Russian Chemical Reviews **65** (8) 725 – 734 (1996)
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UDC 678.674.576.851

Bacterial polyesters. Synthesis, properties, and applications

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Abstract. The mechanism of bacterial synthesis of polymers and copolymers of hydroxyalkanoic acids is described. The structural and physicochemical properties of polymers obtained by biosynthesis are identical to those of the polymers obtained by polymerisation of lactones. Polymers and copolymers of hydroxyalkanoic acids are readily crystallisable flexible-chain polymers, capable of forming films and fibres when the degree of polymerisation is higher than 500 – 600. These compounds are biodegradable. The rate of biodestruction, which leads mainly to oligomeric products, depends on the chemical nature of the compound, on the degree of tacticity in the polymeric chain, and on the degree of ordering in the polymeric substrate. The bibliography includes 105 references.

I. Introduction

The increase in the production and consumption of various polymeric materials has led to increased environmental hazard, including the greenhouse effect. There are some other reasons for this phenomenon, for example:

– the increase in the amount of organic fuel being combusted;

– the increase in the amount of organic raw materials being processed to target products including processing of waste materials;

– the decrease in forest area due to the increase in tree-felling and in the processing of wood;

– the accumulation of waste polymeric materials incapable of biodegradation.

Therefore, the challenge arises to develop new energy-saving methods for the synthesis and processing of polymers accompanied by minimal amounts of CO_2 discharged to the atmosphere. This problem can be solved, for example, by developing biotechnological processes for the synthesis of film-forming and fibre-forming polymers.

Conventional technological schemes for the production of basic polymers [for instance, cellulose, poly(ethylene terephthalate), polypropylene, polyhexanamide, and copolymers of acrylonitrile] require 18 – 55 GJ of energy per ton of the product. Similar energy expenditure is needed to isolate the target products of microbiological synthesis; however, in this case, the amount of CO_2 liberated decreases sharply, and the biomass obtained can be included in the hydrocarb energy-chemical cycle.[1]

Among the film-forming and fibre-forming polymers that can be synthesised using the microbiological approach, polyesters based on hydroxyalkanoic acids are of considerable interest. Recently, it has been found that some microorganisms are capable of synthesising thermoplastic regular homopolymers and copolymers of β-hydroxyalkanoic acids containing $C_1 - C_{11}$ side groups. Such polymers can undergo biodegradation at a controlled rate.

Comparison of the physicochemical properties of poly(hydroxyalkanoates) (PHA) synthesised by the microbiological method with those of the PHA obtained by polymerisation of the corresponding lactones demonstrated that they are identical, although the primary structures of the polymers are somewhat different. The characteristic features of the polymerisation of lactones were analysed for the first time by Sazonov;[2] later, studies along these lines were continued.[3–6] It should be noted that many of the PHA synthesised proved to be biodegradable,[7] which is very important from the ecological standpoint. PHA are of considerable interest as film-forming and fibre-forming polymers and also as molecular plasticisers compatible with many synthetic and natural polymers.

II. Microbiological synthesis of poly(hydroxyalkanoates)

The target-directed synthesis of polymers based on hydroxyalkanoic acids and their lactones was undertaken for the first time by Van Natta et al.[8] At present, PHA can be obtained by ionic, hydrolytic, radiation-induced, and UV-induced polymerisation or copolymerisation of β-lactones, δ-lactones, and some other lactones.[2] Some PHA, for example, poly(ε-hydroxyhexanoate) [P(6HH)], are industrial products.

The possibility of using bacterial methods to synthesise PHA was recognised more than 20 years ago, when the accumulation of poly(3-hydroxybutanoate) [P(3HB)] granules in the cytoplasm of the *Alcaligenes eutrophus* bacterium was observed. It was shown that this polymer acts as a reserve compound, i.e. as a source of carbon and energy, for this bacterial culture.[9–11]

The bacterial P(3HB) is a high-molecular-weight ($\bar{M}_w > 10^5$), readily crystallisable, thermoplastic, biocompatible, and biodegradable polymer.[12] The industrial production of this polymer based on biotechnological processes using *A. eutrophus*[13] and *A. latus*[14] bacteria was established by the ICI company (UK). By now, procedures for the preparation and properties have been reported for many other polymers built of units of the general formula $\sim[O\text{–}CH(R)\text{–}CH_2\text{–}C(O)]\sim$, where R is a $(C_1 - C_{11})$ saturated,[15–17] linear unsaturated,[18–19] or branched unsaturated

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Received 13 December 1995
Uspekhi Khimii **65** (8) 782 – 793 (1996); translated by Z P Bobkova

alkyl,[19] haloalkyl,[20–22] or aryl[23] radical. It has been shown that microbiological synthesis can be used for the preparation of thermoplastic aliphatic polyesters not only from β-hydroxycarboxylic acids, but also from α-,[24] γ-,[25,26] and δ-hydroxycarboxylic acids.

1. Synthesis of poly(hydroxyalkanoates)

Biosynthesis of homopolymeric and copolymeric PHA by various bacterial strains has been studied in detail by many researchers (Table 1).

Table 1. Bacterial synthesis of various homopolymeric and copolymeric PHA.

Strain	Polymer	Ref.
Alcaligenes eutrophus	(3HB)	27
	(3HB-co-3HP)	24
	(3HB-co-4HB)	26, 28–30, 35
	(3HB-co-3HV)	14, 17, 31, 32
	(3HB-co-5HV)	33
Alcaligenes latus	(3HB-co-3HP)	34
Pseudomonas oleovorans	(3HO)	18
	(3HH-co-3HO)	22

Note. 3HP is 3-hydroxypropionate, 4HB is 4-hydroxybutyrate, 3HV is 3-hydroxyvalerate, 5HV is 5-hydroxyvalerate, 3HH is 3-hydroxyhexanoate, and 3HO is 3-hydroxyoctanoate.

The physicochemical and structural characteristics of PHA obtained using different bacterial strains are very similar.

The biosynthesis pathway of PHA has been considered in detail in relation to the generation of P(3HB) by the *A. eutrophus* culture.[17,28,31] The block diagram of this process is shown in Fig. 1. The formation of aliphatic acids and their derivatives in organisms is known[36] to result from the metabolism of carbohydrates, and the first stage of their formation is one of the last stages of the biodegradation of monosaccharides. The hydroxy-acids thus obtained are subsequently biopolymerised.

