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**HETEROGENEOUS SYSTEMS OF  
POLY-  $\beta$ - HYDROXYBUTYRATE**

Summary of the Thesis to  
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Current work is carried out in Riga Technical University,  
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### ***General characterization of thesis***

Production of organic polymeric materials is currently one of the principal areas of chemical industry. In recent years the total volume of yearly produced plastic materials exceeds 40 million tons, majority of which is used for the production of packaging materials and consumer goods. The percentage of used polymeric materials in waste increases constantly and their slow biodegradation process causes serious environmental problems. This aspect explains the lately observed rapid increase of the significance of biodegradable polymeric materials and materials produced on their basis.

The research on the production and application possibilities of such polymers was commenced at the beginning of the 1970-ies, when the economically developed western countries encountered very serious problems caused by the amount of polymeric materials of everyday application in waste. The biotechnologically produced poly-p-hydroxybutyrate (PHB) turned out to be one of the most promising biodegradable polymers. It is a linear, stereoregular polyester synthesized by individual bacteria from glucose and accumulated as a reserve of carbon and energy, similarly to how starch is accumulated in plants or fat in human and animal bodies.

**PHB** is a thermoplastic material that has attracted much attention due to such properties as biocompatibility and biodegradability. Due to the good dissolubility in several organic solvents and thermoplasticity, **PHB** articles can be produced from both solution and fusion. A significant advantage of PHB is that it can be produced from local renewable resources.

During the last decade certain research of the PHB structure has been performed. Problems related to both processing and **low** deformability of **PHB** are being solved, but at the same time information on **PHB** structure, its effect on the most important properties of a polymer (including the characteristics of strength-deformability) remains fragmentary and incomplete, thus systematic research in this area should be considered a **topical** issue.

**The main purposes of the work:**

to perform detailed analysis of the PHB molecular structure and properties,

to search for possibilities of purposeful modification of the structure of a polymer in order to obtain materials that would allow their application in such areas as medicine, agriculture and manufacturing of packaging materials.

The **scientific novelty** of the thesis is characterized by several aspects.

The characteristics of the PHB structure largely depend on the nature of the bacteria performing the synthesis. The Institute of Microbiology and Biotechnology of the University of Latvia has developed the technology of PHB synthesis where *Azotobacter chroococcum* 23 is used as a producer. The Institute of Polymer Materials of the Riga Technical University has developed an original methodology of obtaining this polymer from a biomass. The obtained material differs from the known materials with an unusually high molecular weight - up to 2500 kDa (normally the molecular weight of PHB produced as a mass production does not exceed 200 kDa).

Due to these properties this polymer becomes an interesting subject of research in regards of both its structure and properties, and also the input for obtaining PHB samples with a lower molecular weight by performing a purposeful radiation splitting of macromolecules with the help of  $\gamma$ -radiation. The performed structural research indicated that transformations caused by the radiation affect not only the amorphous part of a polymer (as it is considered), but also the crystalline structures of a PHB.

This thesis demonstrates also the possibilities of obtaining a PHB/HV copolymers using the above-mentioned *Azotobacter chroococcum* 23 as a producer. The structure and properties of the obtained products (depending on the composition of the copolymer) were analyzed with the purpose of developing a polymer with improved strength-deformability characteristics.

Special attention has been paid to the biomass containing PHB as a potential product for obtaining polymer materials. Biomass should be considered a semi-finished product of PHB, the application of which for the production purposes of finished articles would considerably reduce the production cost of such products. The thesis shows that the

application of appropriate plasticizers and production technologies allows obtaining materials with a rather wide range of properties (consequently - also applications).

Such plasticized PHB systems were developed and analyzed. These systems demonstrated not only high plasticizing effect (increase of the relative elongation at break, reduction of the glass transition temperature of the amorphous part, etc.), but also retained the positive PHB „bio" properties.

The **practical use** of the results is determined by the fact, that PHB is an environmentally safe material and its degradation products in both soil and human body are nontoxic. Developing and testing PHB based materials the following potential areas of application were considered: medicine (therapeutic systems containing antiseptic preparations; burn treatment bandages; gradual medication dispense systems; systems used in endoprosthetics, etc.), agriculture (ecological incrustation materials of seeds and other agricultural products; materials with controlled release of pesticides or fertilizers, etc.), environmentally friendly packaging materials (disposable dishes, various packaging materials).

Application of such PHB structure modification methods as plasticizing and  $\gamma$ -irradiation, PHB/HV copolymers with an optimal proportion of comonomers in macromolecule and certain PHB biomass systems, allows production of materials that may be used in production of the above mentioned areas in Latvia.

**Discussion of the results of the research.** The main results of the research were reported at the student conferences of the Riga Technical University (Riga, 1997, 1998, 1999), at the conference "Timber and flora based raw materials" of the LZP program (Jelgava, 1999), international conferences: "Chemistry and Chemical Technology" (Kaunas, Lithuania, 1997), "Mechanics of composite materials" (Riga, 1998), "Biodegradable plastics and polymers" (Stockholm, Sweden, 1999), "Property tailoring of thermoplastics - based blends and composites" (Bratislava, Slovakia, 2000), "Baltic Polymer Symposium" (Jurmala, 2003).

**Publications.** The results of the research have been described in 15 publications, incl. 6 articles in scientific magazines and 9 articles in the collections of conference materials.

**Structure and volume of the thesis.** The thesis consists of the introduction, review of the literature, methodological part, discussion of the results (5 sections), conclusions and the list of used literature. The content consists of 108-pages. The paper contains 36 figures, 15 tables and the list of used literature that consists of 141 sources.

## METHODOLOGY OF THE RESEARCH

The main subject of the research performed for the purposes of this thesis is the PHB synthesized in the Institute of Microbiology and Biotechnology of the University of Latvia (producent *Azotobacter chroococcum* 23), and the corresponding biomass. For comparison the product of the company ICI (producent *Alcaligenes eutrophus*) with molecular weight 145 kDa was used. 50+5 µm films were obtained from a diluted (1%) PHB solution in chloroform allowing slow evaporation of the dissolvent at a room temperature. 100+10 µm films were obtained with the hot pressing method. The pressing process lasted for 1,5 minutes at 190±2°C.

A number of low molecular compounds with similar molecular weight and comparable molecular polarity, as well as a nonpolar polymer polyisobutylene (PIB) were selected as PHB and its biomass plasticizers.

Systems with plasticizer content not exceeding 50% are developed by mixing appropriate amounts of 1% PHB and the solutions of the appropriate plasticizer in chloroform. Then upon evaporation of the solvent at a room temperature 50+5 µm films were obtained which before further testing were stored at a room temperature for at least 10 days in order to achieve balanced structure.

γ-irradiation was used as a method of a purposeful reduction of the molecular weight of the polymer. Irradiation of the polymer samples (powder, films) was performed in the air at an atmospheric pressure and in vacuum ( $10^{-3}$  tor) using  $\text{Co}^{60}$  as the radiation source that ensures irradiation with a permanent capacity of 0,78 Mrad per hour.

