

# The Influence of 4HB Content on the Properties of Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) Based on Melt Molded Sheets

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**ABSTRACT:** A series of poly(3-hydroxybutyrate-co-4-hydroxybutyrate)s [P(3HB-co-4HB)s] sheets with the 4HB contents from about 9 to 34 mol % were prepared via melt molding. Their crystallinity, crystalline structures, thermal and mechanical properties were characterized by differential scanning calorimetry, X-ray diffraction, thermogravimetric analysis, dynamic mechanical analysis, and tensile test. It was found that the melt temperatures ( $T_m$ ), glass transition temperatures ( $T_g$ ), and storage modulus ( $E'$ ) of all the [P(3HB-co-4HB)s] copolymers investigated decreased continuously with increasing the amount of the 4HB; the yield stress and breaking stress nearly decreased

with the increase of the 4HB contents while the elongation at the yield and break points increased; and the thermal stability of the P(3HB-co-4HB)s improved with increasing 4HB contents. The results suggest that the mechanical properties and crystal lattice parameters of the melt molded sheets are somewhat different from those of the solution cast films. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 109: 1962–1967, 2008

**Key words:** biomaterials; crystal structures; mechanical properties; thermogravimetric analysis; thermal properties

## INTRODUCTION

Since Poly(3-hydroxyalkanoate)s [(PHA)s] were discovered, they have attracted wide attention because of their biodegradability and biocompatibility.<sup>1–3</sup> They are extensively used as a degradable thermoplastic material for agricultural, marine, and medical applications.<sup>4</sup> They can be accumulated under unbalanced growth conditions by lots of bacterial as carbon and energy storage materials.<sup>5–7</sup> The most familiar types of PHAs are the homopolymers and copolymers of Poly(3-hydroxybutyrate) (PHB). PHB has an unusually high degree of crystallinity because of the stereoregularity of the perfectly isotactic chains. This high crystallinity leads to a rather brittle and hard material that is not very useful for many applications, and its very high melting point (>170°C) makes it difficult to use melt processing equipment.<sup>8</sup> The incorporation of a comonomer unit, 4-hydroxybutyrate, can decrease the excessively high

$T_m$  and control efficiently the degree of crystallinity for 4HB unit cannot crystallize in the sequence of 3HB units and act as defects in the P(3HB) crystal lattice.<sup>9,10</sup>

The properties of P(3HB-co-4HB) were significantly affected by unit composition of comonomers. As the 4HB content increase, the melting temperature ( $T_m$ ), glass transition temperature ( $T_g$ ), enthalpy of fusion ( $\Delta H_m$ ), and crystallinity degree of copolymer first decreased in low 4HB content range, and then increase in high 4HB content range for the incorporating units acting as defect in the matrix crystal lattice. The tensile strength of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] solution casting films with composition of 0–16 mol % 4HB decreased from 43 to 26 MPa, while the elongation at break increased from 5 to 444%,<sup>11</sup> and the tensile strength of 64–100% 4HB increased from 17 to 104 MPa.<sup>12</sup> The rate of biodegradation also greatly vary with varying the 4HB content.<sup>12–14</sup>

Up to now, nearly all the solid experiments about P(3HB-co-4HB) including X-ray diffraction (XRD), biodegradation, and tensile test are based on the  $\text{CHCl}_3$  casting films. It is well known that the remnant of solvent will spoil the properties of material. Compared to solution casting, compression molding can avoid the remnant of solvent and is close to the real practice process. However, there is few research

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TABLE I  
Compositions and Molecular Weights of P(3HB-co-4HB)s

Sample	Composition (mol %) <sup>a</sup>		Molecular weight <sup>b</sup>	
	3HB	4HB	$M_n$ ( $10^{-3}$ )	$M_w/M_n$
P(3HB-co-9 mol % 4HB)	80.45	9.55	474	1.92
P(3HB-co-11 mol % 4HB)	88.09	11.91	457	1.89
P(3HB-co-17 mol % 4HB)	82.66	17.34	432	1.88
P(3HB-co-20 mol % 4HB)	79.05	20.95	589	1.66
P(3HB-co-34 mol % 4HB)	65.26	34.74	414	1.99

<sup>a</sup> Calculated by <sup>1</sup>H-NMR analysis.