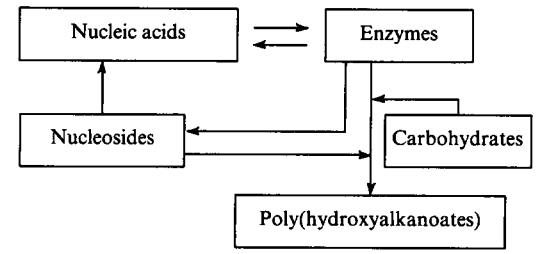
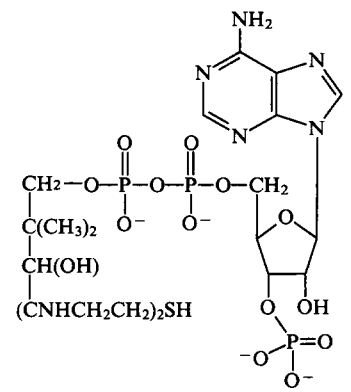


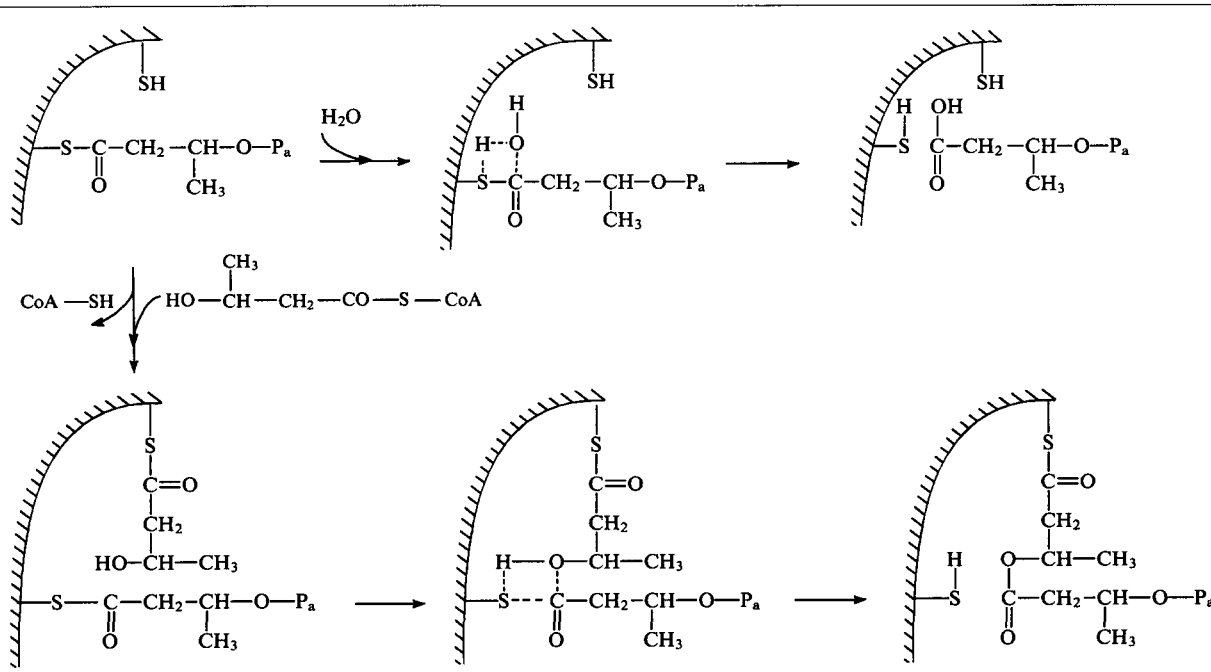
Figure 1. Block diagram of the biosynthesis of poly(hydroxyalkanoates).

The metabolism of carbohydrates occurring with the active participation of the nucleotide adenosinetriphosphate leads to the formation of the compound



coenzyme A (CoA–SH). The acetate of this coenzyme [CoA–$SC(O)CH_3$] plays an important role in the synthesis of poly-(3-hydroxybutanoate).

P(3HB) and other bacterial PHA are synthesised at 30–35 °C via a series of heterogeneous reactions occurring on the surface of enzymes (Fig. 2).[27] Recently, blocks of DNA codons responsible for the synthesis of these enzymes have been determined.[29,30,33] The mechanism of the action of the corresponding enzymes on the



P_a is poly(oligo)hydroxyalkanoates.

Figure 2. Scheme for the enzymic synthesis of P(3HB) with participation of D-(−)-3-hydroxybutyryl−CoA.

dynamics of the synthesis and depolymerisation of P(3HB) has also been considered.[37] The polymerase generated by *A. eutrophus* was found to be a polypeptide with $\bar{M}_w$ = 63 940 containing five cystine units, three of which ensure the conformational stability of the enzyme and the other two provide the binding of a hydroxyalkanoic acid molecule on the enzyme surface through the HS groups (Fig. 2). The scheme for this process in relation to the synthesis of P(3HB) is presented in Fig. 3. In the first stage, the enzyme 3-ketothiolase catalyses the reversible condensation of two CoA – $SC(O)CH_3$ molecules to give the acetoacetyl derivative CoA – $SC(O)CH_2C(O)CH_3$.

In the second stage, the NADPH-dependent enzyme, acetoacetyl-CoA-reductase (hydrogenase), catalyses the reversible reduction of the acetoacetyl derivative of coenzyme A to D-(−)-3-hydroxybutanoyl – CoA.

The third stage involves a reversible redox reaction catalysed by the NAD-specific dehydrogenase, in which the L-form of 3-hydroxybutanoic acid arising via an independent pathway is converted into the acetoacetyl derivative. The reaction mixture is thus 'fed' with the monomer.

In the fourth stage, the enzyme P(3HB)-polymerase (synthase) catalyses the heterogeneous stereospecific synthesis of P(3HB). The activity of the P(3HB)-polymerase remains virtually invariable during the synthesis of the polyester.

In the fifth stage, depolymerisation of P(3HB) to D-(−)-3-hydroxybutyric acid can occur under the action of the enzyme P(3HB)-depolymerase.

The sixth stage involves the oxidation of the hydroxy-acid with NAD-specific-dehydrogenase to acetoacetic acid, which reacts in the seventh stage with CoA – SH to give CoA – $SC(O)CH_2C(O)CH_3$.

Evidently, the acetoacetyl derivative of coenzyme A arises as an intermediate both in the synthesis and in the biodegradation of

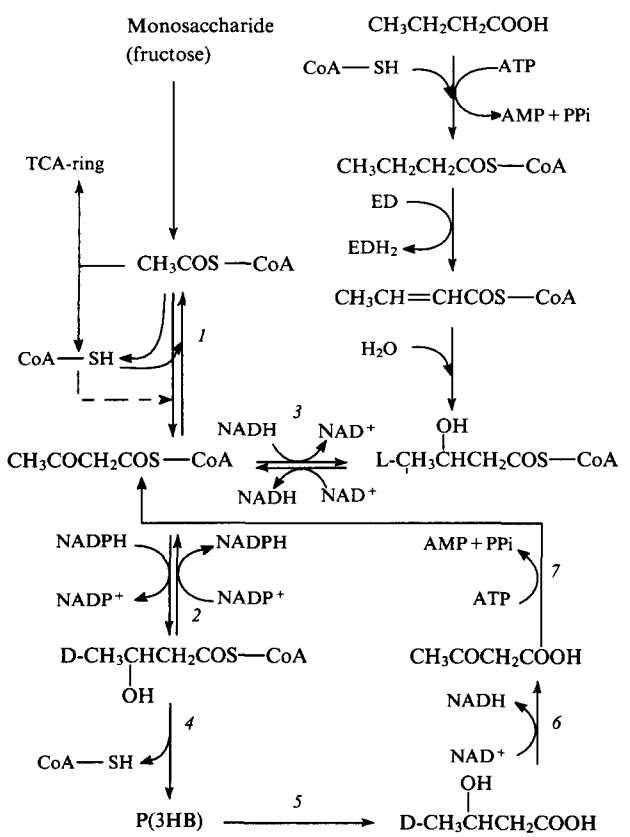


Figure 3. Scheme for the biosynthesis and biodestruction of P(3HB)[27] (fructose and butyric acid as the medium); PP*i* is the amide of nicotinic acid; 'TCA-ring' is the ring of tricarboxylic acids; and ED is the dehydrogenase.

