidly. After 5 days, the polymer was not visible any more and the colour had diffused into the culture medium. Similar results were obtained within 1 day when thin sections were exposed to purified depolymerase.

SEM revealed that the surface of control polymer, which had been incubated in sterile medium for 3 weeks, was rather smooth, whereas the surface of partly degraded polymer was very rough (*Figure 9*). No difference in surface appearance was observed between polymer samples that had been incubated with *P. fluorescens* for 1, 2 or 3 weeks. Cross-sections of the samples demonstrated the presence of holes at the surface only. Polymer surfaces that had been exposed to purified depolymerase showed large spherical holes. These results demonstrate that the polymer is degraded by enzymatic hydrolysis and subsequent surface erosion similar to the degradation of PHB^{29,40}.

A 17 mg polymer sample was partly degraded (24 wt%) by purified depolymerase. When the composition of the remaining material was analysed using gas chromatography, no difference from the original polymer was found. The soluble degradation products were analysed.

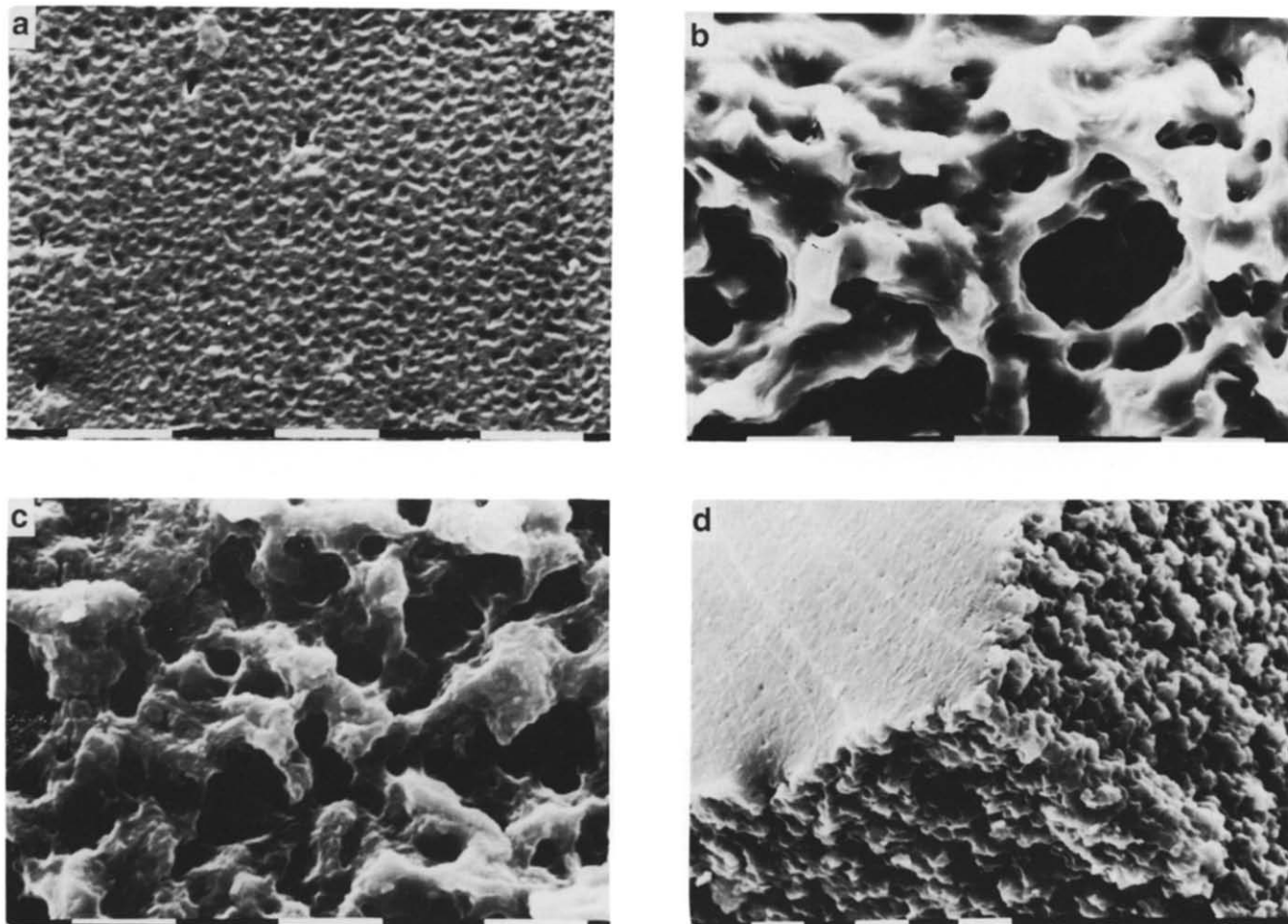


Figure 9 SEM micrographs (scale bar 10 μm) of MCL-PHA containing 15 mol% unsaturated monomer and irradiated with 100 kGy e.b. incubated for 70 days with (a) sterile medium, (b) purified depolymerase or (c), (d) *Pseudomonas fluorescens* GK13 ((c) superficial view, (d) cross-section))

As main products, 3-hydroxyoctanoic acid ($\text{C}_{8:0}$) and the dimeric esters $\text{C}_{8:0}\text{-C}_{8:0}$, $\text{C}_{8:0}\text{-C}_{6:0}$ and $\text{C}_{6:0}\text{-C}_{6:0}$ were identified. In addition, minor amounts of 3-hydroxyhexanoic acid ($\text{C}_{6:0}$), 3-hydroxyhexenoic acid ($\text{C}_{6:1}$) and 3-hydroxyoctenoic acid ($\text{C}_{8:1}$) as well as all related dimers were detected. The same products were found when the unsaturated, but non-crosslinked, polymer was hydrolysed by purified depolymerase. Apparently, the depolymerase is rather unspecific and hydrolyses ester bonds of saturated and unsaturated 3-hydroxy fatty acids consisting of six or eight carbon atoms.

CONCLUSIONS

MCL-PHAs have been classified as thermoplastic elastomers⁶⁻⁸. However, their low T_m and low crystallization rate seriously limit their applicability. This paper shows that these limitations can be overcome by crosslinking using e.b. irradiation. Unsaturated bonds were deliberately introduced by tailoring the carbon source for biosynthesis. These prevent crystallization of the polymer and enhance crosslinking upon irradiation. This process yielded a true rubber with constant properties over a wide temperature range from T_g up to the degradation temperature (170°C). The crosslink density and thus the rubber modulus could be controlled by varying the fraction of unsaturated monomer units and the radiation dose.

The scope of possible applications is directly related to the most attractive feature of PHAs, which derives from their natural origin: biodegradability. Importantly, even after crosslinking the MCL-PHA remained biodegradable. The crosslinked polymer is degraded by enzymatic hydrolysis and subsequent surface erosion similar to the degradation of the crystalline MCL-PHA. Degradation proceeds to completion within experimental error. To the authors' knowledge, this is the first microbially produced biodegradable rubber.

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