<sup>b</sup> Obtained from GPC.

about P(3HB-co-4HB) melt molded sheets. In this article, we investigate the effect of 4HB fraction content on the properties of P(3HB-co-4HB) melt molded sheets with 4HB content from 9 to 34 mol % by differential scanning calorimetry (DSC), XRD, thermogravimetric analysis (TGA), dynamic mechanical analysis (DMA), and tensile test.

## EXPERIMENTAL

### Sample preparation

P(3HB-co-4HB) copolymers were supplied by Tianjin Green Bioscience Company by large scale. The Company constructs a recombinant strain GBC 2003 capable of producing poly(3HB-co-4HB). The recombinant strain contains the entire polyhydroxyalkanoate synthase gene and 4-hydroxybutyrate-CoA transferase gene and vitreoscilla hemoglobin gene. The extraction process of P(3HB-co-4HB) is similar with that of PHB with the solvents such as chloroform and dichloromethane. The copolymers compositions were determined by <sup>1</sup>H-NMR spectroscopy and the molecular weights were obtained by gel permeation chromatography (GPC). Table I lists the compositions and molecular weights of the P(3HB-co-4HB) copolymers. All of the testing samples were molded into sheets of about 2 mm thick at 140°C under 15 MPa pressure in 10 min, then cooled, and stored at room temperature over 2 weeks.

### Characterization

The <sup>1</sup>H-NMR analysis of P(3HB-co-4HB) copolymers was carried out on a Bruker AV600 spectrometer. The 600-MHz <sup>1</sup>H-NMR spectra were recorded at 30°C in CDCl<sub>3</sub> solution of P(3HB-co-4HB) (5 mg/mL). The GPC experiments were conducted at 50°C by using a waters 515-2410 system GPC system. Tetrahydrofuran was used as eluent at a flow rate of 0.5 mL/min, and a sample concentration of 1.0 mg/mL was used. Polystyrene standards with a low polydispersity were used to make a calibration curve.

The DSC data for the copolymer were recorded on a NETZSCH DSC 204 under a nitrogen flow of 30 mL/min. The samples of 5 mg were encapsulated in aluminum pan and rapidly cooled to -50°C. They were heated from -50 to 150°C at heating rate of 10°C/min. The glass transition temperature ( $T_g$ ) was taken as the midpoint of the heating capacity change. The melting temperature ( $T_m$ ) and enthalpy of fusion ( $\Delta H_m$ ) were determined from DSC endotherms.

Wide-angle X-ray diffraction measurements of P(3HB-co-4HB) samples were made on a D/max IIIA system. Cu K $\alpha$  radiation ( $\lambda = 0.1542$  nm) was used as the source. The XRD patterns were recorded at 25°C in the range  $2\theta = 5-70^\circ$  at a scan speed of 7°/min. The quantitative analyses on the crystallinity of the samples were performed using a multipeak separation program (MDI Jade 5.0, Materials Data, Livermore, CA).

TGA measurements were performed with a Netzsch 209C apparatus under nitrogen flow (60 mL/min) at a heating rate of 10°C/min from 30 to 350°C.