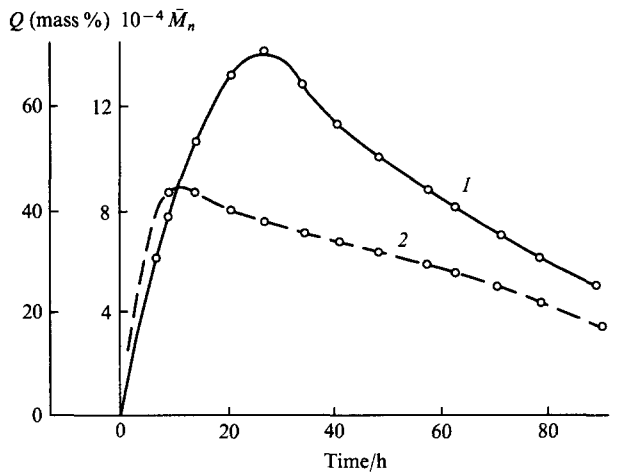


Figure 4. Dynamics of the synthesis of P(3HB) (Q is the polymer yield) with the *Alcaligenes eutrophus* bacteria (*1*), and of the variation of the molecular weight ($\bar{M}_n$) of the polymer (*2*).[27]

P(3HB). The dynamics of the synthesis of P(3HB) and of the change in the molecular-mass characteristics of the resulting polyester are illustrated in Fig. 4.

The sequence of chemical transformations occurring during the biosynthesis of P(3HB) includes three stages:

The generation of the polymeric chain:

$$E\text{—}SH + D\text{-}CH_3CH(OH)CH_2COS\text{—}CoA \xrightarrow{k_i} \longrightarrow D\text{-}CH_3CH(OH)CH_2COS\text{—}E + CoA\text{—}SH\,.$$

The growth of the macromolecule:

$$(3HB)_{n-2}OCH(CH_3)CH_2COS\text{—}E + + D\text{-}CH_3CH(OH)CH_2COS\text{—}CoA \xrightarrow{k_g} \longrightarrow (3HB)_{n-1}OCH(CH_3)CH_2COS\text{—}E + CoA\text{—}SH\,.$$

The termination of the polymeric chain:

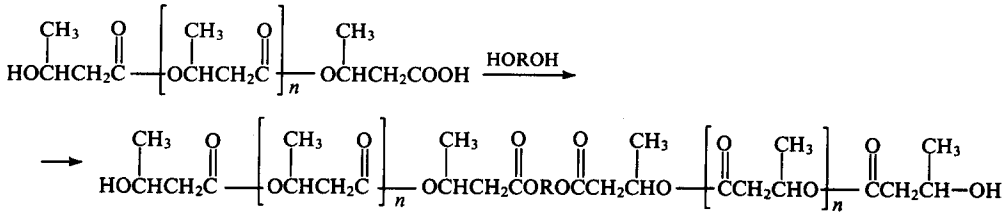
$$(3HB)_{n-1}OCH(CH_3)CH_2COS\text{—}E + H_2O \xrightarrow{k_t} \longrightarrow (3HB)_nOH + E\text{—}SH\,.$$

Here, E is the enzyme and k_i, k_g, and k_t are the rate constants for the chain generation, chain growth, and chain termination, respectively. All three stages are considered as pseudo-first-order reactions.

Under the isothermal conditions of the synthesis, the rate constants k_g and k_t depend on the presence of monomeric carbon sources in the culture medium in which the bacterial strains are grown. As applied to the biosynthesis of P(3HB), this is illustrated by the following data:[27]

Content/ g dm^{-3}	Rate constants/ h^{-1}	
	$10^3 k_g$	$10^2 k_t$
Fructose		
5	5.8	28
10	6.6	28
20	6.0	72
Butyric acid		
5	11.2	116
10	8.0	58
20	4.4	150

Scheme 1



HOROH = $HOCH_2C{\equiv}CCH_2OH$, $HO(CH_2)_4OH$, $HOCH_2C(CH_3)_2CH_2OH$, *cis*-$HOCH_2CH{=}CHCH_2OH$.

With the assumptions that the duration of the chain generation stage is much shorter than those of the other stages of the synthesis and that the number of enzyme molecules remains constant during the synthesis, the overall kinetics of the synthesis can be described by the following equation:

$$\bar{P}_n = \frac{\int_0^t R_g dt}{[E] + \int_0^t R_t dt} = \frac{k_g[E]t}{[E] + k_t[E]t}, \quad (1)$$

where $\bar{P}_n$ is the number-average degree of polymerisation, R_g is the rate of the polymeric chain growth, $R_g = k_g[E]$; R_t is the rate of the polymeric chain termination, $R_t = k_t[E]$; and [E] is the concentration of the polymerase. All the kinetic parameters of the process are assumed to be constant during the synthesis. It is clear that the number of P(3HB) molecules produced over the period of time t is

$$N_t = k_g[E]t\bar{P}_{n,t}^{-1} = [E] + k_t[E]t\,. \quad (2)$$

It follows from equation (2) that [E] can be evaluated from the plot of N_t against t by extrapolating the N_t value to the region $t \to 0$. It was found that the concentration of the polymerase [E] does not depend on the nature and on the initial concentration of monomeric carbon sources present in the culture medium. Calculations showed that the number of P(3HB)-polymerase molecules is 18 000 per cell.

The accumulation of the polymer in the biomass reaches 75 mass %. Initially, products with $\bar{M}_w = 2 \times 10^6$ are formed; however, during the polymerisation, the $\bar{M}_w$ value decreases to 6×10^5, whereas the polydispersity of the polymer remains virtually invariable during the whole enzymic cycle: $\bar{M}_w/\bar{M}_n = 2.0 \pm 0.2$. When carbohydrates, hydrocarbons, and acids are no longer present in the culture medium, biodegradation of the polyester begins.

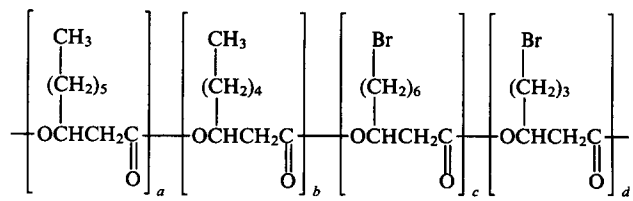
2. Biosynthesis of copolymers of poly(hydroxyalkanoates)

The composition and properties of copolyesters obtained by copolymerisation of various hydroxyalkanoates depend only slightly on the bacterial strain chosen. However, the introduction of various monosaccharides as well as alkanes,[18] alkenes,[18,33] alkanoic acids,[16,40,41] or alkenoic acids[19,42] into the culture medium makes it possible to achieve the reproducible synthesis of various PHA copolymers (Table 1). The biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxypropionate) [P(3HB-co-3HP)], containing 0% to 80% of 3HP units, has been reported.[26] The structure and physical properties of the P(3HB-co-3HP) copolymers were studied by ^1H and ^{13}C NMR spectroscopy, X-ray analysis, gel-permeation chromatography, differential scanning calorimetry (DSC), and dielectric and dynamic mechanical spectroscopy.

In the primary structure of the copolyesters obtained, the 3HB and 3HP units were distributed randomly along the polymeric chain; the molecular weight of these copolymers lies in the range $(1.1-3.5) \times 10^5$.

