Biotechnological Production and Characterization of Polyesters Containing 4-Hydroxyvaleric Acid and Medium-Chain-length Hydroxyalkanoic Acids[†]

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ABSTRACT: A two-stage aerobic fed-batch process for the biotechnological production of polyesters containing 4-hydroxyvaleric acid (4HV) and medium-chain-length hydroxyalkanoic acids by a recombinant strain of *Pseudomonas putida* was developed in mineral salts medium with octanoic and levulinic acid as carbon sources at a 15-L scale. The cells were first grown to high densities on octanoic acid at a pH between 7 and 8 and at a temperature of 30 °C. Accumulation conditions were induced in the second stage by nitrogen starvation at pH 7.0 and at 35 or 37 °C while levulinic acid was continuously supplied. At the end of the accumulation phase, 4HV-containing polyesters, contributing up to 50% (w/w) of the cellular dry weight, were cast into films after extraction with chloroform and precipitation with ethanol, and were spun to fibers. The unprocessed as well as the processed polyesters were characterized with respect to their molecular weight and their thermal, rheological, and mechanical properties. These polyesters showed a distinctly elastomeric behavior resulting from the low content of medium chain-length hydroxyalkanoic acids. The polyester revealed an extremely high elongation at break of approximately 1000%; the molecular weights (M_w) were between 3.3×10^5 and 9.4×10^5 g/mol and decreased during the melt spinning process.

Introduction

Polyhydroxyalkanoic acids (PHA) represent a complex class of polyesters exhibiting a broad spectrum of properties. The large number of available building blocks and combinations in these polyesters provide a wide range of different polyesters for various technical applications.[1] Most of these polyesters are synthesized by many bacteria as storage compounds from renewable resources or precursor substrates as carbon sources if they are provided in excess and if growth of the bacteria is impaired by the lack of at least one other nutrient.[1−3] The properties of PHA can be varied to a great extent by choosing comonomers with different alkyl side chain length, different positions of the hydroxyl group relative to the carboxyl group, or different chemical structures of the alkyl side chain with double bonds, methyl branches, or functional groups. The properties of the resulting polyesters vary with respect to hydrophobicity, resistance to hydrolytic degradation, crystallinity, brittleness, and the elasticity, as well as other properties, without losing biodegradability, which is one of the characteristic features of these polyesters.[4] First examinations focused on the biotechnological production of the homopolyester of 3-hydroxybutyric acid (3HB) as well as copolyesters of 3HB and 3-hydroxyvaleric acid (3HV). Recently, PHA consisting of hydroxyalkanoic acids of medium-chain length (C_6−C_{12}) were analyzed.[5,6] They show elastomeric properties and can be integrated into the class of thermoplastic elastomers which represent one of the most interesting developments in modern material research.

In previous studies, polyesters containing 4-hydroxyvaleric acid (4HV) were synthesized and accumulated by several strains of *Pseudomonas* and *Alcaligenes*[7,8] from 4-hydroxyvaleric acid as carbon source; in these polyesters 4HV contributed up to 30 mol % of the hydroxyalkanoate constituents. Biodegradability of 4HV-containing polyesters has not yet been shown but it can be assumed that these polyesters are biodegradable since this has been shown for other biotechnologically produced polyesters.[4] The bacterial strain employed in this study used a wide range of different substrates and synthesized polyesters with unusual compositions.[9] Since 4-hydroxyvaleric acid is not commercially available, levulinic acid (4-ketovaleric acid) was used as a precursor substrate for the accumulation of 4HV-containing PHA. The aims of this study were to produce such polyesters from levulinic acid and to demonstrate the processing of these polyesters by methods commonly applied in thermoplastic manufacturing; additional goals were to characterize the molecular weight of the polyesters and their thermal, rheological, and mechanical properties and to investigate the spinnability and the fiber properties. The results were related to former investigations of a 3HV-homopolyester,[10] P(3HV), and aimed at the characterization of the influence of 4HV and medium-chain-length hydroxyalkanoates on the properties of the polyester.

Materials and Methods

Bacterial Strains. A recombinant strain of the PHA-negative mutant GpP104 of *Pseudomonas putida*, which harbored the plasmid pHP1014::E156 encoding the PHA synthase from the anoxygenic phototrophic bacterium *Thiocapsa pfennigii*,[9] was used.

[†] This manuscript is dedicated to Professor Jacobasch on the occasion of his 60th birthday.