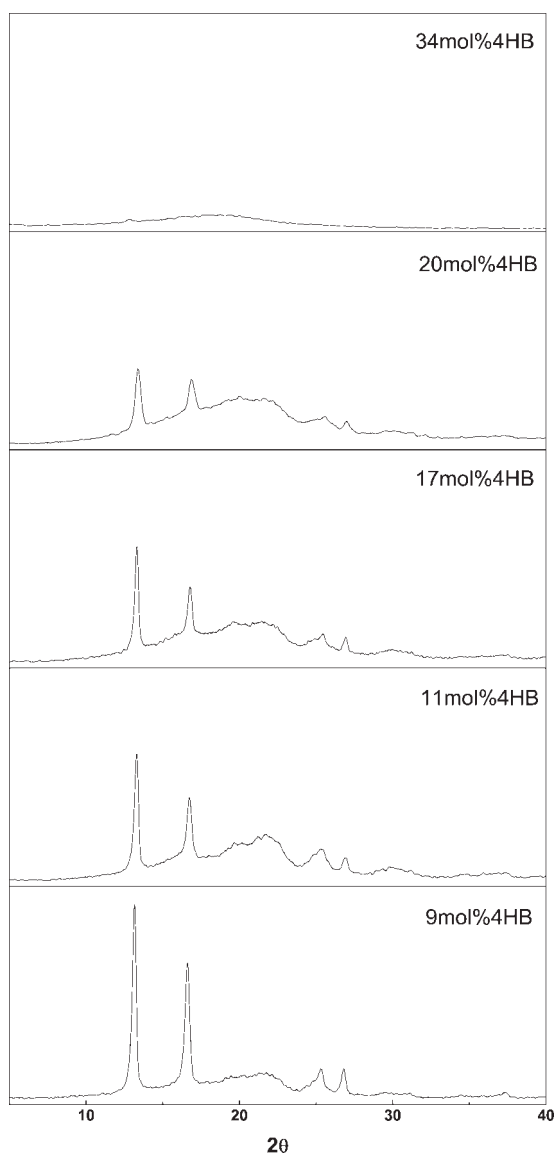
Dynamic mechanical analysis (DMA) was performed by using a Netzsch DMA242C dynamic mechanical analyzer to evaluate the storage modulus and mechanical loss factor ( $\tan \delta$ ) of the copolymers over the temperature range from -80 to 60°C at a heating rate of 5°C/min. The frequency was set to 10 Hz, respectively. The specimen size was  $30 \times 4 \times 2$  mm<sup>3</sup> in length, width, and thickness, respectively.

Tensile properties were measured on Instron1185 tester with 10 mm/min strain rate at room temperature. The sheets were cut into dumb-bell shaped specimens, with an overall length of 50 mm, the width of 4 mm at the narrow parallel portion, and the thickness of 2 mm. All data were calculated on an average of five specimens.

## RESULTS AND DISCUSSION

### XRD analysis of P(3HB-co-4HB) copolymers

Figure 1 shows the XRD patterns of melt molding P(3HB-co-4HB) sheets containing from 9 to 39 mol % 4HB. Only one crystalline form of P(3HB) lattice is



**Figure 1** X-ray diffraction patterns of P(3HB-co-4HB) molded sheets (sheets had been aged for at least one month at room temperature).

observed for the P(3HB-co-4HB) copolymers with compositions from 9 to 34 mol %. The degree of X-ray crystallinity decreases from 37.72% to 1.39% as

the 4HB content increases from 9 to 39 mol % (Table II). The reasons may be that the incorporation of 4HB, as defects in the P(3HB) crystal lattice, breaks out the stereoregularity of the P(3HB) chains, which results in the decrease of the crystallization of P(3HB-co-4HB). Furthermore, the XRD pattern of P(3HB-co-34%4HB) is nearly featureless because of the earlier reason. The similar results were reported by Kunioka et al.<sup>10</sup> Nevertheless, there are some differences. The crystallinity degree of copolymer in our study is lower than that of the solution-cast films reported. Especially, the crystallinity degree of the copolymer with 34 mol % 4HB is only 1.39% smaller than  $23\% \pm 5\%$  of the solution-cast P(3HB-co-42% 4HB) film reported by Kunioka et al.<sup>10</sup> This is due to the difference of the crystallization time and the viscosity in polymer melt or solution. The crystal form in solution had more time to arrange its chain than that formed in melt because the speed of solvent ( $\text{CH}_3\text{Cl}$ ) evaporation is slower than the speed of melt cooled. In addition, the viscosity of solution is smaller than that of melt system, so that macromolecule chain of solution is easier to form crystal than that of melt.