Statistical P(3HB-co-4HB) copolymers with compositions varying over the whole range have been obtained by adding 4-hydroxybutyric acid, butane-1,4-diol, and γ-butyrolactone to the culture medium.[25,26] The biosynthesis of P(3HB-co-3HV) has been carried out, and characteristic features of its primary structure have been studied.[9,43-48]

Copolymers of PHA containing brominated repeating units (up to 2–3 mass %) have been prepared[22] by cultivation of *Pseudomonas oleovorans* in culture media containing ω-bromoalkanoic acids and octanoic and nonanoic acids. It was noted that in the presence of a bromoalkanoic acid alone, the synthesis of PHA does not occur.



The proportion of bromine-containing units in the PHA remains virtually constant during the synthesis, whereas the yield of PHA initially increases and then decreases asymptotically as the content of carboxylic acids in the culture medium decreases.

The distribution of different units along the chain in all the copolymers of PHA studied is described adequately in terms of Bernoulli probability statistics, and the mole fractions of the copolymers in the polymeric product are described by the Leibnitz formula. For example, the following polynomial is valid for the estimation of the distribution of units in P(3HV-co-3HH-co-3HU), where 3HU is the residue of 3-hydroxyundecanoic acid:[49,50]

$$P_i = \frac{x + y + \ldots z}{x! \cdot y! \ldots z!} P_{HV}^x P_{HH}^y \cdots P_{HU}^z\,, \quad (3)$$

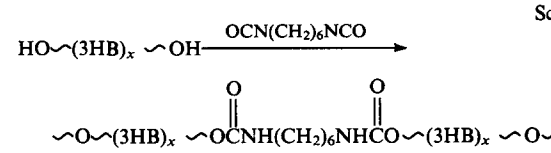
P_i is the mole fraction of a comonomer; P_{HV}, P_{HH}, and P_{HU} are the mole fractions of the corresponding comonomers in the PHA.

The polynomial coefficients x, y, ... z in the formula (3) characterise the number of probable combinations of the units in the copolymer. The legitimacy of this approach to the description of the primary structure of the macromolecules of PHA copolymers has been confirmed by computer analysis.

Block copolymerisation opens up additional opportunities for varying the primary structure of the PHA. A variant of such synthesis has been described.[51,52]

The synthesis of a $(3HB)_x$ block copolymer is shown in Scheme 1, and the synthesis of a polyurethane is presented in Scheme 2.

Scheme 2



The resulting PHA derivatives exhibit elastomer properties over a fairly broad temperature range and are biodegradable.

The possibility of the enzymic synthesis of high-molecular-weight block copolymers of aliphatic polyesters has recently been considered.[53]

III. Structure and properties of poly(hydroxyalkanoates)

The possibilities for technical application of PHA depend on the sum of their physicochemical characteristics and on the physicomechanical properties of the polymeric materials based on them (films, fibres). The presence of the ester bond in the polymeric chain and weak interchain interaction account for a series of specific properties of these polymers. Some physicochemical properties of fibre-forming polymers with $\bar{M}_n = 2 \times 10^4$ based on poly(ε-hexanamide) [P(6HA)] and poly(ε-hydroxyhexanoate) [P(6HH)] are presented in Table 2.

Table 2. Glass transition temperature (T_g), melting point (T_m), density, and heat capacity at 25 °C of P(6HA) and P(6HH).[54]

Polymer	T_g /°C	T_m /°C	ρ_{25}/kg m^{-3}		C_p /kJ kg^{-1} K^{-1}
			amorphous state	crystalline state	
P(6HA)	52	220	1090	1230	1.512
P(6HH)	−70	69	1081	1200	1.343

The polymeric chains of poly(hydroxyalkanoates) are markedly more flexible than those of the analogous polyamides. PHA obtained by both chemical and microbiological methods are readily crystallisable thermoplastic materials.[48] Some physicochemical characteristics of various homopolymeric PHA are given in Table 3. These polymers are readily soluble in dichloromethane, dimethylformamide, and some other solvents. The specific features of the supermolecular organisation of a polymeric substrate, including its self-ordering (crystallisation), depend on the special conditions of its synthesis[5] or biosynthesis,[7,55,59] on the tacticity of the primary structure of the macromolecules,[7] and on the conditions of annealing.[60,61]

For example, rapid cooling of P(3HB) melts leads to its transition to the amorphous state.[62] However, this polymer readily crystallises, the rate of the accumulation of the crystalline phase being substantially dependent on the temperature of crystallisation (Fig. 5); more precisely, the rate at which spherulites grow increases with temperature and passes through a maximum at T_c = 80–90 °C. Crystallographic characteristics of P(3HB), P(3HV), and of P(3HB-co-3HV) copolymers of various compositions have been reported.[32] The influence of the degree of crystallinity of PHA on the structural and mechanical properties of these thermoplastic elastomers has been considered.[63] These polymers exhibit highly elastic properties both above and below the temperature range of transition to the plastic state (T_p).[64] When the polymeric substrate is heated above T_p, the proportion

Table 3. Physicochemical properties of poly(hydroxyalkanoates).

Polymer	T_g /°C	T_m /°C	T_{mc} [a] /°C	ρ_{25} /kg m^{-3}	$\delta/10^3$ [b] (J m^{-3})$^{\frac{1}{2}}$
P(3HB)	15–16	184	62	1193.4	19.57
P(3HB)	4.0 to −4.5	176	57	1171.2	19.12
P(3HV)	−19 to −20	107	50	1159.7	19.45
P(3HO)	−35 to −37	55	30	1098.1	19.29
P(4HB)	−50 to −51	54	20	1184.2	20.01
P(6HH)[c]	−69 to −70	63	25	1149.3	20.15
P(10HD)[d]	−77 to −78	68	27	1063.2	19.32
P(3H3MB)[e]	76 to 77	245	120	1221.5	22.28

[a] T_{mc} is the temperature at which the rate of crystallisation is a maximum; [b] δ is a solubility parameter; [c] P(6HH) is poly(6-hydroxyhexanoate), [d] P(10HD) is poly(10-hydroxydecanoate); [e] P(3H3MB) is poly(3-hydroxy-3-methylbutyrate).

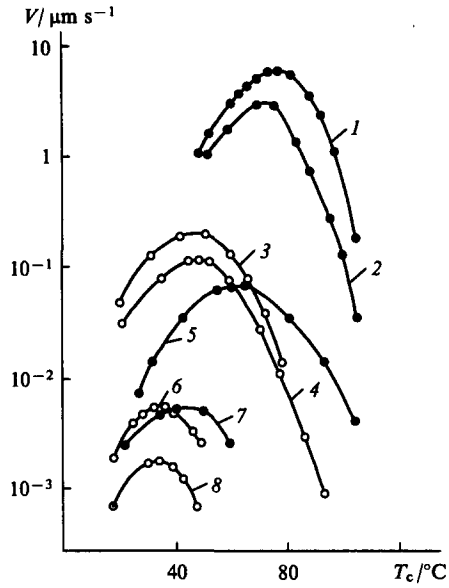


Figure 5. Influence of the temperature of crystallisation (T_c) on the rate of growth of spherulites (V) for P(3HB-co-3HV) copolymers; proportion of the 3HV units, mass %: (*1*) 0; (*2*) 8; (*3*) 95; (*4*) 82; (*5*) 19; (*6*) 71; (*7*) 34; (*8*) 55.

of the highly elastic component in the total deformation decreases. On cooling below T_p, the segments of the PHA macromolecules are capable of self-ordering due to the formation of crystalline domains. The appearance of the crystalline domains in the amorphous PHA determines the effect of 'physical links' between the chains in amorphous regions.

