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Biodegradable Materials from Plasticized PHB Biomass

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Summary: Biodegradation rate in different environments as well as deformation characteristics of poly-β-hydroxybutyrate (PHB), PHB biomass and of plasticized systems thereof have been investigated. Polyethylene glycol (PEG) and oxypropylated glycerine or laprol (LAP) were selected as plasticizers. Increase of the content of plasticizer from 5 to 50 % gave rise of the elongation at break from 4 to 25 % for LAP and from 2 to 9 % for PEG, respectively. No significant changes of strength were recorded. It remained comparatively small - around 2.5 MPa. PHB, biomass and the PHB composition containing 10 % PEG completely decomposed in soil during 30 days. PHB containing 33 % of biologically stable LAP additives lost half of its mass at the same period. Structural changes of plasticized biomass are also shown.

Introduction

Microbiologically synthesized polyester polyhydroxybutyrate (PHB) produced by various bacteria as intracellular carbon and energy storage material[1] is of special scientific and practical interest mainly due to possibility of its production from cheap renewable raw materials, thermoplasticity and specific properties - biodegradability and biocompatibility. These features form a serious background for a wide range of applications (medical, agricultural, marine, packaging) of the polymer[2,3]. Microbial PHB can be processed like other synthetic thermoplastics, however, some characteristics of the polymer, such as brittleness, narrow temperature window of processability and relatively high cost, are shortcomings seriously limiting its processing and use. In order to change and control properties of PHB and make it viable and cost competitive in a high-volume market of polymers, the modification of PHB through blending with different high- and low molecular components has been done. The last is comparatively inexpensive and simple but less exploited means of tailoring material properties.

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The aim of the present work was (i) to develop a process of making plasticized *Azotobacter chroococcum 23* PHB and biomass composite blends with some low molecular polyethylene glycol (PEG)[4] and oxypropylated glycerol (laprol), and (ii) to study physical, thermal, mechanical properties and biodegradability of the obtained composite materials. Special attention was paid to *Azotobacter chroococcum 23* PHB biomass as an intermediate product of PHB production. Dry biomass of PHB contains 70 to 90 wt % of PHB, the rest being composed of cell debris[1]. The cost of PHB has mainly been fixed by outlay for PHB extraction process from biomass, therefore, the possibility to use a cheaper untreated biomass instead of pure PHB for some kinds of the products (with lower demand for purity and strength - deformation characteristics) is particularly attractive. Potential application areas of PHB biomass items could be agrochemicals containing matrices and seed immobilization films for agriculture. As biomass is more brittle than PHB, plasticization is necessary. Thermal and mechanical destruction was prevented by using the film-casting technology for sample preparation. The conditions for controlling the submolecular structure were provided elsewhere[5, 6].

Materials and methods

Biomass was obtained by fermentation of glucose in the presence of a producer *Azotobacter Chroococcum 23*[7-9]. PHB content in biomass is 75 wt.%. To obtain pure PHB polymer was extracted from biomass with chloroform. Solution was filtered and PHB precipitated with isopropanol. PHB with the average viscometric molecular weight 1.9×10^6, glass transition temperature of amorphous part $T_g = 5$ °C, melting temperature $T_m = 183$ °C, crystallinity $\alpha = 58$ % was obtained.

Polyethylene glycols (PEG) and oxypropylated glycerol or laprol (LAP) as plasticizing additives were used. Molecular weight of PEG is 300, glass transition temperature $T_g = -4.8$ °C, melting temperature $T_m = -16.5$ °C. Molecular weight of LAP is 5000, $T_g = -66.6$ °C. Systems with plasticizer content of 50 % were produced by mixing the respective amounts of 1 % solutions of PHB biomass and corresponding plasticizers in chloroform. By slow evaporation of the solvent at room temperature 30-40 μm thick films were obtained. In order to obtain an equilibrium submolecular structure, samples were kept in a dessicator at room temperature for at least 10 days before testing to reach complete crystallization[10].

Obtained films were cut into specimens in the form of flat dumbbells (width of the test area 5 ± 0.05 mm, length 10 ± 0.2 mm). Tensile strength σ (the maximum stress of the stress/strain curve) and the relative elongation at break ε were determined. The test was performed by using a UTS-100 device at room temperature with strain rate 2 mm/min.