The molecular weight of PHB and PHB/HV samples was determined with the help of viscosimetric measurements. Polymer solution in chloroform was used. The temperature of the solution was 30°C. The viscosity of the solution was measured by Ubbelohde's dilution viscosimeter. The molecular weight was calculated according to the Mark-Howink equation  $[\eta] = KM^\alpha$ , where  $[\eta]$  is the intrinsic viscosity and K and  $\alpha$  the constants for the given polymer-solvent system ( $K = 1,18 \times 10^{-4}$ ,  $\alpha = 0,78$ ).

The mechanical properties were characterized by elongation tests of the samples. Dumbbell-shaped samples of PHB-based films (base width  $5 \pm 0,05$  mm, base length  $10 \pm 0,2$  mm) were used for mechanical tests. The samples were tested using a Universal Testing Machine UTS-100 at a room temperature at the extension rate of 2mm/min. From the obtained tension ( $\sigma$ ) - relative deformation ( $\epsilon$ ) curves the tensile strength ( $\sigma_B$ ) and the relative elongation at break were determined.

The microscopic analysis of the systems allowed additional characterization of the molecular structure of PHB systems. For the purposes of the research the optical microscope Leitz Laborlux 340, equipped with sample heating system was used. Color microphotographs were obtained using the video printer Sony UP-1200 EPM.

Thermal analysis of the systems was performed using a differential scanning calorimeter METTLER DSC-30. The samples were scanned at a rate of 10°C/min. under nitrogen flow from 25° to 220°C (first heating cycle). This allowed evaluation of the melting parameters of the PHB crystalline phase (melting peak maximum temperature  $T_{m1}$ , melting temperature range  $\Delta T_{m1}$  and the degree of crystallinity  $\alpha_1$ )- Then the samples were rapidly cooled, which caused the increase of the amorphous part.

Then the samples were scanned again at the same speed at a temperature -50 - 220°C (second heating cycle). During the repeated heating cycle it was possible to register much more precisely the glass transition processes of the PHB amorphous part, as well as to obtain additional information on the structure of the polymer by analyzing the "cold" crystallization process and the melting of the newly formed crystalline phase. Parameters to be determined: PHB glass transition temperature  $T_{gl}$ , the maximum temperature of the "cold" crystallization

peak  $T_C$ , temperature range  $\Delta T_C$  and the crystallinity *etc*, the melting peak maximum temperature  $T_{m2}$  of the newly developed crystalline phase, the range of melting temperature  $\Delta T_{m2}$  and the crystallinity  $\alpha_2$ , for plasticized systems also the plasticizer glass transition temperature  $T_{gl}$  and in several cases also the melting temperature of plasticizer  $T_{mpl}$

All parameters are determined using the original program METTLER GraphWare TA72PS.5. The crystallinity of PHB was determined by the heat effect of the crystalline phase melting, assuming that for a 100% crystalline PHB this value constitutes 146 J/g.

In order to determine the degradation temperature for PHB systems and the content of the low molecular products that might accumulate in a polymer as a result of irradiation of a polymer, the thermogravimetric method (TG) was used. The measurements were done using the system METTLER TG-50 within the temperature range 25-400°C at a permanent heating speed of 10°C/min. The value that corresponds to the maximum speed of the loss of weight of the polymer sample was assumed as the degradation temperature  $T_{destr.}$  The content of the low molecular products was characterized by the losses of weight within the temperature range that does not exceed 250°C.

The biodegradability of materials was characterized with the help of laboratory-accelerated soil biodegradation studies. PHB and biomass film samples (weight  $0.15 \pm 0.05$  g, thickness 30-40  $\mu\text{m}$ ) were placed in glass containers filled with microbially active soil (garden soil with 2% humus content, 22-24% water content, pH 6,5) and stored at a 25°C temperature. After 3, 7, 14, 22 and 30 days the samples were removed from the containers, washed in distilled water, dried and weighed in order to determine the loss of weight during the respective period of time.

The hydrolytic stability of systems was characterized by subjugation of samples to an impact of phosphate buffersolution with pH 7,4 at 70°C. 20x20 mm samples (thickness 30-40  $\mu\text{m}$ ) were placed each in a separate closed glass container with 20 ml buffersolution and stored in a thermostat at 70°C. With an interval of approximately 4 days the samples were removed from the buffersolution, washed, dried (after drying up the samples were for several days stored in a

desiccator in order to reach a balanced weight) and weighed, registering the changes in weight.

## RESULTS OF THE RESEARCH

### ***Isolation of PHB and characterization of the product***

The PHB used for the purposes of this research was synthesized in the Institute of Microbiology and Biotechnology of the University of Latvia using the method of controlled fermentation and the producer *Azotobacter chroococcum* 23 that ensures high polymer content in biomass - up to 80%. As a result of the research the technology of isolation and purification of a PHB developed by the Institute of Polymer Materials of the Riga Technical University was improved. In order to isolate the polymer from the bacteria cell remnants the solvent-casting technique was used. This process consists of three basic stages:

- 1) degradation of cells (in order to increase the penetrability of cell walls) and separation of the lipid fraction;
- 2) extraction of a polymer;
- 3) purification of a polymer.

By optimization of these stages it is possible to influence the level of degradability of a polymer, to increase the purity of the obtained polymer and to rationally regenerate the used solvents. Combined mechanical and chemical impact was used during the stage of primary processing of cells in order to accelerate the degradation of the cell shells. During the extraction phase of the product the optimal combinations solvent/non-solvent and their most beneficial proportions for the extraction were searched for. During the purification phase of a polymer the main attention was paid to the methodology of filtration and the selection of an appropriate filtration material.

As a result of the research a pilot-plant for the isolation of PHB from biomass after the fermentation process was developed, ensuring also the regeneration and recycling of the solvents. It should be noted that extraction of polymer and modification of its properties with the purpose of obtaining a product with defined properties may be combined into one technological process. In order to determine the possibilities of its application in specific articles the main properties of

the obtained PHB and its modification possibilities were determined (table 1).

The values of characteristic intrinsic viscosity  $[\eta]$  of a polymer obtained in laboratory scale using the pilot-plant are similar and vary between 8-12, respective values of polymer molecular weight (M) were 1500 - 2500 kDa. The selected producent and extraction method allows obtaining a polymer with a very high molecular weight that provides a wide range of possibilities for the regulation of the molecular weight and modification of PHB properties.

**Tab. 1. Main characteristics of PHB obtained**

1. Chemical structure	$\text{H}-\left[ \begin{array}{c} \text{CH}_3 \\   \\ -\text{O}-\text{CH}-\text{CH}_2-\text{C}- \\   \\ \text{O} \end{array} \right]_n-\text{OH}$ $n=17500 - 30000$
2. Intrinsic viscosity	8 - 12
3. Molecular weight	1.500 - 2.500 kDa
4. Admixture content	<1%
5. Density	1,25 g/cm <sup>3</sup>
6. Crystallinity	69%
7. Melting point	180°C
8. Glass transition	4,3°C
9. Tensile strength	31 MPa
10. Tensile modulus	2,7 GPa
11. Elongation at break	3%

Repeated dissolution and sedimentation of a polymer indicated that the PHB molecular weight remains unchanged, and the solvent-casting technique for preparation of PHB-based films may be used as a safe method for the preparation of PHB test samples not worrying about the degradation of a polymer.