It has been suggested[65] that the hardening of thermoplastic elastomers occurring below T_p can be represented as a 'physical' gel formation. In conformity with this approach, as the degree of crystallinity (α_c) of the polymeric substrate increases, the crystalline domains are converted into crystalline 'clusters'. The size distribution of these 'clusters' is fairly broad. When the critical level of self-ordering ($\tilde{\alpha}_c$) is reached, the polymer transforms from the liquid state into the solid state, and the highly elastic component of deformation somewhat decreases. This effect of hardening of the PHA polymeric substrate is referred to as 'the gel formation point' (GP), and its structure is defined as the 'critical gel'. The above description of the hardening of readily crystallisable elastomers (PHA) is similar to that of 'chemical' gel formation studied in detail previously.[66,67] It is clear that the GP characterises the stage of molecular structuring, during which $\bar{M}_n \to \infty$, i.e. the kinetic individuality of the chains is lost.

The crystallisation of PHA on cooling has been studied.[65] The dynamics of the variation of the modulus of elasticity $G(t)$ (defined as the ratio of the stress to the deformation at constant deformation), of the modulus $G''(\omega)$ (defined as the ratio of the stress component that is in phase with the sinusoidally varying deformation component to the magnitude of this deformation), and of the modulus of loss $G''(\omega)$ (defined as the ratio of the stress component phase shifted by 90° with respect to deformation to the magnitude of this deformation) were studied. At constant deformation amplitudes, the $G''(\omega)$ value is a measure of energy dissipation. Since $G(t)$ and $G'(\omega)$ correspond to the stored elastic energy and since the dynamic change at frequency ω is qualitatively equivalent to the change in the nonequilibrium properties at $t = \omega^{-1}$, the $G(t)$–t and $G'(\omega)$–ω dependences plotted in logarithmic coordinates are, to a first approximation, mirror images with respect to the modulus axis of the corresponding plots describing stress relaxation.[68]

According to Winter and Chambon,[69] in the GP region for $t_0 < t < \infty$, one can write

$$G(t) = St^{-n}. \quad (4)$$

Here, S is the strength of the 'gel' and n is the 'relaxation exponent'. These parameters are the characteristics of a critical 'gel'. At the point of gel formation, the relation $G'(\omega) \sim G''(\omega) \sim \omega^n$ holds at $0 < \omega < t_0^{-1}$. In other words, the above dependences plotted in logarithmic coordinates are similar. The loss angle (δ_k) in the GP is independent of ω. Since

$$\delta_k = \frac{\pi}{2} n,$$

then

$$\tan\left[\frac{G''(\omega)}{G'(\omega)}\right] = \tan\left(\frac{\pi}{2} n\right). \quad (5)$$

The latter dependence permits evaluation of the GP of 'physical' gels from $\tan\delta_k$, which has been demonstrated in relation to various crystallisable polymeric systems.[70] A study of these dependences for P(3HO) showed that the dynamics of crystallisation of PHA depend substantially on the initial temperature of the melt (T_i) and on the temperature of crystallisation of the amorphous polymer obtained by fast cooling (Fig. 6). For example, at 15 °C and 20 °C, the crystallisation of a thermoplastic P(3HO) elastomer occurs by the 'physical' gel formation mechanism with the manifestation of the GP. However, crystallisation of this polymer at 30 °C occurs by a different mechanism. When a P(3HO) melt heated to 90 °C or above is abruptly cooled, the GP is clearly manifested. At lower temperatures of the melt, no GP is observed. Recently,[71] kinetic and thermodynamic aspects of the flexibility and self-ordering of P(3HO) macromolecules in a block have been studied by means of ^{13}C NMR spectroscopy. It should be noted that the kinetic features of crystallisation of PHA of identical compositions are somewhat different depending on whether the polymer has been obtained by chemical synthesis or by biosynthesis: in the latter case, the rate of self-ordering is somewhat higher. This is believed[72] to be due to the plasticising effect of the residual lipids and proteins present in PHA preparations isolated from the biomass.

The crystallographic lattice parameters of P(3HB-co-3HP) specimens with compositions varying over the whole range have been reported.[34] It was shown that the rate of radial growth of the copolymer spherulites, containing 11 mol % of 3HP units, under isothermal conditions is approximately 100 times lower than that for homo-P(3HB). Under the conditions of isothermal crystallisation, the degree of crystallinity of the polymeric substrate decreases as the content of the 3HP units in the polymer increases. The crystallographic lattice parameters remain almost constant as the content of 3HB units increases up to 37 mol %. Crystallisation and structural features of the crystalline phase of P(3HB-co-3HV) copolymers have been studied.[32,43,44,72,73] Crystallisation of these copolymers has been investigated using films obtained from both melts[73-75] and solutions.[43]

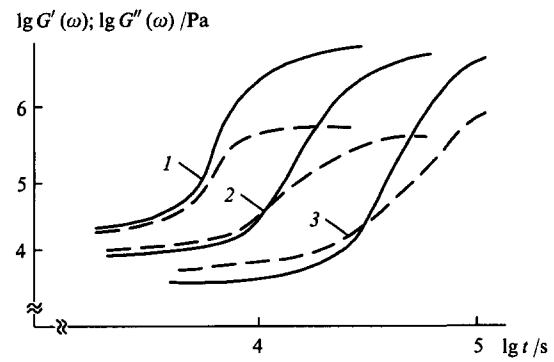


Figure 6. Variation of the $G'(\omega)$ (continuous line) and $G''(\omega)$ (dashed line) moduli as functions of the temperature of crystallisation of P(3HO); T_i = 90 °C; ω = 1 rad s^{-1}; T_c/ °C: (*1*) 15; (*2*) 20; (*3*) 30.

Crystallographic characteristics of the P(3HB-co-3HV) copolymers of various compositions are listed in Table 4.

Table 4. Structural characteristics of bacterial copolymers P(3HB-co-3HV).[32]

Specimen	Type of the lattice	Parameters of the lattice/ nm			α_c (%)	ΔH_0 [a] /J g^{-1}
		a	*b*	*c*		
P(3HB)	P(3HB)	0.573	1.315	0.596	73	130
P(3HB-co-19% 3HV)	P(3HB)	0.580	1.330	0.599	54	115
P(3HB-co-34% 3HV)	P(3HB)	0.604	1.353	0.600	52	92
P(3HB-co-55% 3HV)	P(3HV)	0.953	0.995	0.559	60	95
P(3HB-co-71% 3HV)	P(3HV)	0.960	1.004	0.558	64	111
P(3HB-co-82% 3HV)	P(3HV)	0.959	1.008	0.560	65	128
P(3HB-co-95% 3HV)	P(3HV)	0.958	1.017	0.558	68	128
P(3HV)	P(3HV)	0.952	1.008	0.556	–	–

[a] ΔH_0 is the enthalpy of melting.