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Table 1. Composition of 4HV-Containing Polyesters Obtained from Levulinic Acid with the Recombinant Strain of *P. Putida*

sample	octanoic acid (g)	levulinic acid (g)	PHA content % (w/w) of CDW	composition of PHA (mol %) 3HB	3HV	4HV	3HHx	3HO
F-5	75	160	52.3	18.0	52.9	23.9	4.7	0.5
F-6	60	720	28.7	22.9	52.3	17.8	5.9	1.1
F-8/1	300	2000	32.3	10.7	64.5	21.2	3.6	n.d.
F-8/2	300	2000	32.3	13.2	62.1	22.0	2.7	n.d.
F-11	555	1850	32.1	20.0	47.0	16.0	15.0	2.0

Abbreviations: 3HB, 3-hydroxybutyric acid; 3HV, 3-hydroxyvaleric acid; 4HV, 4-hydroxyvaleric acid; 3HHx, 3-hydroxyhexanoic acid; 3HO, 3-hydroxyoctanoic acid; n.d., not detectable. Experimental conditions: growth phase: T 30 °C, pH 8.0, aeration 1 vvm; tetracycline 50 μg/mL; accumulation phase: F-5 and F-6: pH 7.0, T 37 °C, aeration 1 vvm; F-8/1 and F-8/2: pH 6.5, T 35 °C, aeration 1.5 vvm; F-8/1 and F-8/2: products F-8 after 24 or 96 h of extraction, respectively; F-11: similar to F-8, but aeration 0.75 vvm.

Media. A mineral salts medium (MSM) described by Schlegel et al.[11] was used. Stock solutions of all media components, carbon sources octanoic and levulinic acid, and tetracycline were sterilized separately and added to the basic medium as indicated in the text.

Cultivation of *P. putida*. Cultivation of *P. putida* at a 15-L scale was done in a stirred (at 1000 rpm) and aerated (at 15−20 L air/min) 27-L stainless steel fermenter (Apparate- und Behältertechnik Harrislee GmbH, Germany). Fermentations were carried out in MSM containing octanoic acid, which was fed in portions of 1 and 2 g/L (from 60 to 555 g in total) during the growth phase, and levulinic acid (from 160 to 2000 g), which was fed during the following accumulation phase (see Table 1 for details). Solutions of NaOH (2 N), HCl (2 N), or levulinic acid (500 g/L) were used to adjust the pH to the values indicated in the text. Tetracycline (50 μg/mL) was used for plasmid maintenance. (Details of each cultivation are given in Table 1.)

Polyester Analysis. The polyester content of the bacteria was determined by methanolysis of 3−5 mg lyophilized cells in the presence of 15% (v/v) sulfuric acid according to Brandl et al.[12] The resulting methyl esters of the constituent hydroxyalkanoic acids were characterized by gas chromatography according to Brandl et al.[13] and as described in detail by Timm et al.[14]

Isolation of PHA. PHA was isolated from lyophilized cells by extraction with chloroform in a Soxhlet apparatus. The polyester was precipitated from the chloroform solution (concentration of the polyester was approximately 80 g/L) by the addition to 10 vol ethanol. The precipitate was separated from the solvents by filtration. Remaining solvents were removed by exposure of the polyester to a stream of air.

Processing of the Polyesters. The melt spinning experiments were carried out with a self-constructed spinning apparatus, which had a capacity of 10 cm^3. It consists of a piston extrusion equipment with melt filtering and a wind-up device for monofilament. The polyester was melted under a nitrogen atmosphere and was kept at a temperature of 190 °C for 5 min. At this temperature the samples revealed an adequate molding property. The spinning conditions were as follows: 0.4 cm^3/min volume flow rate, 0.3 mm hole diameter, up to 400 m/min spinning velocity, and 2 was the relation of length to diameter.

Characterization of the Polyesters. The water content of the polyester was determined by coulometric titration at 120 °C in a Karl Fischer Coulometer 684 (Deutsche Metrohm GmbH & Co., Filderstadt, Germany).

The thermal stability of the polyester was characterized thermogravimetrically under a nitrogen atmosphere using a TGS 2 thermobalance (Perkin-Elmer, Überlingen, Germany). The weight loss of the samples was measured while the temperature was increased from 20 to 600 °C at a rate of 20 °C/min.

The relative viscosity of the samples was determined for 0.15, 0.3, and 0.5% (w/v) solutions of the polyester in chloroform at 20 °C in a Ubbelohde capillary viscometer (Schott-Geräte GmbH, Hofheim a. Ts., Germany). The intrinsic viscosities (η_{int}) of the unprocessed and the processed polyesters were calculated from the relative viscosities (η_{rel}), according to an empirical relationship of Huggins:[15]

$$\frac{\eta_{rel}{}^{-1}}{c} = \frac{\eta_{sp}}{c} = A_1 + A_2 c, \quad \lim c \to 0, A_1 = \eta_{int} \tag{1}$$

Size-exclusion chromatography (SEC) was performed at room temperature in an HPLC apparatus, which was equipped with a differential refractometer (Knauer, Berlin, Germany). A Zorbax PSM Trimodal-S column (Rockland Technologies Inc., New Port, DE) was used. The polyester was dissolved in chloroform, and a flow rate of 1 mL/min was applied. Calibration was done with polystyrene molecular weight standards (Polymer Standards Service, Mainz, Germany).