Biodegradability of materials was characterized by a biodegradation test in soil. Specimens of PHB and biomass films (weight 0.15 ± 0.05 g, thickness 30-40 μm) were placed to the glass containers with microbially-active soil (garden soil with 2 % humus content, 22-24 % water content, pH 6.5) and held at 25 °C. At a given time (3, 7, 14, 22 and 30 days) specimens were taken out from containers, washed in distilled water and dried. Weight loss of samples was determined gravimetrically[11].

Hydrolytic stability of systems was characterized by subjection of samples to the phosphate buffer solutions at pH 7.4 and temperature 70 °C. Sheets (20×20 mm, thickness 30-40 μm) were placed separately from each other in a closed glass container with 20 ml of buffer solution and held in the oven. In 4 days interval, specimens were withdrawn from the buffer solution, washed and dried. To achieve weight equilibrium after drying specimens were held in the dessicator for some days and then weighed.

Calorimetric measurements were carried out by differential scanning calorimetry on a METTLER DSC-30 instrument at a constant rate of heating 10 °C/min in a nitrogen atmosphere in the temperature range 25-220 °C (the first heating cycle). Then the specimens were rapidly cooled (about 100 °C/min) and scanned again at the same rate in the temperature range -50-220 °C (the second heating cycle). Upon rapid cooling from the molten state, the polymer has time to crystallize just partially. The content of its amorphous part considerably increases. The second heating cycle makes possible to record the transition of the amorphous part of the polymer from the glassy state as well as to observe the subsequent process of "cold crystallization" and next melting of the crystalline phase (evaluated parameters: T_{gII} - glass transition temperature of PHB; T_c - crystallization temperatures; T_{m2} - melting temperature (position of melting peak maximum) of newly developed crystalline phase; ΔT_{m2} - temperature interval of melting; α_2 - degree of crystallinity; T_{gI} - glass transition temperature and T_{mpl} - melting temperature of plasticizers).

All the characteristics were calculated by using a software METTLER GraphWare TA72PS.5. The degree of crystallinity α of PHB was evaluated from the heat effect of melting the crystalline phase (per weight unit of PHB), assuming that for a 100 % crystalline PHB this quantity is equal to 146 J/g[12].

Results and discussion

Our preliminary experiments showed that untreated biomass is unsuitable for film forming and mechanical characteristics of brittle biomass samples are extremely poor. As it was previously mentioned, biomass contains 75 % PHB – the film forming component, which provides its mechanical strength of the whole system. The remaining part of biomass consists of cell residues – mainly membranes of cell walls containing high molecular proteins. This component should be considered as a filler of PHB matrix. Small amounts of some low molecular lipids and proteins may be integrated in the structure of polymer matrix and could influence its properties.

Filtration of PHB biomass solution through fine-spun polycaproamide cloth sieve allows for removal of the rough cell residues and to obtain a uniform polymer films. However their strength and deformability were too poor for practical considerations. Introduction of plasticizers in biomass results in significant increase of deformability of the film (Fig.1). Since biomass actually is a PHB matrix which contains particles of cell residue as a filler, it seems reasonable to compare properties of plasticized biomass systems with ones of PHB based[5].

Just like in a case of pure PHB, biomass systems with LAP provide higher values of deformation than with PEG. Even 5 to 10 % of LAP additive provides 5 % of elongation at break. It is enough for some mechanical manipulations with the polymer film. The same values of elongation at break of PEG containing films can be reached only within the content range of plasticizer φ_{pl} equal 30 to 40 % (Fig.1).

The ε (φ_{pl}) curves are similar for plasticized systems of both pure PHB and biomass. However, increase of elasticity for pure PHB is more pronounced than for biomass at the same level of plasticizer.

As mentioned before, the reason probably is the presence of cell residue agglomerates in the biomass system which cause structural defects. Certain confirmation of this assumption is a fact that values of tensile strength in contrast to pure PHB do not practically change with the content of plasticizer and are equally low for all plasticized biomass systems (Fig.2).