When the content of a comonomer is low, the dynamics of crystallisation of PHA copolymers and the structure of the crystalline phase hardly differ from the corresponding characteristics of the homopolymers. In this case, the units of the comonomer act as 'defects' in the crystallographic lattice of the polymeric substrate. However, in some cases, when the distinctions between the sizes and structures of different units are small, the effect of isomorphism is observed.[76] The ability of the P(3HB-co-3HV) copolymers to form common crystallographic lattices over a wide range of comonomer ratios has led Bluhm et al.[44] to the hypothesis of diisomorphism, i.e. of the possibility for 3HV units to be incorporated in the crystallographic lattice of P(3HB) and for 3HB units to be incorporated in the lattice of P(3HV). This conclusion was made in a study of the isothermal crystallisation of PHA copolymers in the temperature range from 0 °C to 80 °C by high-resolution ^{13}C NMR spectroscopy. It was found that an increase in the temperature of crystallisation results either in a decrease in the degree of crystallinity of the polymeric substrate, when the content of the 3HV units is low, or in its increase, when the content of these units is high (> 50%), which is in agreement with the Bluhm hypothesis.[77] The rate of radial growth of spherulites of P(3HB-co-3HV) of various compositions in the temperature range from 0 °C to 120 °C is shown in Fig. 5. The maximum rate of growth of the copolymer spherulites is lower than that for P(3HB) and is observed at lower T_c values. It is noteworthy that the physical characteristics of these copolymers depend on the stress of the polymeric material (Table 5).

Table 5. Crystallinity and melting points of P(3HB-co-3HV).

Specimen	Deformation,	T_m/ °C		α_c (%) (25 °C)		
	ε_0 (%)	DSC	IGC	DSC, $\Delta H/\Delta H_0$	X-ray analysis	IGC
P(3HB)	0	179	–	59	64	–
P(3HB-co-7% 3HV)	0	155	–	53	64	68
	4	154	159	49	–	–
	9	154	156	51	–	–
P(3HB-co-21% 3HV)	0	121	–	32	62	65
	4	123	–	35	–	–
	9	121	125	31	–	–
P(3HB-co-27% 3HV)	0	–	–	28	63	60
	4	114	117	23	–	–
	9	112	113	24	–	–

A study of the mechanism of crystallisation of P(3HB-co-3HV) carried out by DSC and by inverse gas chromatography (IGC) has shown[74] that the melting point of the copolymers decreases with an increase in the content of 3HV units even in the case where the degree of crystallinity remains the same. Apparently, the differences between the melting points (T_m) determined by DSC and IGC are due to the differences in the morphology of PHA specimens. The authors cited[74] believe that the evaluation of the crystallinity of PHA specimens from their density, which has been proposed in another study,[78] does not permit one to obtain correct characteristics of the self-ordering of these polyesters.

The physicochemical and mechanical properties of P(3HB-co-4HB) copolymers have been studied over the whole range of comonomer ratios.[26] Some physicochemical characteristics of these copolymers are given in Table 6.

Table 6. Physicochemical properties of copolymers P(3HB-co-4HB).[26]

Proportions of comonomers (mol %)		$10^{-3}\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	T_c /°C	T_m /°C	ΔH_0 /J g^{-1}	α_c (%)
(3HB)	(4HB)						
100	0	768	1.9	4	177	130.0	59 ± 5
94	6	494	2.1	−1	162	96.2	56 ± 5
90	10	395	3.0	−3	159	80.1	46 ± 5
72	28	252	2.6	−12	–	–	23 ± 5
15	85	168	3.7	−34	46	62.3	29 ± 5
10	90	327	2.1	−44	48	70.2	–
6	94	315	3.0	−46	48	86.2	42 ± 5
0	100	93	2.4	−50	54	89.7	–

Copolymerisation may lead to a sharp decrease in T_m, in the enthalpy of self-ordering ΔH_0, and in the degree of crystallinity of PHA (α_c). The mixed amorphous and crystalline nature of the polymeric substrate (for both homopolymeric and copolymeric PHA) has been illustrated by the results of studies of the temperature dependences of relaxation spectra.[34, 39, 62, 79] The apparent activation energy of the relaxation processes (ΔE_a) is ~ 180 kJ mol^{-1} (see a published study[34]). The thermal stability of PHA depends largely on the primary structure of the polymeric substrate. The dynamics of thermal decomposition of linear polyesters of various structures have been analysed.[80] A kinetic model for the thermodestruction of PHA has been suggested.[81] At 200–300°C this process can formally be described by the following first-order equation:

$$\bar{P}_{n,t}^{-1} = \bar{P}_{n,0}^{-1} + k_d t, \quad (6)$$

where $\bar{P}_{n,0}$ and $\bar{P}_{n,t}$ are the number-average degrees of polymerisation of PHA at the beginning of the isothermal heating and after a period of time t, respectively. The polydispersity of the polymer hardly changes during the process: the ratio $\bar{M}_w/\bar{M}_n$ remains constant.

It has been shown that the thermodestruction decelerates as $\bar{P}_{n,0}$ decreases and that the mechanism of the process depends on the presence of free terminal COOH groups; in the presence of terminal COOH groups, the thermodestruction proceeds by a thermal depolymerisation mechanism, the yield of the corresponding lactone reaching 99% and that of oligomers being only 1%.[82]

Melts of poly(3-hydroxy-3-methylbutyrate) P(3H3MB), which is a polyester with T_g = 76–77 °C and T_m = 245 °C (see Table 3), are fairly stable under a nitrogen atmosphere up to 260–270 °C. As for poly(3-hydroxy-2,2-dimethylpropionate P(3H2MB), it is thermostable under these conditions up to 300 °C. The maximum rate of thermal depolymerisation (T_{md}) of this polymer has been observed at 450 °C.[82] When the methyl group is substituted by an alkyl group, the T_{md} value decreases. The larger the alkyl side chain in PHA, the lower the T_{md} value. The following results were obtained for ~[OCH$_2$–C(CH$_3$). .(R) C(O)]$_n$~ polymers:

R	T_{md}/ °C
CH_3	~450
CH_2CH_3	415
$CH_2CH_2CH_3$	405
$CH_2C(CH_3)_3$	445
$CH(CH_3)CH_2CH_3$	390

It has been found[26] that when the P(3HB-co-94%4HB) copolymer is kept under nitrogen at 100 °C, its $\bar{P}_{n,0}$ value remains constant. At higher temperatures, thermodestruction becomes more intense, but the $\bar{M}_w/\bar{M}_n$ ratio still remains virtually invariant. The rate of the destruction (k_d) [Eqn (6)] of P(3HB-co-94%4HB) increases from 1.1×10^{-5} to 4.5×10^{-5} min^{-1} as the temperature is raised from 150 °C to 180 °C. The thermodestruction of this copolymer in this temperature range is also described as statistical depolymerisation. The temperature range in which the thermal depolymerisation occurs shifts depending on the primary structure of the PHA.

The physicochemical properties of films and fibres based on PHA depend essentially on the primary structure of these polyesters and on their molecular-weight characteristics. By analysing the 'load-elongation' curves recorded at 23 °C, Nakamura et al.[26] have found the following parameters for P(3HB-co-94%4HB) films: modulus of elasticity 5 MPa, rupture stress (G_r) 39 MPa, ultimate elongation (ε_u) 500%. In relation to poly(11-hydroxyundecanoate), it has been shown[83] that at $\bar{P}_n \approx 100$, the formation of films and fibres proceeds unsatisfactorily, whereas at $\bar{P}_n \geqslant 600$, high-strength fibrous and film materials can be obtained.

