Assessment of Different Plasticizer – Polyhydroxyalkanoate Mixtures to Obtain Biodegradable Polymeric Films

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The elaboration of films from biodegradable polymer polyhydroxybutyrate was studied with 4 main plasticizers at variable concentrations: polyethyleneglycol, lauric acid, glycerol and epoxidized soy bean oil. An evaluation of the state of the art in the properties of commercial PHB (polyhydroxybutyrate) and necessary additives was elaborated in order to establish the experimental design to develop a polymeric film. Also, the qualitative evaluation of overall brittleness showed a variability due the nature and concentration of plasticizer. The influence of plasticizers on polymeric films was evaluated with gas chromatography (GC/MS) and Infrared Spectroscopy. The measurements were made on samples from different stages of the process in order to evaluate PHA (polyhydrixyalkanoate) purity. Measurements from the gas chromatography studies show the purity of the inicial fermentation broth (59.87%) , after the extraction with SDS and sodium hypochlorite (65%) and in mixtures with PEG (58.15%). From a general perspective, polyethyleneglycol (PEG) lower the material frailness and does not modify the Infra Red Spetrum as well as the Crystallinity Index (CI) in comparison with a standard commercial sample (70%+)

1. Introduction

Among polymer production from renewable resources, polyhydroxyalkanoates are a very promising alternative due to its high biodegradability, similarities with polypropylene regarding vitreous transition temperatures, and fusion point among others (Innocentini-Mei, 2003). Polyhydroxyalkanoate is useful for short life span applications due to its high degradability and thermal properties which make it a good alternative to polymers from petrochemical sources. However, PHA from bacteria shows high variability in its polymeric chains, which affects its physical properties. On top of that, such polymers tend to have high brittleness, therefore in order to ensure properties for its applications it must be modified with additives such as plasticizers or co-polymerization (Shin-wo Hong, 2013).

The use of plasticizers in with polyhidroxyalkanoates has been studied in order to reduce brittleness and poor mechanical properties that limit the applicability for this material. Additives for polymers are usually organic molecules that are added to in quantities ranging from 0,01% -10% w/w during the process of manufacturing or pelleting in order to modify its properties (Wiley, 2007). These properties can be determined analyzing the generated mixtures with the use of different characterization techniques that allow to determine the composition, as well as the chemical properties of the material generated, such as the use of gas chromatography, and infrared spectroscopy (IR).

Three main group of additives can be used: performance enhancers, polymer modifiers and process supporting additives. The main focus of this study is on plasticizers, which are performance enhancers for PHAs. Plasticizing agents are the most produced type of additives and applied for PHAs in order to raise

flexibility. Plasticizers usually studied for this purpose are also biodegradable to maintain PHAs high degradability (Choi, 2003). Mixtures of PHB and PEG have been described by multiple authors to better thermal properties yet affect negatively tensile resistance, so is necessary to be careful with the percentage of this plasticizer present in the mixture (Parra, 2006). Other fusions with commercial PEMAGMA (dpoly(ethylene-co-methyl-acrylateco-glycidyl-methacrylate)), TEG (triacetyl glycerol, TEC bis(2-ethylhexanoate) and TBC (trybutyl citrate) were reported to have also negative impact on mechanical performance and showed that mechanical processing affected its properties as well. (Kurusu, 2015). Experimental procedures on evaluation of plasticizers and PHA vary from one author to another. Generally authors tend to do plasticizer evaluations via chloroform casting. The PHA and the plasticizer are dissolved in heated chloroform (around 60°C) afterwards the mixtures is left to air dry the chloroform in order to remove the remaining solvent. This procedure was done by numerous sources (Choi, 2003) (Parra, 2006) (Yoshie, 2000). The result of this procedure is of a fragmented material than must be thermoformed. In these sources it's shaped with heated press or by extrusion. Processing the plasticized polymer is enhanced due to its higher resistance (Wiley, 2007).

The mixtures made can be studied by a number of tests for characterization. In this work the influence of different plasticizers and mixture techniques on the polymeric material obtained is evaluated. The PHA obtained using the Burkholderia cepacia was characterized by IR characterization for the analysis of specific functional groups (Bagheriasl, 2013) and Gas Chromatography with previous treatment of acid methanolysis (Braunegg et al., 1978) in order to evidence the purity and confirm the presence of the PHA in the materials obtained.