In order to obtain a polymer with a defined molecular weight a fractional sedimentation may be performed. Control of the PHB molecular weight is possible also during the extraction process by adding mild hydrolyzing agents to the blend of polymer dissolvents. Using this method a polymer with M 30 - 1 000 kDa may be obtained.

Important are also the properties of a polymer fusion that were investigated by means of thermal analysis. The melting temperature of

the obtained PHB is 175-180°C. Investigating the flowability of the polymer fusion it was indicated that its processing by traditional methods is possible only within a narrow range of temperatures when the temperature of the fusion exceeds the PHB melting temperature by 5-10°C. This means that PHB can be processed with the injection moulding method provided that all necessary measures are taken to reduce the melting temperature and minimize the processing period in order to avoid rapid thermal degradation. The development and optimization of PHB obtaining methods was performed keeping in mind the specific areas of application of a polymer and its composites.

#### *Plasticized PHB systems*

A number of low molecular compounds with similar molecular weight and comparable molecular polarity were selected as PHB plasticizers: dibutylsebacate (DBS), dioctylsebacate (DOS), polyethylene glycol (PEG), polyethers: laprol 503 (a product obtained by polymerization of the propylene oxide and glycerin) and laprol 5003 (a product obtained by polymerization of the propylene oxide and glycerin and a following copolymerization with ethylene oxide).

All of the used products (with the exception of laprol 5003) are nontoxic biodegradable substances. For comparison, the PHB blends with polyisobutylene (a nonpolar polymer that is *a priori* incompatible with PHB) were also investigated.

Plasticized **PHB** systems (PHB-based films) were obtained from solutions, thus completely excluding the possibility of thermal and mechanical degradation of PHB and ensuring repeatable and easily controllable conditions for the development of molecular structures within the systems PHB-plasticizer. Besides such technique of test sample preparation was selected also keeping in mind the requirements of the production of film-based articles made of plasticized PHB that might find wide application in medicine, agriculture and manufacturing of packaging materials.

As it could be expected, the strength of compositions decreases with the increase of the plasticizer content ( $\phi_{pl}$ ). The curves  $\sigma_B(\phi_{pl})$  are similar for all plasticizers, and for the interval of the plasticizer content that does not exceed 0,5 it can be satisfactorily approximated by a linear coherence  $\sigma_B = \sigma_B^* - k\phi_{pl}$ ;  $\sigma_B^* = 30,8$  MPa, tensile strength of a

pure PHB;  $k=55,3$ ). Presuming that approximately 10 MPa is a minimum permissible value  $a_B$  of a plasticized polymer (for the majority of application fields this is a sufficient value) the maximum permissible value may be determined  $\phi_{pl} = 0,2 - 0,3$ .

The most interesting point is the variation of the elongation at break  $\epsilon_B$  with the plasticizer content in the system. A similar dependence can be observed for all structures. At a low concentration of a plasticizer comparatively small changes in  $\epsilon_B$  can be observed, though already at a plasticizer content  $\phi_{pl}$  around 0,1 the value of  $\epsilon_B$  for practically all systems has increased several times in comparison with the initial value  $\epsilon_B = 3\%$  for a pure PHB. Further increase of the plasticizer content causes a rapid increase of  $\epsilon_B$ , but starting with  $\phi_{pl} > 0,3$  we can again observe only an insignificant increase of  $\epsilon_B$  (Fig. 1).

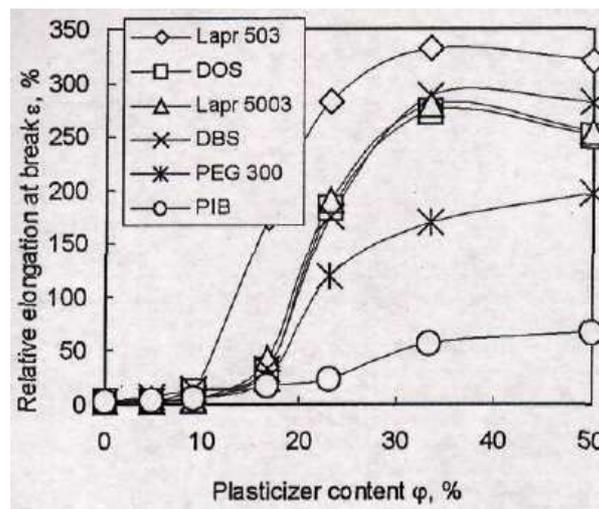


Fig. 1. Relative elongation at break  $\epsilon_B$  of plasticized PHB systems as function of the plasticizer content  $\phi$ .

Individual plasticized systems differ from each other by the maximum values of  $\epsilon_B$  (with  $\phi_{pl} > 0,3$ ) and the plasticizer content at which a rapid increase of the elongation at break values starts. The highest  $\epsilon_B$  values are achieved in the case of L503 ( $\epsilon_B > 300\%$ ). Even the content  $\phi_{pl} > 0,1$  of this plasticizer causes a significant increase in

the deformability. The group of plasticizers DBS, L5003 and DOS allows to achieve elongation values of about 250-300%. A rapid increase in deformability of these systems takes place at  $\phi_{pl} > 0,10-0,15$ . PEG causes more or less significant increase in the deformability of PHB only at  $\phi_{pl} > 0,2$ . As it could be expected, PIB causes the less noticeable changes in the  $\varepsilon_B$  values, though also this plasticizer ensures a  $>60\%$  elongation at the content  $> 0,3$  (Fig. 1).

According to their ability to plasticize PHB the additives may be arranged as follows (content  $\phi_{pl} = 0,2$  being the point of reference): PIB < PEG < (DBS, L5003, DOS) < L503.

The mechanical properties of plasticized systems are directly related to the structural changes of PHB. Depending on the level of compatibility of the specific plasticizer with the polymer, these changes may affect both the amorphous phase and the crystalline part of the PHB. In order to obtain information on the character of these transformations, a careful analysis of the structural changes of plasticized systems was performed, which was based on the differential scanning calorimeter (DSC) data.

First of all it was interesting to observe how the presence of plasticizers affect the crystallinity of PHB. During the formation of the structure of the system PHB-plasticizer from a solution a fully balanced crystalline phase is obtained. This is confirmed by the fact that the DSC  $T_m$  data of the first heating cycle do not indicate the exothermic "cold" crystallization peak. The level of crystallinity  $\alpha_1$  that is determined from the values of melting heat effect  $\Delta H_{m1}$  with the increase of the plasticizer content increases only a little and remains within the range 0,55-0,75.

Slight increase of  $\alpha_1$  values with the growth of the contents of such plasticizers as L503, DOS and DBS is determined by the increase of the macromolecular mobility of a polymer as a result of crystallization within the presence of the above plasticizers.