The glass transition temperature (T_g), melting point temperature (T_m), melting enthalpy (ΔH_m), and heat capacity (c_p) of the polyesters were determined by means of differential scanning calorimetry (DSC) using a DSC 7 calorimeter (Perkin-Elmer, Überlingen, Germany) for a temperature range between −50 and 150 °C. The first heating was carried out at a rate of 20 °C/min from −50 to 150 °C; the temperature was kept for 5 min at this level before the polyester was cooled to −50 °C at a rate of 20 °C/min. The second heating was done at the same rate as the first heating.

The dynamic mechanical measurements (DMA) were done on unprocessed samples by means of a rheometer (model RMS 800, Rheometrics Inc., Piscataway, NJ). The geometry chosen for these measurements was a cone−plate arrangement (25 mm diameter, 0.1 rad cone angle). The frequency (ω) was in the range 10^{-1} and 10^2 rad/s. The measurements were performed under nitrogen at 140 °C and the melt viscosity η^*, shear storage modulus G', and shear loss modulus G'' were determined.

The mechanical properties of the original film castings (40 mm length) and the fibers (20 mm length) were measured by means of a stress−strain universal tester (TF−CM, Instron, Offenbach, Germany) at a strain rate of 100 mm/min. The physical break stress σ was calculated from the maximum strength R_H and the elongation ϵ_H according to eq 2.

$$\sigma = \boldsymbol{R}_{\mathbf{H}}\,(\mathbf{1} + \epsilon_{\mathbf{H}}/\mathbf{100}) \tag{2}$$

Results and Discussion

Fermentative Production of Polyesters with *P. putida*. *P. putida* accumulated a polyester with 4HV as one constituent, if the cells were cultivated in the presence of 4HV acid.[16] Because this compound is rather expensive, we used levulinic acid which can be obtained by chemical conversion of renewable resources such as fructose or from waste of the wood-processing industry.[17] The PHA-negative mutant GpP104 of *P. putida* harboring the PHA synthase structural genes of *T. pfennigii* accumulated a terpolyester consisting of 3HB, 3HV, and 4HV if levulinic acid was provided as a precursor substrate for 4HV.[18] In order to obtain large quantities of this polyester, we established a two-phase fed-batch fermentation process at a 15-L fermentation scale with octanoic acid as carbon source in the growth phase. Accumulation of PHA was induced by nitrogen starvation and by continuously supplying an excess of levulinic acid. In the growth phase the pH was maintained at 8.0, the temperature was kept at 30 °C, and the cells were aerated with up to 1 vvm. In the accumulation phase the pH was shifted to 7.0 and the temperature was kept at 35 or 37 °C. To adapt the cells for the utilization of levulinic acid, small amounts

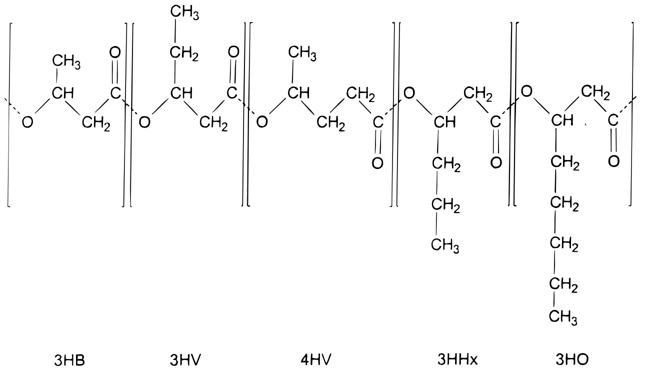

Figure 1. Chemical structure of poly(3HB-*co*-3HV-*co*-4HV-*co*-3HHx-*co*-3HO).

(0.05−0.1% w/v) of this organic acid were already provided during the growth phase in experiments F-8 and F-11. Thus, it was possible to cultivate the recombinant *P. putida* strain to cell densities of up to 20 g/L. The PHA content of these cells amounted up to 50% (w/w) of the cellular dry weight. In addition to the three monomer units mentioned above, the polyester regularly contained some 3-hydroxyhexanoic acid (3HHx) which resulted from the degradation of the octanoic acid that was used as substrate during the growth phase. In addition, some PHA samples contained traces of 3-hydroxyoctanoic acid (3HO). Figure 1 shows the chemical structure of the accumulated polyester. The cultivation conditions and the resulting molar compositions of the polyesters extracted from the cells obtained during five independent experiments are shown in Table 1. The time course of a representative fermentation is shown in Figure 2.