The (020), (110), and (121)  $d$ -spacings of the P(3HB-co-4HB) samples from the XRD figures are list in Table II. All the  $d$ -spacings apparently decrease with the composition range from 9 to 20 mol % 4HB. This result indicates that the crystal lattice parameters of the P(3HB) unit cell are slightly reduced by the incorporation of 4HB.

#### Thermal properties of P(3HB-co-4HB) copolymers

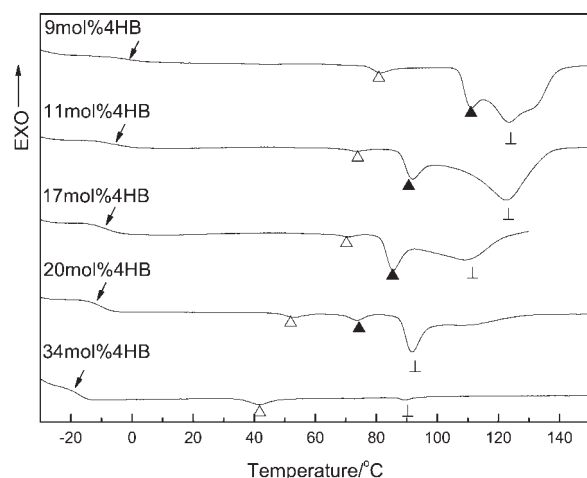
Figure 2 shows the DSC curves of the melt molded sheets derived from P(3HB-co-4HB) copolymers with the 4HB content from 9 to 34 mol %. It can be observed from these curves that all the investigated copolymers exhibit three distinct peaks. The peak at the lowest temperature ( $T_{m1}$ ) corresponds to the crystalline phase of 4HB-rich fractions; the middle one ( $T_{m2}$ ) belongs to the melting peak of 3HB-rich fractions; while, the highest one ( $T_{m3}$ ) is believed to

**TABLE II**  
Degree of Crystallinity, Crystalline Structure, and  $d$ -Spacing of P(3HB-co-4HB) Samples from X-ray Diffraction

Sample	Crystallinity <sup>a</sup> (%)	Crystalline structure	$d$ -Spacing ( $\text{\AA}$ )			
			(020)	(110)	(121)	(040)
P(3HB-co-9 mol % 4HB)	37.72	P(3HB)	6.7319	5.3359	3.5173	3.3238
P(3HB-co-11 mol % 4HB)	25.64	P(3HB)	6.6516	5.2856	3.5119	3.3068
P(3HB-co-17 mol % 4HB)	20.18	P(3HB)	6.6419	5.2790	3.4956	3.3068
P(3HB-co-20 mol % 4HB)	13.37	P(3HB)	6.6119	5.2481	3.4742	3.2996
P(3HB-co-34 mol % 4HB)	1.39	P(3HB)	— <sup>b</sup>	—	—	—

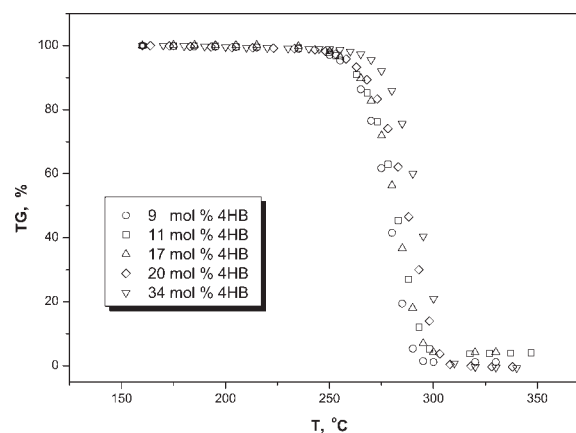
<sup>a</sup> Determined by X-ray diffraction.