The maximum melting temperature peak  $T_{m1}$  decreases a little due to the simultaneously observed certain loss of arrangement in the crystalline part of PHB.

The DSC data concerning the glass transition indicate that the compatibility between the plasticizers and the PHB is limited. Two glass transition temperatures are observed on the DSC curves of systems with high plasticizer content. Temperature  $T_{glb}$ , which is lower

than the glass transition temperature of a nonplasticized PHB corresponds to the transition of the amorphous part of PHB containing plasticizer. The second temperature -  $T_{gl}$ , which is close to the glass transition temperature  $T_g$  of a plasticizer itself, probably corresponds to the plasticizer phase that possibly contains admixtures of low molecular fractions of PHB.

All investigated systems exhibit a number of common tendencies. The values of  $T_{gl}$  decrease with the rise of the plasticizer content to a certain value  $\phi_{pl}^*$  (approximately 0,1-0,2). When the  $\phi_{pl}$  increases even further,  $T_{gl}$  remains practically unchanged.

If  $\phi_{pl} < \phi_{pl}^*$ , only one transition temperature is registered -  $T_{gl}$ . This confirms complete compatibility of a plasticizer with PHB within this range of plasticizer content.

The investigated plasticizers cause a diverse decrease of  $T_{gl}$  that develops as a result of weakening of intermolecular influence in the amorphous part of the PHB. The most effective  $T_{gl}$  reduction is caused by L503. Other polar plasticizers - DBS, PEG and DOS - cause similar reduction of  $T_{gl}$ . At the same time the presence of L5003, which is higher molecular and less polar (consequently also less compatible with the PHB) reduces  $T_{gl}$  only a little.

Plasticizers that are able to reduce the values of  $T_{gl}$ , significantly were expected to cause also substantial significantly increase of the deformability of PHB. The majority of the investigated plasticizers did exhibit this regularity.

### ***The impact of Gamma radiation***

PHB synthesized in the Institute of Microbiology and Biotechnology of the University of Latvia was used for the purposes of this research, (hereinafter referred to as PHB-1). The average intrinsic viscosity and molecular weight of the PHB-1 was 1900 kDa. PHB-1 was compared with the product of the company ICI (producent *Alcaligenes eutrophus*) whose molecular weight was 145 kDa (hereinafter referred to as PHB-2).

Complete dissolution of irradiated samples and decrease of the characteristic viscosity values as a result of increase of the dose of radiation indicate that the effect of  $\gamma$ -radiation upon PHB is purely degrading.

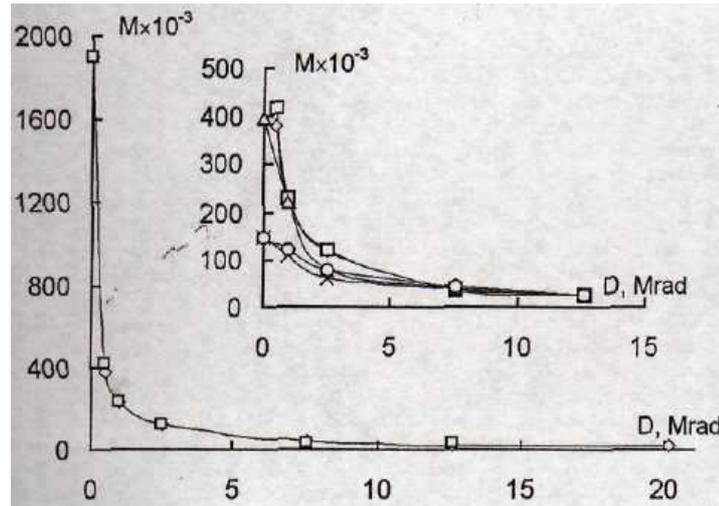


Fig. 2. Molecular weight  $M$  as function of irradiation dose  $D$ : for PHB-1 powder, irradiated at atmospheric pressure ( $\diamond$ ) and at pressure  $10^{-3}$  torr ( $\square$ ); for melt pressed PHB-1 with spherulite structure, irradiated at atmospheric pressure ( $\Delta$ ); and for PHB-2 powder, irradiated at atmospheric pressure ( $\times$ ) and at pressure  $10^{-3}$  torr ( $\circ$ ).

The dependency of molecular weight of PHB-1 and PHB-2 on the dose of radiation is reflected in Fig. 2. As we can see, even small doses cause a significant reduction in the molecular weight of both polymers. For example, if the dose of radiation constitutes 2,5 Mrad, the molecular weight of PHB-1 decreases from 1900 to 116 kDa (more than 16 times), but that of PHB-2 - from 145 to 61 kDa (more than 2 times). The dose of 1-2 Mrad corresponds to the regular sterilization doses in medicine. The obtained data indicate that the radiation sterilization of PHB articles should be performed rather carefully.

Linear dependence of the inverse value of the average statistical molecular weight on the dose of radiation  $D$  is observed:

$$\frac{1}{M(D)} = kD + \frac{1}{M(0)} \quad (1)$$

where  $M(D)$  - molecular weight of a polymer after the dose of radiation  $D$ ,  $M(0)$  - the initial molecular weight of a polymer,  $k$  - coefficient, that corresponds to the probability of the bond breaking, that is referred to the unit of the radiation dose and characterizes the intensity of the degradation process caused by the radiation.

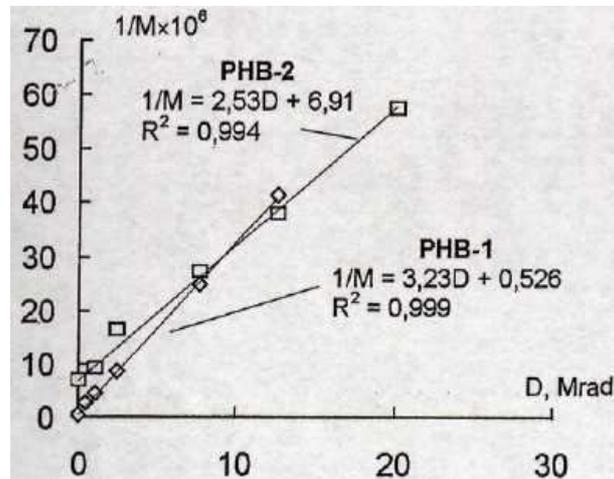


Fig. 3. Reciprocal values of molecular weight  $1/M$  as function of irradiation dose  $D$  for PHB-1 and PHB-2 powder, irradiated at atmospheric pressure.

PHB-1 degrades a little more intensively,  $k$  values that are calculated according to the obliquity of the straight lines drawn through experimental points constituted  $3,23 \times 10^{-6} \text{ Mrad}^{-1}$  for PHB-1 and  $2,53 \times 10^{-6} \text{ Mrad}^{-1}$  for PHB-2 respectively.

The obtained data indicate that the changes in the molecular weight of PHB taking place as a result of irradiation can be predicted with a fair degree of precision and  $\gamma$ -radiation can be used as a safe method for obtaining PHB with the required average statistical chain length.

