

CROSSLINKING POLY-3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE WITH
DICUMYL PEROXIDE FOR PROPERTIES MODIFICATION

A Thesis

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Science

with a

Major in Natural Resources

in the

College of Graduate Studies

University of Idaho

by

Dikshya Pokhrel

Major Professor: Armando McDonald, Ph.D.

Committee Members: Lili Cai, Ph.D.; Erik Coats, Ph.D.

Department Chair: Charles Goebel, Ph.D.

May 2021

Authorization to Submit

This thesis of Dikshya Pokhrel, submitted for the degree of Master of Science with a Major in Natural Resources and titled “Crosslinking poly-3-hydroxybutyrate-co-3-hydroxyvalerate with dicumyl peroxide for properties modification,” has been reviewed in final form.

Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____
Armando G. McDonald, Ph.D.

Committee Members: _____ Date: _____
Erik R. Coats, Ph.D.

_____ Date: _____
Lili Cai, Ph.D.

Department Chair: _____ Date: _____
Charles Goebel, Ph.D.

Abstract

PHAs are new generation bioplastics. Production from cheaper carbon sources by using mixed microbial cultures, accompanied with various bio-compatible characteristics has heightened research interest in PHA-based polymers. PHBV is the copolymer of PHB and PHV, tailored to overcome the drawbacks like brittleness, low melt strength of PHB homopolymer. Using DCP as a crosslinking agent was found effective in improving PHB and PHBV properties in laboratory scale experiments. Therefore, PHBV-L (laboratory), extracted from MMC fed fermented dairy manure in pilot scale production was tested to see the effect in thermal and mechanical properties due to crosslinking. The shorter reaction time of 3-min and 1% DCP improved melt strength, thermal and mechanical properties for PHBV-C (commercial). Material characterization, molecular weight calculation, rheological characterization, tensile properties, and DSC analysis were done. PHBV-L with 21% HV and M_w , 8.9×10^5 g/mol was extracted with chloroform. Double melting points (T_{m1} at 126 °C and T_{m2} at 150 °C), crystallization temperature (T_c at 85 °C), and cold crystallization temperature (T_{cc} at 65 °C) and % crystallinity ($\%X_c$ at 25%) as obtained from DSC analysis. Gel content measurement was done after Soxhlet extraction show that crosslinked gel network was not initiated by DCP addition for PHBV-L_{0-0.5} and negligible (<1%) gel content was obtained for PHBV-L₁. T_{m1} and T_{m2} and $\%X_c$, changed negligibly for PHBV-L_{0-1sol}, whereas PHBV-L_{1gel} show the reduction of $\%X_c$ to 9% and T_{m1} and T_{m2} decreased to 126.7 °C and 144.4 °C, respectively. 1% DCP increased tensile strength with a significant difference in modulus and increase in energy at the break. Injection molding of PHBV-L decreased its M_w by ~50% due combined effect of temperature and DCP as shown by decreased M_w and complex viscosity (η^*) as % DCP increased. Processing PHBV-L at 155 °C and 175°C did not show a significant change in thermal properties however, a significant change in tensile property was observed for higher processing temperature. Therefore, the strength of PHBV-L did not change significantly due to crosslinking, but toughness and flexibility can be improved by 0.5-1% DCP and high processing temperature can be used for PHBV-L processing without thermal, degrading mechanical property while crosslinking effect was more favorable for 0.5-1% DCP content.

Keywords: *Poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV), Dicumyl Peroxide (DCP), Crosslinking*

Acknowledgement

First and foremost, I would like to acknowledge my major professor Dr. Armando G. McDonald for providing me with the opportunity to work with him. This work would not have been possible without his continuous supervision, guidance, and constructive suggestion throughout the academic period. I am very thankful to my committee members Dr. Erik Coats and Dr. Lili Cai for their support and feedbacks.

I am also very thankful to my father and my mother Mr. Surya Prasad Pokhrel and Mrs. Bhagawati Pokhrel, for your unconditional support on my endeavors. I would like to remember my grandmother. I am truly to a blessed to have my elder sister Ms. Jayanti Pokhrel and brother-in-law Dr. Raju Khatiwada, kept me motivated and positive throughout this period. My younger sisters, Indira, Swostika, Saru and my dearest friends Shilu, Yagya, I am very grateful to you all. I am also thankful to dear brothers, Bishal Thapa and Oshan Singh Karki for being there. I am very extremely thankful to my colleagues and friends, Maryam Abbasi, Berlinda Orji, Gurkeerat Kukal, Lucky Ewurum, Endalkachew Mengistie, Farid Sotoudehniakarani who were there to suggest me, help me and support me at the time of need.