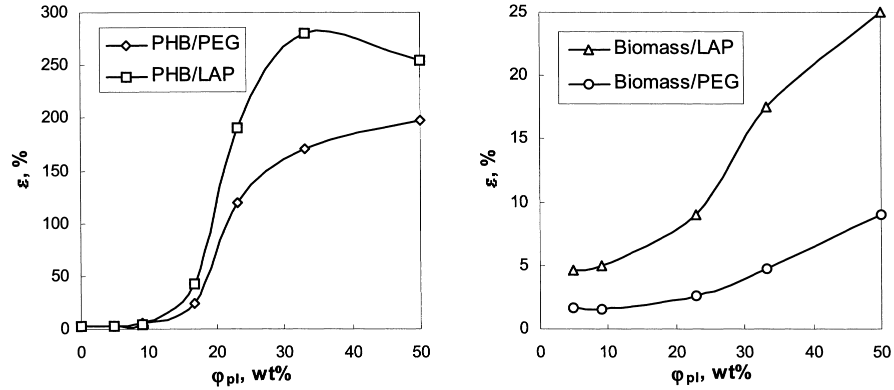

Fig. 1: Elongation at break ε of plasticized PHB and biomass systems as a function of the plasticizer content φ_{pl}.

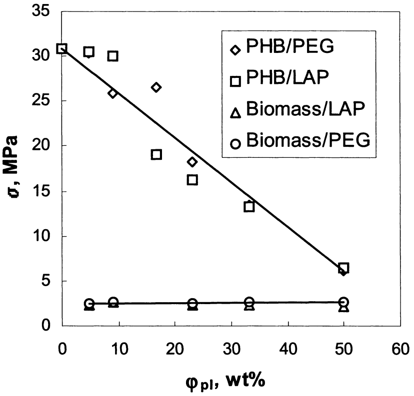

Fig. 2: Tensile strength σ of plasticized PHB and biomass systems as a function of the plasticizer content φ_{pl}.

Although plasticized biomass systems can not be considered to be identical to pure PHB in respect to deformability and mechanical strength, biomass composites with plasticizer over 30 % are suitable for some application fields, for example as biodegradable matrices for agrochemicals and seeds incrustation[13]. The most important environmental characteristic of PHB containing systems is full microbial degradability to CO_2, water and energy[14]. Since the studied composite materials are foreseen for incubation in a soil, the most exact evaluation of this kind of degradability is a direct simulation of this processes, *i.e.*, testing biodegradation of materials in a soil under controlled laboratory conditions[11].

Complete kinetics of biodegradation of pure PHB, biomass and two plasticized model systems, PHB/LAP with 33 % of LAP and PHB/PEG with 10 % of PEG, shown in Fig. 3 indicate that the rate of biodegradation of tested systems is significantly influenced by the type of plasticizer. The highest weight loss was observed for biomass and PEG containing samples (100 % biodegradation after 22 days of incubation in soil). This can be explained by the hydrophilicity and solubility of PEG as well as high biodegradability of cell residues in biomass, which leads to formation of microvoids in polymeric matrix and to increase of the specific surface area of the samples. This, consequently, facilitates the degradation activity of microorganisms.

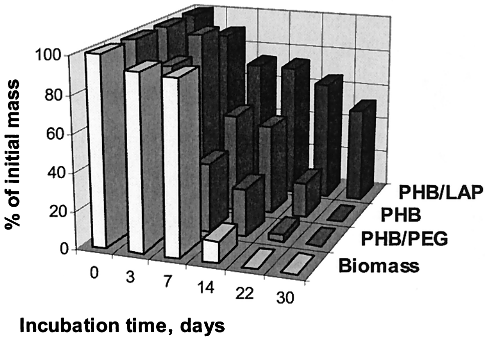

Fig. 3: Biodegradation kinetics of PHB, biomass and plasticized PHB systems in soil.

Complete biodegradation of pure PHB films occurred after 30 days of exposure. PHB/LAP samples containing 33 % of LAP exhibit only half of weight loss after the same exposure time in soil. Because the chemical structure of laprol is more resistant to the microbial degradation than PHB, it can be assumed that during formation of PHB plasticized systems laprol somehow encapsulates the structural domains of PHB and thus retards biodegradation of the composite. An average life-time of PHB/LAP film was 6-8 months.