Significant differences in the degradation kinetics for various types of PHB-1 samples subjected to irradiation (PHB powder isolated from the biomass, PHB-based films obtained from a fusion and from a chloroform solution by the solvent-casting technique) were not identified. Coefficient  $k$  values in an equation (1):  $3,23$ ;  $3,26$  and  $3,33 \times 10^{-6} \text{ Mrad}^{-1}$  respectively.

The impact of the molecular weight and the molecular structure upon the mechanical properties of PHB can be traced by irradiating the polymer powder with subsequent casting the films from the solution. Structural formations of the powder particles get completely degraded and a structure determined by the molecular weight of the polymer after irradiation in identical evaporation conditions of the solvent develops.

Attention should be paid to the fact that the molecular structure that develops by casting the films from the PHB solution is not a balanced structure. As a result of reorganization of the structure during the first 6-10 days after the film is cast, a rapid decrease of  $\sigma_B$  values takes place that stabilizes during the next 40-50 days;  $\sigma_B$  values at the same time increase a little. The reduction of deformability and a certain increase of strength apparently should be related to the post crystallization process that takes place at a room temperature (the crystallinity of a PHB balanced structure is 68%, the value demonstrated by the samples immediately after their preparation - only 58%), as well as to certain processes in amorphous part of a polymer (glass transition temperature determined immediately after the preparation of a polymer film constitutes  $-4,8^{\circ}\text{C}$ , but the glass transition temperature for films stored at a room temperature until they reach a balanced structure constitutes  $4,3^{\circ}\text{C}$ ).

$\varepsilon_B$  values of films cast from an irradiated polymer solution are considerably reduced with the reduction of the molecular weight of PHB.  $\sigma_B$  values start to decrease significantly only when the molecular weight becomes lower than 250 kDa (radiation dose  $>2,5$  Mrad).

Irradiation of samples that are obtained from polymer films stored until they have reached a balanced structure demonstrate a small reduction in  $\sigma_B$  and a reduction of  $\varepsilon_B$  values by 20-40%. Notwithstanding the relatively mild pressing conditions considerable thermodegradation of PHB-1 takes place if the samples are obtained with the help of hot pressing. The molecular weight decreases from 1900 to 400 kDa.

The molecular structure of a PHB developed from a fusion largely depends on the thermal prehistory. As a result of slow cooling process a defective crystalline phase is developed, that is characterized by the content of large spherolytic structures (the spherolyte dimensions are within the range of 200 - 1500 mkm, see Fig. 4) and a high crystalline

phase content (62%). As a result the strength and deformability of the samples is reduced. Irradiation of such samples leads to a further decrease of  $\sigma_B$  and  $\epsilon_B$  values.

Rapid cooling causes considerable super cooling and amorphisation of samples (the level of crystallinity practically decreases to 0). As a result the  $e_a$  values increase almost by two orders (the strength is correspondingly somewhat reduced). If these samples are stored for several weeks at a room temperature, the "cold" crystallization takes place and the level of PHB crystallinity reaches its usual values of 60-70% (spherulyte dimensions: 100 - 1200 mkm).



*Fig. 4.* Spherulitic structure of PHB (magnification 40 $\times$ ).

Taking into account the crucial effect of PHB molecular structure on its properties, calorimetric measurements of the polymer samples were performed before and after the irradiation. The obtained results indicate that when the radiation dose increases and the molecular weight of the polymer decreases correspondingly, the content of the

crystalline phase increases a little. Evidently during the irradiation process and as a result of breaking of chains a structural rearrangement, having a phase transformation character, takes place in the amorphous part of the polymer. The smaller the length of the macro chains (bigger dose of radiation), the more significant is the increase of the level of crystallinity, i.e. the bigger the effective mobility of chains. Here it should be noted that the temperature in the irradiation camera was 60°C. The mobility of macro chains under these conditions is sufficient for certain structural changes.

The level of crystallinity characterizes only the content of the crystalline phase, but the melting temperature  $T_m$  - the level of its arrangement. As we can see from the obtained data, the  $T_m$  values reduce with the reduction of molecular weight as a result of irradiation, due to the increase of configurative heterogeneity of the macro chains that constitute a part of the crystalline formations. A canonic dependence of the glass transition temperature  $T_g$  on the molecular weight of the irradiated polymers and a close to linear correlation between the content of the amorphous part of the polymer and the changes in the heat capacity of the system  $\Delta C_p$  were registered.

There is a generally accepted view that the processes caused by the radiation are mainly taking place in the amorphous part of the polymer and practically do not affect the crystalline phase. If this would be the case also with PHB, which is subjected to the degradation by radiation at 60°C, then the average decrease in the molecular weight should be only taking place in the amorphous part, which constitutes less than 40%. As a result there would be a significant widening of distribution of the molecular weight of the polymer (unfortunately in this study the distribution of the molecular weight was not experimentally determined) and consequently also the dispersion of the level of (arrangement of the crystalline structures, determined by the temperature difference between the end and the beginning values of the melting ( $T_{em}-T_{bm}$ )) would increase. Our data prove that the values of ( $T_{em}-T_{bm}$ ) practically do not alter by the radiation dose. This fact allows to assume that the radiation degradation affects also the crystalline part of a polymer.

Thermogravimetric research indicated that within the interval 20 - 2000 kDa of the molecular weight the PHB degradation temperature practically does not change and constitutes 295-300°C. At a

temperature that does not exceed 250°C, with the reduction of the molecular weight of a polymer the loss of weight increases almost twice, which can be explained by the increased hydrophilicity of the PHB at the expense of the increase of the number of end groups and by the increase of the content of low molecular degradation products in the polymer.

### ***PHB/HV copolymers***

To study an effect of phosphate concentration and carbon sources on the PHB/HV composition and on physical and mechanical characteristics, cultures of *Azotobacter chroococum* 23 were grown in glucose or sucrose media with valerate or without it for 48 hours. The highest biomass yields and values of the polymer production under growth conditions were reached upon *Azotobacter chroococum* 23 cultivation in glucose without valerate. Addition of valerate depressed culture growth both in the glucose and in the sucrose medium, but with sucrose to a greater degree than with glucose.

*Azotobacter chroococum* 23 cultures demonstrated different growth speed in different media. The highest PHB cellular concentrations were found in sucrose and the lowest PHB/HV copolymer concentrations in sucrose with valerate. Also the PHB/HV copolymer with the highest HV concentrations was produced in the sucrose medium. Both, in sucrose and in glucose the HV concentration in copolymer increased with the increase of the phosphate concentration in the media. Depression of PHB production and accumulation of phosphate in cells of *Azotobacter chroococum* 23 was observed under conditions of high  $\text{PO}_4^{3-}$  level in medium. It is possible that the observed differences in the HV molar concentration in the copolymer produced by *Azotobacter chroococum* 23 under growth conditions are connected with culture growth phase and a PHB/HV copolymer with the highest HV concentration is synthesized in slower growing cultures.