I would like to acknowledge the U.S. Department of Agriculture National Institute of Food and Agriculture under Grant Number 2018-67022-27894 and the Idaho Global Entrepreneurial Mission (IGEM) Grant Number IGEM19-001 for their financial support.

Dedication

I would like to dedicate this work to my parents Mr. Surya Prasad Pokhrel, Mrs. Bhagawati Pokhrel, and my Didi-Bhinaju, Jayanti Pokhrel and Dr. Raju Khatiwada.

Table of Contents

| | |
|--|-------------|
| Authorization to Submit..... | ii |
| Abstract..... | iii |
| Acknowledgement..... | iv |
| Dedication | v |
| Table of Contents | vi |
| List of Abbreviations and Symbols | viii |
| List of Figures..... | x |
| List of Tables | xii |
| 1. Literature Review | 1 |
| 1.1. Market and Production of Bioplastics | 1 |
| 1.2. Properties of biopolymer: | 2 |
| 1.3. Production of PHAs: | 2 |
| 1.4. Production of PHBVs:..... | 3 |
| 1.5. Structure of PHBV: | 3 |
| 1.6. Properties of PHBV | 4 |
| 1.6.1. Rheological properties:..... | 4 |
| 1.6.2. Thermal properties of PHBV:..... | 6 |
| 1.6.3. Tensile Properties | 7 |
| 1.7. Methods of PHBV properties modification: | 8 |
| 1.8. Crosslinking of PHBV: | 10 |
| 1.9. Crosslinking PHBV using DCP: | 11 |
| 1.10. Effect of Dicumyl peroxide on material properties..... | 12 |
| 1.11. Recent Studies of PHBV: | 12 |
| 2. Research Goals..... | 14 |
| 3. Materials and methods..... | 15 |

| | |
|---|-----------|
| 3.1. Materials | 16 |
| 3.1.1. PHBV production and extraction..... | 16 |
| 3.2. Material Characterization | 17 |
| 3.2.1. Determination of HB/HV content:..... | 17 |
| 3.2.2. Molecular Weight determination | 18 |
| 3.2.3. Thermal Properties..... | 18 |
| 3.3. Sample Preparation..... | 19 |
| 3.3. Mechanical analysis | 19 |
| 3.3.1 Tensile testing:..... | 19 |
| 3.4. Melt Viscosity | 19 |
| 3.5. Properties of crosslinked PHBV..... | 19 |
| 3.5.1. Gel content of crosslinked PHBV..... | 19 |
| 3.5.2. Thermal analysis..... | 20 |
| 3.6. Surface Chemistry | 20 |
| 4. Results and Discussion | 21 |
| 4.1. Extraction and analysis of PHBV-L:..... | 21 |
| 4.2. Crosslinking by DCP:..... | 23 |
| 4.3. Thermal Properties: | 24 |
| 4.3.1. Thermal properties of PHBV-C-175 °C..... | 24 |
| 4.3.2 Thermal properties of PHBV-L: | 27 |
| 4.4 Tensile properties: | 31 |
| 4.6. Melt viscosity Molecular weight..... | 35 |
| 4.8. FTIR spectral analysis: | 39 |
| 5. Conclusions..... | 42 |
| 6. Limitations and Future Recommendations..... | 43 |
| References..... | 44 |

Abbreviations and Symbols

Abbreviations

| | |
|--------|--|
| DCP | Dicumyl Peroxide |
| DMA | Dynamic Mechanical Analysis |
| DSC | Differential scanning calorimetry |
| EAB | Energy at Break |
| FTIR | Fourier Transformation Infra-red Spectroscopy |
| GCMS | Gas chromatography-mass Spectrometry |
| HB | Hydroxybutyrate |
| HV | Hydroxyvalerate |
| MMC | Mixed Microbial Consortia |
| PBAT | Poly (butylene adipate-co-terephthalate) |
| PBS | Poly-Butyl Succinate |
| PBSebT | poly (butylene sebacate-co-butylene terephthalate) |
| PCL | Poly-caprolactone |
| PDI | Polydispersity Index |
| PE | Polyethylene |
| PHA | Poly-hydroxyalkanoate |
| PHB | Poly-3-Hydroxybutyrate |
| PHBV | Poly-3-hydroxybutyrate-co-3-hydroxyvalerate |
| PHBV-C | Poly-3-hydroxybutyrate-co-3-hydroxyvalerate-Commercial |
| PHBV-L | Poly-3-hydroxybutyrate-co-3-hydroxyvalerate -Lab |
| PHV | Poly-3-Hydroxyvalerate |
| PLA | Poly-Lactic Acid |
| PLLA | Poly-L-Lactic Acid |
| TGA | Thermogravimetric analysis |
| VFA | Volatile Fatty acid |