For more complete characterization of biomass degradability, the hydrolytic stability of systems was studied. In this case the enzymatic influence was excluded. It was found (Fig.4) that hydrolytic degradation of PHB at experimental conditions was negligible and could be related to the high crystallinity (58 %) and high molecular weight (1.9×10^6) of the polymer resulting in small content of hydroxyl end groups and increased hydrophobicity of the polymer.

Thereby weight loss of biomass can be related to the dissolution of low molecular compounds. As weight loss of biomass composites does not exceed 10 % it is clear that such a weight loss can be attributed to the dissolving of plasticizer from the system. This process is completed after a week of exposure. Experimental results show that only 5 to 10 % of PEG and 25 % of LAP amount introduced have remained in composites (Fig.4).

Obviously a better migration of PEG is due to its smaller molecular weight, hydrophilicity and solubility. Such results are quite comparable with data previously obtained by the soil degradation tests.

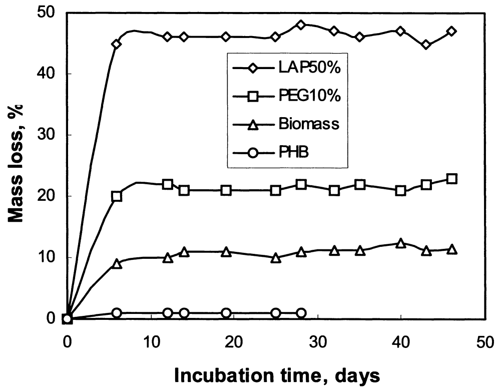

Fig. 4: Hydrolytic degradation of PHB, biomass and plasticized PHB systems.

Structural changes of PHB in the biomass caused the by presence of plasticizer were studied by a DSC method (Table 1). Comparison and analysis of previously obtained DSC data of the plasticized PHB[5] with DSC data of biomass shows that both systems behave similarly. Crystallinity and degree of arrangement macromolecules of PHB in biomass are found to be somewhat lower than those of pure PHB. Substantial differences are observed in the dimensions of the exothermal peaks of cold crystallization, which characterize the rate of crystallization and the mobility of the macromolecular chains. A lower intensity of this peak in comparison with the melting peak expresses a higher tendency to crystallize. When 75 %

crystallinity of PHB is attained upon rapid cooling, only 50 % crystallinity is obtained for PHB in biomass. Glass transition temperatures of PHB in biomass are also found to be somewhat lower.

The above mentioned tendencies are significantly more intense at the presence of plasticizers in the biomass composites. The degree of crystallinity of PHB in plasticized biomass is found to be in the range of 41 to 54 %, which is by 10 to 20% lower than the plasticized systems of pure PHB. Similar results are observed for melting temperatures, which is associated with the extent of crystalline formations of the polymer (Fig. 5). Obviously, the components of biomass prevent crystallization of PHB and hinder the development of a more arranged crystalline phase.

It is important to note that in the presence of LAP in biomass the glass transition temperature of PHB considerably decreases (Fig. 6). It could be explained by both a synergetic effect of the plasticizer and low molecular admixtures in biomass, which weaken intermolecular interaction.

It was not possible to obtain the glass transition temperatures of PHB for biomass systems with PEG content over 10 % because the region of glass transition is covered by the endothermal peak of melting temperature of non - compatible plasticizer.

Comparison of the efficiency of selected plasticizers shows a noticeable influence of low molecular PEG on the melting temperature and rate of crystallization of PHB (Fig.5, Table 1). At increased content of plasticizer (50 %), a marked increase in the chain mobility occurs, full crystallization arises which is confirmed by the absence of the cold crystallization at the second heating cycle (Table 1).

Different changes of PHB crystallinity in biomass are observed in the presence of plasticizers. An increase in the LAP content from 10 to 50% results in the crystallinity of PHB 42 – 54 %, whereas PEG causes a decrease in crystallinity from 50 % to 41 % (Table 1). It could be explained by essentially different chemical structure and values of molecular masses of plasticizers used and by a different degree of compatibility with PHB. Besides, PEG partly crystallizes, hence, the system contains at least two different amorphous and crystalline phases. It is quite possible that crystallinity of PEG is a reason for a diminished deformability in comparison with the LAP plasticized systems.