Processing of the Polyester. The water content of the samples obtained from the isolation process was about 0.19% (w/w) and was reduced to 0.06% (w/w) by 24 h of drying under vacuum at a temperature of 20 °C. The dried polyester was subsequently stored in a desiccator and was used for all experiments. Five different samples of the polyester obtained from four independent cultivations of the recombinant *P. putida* were investigated and processed by melt spinning. Since the samples degraded during the spinning process, the viscosity decreased, and the spinning velocity had to be adjusted, particularly at the end of the process. The relative low glass transition temperature (Table 2) and low rate of crystallization prevented a sufficient solidification and crystallization of the fibers after leaving the spinneret. Therefore, the fibers stuck together on the bobbin and could not be easily separated afterward. This behavior could not be avoided, even by cooling the fibers with a methanol/dry ice mixture at −30 °C.

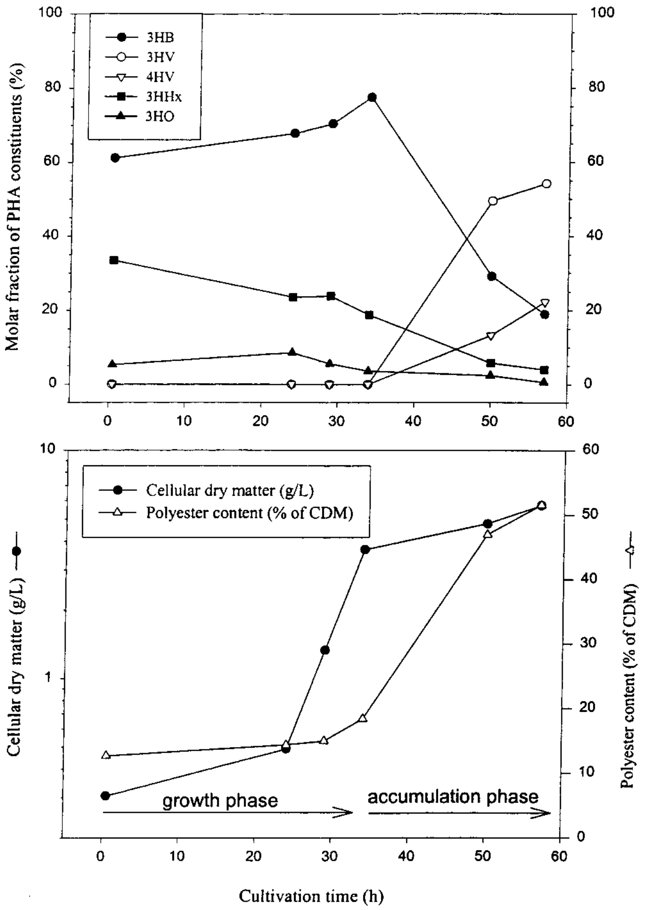

Figure 2. Time course of cultivation of *P. putida* GpP104 (pHP1014::E156) to obtain a sample (F-5) of 4HV-containing polyester. Accumulation conditions were induced at 35 h by nitrogen limitation, temperature shift from 30 °C to 37 °C, pH-shift from 8.0 to 7.0, and by continuous feeding of levulinic acid as the sole carbon source.

Molecular Weights of the Unprocessed and of the Processed Polyesters. To evaluate the properties of the new polyesters, P(3HV) was produced as a reference.[10] The intrinsic viscosities of the polyesters were significantly higher than the viscosities of the P(3HV) sample, and the fibers exhibited a lower viscosity than the unprocessed samples (Table 3), although thermogravimetric studies had demonstrated a weight loss of the samples as a result of vaporization of volatile compounds only at temperatures above 260 °C (not shown in detail). This indicated that the polyesters were degraded during the melt spinning process. This conclusion was confirmed by the analysis of the molecular weights employing size exclusion chromatography, which was done for the unprocessed polyester samples and for the fibers, respectively, to show the influence of the thermal strain during the spinning process on the molar mass distribution. The number-average molar mass (M_N), the weight-average molar mass (M_W), and the Z-average molar mass (M_Z) were determined by using calibrations with polystyrene standards (Table 3). The analysis revealed relatively narrow polydispersity indexes (M_W/M_N) for the unprocessed samples, which were between 2.5 and 3.6. A comparison of the molecular weight of the unprocessed samples and of the fibers revealed lower M_Zs for the fibers (Table 3); this indicated that degradation is related mainly to the higher molecular proportions of the polyester. In addition, the increase in the degradation index (DI) confirmed this assumption. The DI[19] is equal to the average number of chain scissions per initial number-average macromolecule. The calculation according to eq 3 showed that all polyesters were, in comparison to P(3HV), subjected to degradation, which had a negative effect on the stability during the spinning process.

$$\mathrm{DI} = (M_{\mathrm{NO}}/M_{\mathrm{N}})^{-1} \qquad (3)$$

M_{NO} and M_N are the initial and the final values, respectively, of the number-average molar mass.