The polymers were recovered from the *Azotobacter chroococum* 23 cultures and purified to determine the intrinsic viscosities of the polymer samples. The intrinsic viscosity and the mean molecular weight of homopolymers were in general higher than that of copolymers and ranged within 7,7 (molecular weight - 1488 kDa) - 12,3 (2713 kDa), for a copolymer from 6,4 (1174 kDa) - 8,7 (1740

kDa) correspondingly. The lowest values of the mean molecular weight of PHB and PHB/HV were observed in the cultures grown in the medium with the lowest phosphate concentration (0,5 g/l). These differences could be explained by differences in growth stages of *Azotobacter chroocooom* 23 caused by the phosphate concentration. Similarly a decrease in the PHB molecular weight during its accumulation has been observed under conditions of *Alcaligenes eutrophus* phosphate exhaustion.

In order to determine the structural properties of PHB and PHB/HV synthesized by *Azotobacter chroocooom* 23, thermal analysis of the samples with the HV content within the range from 0 to 20 mol% was performed. The total crystalline phase in PHB/HV samples was significantly lower than that in pure PHB, and upon an increase in the HV content from 0 to 20 mol%, it decreased from 63 to 31%. Similarly, the temperature values of glass transition also decreased from about +4° to - 6°C, which indicates an increase in the flexibility of the macromolecular chains and allows to achieve the desirable increase in the deformability of a polymer. The values of the melting temperature ( $T_m$ ) of the analyzed PHB/HV copolymer decreased from 180° to 116°C (Fig. 5), reaching the lowest value at 20 mol % HV content in the copolymer. The lowest melting temperature for a PHB/HV copolymer reported in the literature is 75°C at approximately 40 mol%.

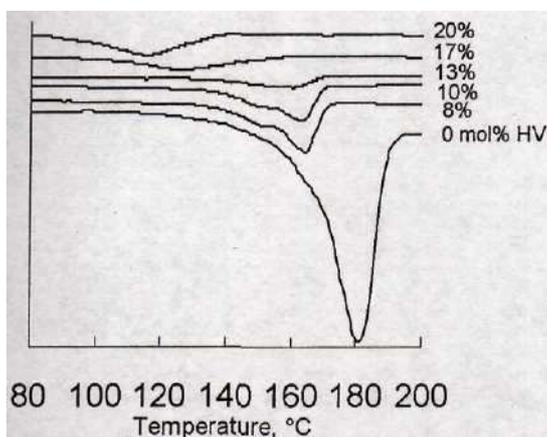


Fig. 5. Thermograms of PHB and PHB/HV with different HV content.

Reduced melting temperature in comparison with pure PHB is a significant advantage of the obtained copolymer since the temperature at which considerable degradation of chains of this polymer starts taking place, changes only a little with the increase of HV content. Thus the processing of PHB/HV copolymer with the traditional methods of thermoplastics is considerably facilitated, since the range of processing temperatures in comparison with pure PHB is much wider.

It is obvious that the, above described, structural characteristics have a direct impact also on the strength - deformation properties of a polymer. Therefore the mechanical properties of obtained copolymer samples with different content of HV groups were measured by testing the tensile strength after 10, 28, 224-day storage at a room temperature. It was done because the deformability of PHB and its copolyesters tends to decrease during storage at a room temperature. It is also possible, that this phenomenon is the reason for the considerable differences in the PHB mechanical characteristics determined by various researchers.

The obtained mechanical properties of a PHB are typical for a polymer isolated from *Azotobacter Chroococcum* 23. The value of tensile strength  $\sigma_B$  of PHB samples measured after 10 days since their preparation was about 30 MPa and increased during the next 18 days to about 31 MPa only. The value of elongation at break  $\varepsilon_B$  of the PHB samples after 10-day storage was about 6% and decreased to 3,3% during the next 18 days. Further storage of the PHB samples did not lead to significant changes in the mechanical properties of a polymer, which corresponds with the data obtained as a result of earlier research.

The tensile strength  $\sigma_B$  of PHB/HV copolymers synthesized from *Azotobacter Chroococcum* 23 is by 40-50% lower than the tensile strength of pure PHB obtained under identical conditions and it differs within the range of 15-23 MPa. At the same time, the values of elongation at break  $\varepsilon_B$  of copolymers were considerably higher than those of the homopolymer and increased markedly with increasing HV content. High values of elongation at break  $\varepsilon_B$  were shown for systems obtained using sucrose: P1/SV, P2/SV and P3/SV, reaching even 800% if the samples are tested after 10 days since their preparation (upon reaching a balanced structure,  $\varepsilon_B$  reduces to 182%). Evidently, high levels of elongation at break for systems with a high HV

concentration are to be associated with low crystallinity and glass transition temperature values.

The deformability of both pure PHB and copolymers reduces considerably during the period after their preparation, especially this is the case with the PHB/HV copolymer and the samples under observation demonstrated a 5-10 times decrease of  $\epsilon_B$  values within the period of 224 days since their preparation. At the same time it is clear that a copolymer requires a much longer period of time in order to reach a balanced structure than a pure PHB (approximately 30 days at a room temperature), which is related to difficult forming conditions of crystalline lattice in a copolymer. However, even after reaching a balanced structure, a PHB/HV copolymer demonstrates a much higher deformability in comparison with the PHB homopolymer. For a copolymer synthesized from *Azotobacter Chroococcum 23*, the  $\epsilon_B$  values differ within the range 25-182% depending on the HV content

Therefore a conclusion may be drawn that a PHB/HV copolymer may be produced by *Azotobacter chroococcum 23* and different conditions of synthesis ensure obtaining of a polymer with a wide range of physical and mechanical properties. At the same time, of course, a polymer remains highly biodegradable and biocompatible.

### ***PHB biomass as an independent product***

As a result of evaluation of the mechanical properties of the PHB biomass samples a conclusion was drawn that pure biomass practically cannot be used for obtaining film-based articles from a solution. As it was mentioned earlier, biomass contains only 75% of PHB that is able to create a homogeneous film and ensure its mechanical strength. The rest of the biomass consists of cell remnants, which are very heterogeneous and contain both high molecular proteins that act as inert fillers. Besides, the biomass contains a number of low molecular lipids and proteins that are able to integrate within the structure of a polymer and affect its properties. It should be noted that the PHB content in biomass and the content of cell remnants may vary significantly depending on the selected fermentation technology and the type of the bacteria used as a producent.

During the process of a polymer film developing from a solution, the cell remnants tend to associate in irregular agglomerates. They

spread unevenly in the film and create areas with decreased deformability and strength, if the deformability of a polymer film is insufficient, it disintegrates already during the drying process as a result of uneven shrinking. By filtering the rough fractions of the cell remnants, a uniform polymer film can be obtained easily. Though its strength and deformability is so low that it is practically impossible to determine their values.

Adding plasticizers to the biomass, the deformability of the obtained films can be significantly increased. As a biomass is essentially a PHB system where the particles of cell remnants act as fillers the most effective characterization of the properties of plasticized systems can be provided comparing them with the corresponding pure PHB systems.

