

Environmentally Degradable Polymeric Composite Materials
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Abstract

The present study was focused on improvement of the competitiveness of *Azotobacter chroococcum* 23 produced PHB in two directions: 1) seeking for convenient raw materials for PHB biosynthesis; 2) PHB blending with plasticizers in order to obtain PHB composite materials with controllable physical, thermal properties and biodegradability. Corn starch syrup (PHB content 10,2g/l and 9,8g/l, $Y_{p/s}$ 0,26g/g and $Y_{p/s}$ 0,28g/g on glucose and corn starch syrup consequently) was most convenient unrefined carbon source for PHB production. The effect of blend composition on the changes of most important factors of polymeric systems, influencing biodegradability, crystallinity and intrinsic viscosity was evaluated. An essential increase in PHB-based films flexibility was obtained using lower molecular weight plasticizers - dibutylsebacate (DBS), dioctylsebacate (DOS), polyethylen glycol (PEG) 300 upon plasticizer content 20 - 30 w.%.

Introduction

Use of biodegradable polymers as a part of recycling can be offered as a sound argument and a partly solution for the plastic waste problem. Polyhydroxyalcanoates (PHAs) are natural biodegradable thermoplastics. One of such polymers is poly-b-hydroxybutyrate (PHB), accumulated by many bacteria as carbon and energy reserve material [1]. The production of PHA from renewable resources, offers additional ecological advantages of the polymer as compared to thermoplastics and elastomers produced from fossil carbon sources [2]. The suitability of a bacterium for the PHA production depends on many different factors such as stability and safety of the organism, growth and accumulation rates, achievable cell densities and PHA contents, extractability of PHA, molecular weights of accumulated PHA, range of utilizable carbon sources, costs of the carbon source and other components of the medium, and occurrence of by-products [3]. As thermoplastic polymer PHA can be processed like other synthetic thermoplastics however some polymer properties thermal stability, brittleness and relatively high cost - are reasons limiting its processing and use.

Modification of PHB through blending with high and low molecular components provides a potentially relatively inexpensive and simple route to change and regulate properties of polymeric material and offers opportunities to obtain cost - competitive polymers as compared to traditional polymers and processable materials simultaneously preserving their specific property - ability to biodegrade. Various attempts have been made to blend PHB with synthetic and natural polymers [4-8] and determine the influence of the second component on the physical properties and biodegradability of the blends.

With an aim to improve the competitiveness of PHB an effect of unrefined carbon sources on *A. chroococcum* 23 growth, PHB production and polymer properties have been investigated. The influence of plasticizers on PHB physical properties and PHB-based films biodegradability have been investigated as well.

Materials and methods

A. chroococcum 23 producing PHB with a high cellular content (up 70%) and not producing excess of exopolysaccharides was used in this study [9-10]. *A. chroococcum* 23 was cultivated in shake flasks for 48

hours on a modified Burk's mineral salt medium [11] supplemented with carbon sources (4% w/v of carbohydrates). Glucose (reagent-grade chemicals) and unrefined sugars: raw sugar and beet molasses (containing 50-52% w/w sucrose) from Baltic Sugar Company, corn starch syrup (containing 17% glucose, 14% maltose, 58% oligosugars) and potato starch syrup (containing 20% glucose, 15% maltose, 52% oligosugars) from Lickeby Aloja, Latvia were used as carbon sources. Cell dry weight and PHB concentration determined as previously described [11].