Symbols

| | |
|--------------|---------------------------------|
| $\% X_c$ | Percentage Crystallinity |
| ΔH_0 | Theoretical Enthalpy of Fusion |
| ΔH_m | Melting Enthalpy of Pea |
| E' | Storage Modulus |
| E'' | Loss Modulus |
| M_n | Number average Molecular Weight |
| M_w | Weight average Molecular Weight |
| T_c | Crystallization Peak |
| T_{cc} | Cold-crystallization Peak |
| T_g | Glass-Transition Temperature |
| T_m | Melting Peak |
| T_{m1} | First Melting Peak |
| T_{m2} | Second Melting Peak |
| Δ | Delta |
| η^* | Complex Viscosity |

List of Figures

| | |
|---|----|
| Figure 1.1: Chemical Structure of PHBV | 3 |
| Figure 1.2: Crosslinking mechanism in PHBV | 10 |
| Figure 3.1: Flow chart of crosslinking PHBV with DCP for properties modification | 15 |
| Figure 3.2: Production of PHBV from fermented dairy manure | 16 |
| Figure 4.1 : DSC second heating curve for PHBV-C and PHBV-L | 21 |
| Figure 4.2: NMR spectra of PHBV-L | 22 |
| Figure 4.3: NMR spectra of PHBV-L showing HB/HV peaks | 22 |
| Figure 4.4: Graph of gel content versus DCP concentration for cross-linked PHBV-C..... | 23 |
| Figure 4.5: DSC thermograms for second heating cycle for crosslinked PHBV-C processed at 175 °C, DCP 0-2% at 3 min reaction time | 25 |
| Figure 4.6: Relationship between X_c and T_m for crosslinked PHBV-C-175 °C | 26 |
| Figure 4.7: DSC thermograms of the cooling cycle for PHBV-L sol and gel fractions processed at 155 °C..... | 28 |
| Figure 4.8: DSC thermograms (normalized) of the second heating cycle for PHBV-L and crosslinked polymers- 155 °C | 30 |
| Figure 4.9: DSC thermograms of the cooling cycle for PHBV-L sol and gel fraction- 155 °C | 31 |
| Figure 4.10: Tensile stress (MPa) for PHBV-L processed at 155 °C and 175 °C | 33 |
| Figure 4.11: Tensile Modulus (MPa) of PHBV-L processed at 155 °C and 175 °C | 34 |
| Figure 4.12: Melt flow curve complex viscosity versus frequency of PHBV-C at 175°C | 35 |
| Figure 4.13: Melt flow curve of complex viscosity (η^*) versus frequency for crosslinked PHBV-L processed at 155 °C..... | 36 |

| | |
|--|----|
| Figure 4.14: Melt flow curve of complex viscosity versus shear rate for crosslinked PHBV-L processed at 175 °C | 37 |
| Figure 4.15: FTIR spectra of crosslinked PHBV-C processed at 175 °C | 39 |
| Figure 4.16: FTIR spectra of crosslinked PHBV-L processed at 155 °C | 40 |
| Figure 4.17: FTIR spectra of crosslinked PHBV-L processed at 175 °C..... | 40 |

List of Tables

| | |
|--|----|
| Table 1.1: Properties of different polymers compared..... | 2 |
| Table 1.2: Thermal properties of PHB and PHBV | 6 |
| Table 1.3: Tensile Properties of PHB and PHBV | 8 |
| Table 4.1: Thermal properties of crosslinked PHBV-C processed at 175 °C | 26 |
| Table 4.2: Thermal Properties of cross-linked PHBV-L reacted for 3 min processed at 155 °C and 175 °C | 29 |
| Table 4.3: Tensile Properties of crosslinked PHBV-C | 32 |
| Table 4.4: Tensile Properties of crosslinked PHBV-L at 155 °C and 175 °C | 33 |
| Table 4.5: Molecular weight of and complex viscosity of crosslinked PHBV-L-155 and 175 °C | 38 |
| Table 4.6: Crystallinity index of crosslinked PHBV-L and PHBV-C by FTIR analysis..... | 41 |

1. Literature Review

Increasing global concern on the production and use of petroleum based plastics has triggered the research and development of non-fossil based plastics from renewable bio-based resources having biodegradability virtues [1]. Policy shift towards sustainable development demands the cutoff of fossil fuel-based products and the consumer awareness on this matter is creating the market for bioplastics. Bioplastics are being used for variety of applications like packaging applications, carry bags, super-absorbent diapers, medical and dental implants, catering and hygiene products, mulching and agriculture [2]. These plastics should meet various properties before its industrial and commercial application [1].