2. Materials and methods

2.1 Fermentation Process to produce PHA

The PHA is produced from fermentation for 96 h at 32°C with a mutant Bulkhoderia cepacia B27 developed in the IBUN[a] using a mixture of triglycerides from commercial corn oil as a carbon source and nitrogen phosphate as a nitrogen source. The fermentation conditions are describes by Mendez et al., 2016 .To extract PHA, the fermentation broth is digested for one hour at 80°C with sodium dodecyl sulfate (SDS) at a concentration of 0.005mg of SDS per g of biomass. It is subsequently rinsed with distilled water three times each time centrifuged at 5000 rpm for ten minutes to remove lysed cells. The reacted PHB is then whitened for one hour at ambient temperature with sodium hypochlorite at 5.25% v/v. It is then rinsed again three times with distilled water and then dried to obtain PHA.

2.2 Mixtures of PHA and Plasticizers

Experimental procedures to obtain plasticizer- PHA mixtures were elaborated:
-Heating and mixture: The PHA obtain is heated at 120°C and mixed at the corresponding with the plasticizers. It is then mechanically mixed to make a homogenous and compact material.
-Pressure modification and heating: Two different experiments were carried out: Mixtures prepared at constant temperature of 180ºC and at atmospheric pressure in Bogota (Colombia) 560 mmHg and mixtures prepared at temperature of 68ºC and 400mmHg for the mixing under vacuum. These conditions were made for the mixtures of 20% PEG and 80% PHA.
-Chloroform casting: The PHA and the plasticizer is dissolve in a solution of chloroform at 60°C.The mixture is left to evaporate the chloroform.
-Mixing prior to drying. This procedure consists in mixing the plasticizer and the PHA whilst in liquid form after the whitening with sodium hypochlorite then it was left to dry.

2.3 Characterization

Gas chromatography–mass spectrometry (GC-MS) and Infrared Spectroscopy (IR)
30 ml samples of final fermentation broth where prepared for gas chromatography (Braunegg et al., 1978). These samples were made from the fermentation process described above, the mixture processes with the PEG are the following: PHA extracted with SDS, PHA from chloroform casting mixed with PEG, Residual Biomass from fermentation. The prior treatment of the samples was an acid methanolysis with 4,4 ml of acid methanol (3% v/v) and 5,5 ml of Chloroform. The quantity was determined following the procedure described by Hesselman et al. 1999. The samples were then heated to 97ºC for one hour as described by V.RIIS and W.MAI, 1988. They were centrifuged for 10 minutes at 5000 PRM, 0,5 ml of the supernatant was recovered to be injected in the gas chromatographer with the parameter described in Table 1.

The infrared spectrometer PIKE MiRacle ATR was used and all the samples were analyzed at ambient temperature (20ºC) placing them directly on the plaque and the light beam trajectory for 1-2 minutes. The Crystallinity Index was determined following the procedure developed by Bagheriasl (2013).

Table 1: *Instrumental parameters used in the determination of PHA esters by Shimadzu GCMS-QP2010SE*

Parameters	value	Oven program	Value	Mass spectrometer	value
Injecting port	Split	Initial temperature	35º C	Mass range, scan mode	m/z 35-450 amu
Injector temperature	275ºC	Initial time	0,5 min	Solvent delay	2,5 min
Detector temperature	260ºC	Final temp	320 ºC	-	-
Column linear velocity	34,1 cm/s	Final time	6 min	-	-

3. Results and discussion

3.1 Evaluation of mixture methodologies

A complete review was conducted in order to choose the plasticizers with the best behavior in mixtures with PHA. All the mixtures in Table 2 were evaluated qualitatively and using the methodology described in the materials and methods section. The results indicate that through the heating and mixture method there was thermal degradation of the polymer in numerous attempts to make the polymer blend. Additionally, the mixture could never be made homogenous and parts of the PHA did not melt. It is possible that the polymer isn't completely pure at this point and that part of it is still contaminated with biomass therefore, becoming degraded at high temperatures. Chloroform casting allows for the polymer/plasticizer blend to have a more homogenous appearance. It's the one more used in literature. However, the end results is of a brittle and fragmented material. A further thermoforming treatment is necessary in order to obtain a plastic film from the mixture. When mixing prior to drying it can easier be done with liquid based plasticizers, it was evaluated with lauric acid and glycerol however the lauric acid did not have a homogenous mixtures in these conditions. The PHA/Glycerol mixtures did in fact have a more homogenous mixtures (Bagheriasl, 2013). Nevertheless, the best results were obtained from a qualitative point of view, using PEG as plasticizer. Parra, et al (2006) reported presence of this plasticizer until values near to 20 % in the mixture without affecting the tensile resistance of the final material but improving its thermal properties.