A systematic study of the sorption activity of films based on bacterial P(3HB) toward vapours of various liquids has been carried out.[84] It has been found[85] that the presence of impurities in a polymer has an influence on the supermolecular structure of the polymeric substrate and thus leads to changes in moisture absorption properties and in the thermal stability of the polymer. It was shown[85] that diffusion coefficients depend on the vapour concentration and on the thermodynamic parameters of the solvents. For example, the empirical dependence of the diffusion coefficient of acetone on the concentration of its vapour in the sorption of acetone by a P(3HB) film is described by the formula $-\ln D = 9.97\, c^{-0.0975}$.

It has been shown in numerous studies that PHA possess a remarkable compatibility (in solutions, melts, and in the solid state) with various natural and synthetic polymers as well as biocompatibility. However, since this property of PHA is largely specific, it will be considered elsewhere.

IV. Biodegradation of poly(hydroxyalkanoates)

Studies performed at ICI[86] have demonstrated the possibility of effective biodegradation of high-molecular-weight P(3HB) in soil, sea water, bottom-dwelling sediments, and aerobic and anaerobic waste water. Sixteen strains of soil microorganisms were isolated, which are capable of using PHA as an exogenous carbon source and of producing extracellular depolymerases for this purpose. Strains of *Pseudomonas lemoigenes*[87–90] and *Alcaligenes faecalis*[91, 92] bacteria can degrade P(3HB) to give trimers and tetramers in the former case or dimers of 3-hydroxyalkanoic acids in the latter case. Recently, PHA depolymerase has been isolated in a pure state from the *Penicillium funiculosum* fungus,[93] and the degradation activity of this enzyme toward polymeric and copolymeric PHA has been demonstrated.[94, 95] As noted above, biodegradation of PHA begins when monomeric carbon sources (monosaccharides, carboxylic acids and their derivatives) are no longer present in the culture medium. During the biodegradation of PHA, $\bar{P}_n$ and the content of PHA in the biomass decrease. At the same time, the number of polymeric chains N_t remains virtually constant. This is due to the fact that the extracellular

PHA-depolymerase is an exo-hydrolase, which destroys the polymeric chain by cleaving the terminal units of 3-hydroxyalkanoic acids.[27] Moreover, the polydispersity of the PHA polymeric substrate remains virtually constant and lies in the range $\bar{M}_w/\bar{M}_n = 2.0 \pm 0.3$ even at fairly high degrees of conversion.

It has been found[7,96] that soluble extracellular P(3HB)-depolymerase produced by *A. eutrophus* can degrade native P(3HB) granules to give monomeric D-(−)-3-hydroxybutyric acid (see Fig. 2). However, information on the structure and properties of this enzyme is quite scarce. It is believed that P(3HB)-depolymerase is a serine esterase having active HO groups.[92,97] The following sequence of processes has been proposed to describe the destruction of P(3HB) catalysed by this enzyme:[27]

$$E-OH + P(3HB)_n \longrightarrow P(3HB)_{n-1}OE + D\text{-}CH_3CH(OH)CH_2COOH,$$

$$P(3HB)_{n-1}OE + H_2O \longrightarrow P(3HB)_{n-1} + EOH.$$

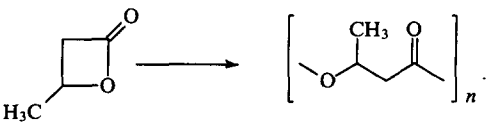
Here, E−OH is P(3HB)-depolymerase. A scheme for this heterogeneous process is shown in Fig. 7. According to a hypothesis,[27] the terminal carboxyl groups in P(3HB) react with the positively charged sites on the enzyme 'surface' to yield an active complex. In the case where fructose and butyric acid are used as initial carbon sources in the culture medium, the rate of anaerobic biodegradation of P(3HB) is illustrated by the following data:

Concentration /g dm^{-3}	Biodegradation rate /g h^{-1} dm^{-3}
Fructose	
5	0.013
10	0.027
20	0.025
Butyric acid	
5	0.023
10	0.023
20	0.034

The rate of biodegradation of P(3HB) depends substantially on the primary structure of PHA: structural regularity of the chain, and the nature and the sequence of the units in the chain.

The influence of stereoregularity of P(3HB) macromolecules on the dynamics of biodegradation of this polymer has been studied.[7] For this purpose, electrophoretically homogeneous P(3HB)-depolymerase was isolated from the *Penicillium funiculosum* fungus. The molecular weight of this protein was 38 000. The protein had an isoelectric point at pH 5.8, and its maximum hydrolytic activity was observed at pH 5.5–6.2 and at 30 to 35 °C.[98] The rate of the biodestruction of PHA is substantially higher than the rate of typical chemical hydrolysis of a polymeric substrate under the same conditions.[99–103]

Stereoregular PHA were obtained by polymerisation of stereoisomers of β-butyrolactone (BL) in the presence of ionic catalysts according to the following scheme:



BL P(3HB)

The polydispersity of the resulting samples of P(3HB) was low ($\bar{M}_w/\bar{M}_n \approx 1.1-1.5$), the proportion of isomeric units in the copolymers ranging from 50% to 100%. The P(3HB)-depolymerase isolated can catalyse hydrolysis of (*R*)-P(3HB) but proves inactive with respect to (*S*)-P(3HB).[7] As the proportion of (*R*)-3HB units in the (*S,R*)-P(3HB) copolymer increases, the rate of biodestruction increases and reaches a maximum when the content of the (*R*)-3HB units is 75 mol% (Fig. 8). Further increase in the content of the (*R*)-3HB units (to more than 90 mol%) results in a decrease in the rate of biodestruction. Biodegradation of a P(3HB) copolymer containing (*S*)- and (*R*)-units in a 1:1 ratio leads to oligomers with $\bar{P}_n = 3 \pm 1$. The biodegradation of PHA copolymers has also been studied by other workers.[95,104]