In comparison with PEG, the plasticizer L5003 ensures a much higher increase in deformability for both pure PHB and biomass systems. Even addition of 5-10 % of L5003 allows achieving of approximately 5% value of elongation at break that is sufficient to ensure free manipulation with the polymer film. PEG is able to ensure such elongation values only around 30-40% (Fig. 6).

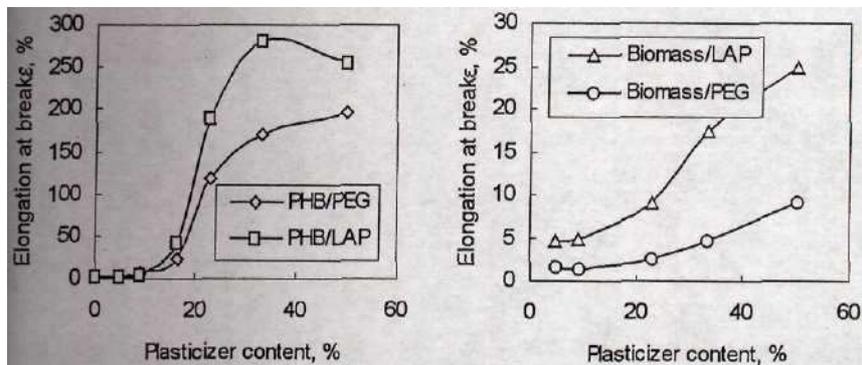


Fig 6. Elongation at break  $\epsilon$  of plasticized PHB and PHB biomass systems as function of the plasticizer content  $\phi$ .

In general plasticizers affect PHB and biomass systems similarly. Though in PHB systems it is possible to achieve much higher elongation values with the same amount of plasticizer. As mentioned

above, this can be explained by the presence of the agglomerates of cell remnants in the biomass, acting as structural defects. This assumption is confirmed also by the tensile strength values of a biomass that, unlike PHB, remain practically unchanged with changes in the content of plasticizer and are identically low in all systems.

Notwithstanding the fact that plasticized biomass systems are unable to match pure PHB neither in the sense of deformability nor strength, blends with the contents of plasticizer >30% can be used as, for example, as agrochemical carriers or seed incrustation materials.

### ***Characterization of plasticized PHB biomass systems***

The most important property of the materials under observation is undoubtedly their full microbial degradability to CO<sub>2</sub> and H<sub>2</sub>O. As these materials are envisaged to work into soil, the most precise information may be provided by the simulation of these processes, i.e. by performing laboratory-accelerated soil degradation studies.

In order to characterize principally the influence of plasticizers, the samples were tested after 3, 7, 14, 22 and 30 days. Full biodegradation kinetics was measured for pure PHB, biomass and two plasticized model systems PHB/LAP33% and PHB/PEG10%.

All systems demonstrated a rapid reduction of weight within a few days. Apparently an intensive colonization process is taking place during the first days, when the soil bacteria acquire the sample surface. Afterwards the PHB and other biodegrading components were degraded, besides the amorphous areas of a polymer degraded significantly faster than the crystalline structures.

Complete biodegradation of pure PHB occurred after 30 days, but after one week the polymer had already lost almost a half of its weight. Biomass degraded even faster. This can be explained by the high biodegradability of the cell wall remnants in the biomass, which allows loosening of the structure of a polymer. As a result the surface of the sample available to the bacteria is considerably enlarged.

PEG acts similarly. Being itself a low molecular plasticizer that demonstrates high biodegradability, it also loosens the PHB structure within a short period of time thus accelerating the degradation of the system. Since soil bacteria practically do not decompose laprol L5003, the slowest degradability demonstrated system, which contains this plasticizer. Apparently L5003 detains access of appropriate bacteria and enzymes to PHB. Though also this system loses half of its weight

within a period of 30 days, which practically results in disintegration of a polymer film (a complete degradation of the system requires 6-8 months). Such degradation of systems can be accelerated by decreasing the contents of the nondegradable component. Thus, for example, reduction of L5003 concentration to 10% twice reduces the period of time required for the system to lose half of its weight.

In order to characterize better the degradability of a biomass, the testing of the system hydrolytic stability was also performed. This experiment differs significantly from the previously described tests, since in this case the influence of enzymes is excluded. The stability of systems in buffer solution with pH 7,4 allows evaluation of the application possibilities of the tested materials in veterinary as matrixes for prolonged medication release or for bandages.

In laboratory conditions PHB practically does not submit to hydrolytic degradation and the hydrolytic degradation of PHB that takes place during a one month period is too insignificant to cause a more or less considerable weight loss. Such a result can be explained by the high level of crystallinity (58%) and high molecular weight (1900 kDa) of a polymer. The latter determines a low content of hydroxyl end groups and increases the hydrophobicity of a polymer.

Thus the biomass losses may be related to the dissolution of low molecular admixtures. Taking into account the fact that PHB practically does not submit to hydrolytic influence, and the biomass weight losses during the period of observation do not exceed 10%, it is clear that the loss of weight of plasticized PHB biomass systems can be mainly explained by the washing out of plasticizer phase from the system. Besides this process has almost come to an end during the first week of the experiment, after which the system weight remains practically unchanged. The obtained data allow conclude that only 5-10% of the initial amount of PEG and approximately 25% of the initial amount of L5003 have remained in the system. Apparently the migration of PEG from PHB matrix is facilitated by its comparatively low molecular weight and a better dissolubility in the appropriate environment. Such a result correlates fully with the previously described soil biodegradation data.

The analysis of the structural changes of polymer systems with the help of differential scanning calorimetric measurements was also performed. The comparison of  $T_m$  data for pure PHB and its biomass indicates that PHB in biomass possesses a little lower level of crystallinity and a lower level of orderliness of crystalline structures (the

Maximum melting peak temperature is by 5°C lower than that for a pure PHB). Melting is taking place within a narrower range of temperature values. A considerable difference can be observed in the dimensions of the "cold" crystallization peak, which characterizes the crystallization speed of a polymer and thus also the mobility of chains. If for a pure PHB 75% of crystalline phases are able to develop during the cooling period, then for a biomass this value constitutes only 50%. Also the PHB glass transition temperature in biomass is somewhat reduced.

As a result of adding plasticizers to the system, the above described tendencies increase even more. The values of PHB crystallinity in plasticized biomass vary between 41-45% and they are by 10-20% lower than those of the corresponding systems of plasticized pure PHB. A similar tendency can be observed also for melting temperatures that reflect the level of orderliness of crystalline compounds of a polymer. Like a nonplasticized pure PHB and PHB biomass, also plasticized systems maintain an explicit difference within the square of the „cold" crystallization peak. Apparently the admixtures in the biomass detain the crystallization of PHB and do not allow the development of such an orderly crystalline phase as in pure PHB.