Table 2: *Most common mixtures to improve mechanical properties of polymers according to the literature*

Substance	N°1	N°2	N°3	Source
Glycerol	18%	22%	26%	Alouia, Khwaldiaa (2011)
Epoxidized Soybean OIL (ESBO)	10%	15%	20%	(Choi, 2003)
PEG	2%	5%	20%	(Parra, 2006)
Lauric Acid	5%	10%	15%	(Yoshie, 2000)

3.2 GC/MS analysis

The method used in the present work operated a mass spectrometer that allowed to identify the methylesters present in the samples as well as the total percentage of these; raising the accuracy of the presented data. The quantity of PHA was defined as the quantity of methylesters present in the sample after prior treatment. (V.RIIS and W.MAI, 1988). The results obtained indicate a variability of the peaks in the chromatogram as function of the downstream processes (Digestion and bleaching) and the plasticizer concentration used in the mixture. Figure 1 shows the chromatogram obtained for the samples after digestion with SDS. The analysis of the raw data obtained through GC/MS, indicate that there was 59.87% of methylesters in the cell mass, the initial sample mass being of 2.7608 g. For the case of the sample treated with SDS and sodium hypochlorite the initial sample weight was 1.9218 g with 65% of identified methylesters. Lastly, the concentration of methylesters from the mixture of PEG and PHA is that of 58.15% with the initial sample weight of 1.033g.

From different results, Table 3 is built and the purity as well as the recovery yield are reported for the original cell mass, the SDS and sodium hypochlorite extraction sample, as well as data for a mixture with made after

the digestion and bleaching. The recovery yield was reported only for the samples obtained during the pretreatment carried out before making the mixtures with the plasticizer.

From these data, is possible to conclude that the best recovery percentage for the samples analyzed is low (75 % wt% solids) if It is compared with previous works which produced PHA from bacteria (89% wt% solids) using the same characterization method (GC/MS) and the same pretreatment process (acid methanolysis) (Hesselmann et al., 1999). The same occurs with respect to the purity (65 wt% solids) where previous works reported purities of 97,9 wt% solids (Yu et al, 2006). Nevertheless, this fact could be due to the different fermentation conditions employed to obtain the material (Mendez et al., 2016) which affect the behavior of the mutant bacteria in regard to the yield of production of PHA. In this case, different fermentation broth obtained through different conditions (initial carbon source concentration) were collected to obtain the final material to be characterized.

It is interesting to remark, that the purity obtained after the mixture with the plasticizer (Step 2) is slightly smaller than the one after the lysis treatment, which indicates that mixtures PEG/PHA (20%/80%) could be applied in a large scale to obtain the polymeric films.
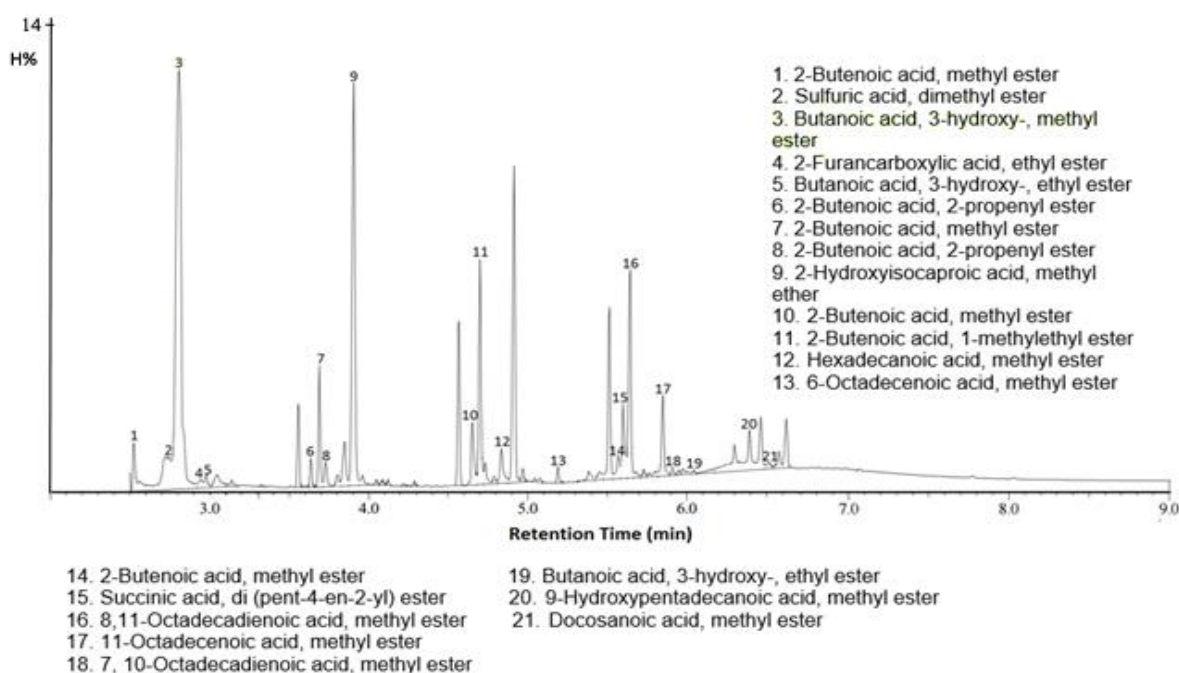