Physical Properties. DSC thermograms of the unprocessed solvent-precipitated polyesters and of P(3HV) are shown in Figures 3a−c. The first heating

Table 2. DSC Analysis of Unprocessed 4HV-Containing Polyesters and P(3HV)

sample	T_g (°C)	melting range (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	ΔH_m (J/g)	T_R (°C)	c_p (J/gK)
F-5	−14.5	30−150	54.5					123.7		15.2	325.7	0.40
F-6	−15.7	30−150	(S)		(S)	93.7	101.3	122.0	133.0	34.1	317.7	0.31
F-8/1	−14.3	25−100	49.3	70.1	(S)					27.6	321.0	0.32
F-8/2	−14.5	25−100	48.0	70.0	81.7					26.5	327.0	0.28
F-11	−15.0	30−150	48.0	70.0	84.0			110.0		25.2	304.0	0.34
PHV	−15.4	50−135						118.5		107.7	318.9	0.57

Abbreviations: T_g, glass transition temperature; T_m, melting temperature; ΔH_m, melting enthalpy; T_R, degradation temperature; S, shoulder; c_p, heat capacity.

Table 3. Molecular Weight Analysis and Viscosity Analysis of Nonprocessed and Processed 4HV-Containing Polyesters and P(3HV)

	extracted PHA						fibers					
sample	M_N 10^3g/mol	M_W 10^3g/mol	M_Z 10^3g/mol	M_W/M_N	η_{int} dL/g	η_0 10^3 Pa·s	M_N 10^3g/mol	M_W 10^3g/mol	M_Z 10^3g/mol	M_W/M_N	η_{int} dL/g	DI
	1	1	1				1	1	1			
F-5	130	325	620	2.5	2.72	37.4	120	290	535	2.4	2.32	0.08
F-6	260	940	1830	3.6	5.45	441.8	110	220	375	2.0	1.78	1.36
F-8/1	175	590	1490	3.4	2.50	69.2	150	470	1050	3.1	2.04	0.17
F-8/2	150	445	1070	3.0	1.89	39.2	120	350	790	2.9	0.96	0.25
F11	165	410	870	2.5	2.68	80.5	120	235	400	2.0	1.13	0.38
PHV	60	155	280	2.6	0.94	1.0	70	160	285	2.3	1.02	−0.14

scans (Figure 3a) revealed T_Gs of about −15 °C for either sample (Table 2), indicating that the unprocessed polyesters were predominantly amorphous. Above this temperature a broad melting range between 30 and 150 °C with several peaks occurred. Therefore, the melting enthalpy (ΔH_m), which indicates the crystalline proportion, could only be calculated in total. The relatively low melting enthalpies of the polyesters (15.2−34.1 J/g) in comparison to P(3HV) (107.7 J/g)[10] confirmed the small crystalline proportion of the polyesters. If the melted polyesters were cooled in the calorimeter at a cooling rate of 20 °C/min, they did not exhibit any crystallization. The cooling scans (Figure 3b), and the second heating scans (Figure 3c) did not show any peaks (except sample F-6 which showed an extremely small peak).

The fibers were cooled at a higher cooling rate during fiber formation and were therefore also amorphous at the beginning. According to a model of Brünig et al.,[20] the cooling rate of the fiber is much higher than the highest possible rate which can be achieved with the DSC apparatus. Subsequent incubation of the fibers for 5 days at 25 °C and 60% humidity caused a crystallization to the same proportion as the unprocessed polyesters as revealed by the first heating scans of the fibers (Figure 4) and the values for melting enthalpy (ΔH_{F-5} = 20 J/g, ΔH_{F-6} = 32 J/g, $\Delta H_{F-8/1}$ = 25 J/g, $\Delta H_{F-8/2}$ = 20 J/g, ΔH_{F-11} = 18 J/g) in comparison to the unprocessed samples (see Table 2 and Figure 3). This incubation gave some form stability to the fibers, but without improving their strength. In comparison to the P(3HV) homopolyester, which exhibited a recrystallization during the second heating, the polyesters showed no recrystallization.