1.1. Market and Production of Bioplastics

Bioplastic market shares only 1% (2.11 of the 360 million tonnes) of total plastics produced globally on annual basis in the year 2019. However, production is expected to rise to 2.43 million tonnes in 2024. PHA covers 1.2% of the total bioplastic market and is expected triple its production capacity by 2024. The major use of bioplastics i.e., 53% (1.14 million tonnes) is in the packaging application However, there is still a large production lag (European bioplastics,2019).

Bioplastics are mainly, starch based, cellulose based, poly-lactic acid (PLA) based and poly-hydroxyalkanoates (PHA) based. Starch based polymers are produced form agricultural feed stocks like potato, corn, wheat and other sources of carbohydrates and are referred to as the first generation of bio-plastics [2][3]. However, the feedstocks for polymer production were shifted from food-based resources to other renewable resources like organic waste material, fatty acids, and lignocellulosic biomass [3]. Different processes are used for the production and synthesis of biopolymers, polysaccharides and lipid-based polymers are produces from agricultural sources by extraction and separation process. PLA, polyethylene (PE), poly-butyl succinate (PBS) is produced by bacterial fermentation and synthesized by a polymerization process. PHAs are produced from microorganisms by a fermentation process [4].

1.2. Properties of biopolymer:

Table 1.1: Properties of different polymers compared

| Material | T _g (°C) | T _m (°C) | Youngs modulus (GPa) | Tensile strength (MPa) | Elongation % |
|--|---------------------|---------------------|----------------------|------------------------|--------------|
| PLA ^[5] | 45-60 | 150-162 | 0.3-3.5 | 21-60 | 2.5-6 |
| PHB ^[5] | 5-15 | 168-182 | 3.5-4 | 40.0 | 5-8 |
| PHB ^[6] | 9 | 175 | 3.8 | 45 | 5 |
| PHBV (HV-11%) ^[6] | 2 | 157 | 3.7 | 38 | 5 |
| PCL (Poly-caprolactone) ^[5] | -60 – -65 | 58-65 | 0.2- 0.4 | 20.7-42.0 | 300-1000 |
| i-polypropylene ^[5] | -17 | 174 | 1.5 | 30 | 10 |

1.3. Production of PHAs:

Main challenge in the commercialization of PHAs is the cost associated with the production [7][8][9][10]. The cost-effective method of producing PHAs is \$4.75/kg when the production scale is increased to million tonnes/year. This cost is very high compared to petrochemical based plastics like PE and polypropylene that can be produced for under \$1/kg. However, the cost of PHAs production compared to other bio-based plastics may be reduced by its biodegradability [8]. Research has been conducted to produce PHA from readily available and cheaper carbon sources and modifying its properties [1].

PHAs are produced from pure cultures as well as mixed microbial cultures (MMC). Pure cultures are mostly used by the industries for commercial production of PHAs [11]. The microbial biosynthesis of carbon substrate required for the PHA production accounts for the 28-50 % of the total cost incurred for the PHBV synthesis [10][9][8]. Therefore, finding cheaper carbon substrates is crucial to lowering production costs [12]. The use of mixed microbial culture is targeted for cost reduction of the biopolymer produced by reducing the cost of substrate [11][7]. PHAs have been produced from MMC obtained from municipal wastewater treatment plant activated sludge using malt waste and soy waste as a carbon source [13]. Taniguchi et al., investigated the use of waste edible oil and tallow for PHA synthesis. The study showed the successful production of PHA where the production of PHB was related to the waste plant oil and PHBV was produced by tallow. PHA accumulation was observed as high as 80% of the cell dry weight [12]. Colombo et al., used fermented cheese

whey containing various proportions of lactic, acetic, butyric, and valeric acids as a carbon substrate with mixed microbial culture to produce PHB more than 60% and PHV up to 40% [14]. The use of agricultural [8] [14] and municipal wastes [7] was seen as an effective measure for reducing the cost of raw materials to improve PHA production costs.

1.4. Production of PHBVs:

PHAs are polyesters synthesized by bacteria that feed on carbon substrates under favorable conditions [1]. PHAs, are linear biodegradable polyesters which are found in bacterial cells. The production of PHAs is based on a biological process with control of the carbon substrate and then isolation of PHA from bacteria cells. The most common type of PHA is polyhydroxy butyrate, PHB. Another type of PHA is a copolymer of hydroxybutyrate (HB) and hydroxy valerate (HV), which is poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) and structure shown in Figure 1 [15].