1. 2-Butenoic acid, methyl ester
2. Sulfuric acid, dimethyl ester
3. Butanoic acid, 3-hydroxy-, methyl ester
4. 2-Furancarboxylic acid, ethyl ester
5. Butanoic acid, 3-hydroxy-, ethyl ester
6. 2-Butenoic acid, 2-propenyl ester
7. 2-Butenoic acid, methyl ester
8. 2-Butenoic acid, 2-propenyl ester
9. 2-Hydroxyisocaproic acid, methyl ether
10. 2-Butenoic acid, methyl ester
11. 2-Butenoic acid, 1-methylethyl ester
12. Hexadecanoic acid, methyl ester
13. 6-Octadecenoic acid, methyl ester

14. 2-Butenoic acid, methyl ester
15. Succinic acid, di (pent-4-en-2-yl) ester
16. 8,11-Octadecadienoic acid, methyl ester
17. 11-Octadecenoic acid, methyl ester
18. 7, 10-Octadecadienoic acid, methyl ester

19. Butanoic acid, 3-hydroxy-, ethyl ester
20. 9-Hydroxypentadecanoic acid, methyl ester
21. Docosanoic acid, methyl ester

Figure 1. Chromatogram Height (%) VS Retention Time (min) generated for the samples after digestion with SDS.

Table 3: PHA content in three stages of treatment

Step	Description	PHA purity (wt% solids)	Recovery yield[a] (wt% solids)
0	Original cell mass	59,87	100
1	Treatment with SDS and sodium hypochlorite	65	75
2	Mixture- Chloroform Casting 20% PEG	58,15	---

[a] Recovery yield is the percentage of PHA solid recovered from original PHA of cells.

3.3 Infrared Spectroscopy

The second method of characterization is the evaluation of different samples in comparison with the results from commercial pure PHB. The advantage in regard to the other characterization methods is that this method is fast, simple and non-destructive of the sample. It tolerates the data recovery, specifically the quantity or types of functional groups in the sample without prior treatment of the sample. It allows the differentiation between the samples with different crystallinity grades. The results obtained for the standard commercial sample (PHB) and the PHA obtained from digestion with SDS (Figure 3) show great similarity. It could be confirmed through the comparison of the peaks showed in Table 4, which indicates that difference between

the values of the main vibrations associated to the material composition are less than 10%, if samples mentioned before are analyzed.