DMA of the unprocessed solvent-precipitated polyester revealed decreasing melt viscosities with increasing frequency (Figure 5a). The melt viscosities (η*) of the polyesters were approximately one order in magnitude higher than the melt viscosity of P(3HV), but their decrease with increasing frequency (ω) was much sharper than that of the P(3HV)-homopolyester[10] and that of, e.g., polyamide,[10] that is widely used by the fiber spinning industry. This indicated that the relaxation times for these polyesters are much longer, most probably because of the relatively high molecular weight and the length of the side chain.

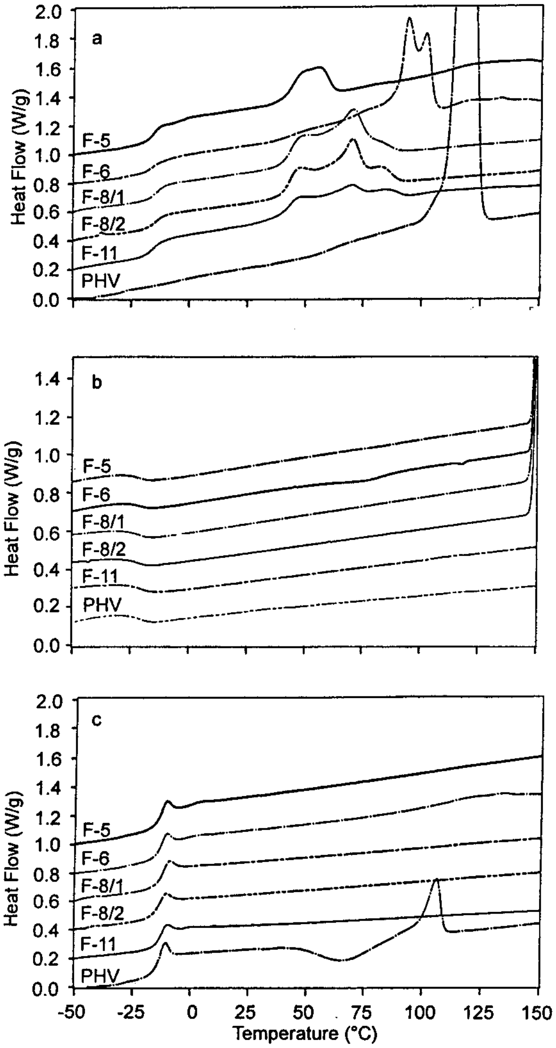

Figure 3. DSC of 4HV-containing polyesters and P(3HV). DSC thermograms were obtained as described under Materials and Methods for different PHA samples as they were isolated from the bacterial cells. (a) First heating; (b) first cooling; (c) second heating.

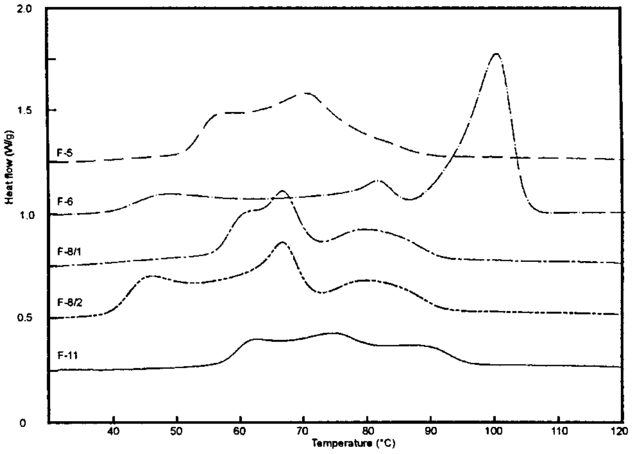

Figure 4. DSC of 4HV-containing polyester fibers, melting range, first heating.

The viscosity function η^* was expressed by the shear frequency ω with an expression containing three parameters according to eq 4; the zero shear viscosity η_0 was determined by extrapolation ($\omega \rightarrow 0$).

$$\eta^* = \frac{1}{c_1 + c_2\omega^{c_3}}, \quad \eta_0 = \lim_{\omega \rightarrow 0} \eta^* \qquad (4)$$

η_0 is independent of ω and increases with molecular weight (M_W) according to eq 5. The polymer-specific values a and b can be determined in close approximation via the correlation of η_0 with the molecular weight, measured by SEC using the Mark–Houwink relationship.

$$\eta_0 = a\, M_W{}^b \qquad (5)$$

The rheological properties of polymers are considerably influenced by the molecular weight and its distribution. The melt viscosity (η^*), shear storage modulus (G'), and shear loss modulus (G'') correlate with these molecular parameters according to eqs 6 and 7.

$$G^*(\omega) = G'(\omega) + i\, G''(\omega) \qquad (6)$$

$$G^*(\omega) = i\, \omega\, \eta^* \qquad (7)$$

DMA of the polyester materials revealed, in comparison to P(3HV),[10] a much higher shear storage modulus (G') and shear loss modulus (G'') at low frequencies (Figures 5b and c). These results emphasize the completely different dynamic mechanical behavior of the polyesters with low content of medium-chain-length hydroxyalkanoic acids in comparison to the brittle homopolyester P(3HV).