PHB was extracted from isopropanol-pretreated biomass with hot chloroform and precipitated with isopropanol. Polyethylene glycols (PEG) of molecular mass 300 (LOBA Feinchemie) and oxypropylated glycerols (laprols) of molecular mass 500 and 5000, dibutylsebacate (DBS), dioctylsebacate (DOS) and polyisobutylene (PIB) (USSR commercial products) were used as PHB plasticizers. PHB-based films, prepared from PHB blends with plasticizers or from PHB containing biomass of *A. chroococcum* 23 were incubated in garden soil (with humus content 2%, pH 6.5) under controlled conditions ($T=25.0 \pm 50C$, moisture - 70% of the soils maximum holding capacity) under laboratory conditions. Degradation of PHB-based films (mass loss) was determined gravimetrically. Changes of PHB physical properties in PHB-based films were determined. The resulting films were dried under vacuum at 500 C up to constant weight and than aged for at least 3 weeks at a room temperature to reach equilibrium crystallinity prior to DSC analysis and biodegradation tests.

PHB intrinsic viscosity [h] measurements were performed in chloroform solution at 30 0C by Ubbelohde dilution viscosimeter [12]. Mettler differential scanning calorimeter (DSC) was used to characterize time - dependent changes of the degree of crystallinity and thermal properties of modified systems studied. The sample size was typically 10-15 mg and they were scanned from -1200 C to +2200 C at the rate 100 C/min under nitrogen flow to cover glass transition and melting temperature ranges of all components used.

Results and discussion

PHB production by *A. chroococcum* 23 and intrinsic viscosity of the recovered PHB.

A. chroococcum 23 growth and PHB production on glucose, raw sugar, beet molasses, corn and potato starch syrups were compared in shake flask cultures (tab.1).

The highest yield of PHB (10,2g/l) in shake flask cultures of *A. chroococcum* 23 was that on glucose (tab.1). Fed-batch fermentation of *A. chroococcum* 23 on glucose resulted in PHB yield 82,5g/l and $Y_{p/s}=0,24$ g/g [11]. The highest $Y_{p/s}=0,28$ g/g and high PHB yield (9,8g/l) on corn starch syrup (tab.1) showed that glucose could be replaced by this unrefined carbon source for the PHB production at a large scale. Carbon sources effect not only the PHB production, but the polymer extractability [13] and it's molecular weight too [14].

Table 1

Effect of carbon sources on the PHB production and intrinsic viscosity of polymer samples

Carbon sources	Biomass, g/l	PHB, %	PHB, g/l	$Y_{p/s}$, g/g	CHCL ₃ dl/g
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Glucose	13.0	78.3	10.2	0.26	11.74
Raw sugar	11.3	73.4	8.3	0.26	8.90
Beet molasses	10.5	51.8	5.4	0.20	3.67
Corn starch syrup	12.6	77.7	9.8	0.28	5.37
Potato starch syrup	13.0	67.0	8.7	0.22	6.05

The highest recoveries of PHB were obtained from biomasses of *A. chroococcum* 23 grown on glucose and the starch syrups (about 79% of total PHB content). The intrinsic viscosity data for all PHB samples except beet molasses sample lie within the range 11,74-5,37 (dl/g), that corresponded approximately to PHB molecular weights 2000-800 kDa required for the polymer treatment [15]. Hence our data offer corn starch syrup, as a most suitable unrefined carbon source for the PHB production and the further work will be aimed at an optimization of the process of the PHB biosynthesis by applying the mentioned unrefined substrate.

Biodegradation of PHB- based films and PHB physical properties.

PHB recovered from *A. chroococcum* 23 biomass was blended with plasticizers and PHB-based films were made from the blends. Data on the extension to break of plasticized PHB (fig.1) indicated that an essential increase of PHB-based films flexibility to occur upon the plasticizers content about 20% w/w and over. It was found that stronger plasticizing effect could be acquired upon using lower molecular weight plasticizers - DBS, DOS, PEG-300, laprol 503.