<sup>b</sup> Not found.



**Figure 2** DSC curves of P(3HB-co-4HB) with the 4HB content from 9 to 34 mol % ( $\Delta$ ,  $\blacktriangle$ ,  $\nabla$ , and  $\perp$  represent  $T_{m1}$ ,  $T_{m2}$ ,  $T_{m3}$ , and  $T_g$ , respectively).

arise from a recrystallization process, in which the lattice of copolymer has enough time to be rearranged at a given DSC heating rate, because the intensity of this peak relative to that of the peak at a lower temperature decreased with the heating rate.<sup>15–17</sup> The main thermal parameters are summarized in Table III. The glass transition temperature ( $T_g$ ), melting temperature ( $T_{m1}$ ,  $T_{m2}$ ,  $T_{m3}$ ), as well as the melting enthalpy ( $\Delta H_{m3}$ ) of the P(3HB-co-4HB) copolymers are shown to decrease with the increase of the 4HB content. The decrease of melting temperature and melting enthalpy can be attributed to the decrease of irregularities in the chain for the incorporation of 4HB unit onto P(3HB) chain. Irregularities in the chain decrease the melting point and the melting enthalpy because the crystal becomes less perfect. The decrease of glass transition temperature can mainly be ascribed to an increase of chain flexibility. The combination of 4HB unit with P(3HB) main chain increases the possibilities for rotation of the main chain and decrease the steric hindrance caused by methyl side group. These two factors make the main chain flexibility increase and the glass transition temperature decrease with the increase of 4HB content.



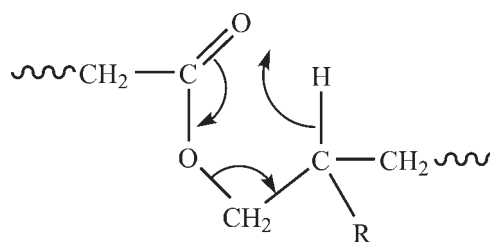
**Figure 3** Thermogravimetric curves of P(3HB-co-4HB) with the 4HB content from 9 to 34 mol %.

### Thermal stability of P(3HB-co-4HB) copolymers

The integral thermogravimetric curves relative to P(3HB-co-4HB)s with various 4HB contents are shown in Figure 3. One can observe that the thermal degradation of the P(3HB-co-4HB)s in this temperature range from 30 to 350°C occurs through one degradation step, which indicates that the increase of the 4HB contents has no effect on the block structures of the P(3HB-co-4HB). It is clearly shown in Figure 3 that the thermal stability of the P(3HB-co-4HB)s improves with increasing 4HB contents, with the maximum volatilization rate of evolution at the temperature from the lowest 281.6°C to the highest 295.9°C. The results reveal that the incorporation of 4HB fractions can significantly promote the thermal stability of P(3HB-co-4HB) copolymer. The reasons can be explained by the  $\beta$ -elimination mechanism causing random chain scission.<sup>18,19</sup> The mechanism involves a  $\beta$ -CH hydrogen transfer process, as shown in Figure 4. The electron-donating group  $-\text{CH}_3$  increases the energies of the H atom at the same carbon atom, so the  $\beta$ -CH hydrogen transfer process is easier to take place in the intramolecule of 3HB unit than in the intramolecule of 4HB unit. In this study, we can also conclude that the thermal degradation temperature of the P(3HB-co-4HB) exceeds