The average values for the tensile strength at break obtained for the fibers were 8–15 MPa and in the same range as for P(3HV). In contrast, the polyesters revealed an extremely high elongation at break, which was approximately 1000%, and the corresponding value for the relatively brittle homopolyester P(3HV) was only 1.4%.[10] Figure 6 shows a typical course obtained for an original film casting of F-11, revealing a tensile strength at break of 45–50 MPa at an elongation of 1000%. This again showed the high elastomeric character of the polyester.

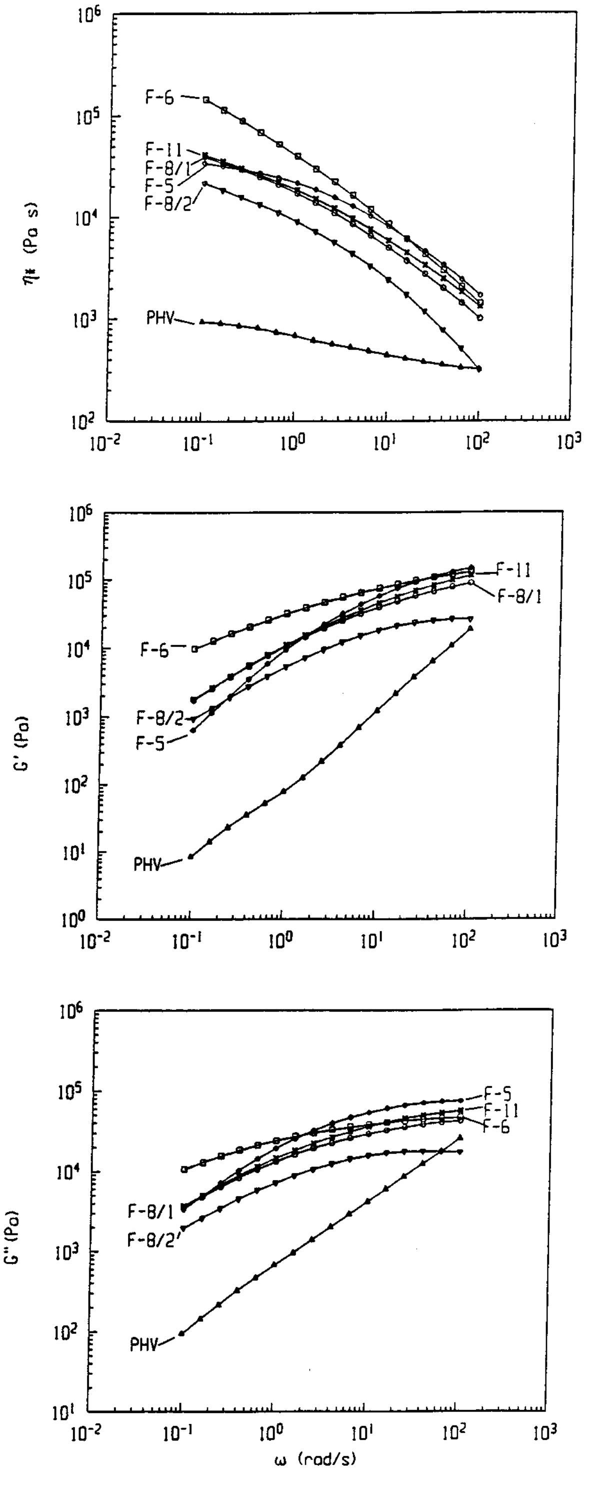

Figure 5. DMA of 4HV-containing polyesters and P(3HV). The variations of the melt viscosities (η^*), shear storage modulus G', and shear loss modulus G'') during the frequency (ω) shift were recorded.