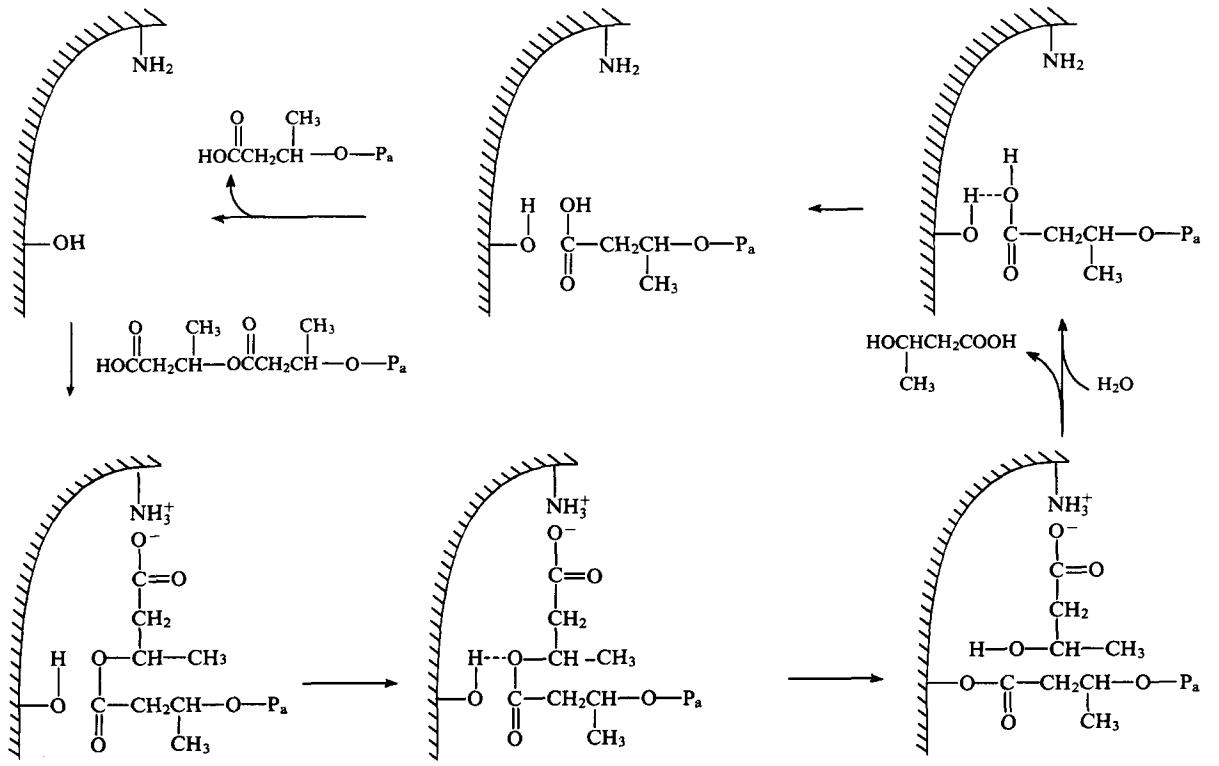


Figure 7. Scheme for the hydrolytic biodestruction of P(3HB) under the action of P(3HB)-depolymerase.[27]

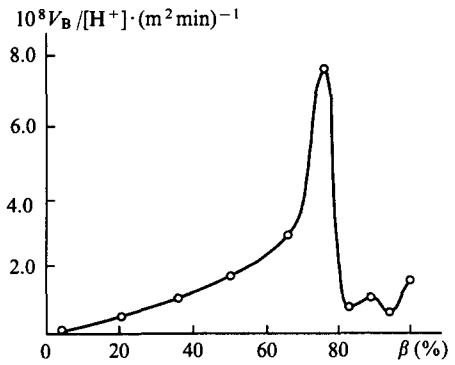


Figure 8. Influence of stereoregularity on the rate of biodegradation of P(3HB);[7] β is the content of the (*R*)-isomer in the stereocopolymer P(3HB), and V_B is the biodestruction rate.

The ability of the extracellular P(3HB)-depolymerase isolated from *Alcaligenes faecalis* to catalyse the hydrolysis of P(3HB-co-4HB) copolymers at 37 °C in a 0.1 M phosphate buffer (pH 7.5) has been studied.[26] Data on the dynamics of the variation in the mass of PHA films are shown in Fig. 9. As the content of the 4HB units in the P(3HB-co-4HB) copolymer increases up to 28 mol %, the rate of film destruction increases; however, when the content of the 4HB units exceeds 85 mol %, the rate of destruction diminishes and becomes lower than that in the case of homo-P(3HB).

The dependence of the rate of biodestruction on the composition of P(3HB-co-3HP) is presented in Table 7.

Biodestruction of the P(3HB-co-3HP) at 37 °C in a 0.1 M phosphate buffer (pH 7.5) in the presence of electrophoretically homogeneous P(3HB)-depolymerase isolated from *Alcaligenes faecalis* has been studied.[34] As the content of the 3HP units in the copolymer increases up to 20 mol %, the rate of the surface bioerosion of P(3HB-co-3HP) films increases. For higher contents of these units, the rate of destruction of copolymers decreases somewhat. The investigators believe[34] that this is due to the specificity of P(3HB) depolymerase.

The rate of the enzymic destruction of PHA has been studied in relation to the P(3HB-co-3HH) copolymer.[105] The influence of the primary structure of the macromolecules of PHA copolymers on the dynamics of biodestruction has been illustrated by Hori et al.[51] The films of polyurethanes synthesised from PHA blocks, various diols, and hexamethylene diisocyanate and having

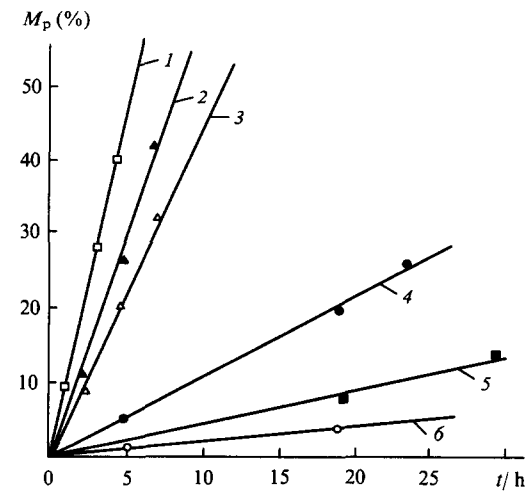


Figure 9. Dynamics of biodestruction of P(3HB)-co-4HB films in aqueous dispersions of P(3HB)-depolymerase at 37 °C and pH 7.5; proportion of (4HB)-units in the copolymer, mol %: (*1*) 28; (*2*) 10; (*3*) 6; (*4*) 0; (*5*) 85; (*6*) 94; M_p is the mass loss.

Table 7. Influence of the composition of P(3HB-co-3HP) on the crystallinity of the polymeric substrate (α_c) and on the loss of mass (M_p) of films during biodestruction.[34]

PHA	α_c (%)	M_p (%)
P(3HB)	60 ± 5	5.1 ± 1.7
P(3HB-co-7% 3HP)	43 ± 5	17.2 ± 3.4
P(3HB-co-11% 3HP)	41 ± 5	21.7 ±3.4
P(3HB-co-20% 3HP)	28 ± 5	31.7 ± 5.1
P(3HB-co-37% 3HP)	19 ± 5	26.6 ± 3.4
P(3HB-co-43% 3HP)	16 ± 5	26.3 ± 3.4

$\bar{M}_n = 1.98 \times 10^5$ and $\bar{M}_w/\bar{M}_n = 2.0$ lost about 70% of their mass following the biodegradation at 30 °C for four weeks, whereas the PHA polymer itself was depolymerised by 93%. The variation of the rate of biodegradation of PHA copolymers as a function of the copolymer composition is due to the difference in the degree of structural ordering of the polymeric substrates: as the degree of crystallinity decreases, the rate of destruction increases (see Table 7). Analysis of the biodegradation dynamics makes it possible to conclude that this process is governed by both the specificity of PHA depolymerase and the ordering of the structural elements of the polymeric substrate.

* * *

Film-forming and fibre-forming PHA can be obtained by biosynthesis in fairly high yields and with relatively low power consumption. By varying conditions of the biosynthesis, one can alter the composition and properties of the resulting compounds over a wide range. Polymers and copolymers of hydroxyalkanoic acids are readily crystallisable thermoplastic polymers with flexible chains. They are capable of forming homogeneous mixtures with various natural and synthetic film-forming and fibre-forming polymers, which permits them to be used as high-molecular-weight plasticisers and as diverse additives that change the relaxation spectra of polymeric materials. The biocompatibility of various PHA is also of considerable interest. Many homopolymeric and copolymeric PHA are biodegradable, which accounts for the efficiency of their practical application for the development of polymeric materials that are biodegraded at a controlled rate.

This work was supported by a grant of the International Soros Science Education Program (ISSEP).

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