**TABLE III**  
Thermal Properties of P(3HB-co-4HB)s

Sample	$T_g$ (°C)	$\Delta C$ [J (g K) <sup>-1</sup> ]	$T_m$ (°C)					
			$T_{m1}$	$\Delta H_{m1}$ (J g <sup>-1</sup> )	$T_{m2}$	$\Delta H_{m2}$ (J g <sup>-1</sup> )	$T_{m3}$	$\Delta H_{m3}$ (J g <sup>-1</sup> )
P(3HB-co-9 mol % 4HB)	-3.8	0.163	81.5	1.271	111.0	8.696	123.4	34.1
P(3HB-co-11 mol % 4HB)	-7.1	0.201	73.8	0.544	91.9	3.677	122.1	25.99
P(3HB-co-17 mol % 4HB)	-6.6	0.374	70.9	0.434	85.4	5.135	109.4	8.571
P(3HB-co-20 mol % 4HB)	-10.9	0.419	53.0	0.895	73.6	1.166	91.6	7.262
P(3HB-co-34 mol % 4HB)	-17.9	0.402	41.7	1.853	72.87	0.09	89.5	0.3678

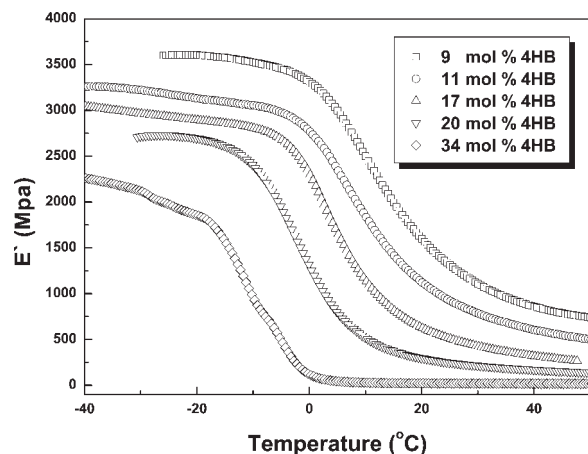


**Figure 4** The scheme of the degradation mechanism involving a  $\beta$ -CH hydrogen transfer process.

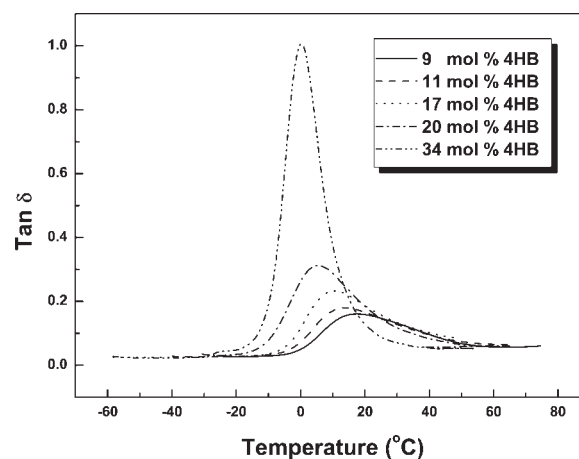
greatly the process temperature, and their thermal stability can meet the requirement of the industrial production.

### Dynamical mechanical properties of P(3HB-co-4HB) copolymers

Figure 5 presents the dynamic mechanical spectra of P(3HB-co-4HB) copolymers with the 4HB content from 9 to 34% mol expressed in terms of storage modulus as a function of temperature. One can observe that the storage moduli of P(3HB-co-4HB) decrease with the increase of the 4HB contents at all the functional temperature range, and the storage moduli at the low temperature range reduce more markedly than those at the high temperature range. It can be also seen from Figure 6 that all the P(3HB-co-4HB) copolymers exhibit a well-resolved single relaxation peak, corresponding to the glass transition temperature ( $T_g$ ). Furthermore, it is still clearly observed that the  $T_g$ s of the P(3HB-co-4HB) copolymers (based on the maximum of the mechanical loss peak) also decrease with increasing the content of 4HB. These results may be ascribed to the plasticization of 4HB unit that the introduction of 4HB unit within P(3HB) main chain decrease the steric hindrance caused by methyl side group, and thus