Outlook. It could be shown that polyesters with even a low content of medium-chain-length hydroxyalkanoic acids (3–15 mol %) belong to the class of thermoplastic elastomers according to their properties. Further efforts will focus on the two-stage fed-batch process in order to gain larger quantities (up to now only 10–50 g quantities were available) of these polyesters with an even molar distribution of the monomers and with low molecular weight to carry out further fiber spinning experiments. The physical relation between molecular weight, temperature, and melt viscosity implies the possibility to reduce degradation during the spinning process by decreasing the spinning temperature until the melt viscosity reaches 100–1000 Pa·s. Larger

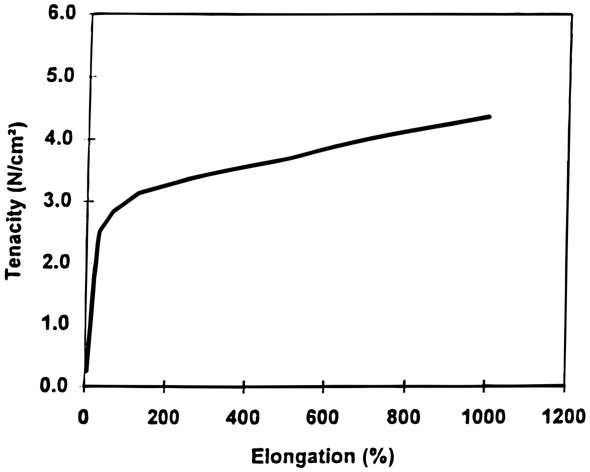

Figure 6. Tensile strength test for a 4HV-containing polyester. Film cast material of sample F-11 was investigated.

quantities of polyester will facilitate a change of the spinning process from a discontinuous to a continuous mode so that shorter spinning times could be reached. In addition, the effect of nucleating agents should be tested in order to reduce processing times and to avoid degradation.

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References and Notes

(1) Hocking, P. J.; Marchessault, R. H. Biopolyesters. In *Chemistry And Technology Of Biodegradable Polymers*; Griffin, G. J. L., Ed., Blackie Academic & Professional: New York, 1994.
(2) Anderson, A. J.; Haywood, G. W.; Dawes, E. A. *Int. J. Biol. Macromol.* **1990**, *12*, 102.
(3) Steinbüchel, A. *Nachr. Chem. Technol. Lab.* **1991**, *39*, 1112.
(4) Jendrossek, D.; Schirmer A.; Schlegel H. G. *Appl. Microbiol. Biotechnol.* **1997,** *46*, 451.
(5) Gagnon, K. D.; Lenz, R. W.; Farris, R. J.; Fuller, R. C. *Macromolecules* **1992,** *25*, 3723.
(6) De Koning, G. J. M.; Van Bilsen, H. H. M.; Lemstra, P. L.; Hazenberg, W.; Withhold, B.; Preusting, H.; Van der Galien, J. G.; Schirmer, A.; Jendrossek, D. *Polymer* **1994,** *35*, 2090.
(7) Valentin, H. E.; Schönebaum, A.; Steinbüchel, A. Appl. Microbiol. *Biotechnol.* **1992,** *36*, 507.
(8) Valentin, H. E.; Steinbüchel, A. J. Environ. Polym. Degrad. **1995**, 3, 169.
(9) Liebergesell, M.; Mayer, F.; Steinbüchel, A. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 292.
(10) Steinbüchel, A.; Schmack, G. J. *Environ. Polym. Degrad.* **1995**, *3*, 243.
(11) Schlegel, H. G.; Kaltwasser, H.; Gottschalk, G. *Arch. Mikrobiol.* **1961**, *38*, 209.
(12) Brandl, H.; Knee, E. J.; Fuller, R. C.; Gross, R. A.; Lenz, R. W. *Int. J. Biol. Macromol.* **1989** *11*, 49.
(13) Brandl, H.; Gross, R. A.; Lenz, R. W.; Fuller, R. C. *Appl. Environ. Microbiol.* **1977**, 54.
(14) Timm, A.; Byrom, D.; Steinbüchel A. *Appl. Microbiol. Biotechnol.* **1990**, *33*, 296.
(15) Huggins, M. L. *Ind. Eng. Chem.* **1943,** *35*, 980.
(16) Valentin, H. E.; Schönebaum, A.; Steinbüchel, A. *Appl. Microbiol. Biotechnol.* **1996**, *46*, 261.
(17) Neumüller, O.-A. Römpps Chemie-Lexikon, **1983.**
(18) Steinbüchel, A.; Gorenflo, V. Biosynthetic and biodegradable polyesters from renewable resources: Current state and prospects. *Macromolecular Symposia*, 1997.
(19) Shyichuk, A. V. J. *Appl. Polym. Sci.* **1996,** *62*, 1735.
(20) Beyreuther, R.; Brünig, H.; Vogel, R. *Polymer Material Encyclopedia*: *Synthesis, Properties and Applications*; CRC Press, Inc., Boca Raton, Florida, 1996, 6, 4061.

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