The main peaks of the IR graphs for the PHA compiled in Table 4, show that the most prominent band for all samples is the carbonyl ester at 1720.5 cm^{-1} which is a characteristic of the PHA spectrums (Yu et al, 2006). For the mixture obtained using vacuum as an operational condition, is important to remark that peak which corresponds to C-O-C at 1100.08 cm^{-1} is more intense, affecting the crystallinity determination. Also, were observed different peaks around the wave length of 1380 cm^{-1} which represent deformations of the methylene (CH$_2$) and the methyl (CH$_3$) in the biopolymer (PHB) (Bagheriasl, 2013). No important differences between the results of the other samples (SDS extraction and PHA/PEG mixtures at atmospheric pressure) and the commercial standard are observed.
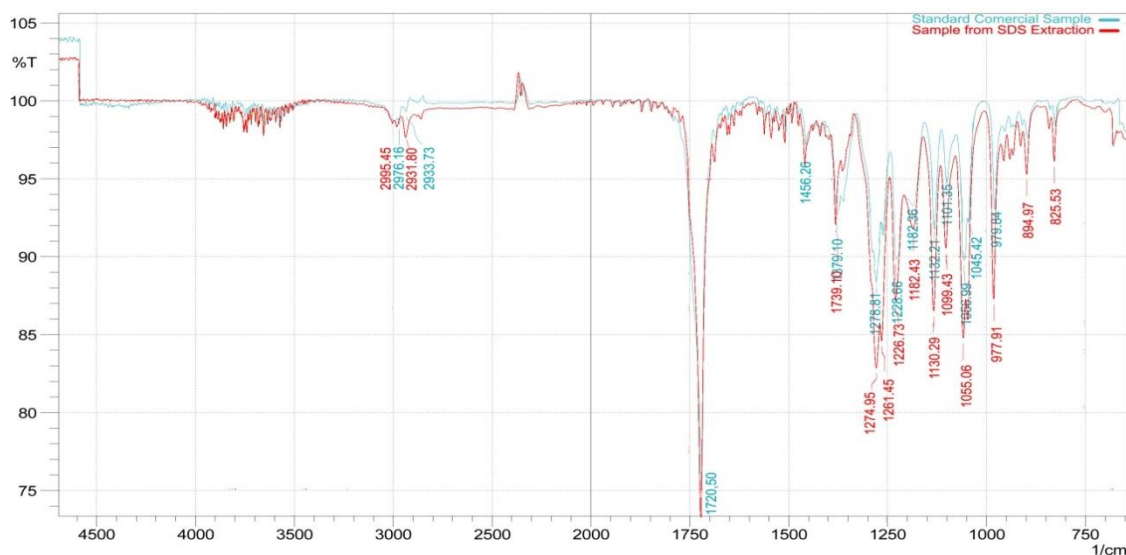

Figure 3. Infrared Spectroscopy graphs generated for the samples studied; Percentage of transmitance in function of cm-1 for Standard commercial Sample and *PHA from fermentation broth extracted with SDS.*

Table 4: Compilation of the main IR spectroscopy peaks (cm^{-1}) from the samples of PHA, the mixtures and the commercial PHA.

Standard commercial Sample	Sample from SDS extraction and hypochlorite whitening	PHA mixed with PEG at vacuum pressure	PHA mixed with PEG at atmospheric pressure	Description
2976	2995,45	2885,51	2976,16	C-H Vibration
1720,5	1720,5	1720,5	1720,5	C=O Vibration
1456,26	-	1465,9	-	CH3 Asymmetric deformation
1379,1	1379,1	1359,82	1361,74	CH Symmetric deformation
1278,81	1274,95	1278,81	1278,81	C-O-C Stretching + CH deformation
1228,66	1226,73	1240,23	1228,66	C-O-C Stretching
1182,36	1182,36	1182,36	1180,44	C-O-C Stretching
1132,21	1130,29	-	1130,29	CH3 Rocking
1101,35	1099,43	1100,08	1099,43	C-O-C Stretching
1045,42	1055,06	1058,92	1055,06	C-O Stretching
979,84	977,91	960,55	979,9	C-CH3 Stretching

The results for the Crystallinity Index are showed in Table 5. These data indicate that the condition which uses vacuum to prepare the mixtures affects negatively this property of the material. This fact is due to the variation of the absorbance values related to the peaks associated to the C-O-C vibration which are sensible for the crystallinity specific value of the sample analyzed (Porter, M. and J. Yu, 2011). Then, mixtures of PHA and PEG obtained under vacuum conditions increase these kind of interactions and subsequently increase the crystallinity of the polymer which is not desired in industrial applications. On the other hand, the mixtures obtained under atmospheric pressure show a lower CI with respect to the commercial standard of PHA, which indicate that the crystallinity of the material is reduced adding the biodegradable plasticizer PEG.