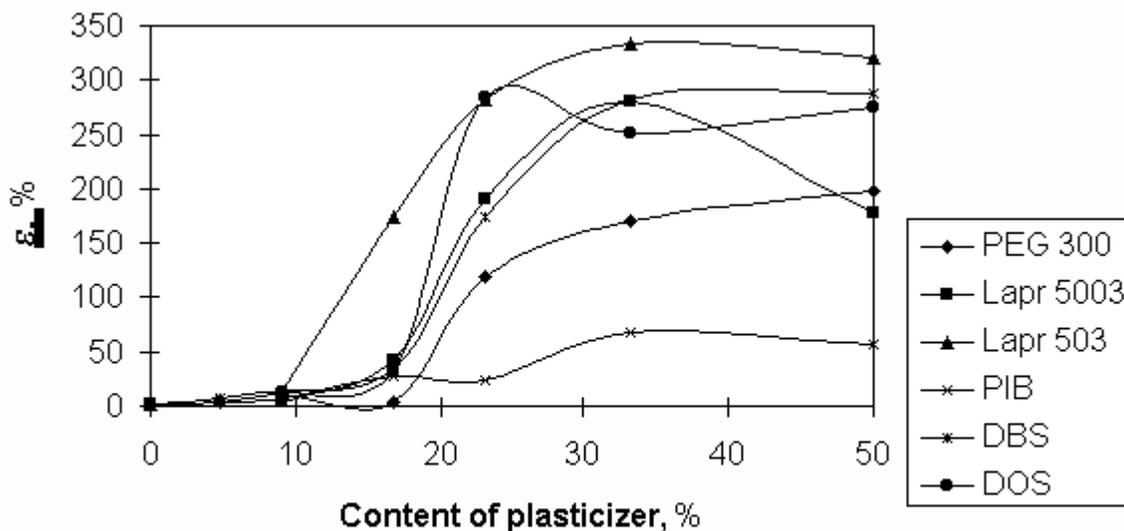


Fig.1. Extension to break of plasticized PHB

Temperature characteristics of plasticized PHB, measured by the DSC method (tab.2), showed that plasticization not to affect the ability of PHB to crystallize. Exothermal crystallization temperature (T_c) of PHB decreased and glass transition temperatures (T_g) of PHB depressed upon an increase of the plasticizer content. The effect could be referred to a higher mobility of macromolecules (PHB). In addition presence of

some plasticizers depressed the PHB melting point (T_m) by 10 -15 0C and as a result the processing window could be enlarged.

Table 2

PHB characteristics	Plasticizers										
	-	PEG-300, %		laprol 503, %		DBS, %		DOS, %		laprol 5003	
	0	16	33	16	33	16	33	16	33	16	33
$T_m, ^\circ\text{C}$	182,6	174,8	168,8	174,5	172,8	175,3	170,9	177,4	170,7	181,5	182,1
$\alpha, \%$	58,3	62,8	69,9	65,8	66,6	66,8	65,6	64,7	66,6	64,7	64,9
$T_g, ^\circ\text{C}$	5,4	-74,3	-73,2	-12,6	-59,6	-8,0	-70,0	-6,7	-6,4	3,3	-17,7

T_m - PHB endothermal melting temperature

α - PHB crystallinity (in plasticized composite material)

T_g - PHB glass transition temperature

Some of PHB-based films: pure PHB, PHB containing biomass of *A.chroococcum* 23 (Biomass), PHB blend with 10% PEG-300 (N-4) and PHB blend with 50% laprol 5003 (Lap-5) were selected to study the process of biodegradation (fig.2.).