**Figure 5** Storage modulus of P(3HB-co-4HB) with the 4HB content from 9 to 34 mol % as a function of temperature.

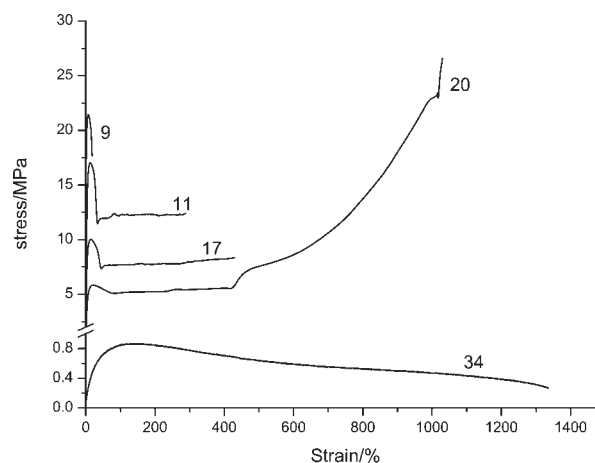


**Figure 6** Mechanical loss factors ( $\tan \delta$ ) of P(3HB-co-4HB) with the 4HB content from 9 to 34 mol % as a function of temperature.

increase the possibilities for rotation of the main chain.

### Mechanical properties of P(3HB-co-4HB) copolymers

The tensile typical curves and tensile properties of the copolymers are showed in Figure 7 and Table IV. The stress at yield and modulus of copolymers decrease as the 4HB content increased while the strain at yield and strain at break increase. The stress at break also decreases as the 4HB content increase except P(3HB-co-20 mol % 4HB). The P(3HB-co-20 mol % 4HB) has highest stress at break about 18.74 MPa comparing with the other copolymer for during tensile process, the crystal deform, and the molecules chain orient. With the 4HB content increasing, modulus of copolymer decrease and elongations at break and at yield increase. The copolymers become



**Figure 7** Typical tensile curves of P(3HB-co-4HB) with the 4HB content from 9 to 34 mol % at room temperature.

TABLE IV  
Stress-strain Results of P(3HB-co-4HB) Melt Molded Sheets

Sample	$\sigma_Y$ (MPa)	$\varepsilon_Y$ (%)	$\sigma$ (MPa)	$\varepsilon$ (%)	$E$ (MPa)
P(3HB-co-9 mol % 4HB)	20.45	11.90	16.93	37.02	206.85
P(3HB-co-11 mol % 4HB)	16.56	17.12	11.63	283.99	113.51
P(3HB-co-17 mol % 4HB)	10.05	20.89	8.025	565.10	67.63
P(3HB-co-20 mol % 4HB)	5.792	32.53	18.74	859.74	29.92
P(3HB-co-34 mol % 4HB)	0.87	159.71	0.82	1173.75	0.58

$\sigma_Y$ , strength at yield;  $\varepsilon_Y$ , elongation at yield;  $\sigma$ , strength at break;  $\varepsilon$ , elongation at break;  $E$ , modules of material.

softer at the same time. The strength of test sheets are smaller than that of solution-cast film for crystallization in bacterial polyesters for the strength depend strongly on the crystallization and annealing temperature.<sup>12</sup> The crystalline degree of the copolymer obtained by XRD, is smaller than that of films reported.

### CONCLUSIONS

A series of [P(3HB-co-4HB)s] sheets with the 4HB contents from about 9 to 34 mol % were prepared via melt molding. With increasing 4HB content, the melt temperatures ( $T_m$ ), glass transition temperatures ( $T_g$ ), and storage modulus of all the [P(3HB-co-4HB)s] copolymers investigated decrease; the stress at yield and the stress at break also decreased while the elongation at yield and at break increase; the thermal stability of the P(3HB-co-4HB)s improves. The result shows that mechanical properties and crystalline lattices are somewhat different with the copolymer solution cast films.

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