Table 5: Crystallinity index for the samples with IR spectroscopy

Sample	Crystallinity Index(CI)
Commercial Standard PHA	0,387
PHA extracted with SDS and sodium hypochlorite	0,381
PHA and PEG vacuum mixed	0,507
PHA and PEG mixed at atmospheric pressure	0,329

3. Conclusions

The acid methanolysis is a good pretreatment method prior GC/MS in order to quantify the PHA content in a sample, being useful to determine purity and recovery yield (wt%) for all the downstream operations during the production of polyhydroxyalkanoates using a mutant bacteria isolated from Colombian soils.

The properties determined for the PHA/PEG mixtures are directly related to the method and conditions of preparation. In this work, the method which involves atmospheric pressure and 180 ºC diminished the CI which is one of the purposes to add plasticizers into biopolymers. Nevertheless, several mechanical characterizations must be performed to evaluate completely the influence of the plasticizer in the material and to find commercial uses for the biopolymer. On the other hand, the results indicate that the digestion and bleaching processes using SDS and hypochlorite doesn't affect the CI with respect to a commercial PHB.

Using the Infrared Spectroscopy technique, was possible to conclude that the polymer produced by the mutant *Bukholderia cepacia* is of the PHB type due to the similarity of the spectrums and the absorbance values for the main peaks found.

References

Bagheriasl, M. 2013, Development and Characterisation of Polyhydroxybutyrate from Selected Bacterial Species. in: School Of Metallurgy And Materials, Phd, University Of Birmingham. United Kingdom, 209.

Braunegg, G., Sonnleitner, B., Lafferty, R.M., 1978, A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass. European Journal of Applied Microbiology and Biotechnology, 6(1), 29-37.

Choi, J. S., 2003. Effect of Biodegradable plazticizers on thermal en mechanical properties of poly(3-hydroxybutyrate). Polymer Testing, 455-460.

Hesselman, R., Fleischmann, T., Hany, R and Zehnder, A. 1999. Determination of polyhydroxyalkanoates in activated sludge by ion chromatographic and enzymatic methods. Journal of Microbiological Methods, 111-119.

Innocentini-Mei, L., 2003, Mechanical and Thermal Properties of Poly(3-hydroxybutyrate) Blends with starch and derivatives. Macromol, 77-87.

K. Sudesh, H. A., 2000. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Progress in Polymer Science, 1503-1555.

Kurusu, S., 2015. Incorporation of Plasticizers in sugar can-based poly(3-hydroxybutyrate)(PHB):Changes in microstructure and properties trough ageing and annealing. Industrial Crops and Products, 166-174.

Mendez, D.A., Cabeza, I.O., Moreno, N.C., Riascos, C.A.M. 2016. Mathematical modelling and scale-up of batch fermentation with burkholderia cepacia B27 using vegetal oil as carbon source to produce polyhydroxyalkanoates, Chemical Engineering Transactions, 49, 277-282.

Noda, I., 2010, Nodax TM Class PHA Copolymers: Their Properties and Applications. In: Plastics from bacteria: Natural Functional and Applications. Berling- Heidelberg: Springer.

Parra, D., 2006. Influence of poly (ethylene glycol) on the thermal, mechanical, morphological and biodegration properties of PHB. Polymer degradation and Stability, 1954-1059.

Porter, M. and J. Yu., 2011, Crystallization Kinetics of Poly(3-Hydroxybutyrate) Granules in Different Environmental Conditions. Journal of Biomaterials and Nanobiotechnology, 301-310.

Riis, V. and Mai, W., 1988, Gas chromatographic determination of poly-β-hydroxybutyric acid in microbial biomass after hydrochloric acid propanolysis. Journal of Chromatography A, 445, 285-289.

Shin-wo Hong, H. W. H., 2013, Thermal Properties and applications of low molecular weight polyhydroxybutyrate. Journal of Thermal Analytical Calorimetry, 1243-1250.

Wiley, 2007, Encyclopedia of Polymer Science and Technology. New Jersey: John Wiley & Sons.

Yoshie, N., 2000, Effect of low molecular weight additives on enzymatic degradation of PHB. Polymer, pp. 3227-3234.

Yu, J., & Chen, L. X., 2006, Cost-effective recovery and purification of polyhydroxyalkanoates by selective dissolution of cell mass. Biotechnol Prog, 22(2), 547-553. DOI: 10.1021/bp050362g.