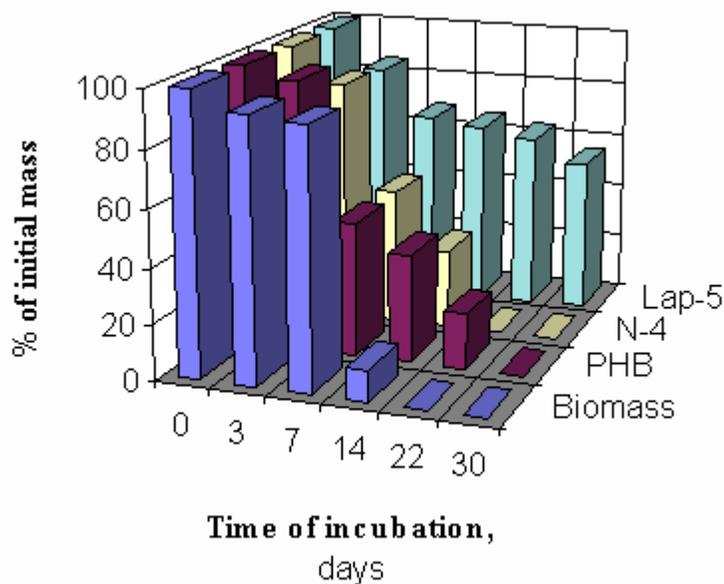


Fig.2. PHB-based films mass changes during incubation in soil

The rate of biodegradation of PHB-based films was significantly influenced by the blend composition. The highest rate of mass loss was observed for Biomass and N-4 films (100% degradation after 22 days incubation in soil). It could be explained by hydrophilicity of PEG (N-4) and components of Biomass films, that led to the microvoids formation in the polymeric matrix, which facilitated degradating effect of microorganisms.

Samples with laprol (Lap-5) demonstrated only 50% mass loss after 30 days of exposure to soil. Laprol due to its chemical structure is stable to microorganisms effect and it could be possible that during formation of a PHB modified system laprol encapsulated partially the PHB component and thus retarding biodegradation of the blend.

Simultaneously with mass changes caused by the attack of microorganisms of PHB-based films some changes of PHB crystallinity were observed (fig.3). Tendencies of crystallinity to increase at the initial stage of biodegradation for all tested PHB-based films could be explained by microbial degradation of the PHB amorphous part of PHB resulted in an increase of crystalline phase. After degradation of the amorphous part of the films degradation of crystalline phase occurred (fig.3). Such biodegradation dependent changes in properties could be regarded as indirect criteria for the degree of biodegradability of modified polymeric systems.

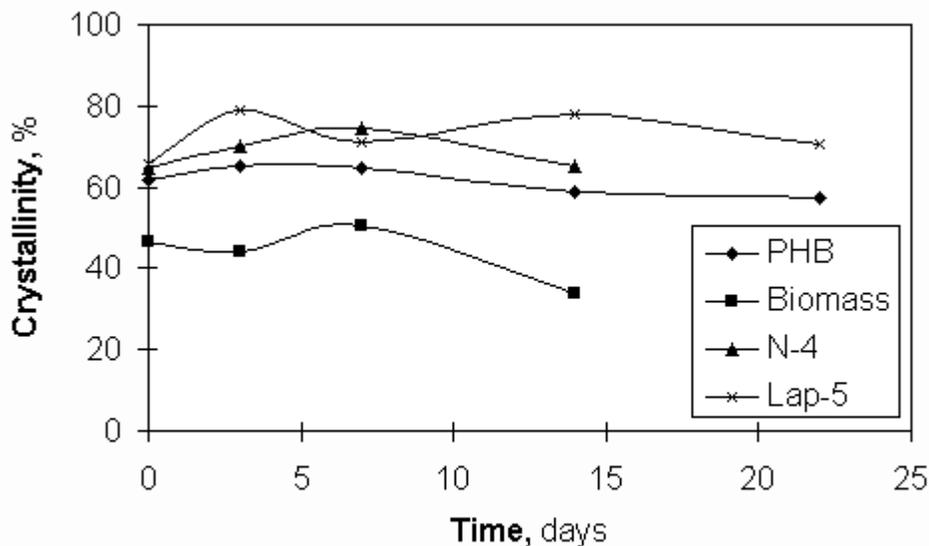


Fig.3. Changes of the PHB crystallinity in plasticized systems during biodegradation in soil

The obtained results indicate to such an approach to the development of modified PHB systems to benefit to designing of composite materials with required and adjustable properties and biodegradability.

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