Tab.1. Main characteristics of plasticized biomass systems determined from DSC thermograms (**bold font represents** first heating run; **normal font represents** second heating run).

Content of plastic. / %	Melting				Crystallization			Glass transition	
	PHB			Plastic.					
	ΔT_{m1},ΔT_{m2}, / °C	T_{m1}, T_{m2}, / °C	α_1, α_2, / %	T_{mpl}, / °C	ΔT_c, / °C	T_c, / °C	α_c, / %	T_{gII}, / °C	T_{gI}, / °C
PHB biomass									
0	**24**	**178**	**51**	-					-
	23	175	51		10	46	25	4.2	
PHB biomass + LAP									
10	**27**	**175**	**42**	-					-
	29	173	46		14	40	15	-9	
20	**28**	**171**	**49**	-					-
	28	167	52		14	45	24	-12	
30	**30**	**168**	**51**	-					
	30	165	56		17	45	26	-14	-61
40	**26**	**164**	**54**	-					
	28	162	54		17	45	26	-15	-61
50	**26**	**161**	**54**	-					
	23	158	56		22	40	22	-14	-62
PHB biomass + PEG 300									
10	**26**	**174**	**50**	-					-
	23	168	50		12	34	17	-13.6	
20	**31**	**166**	**44**					-	
	27	161	49	-12	11	27	20		-78
30	**28**	**162**	**47**					-	
	32	159	52	-13	14	27	21		-73
40	**27**	**159**	**44**					-	
	31	152	50	-13	14	19	10		-73
50	**26**	**157**	**41**		-	-	-	-	
	27	147	48	-13					-73

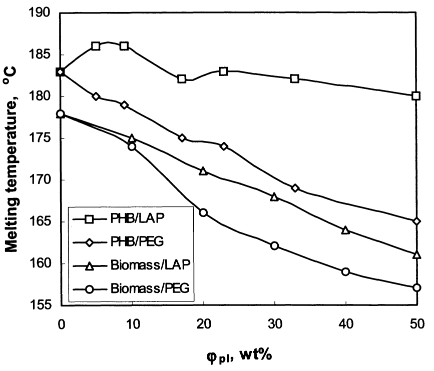

Fig. 5: Melting temperatures T_{ml} of plasticized PHB and biomass systems as a function of the plasticizer content φ_{pl}.

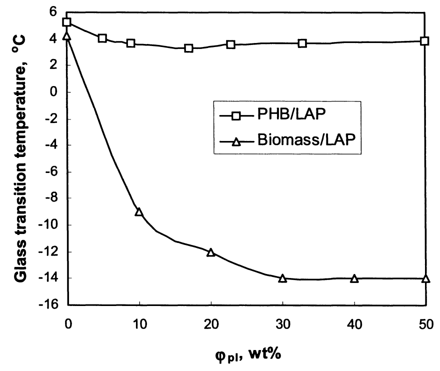

Fig. 6: Glass transition temperatures T_{gII} of plasticized PHB and biomass systems as a function of the plasticizer content φ_{pl}.

Conclusion

It has been shown that PHB containing biomass *Azotobacter chroococcum 23* can be processed into films by the solvent casting technique only in the presence of plasticizers. Due to sufficient mechanical strength, deformability and biodegradability, given composite systems containing both selected plasticizers - LAP and PEG - could be appropriate materials for production of polymeric matrices for agrochemicals with prolonged action. The rate of biodegradation and deformation properties of plasticized PHB and PHB containing biomass films was found to depend on the composition and chemical structure. The presence of PEG and biomass debris (as a biodegradable filler) increase the rate of biodegradation of the plasticized PHB systems when exposed to soil. Non-degradable plasticizer laprol slows down the biodegradability. PHB, biomass and PHB/PEG 10 % completely decomposed in soil after 30 days. It is possible to rise the deformability of plasticized biomass systems to sufficient level by increase in plasticizer content over 30 %. According to the DSC data, the presence of plasticizers decreases the melting temperatures of PHB in plasticized biomass systems by 15 to 20 °C, thus broadening the window for the processing temperatures.

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