Comparison of the influence of the two selected plasticizers indicates that the low molecular PEG is able to reduce the PHB melting temperature and affect the speed of the crystallization process much more significantly. With the 50% plasticizer content the mobility of PHB macromolecules has increased to such an extent that the crystallization can fully take place already during the cooling process, and during the repeated heating cycle the "cold" crystallization peak is no longer observed. Also the crystalline level change tendencies are different. The increase of the L5003 content from 10% to 50%, leads to the increase of the crystallinity of PHB from 42% to 54%. For PEG it reduces from 50% to 41% respectively. Such differences may be explained by the different chemical composition and molecular weight values of plasticizers, consequently - also a different level of compatibility with PHB. Besides PEG'S ability to crystallize should be taken into account, which means that there are several amorphous and crystalline phases within the system, as well as a number of admixtures from the bacteria cell remnants. Probably it is the crystallinity of PEG that does not allow the plasticizers to ensure as high a degradability of a system as L5003 does.

## Summary

1. Poly- $\beta$ -hydroxybutyrate (PHB), microbologically synthesized by use of new efficient producer *Azotobacter chroococcum* 23 was the main subject of this research. This PHB shows a number of specific features, among them extremely high molecular weight (up to 2500 kDa) and low deformability (relative elongation at break  $\epsilon_B < 3\%$ )

2. The methodology of isolation and purification of a PHB was analyzed and respective technique was improved. The most important characteristics of the product were determined.

3. In order to increase the deformability of PHB, plasticized systems were investigated using a number of low molecular compounds as plasticizers: dibutylsebacate, dioctylsebacate, polyethylene glycol (PEG), polyethers (LAP). All these plasticizers are nontoxic biodegradable compounds with similar values of molecular weight and comparable molecular polarity.

The selected plasticizers are fully compatible with a polymer up to a 15 - 20% content and form with it a single phase. The deformability of a polymer can be effectively enlarged ( $\epsilon_B$  at a room temperature reaches 250 - 300%) by rise of the content of a plasticizer within this range. At the same time also a considerable reduction in the strength of the system was observed at plasticizer content above 20%.

In accordance with the data of the DSC measurements, in the presence of the above mentioned plasticizers the proportion between the crystalline and amorphous phase of PHB remains practically unchanged. The changes in the deformability of the system PHB-plasticizer are mainly due to an efficient weakening of the intermolecular interaction within the amorphous part of the polymer. A certain reduction in the level of arrangement in the crystalline part is of a secondary significance.

4. The impact of  $\gamma$ -radiation upon the structure and properties of PHB was investigated. Polymer samples were subjected to 1 - 20 Mrad  $\gamma$ -radiation doses in air and in vacuum. Changes of the molecular weight and of structural characteristics of the crystalline and amorphous phase as well of deformability of a polymer were determined. The reduction of the molecular weight of a PHB with the radiation dose can be described by a simple equation:  $1/M(D) = kD + 1/M(O)$ , where  $M(D)$  - molecular weight of a polymer after the radiation

dose  $D$ , but  $M(O)$  - the initial molecular weight of a polymer. This observation makes the  $\gamma$ -radiation as a safe method of a purposeful reduction of a molecular weight of a polymer.

Elongation at break  $\epsilon_B$  of films that were cast from a polymer solution subjected to a dose of radiation not exceeding 2,5 Mrad, considerably reduces with the drop of PHB molecular weight. The tensile strength  $\sigma_B$  starts to decrease rapidly when the molecular weight has decreased -to a level lower than 250 kDa (radiation dose >2,5 Mrad). A slight reduction of  $\sigma_B$  and reduction of  $\epsilon_B$  by 20-40 % is observed after irradiating of ready samples (that are obtained from cast films and stored until they have reached a balanced structure).

The DSC measurements show that decrease of molecular weight caused by the irradiation leads to the growth of PHB crystallinity. The values of the melting temperature  $T_m$  that characterize the level of orderliness of the crystalline phase diminish with the drop of the molecular weight. In contrary to existing viewpoint that the radiation conversions (degradation, crosslinking) mainly take place in the amorphous part of a polymer, the results of DSC measurements allow to conclude that the degradation caused by the radiation somewhat affects also the crystalline part of a PHB.

5. The possibilities of obtaining hydroxybutyrate-hydroxyvalerate copolymers (PHB/HV) by use of *Azotobacter chroococcum* 23 as a producent were investigated. The effect of phosphate concentration and selected carbon sources on the fermentation process and the composition of the obtained copolymer were ascertained. The isolation and purification of the obtained PHV/HV copolymers was performed.

Most essential characteristics of copolymers were investigated in comparison with a homopolymer PHB obtained under similar conditions. The decrease of melting temperature from 180°C to 116°C and drop of the glass transition temperature from 3,1 to - 6,3°C, respectively, with increase in the HV content from 0 to 20 mol% occurs. The deformability of the copolymer is considerably higher than the deformability of a homopolymer PHB. Elongation at break exceeds 180% at 20mol%of HV.

It is obvious that by change of the synthesis conditions the selected producent *Azotobacter chroococcum* 23 allows to obtain PHB/HV copolymers with a wide range of comonomer ratio and comparatively broad spectra of physical and mechanical properties.

6. It was proved that unpurified biomass together with selected plasticizers (LAP and PEG) can be used for obtaining films by casting from a solution. Thereby both pure PHB and the biomass can be used for production of matrixes for agricultural chemicals and seed incrustation materials.

7. The rate of biodegradation of PHB, respective biomass and corresponding plasticized systems in various conditions was studied. The change of mechanical properties as well structural characteristics of degraded samples was investigated

PEG containing systems are characterized by high biodegradability. They fully degrade in the soil within a period of few weeks. LAP, in its turn, considerably slows down the degradation.

The hydrolytic stability test indicated that biomass contains a number of dissoluble admixtures that demonstrate ability to migrate. Plasticized systems are able to lose the majority of the plasticizer, loosening the structure of the polymer and indirectly making them more degradable. These results, together with the DSC data, confirm that only a certain part of the plasticizer is compatible with the polymer. The rest of plasticizer exists as an autonomous phase within the system. The DCS data indicate that the effect of the biomass admixtures upon the PHB structure is similar to the effect of the plasticizing additives. Though this „plasticizing" effect is not reflected in the mechanical properties. Besides, the analysis of the DSC data allowed to conclude that hydrolysis cause deep and irreversible degradation of the PHB structure detectable long before it can be checked by the changes in weight.

8. PHB and PHB/HV polymers are ecologically safe, their biodegradation products in a soil and human body are harmless and friendly, plasticizing and radiation modification allow to build PHB systems with acceptable strength characteristics and deformability. These systems may be applied in numerous areas in medicine (therapeutic systems containing antiseptic substances; burn treatment bandages; gradual medicine dispense systems; systems used in endoprosthetics), agriculture (ecological incrustation materials of seeds and other agricultural products; materials with controlled release of pesticides or fertilizers) and production of environmentally friendly packaging materials (disposable dishes, various packaging).

## List of the publications

*Main results of the investigations are shown in following publications*

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3. V. Tupureina, A. Dzene, M. Kainins, **I. Bibers**, L. Savenkova. Biodegradable PHB composite materials. Proceedings of the 14<sup>th</sup> Bratislava International Conference on Modified Polymers "Property tailoring of thermoplastics - based blends and composites" Bratislava, October 1-4, 2000, pp. 112-113.
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