Wang et al. extracted PHBV from *Ralstonia eutropha* that was fed levulinic acid (LA) at a yield of 81.2% and with a HV content of 53.9% [10]. The PHBV polymer was flexible due to its high HV content. Chen et al. fed mixed volatile fatty acids (VFAs) as carbon substrate to a MMC and reported 70.4% of intracellular PHA content [16]. A metabolically engineered strain of *Escherichia coli* was able to produce PHBV with HV content between 5-18% [17]. Shang et al. produced PHBV with an HV content between 4-21% using a glucose and valerate substrate on a pure culture of *Ralstonia eutropha* [18]. Therefore, various culture media could be used to alter the production and properties of PHBV.

1.5. Structure of PHBV:

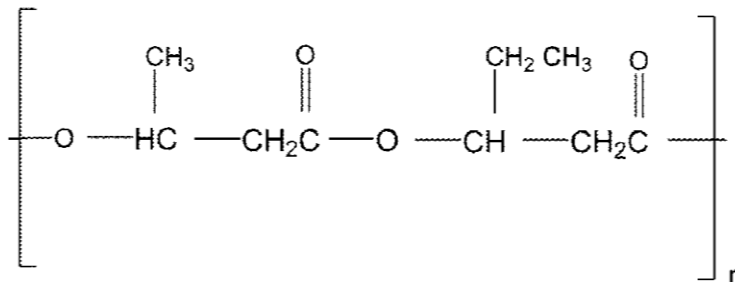


Figure 1.1: Chemical Structure of PHBV

The structure of PHAs (main building blocks (e.g., HB) and co-polymers (e.g., HV)) influence their biodegradability, thermal and mechanical properties. However, brittleness is a serious drawback in PHB, so the use of flexible PHAs for packaging applications is required [15]. PHB is one of the most widely studied polymer in PHA family [19][3]. Properties of PHB is comparable to that of petroleum-based polypropylene and possess gas and moisture barrier properties that are preferred for packaging applications [20]. For PHB, degradation temperature and melting point are very close therefore polymer degrades easily during the melt processing, which limits its processability [21]. Its properties can be altered by copolymerization and by blending with flexible polymers [10]. The copolymer PHBV is produced because of its properties relative to PHB [15][10].

PHBV has improved properties in terms of flexibility, toughness, impact resistance and processability compared to PHB [10][22]. The possible usage of PHB in wide range of application is limited by its brittleness [23][21]. Properties of PHBV like melt viscosity, oxygen barrier, processability during extrusion, and surface tension are better than PHB. Besides all these favorable properties for various applications, it has low impact resistance, poor thermal stability and is very hygroscopic in nature creates problems during processing and the properties of final products [22].

1.6. Properties of PHBV

1.6.1. Rheological properties:

Rheology gives the information about the material deformation behavior under the force applied. Rheological characterization is important to know the viscoelastic behavior of the polymer melts. It mainly gives the material suitability during the processing of the polymers as they have complex and unique flow properties that directly affect the processability [24] [25]. Vera-Sorroche et al. did experiments on HDPE with three different molecular weights and found that polymer viscosity decreased with increasing temperature which was due to the decrease in molecular weight. HDPE having higher shear viscosity showed greater shear thinning behavior at high shear rates that which was associated with higher molecular weight distribution [26]. Similar observation was made by Modi et al., while he studied the properties of PHBV to improve its properties for packaging application by adding high molecular weight natural rubber. Higher molecular weight causes higher molecular chain entanglement and

increased viscosity but increasing temperature and shear rate causes breaking of polymer chains therefore shear thinning is observed [27]. Therefore, higher viscosity was observed at lower temperature whereas at higher temperature viscosity decreased because of chain scission [28]. Ramkumar et al., observed that melt viscosity is also the function of HV content in addition to the molecular weight. PHBV with increased HV content 9-20% showed increase in molecular weight $3.04-4.33 \times 10^5$ g/mol, thus, increasing the viscosity at lower shear rate [29].

Various approaches have been taken by different researchers to improve processibility of PHBV targeted to increase the melt strength of PHBV. Blending of PHBV with PBSebT and PBAT was found to improve the rheological property of the blend by stabilizing the film bubble and increasing the processing window. The properties of the film made from the blend of those material was comparable to that of commercial PBAT and applicable for food packaging [30]. Epoxy-functionalized chain extender was found to increase the melt viscosity of PHBV therefore making PHBV more suitable for sheet extrusion or foaming during the melt processing process [31].

Wei et al. used DCP as a crosslinking agent to study the crosslinking mechanism of PHB and PLLA and found that melt strength of the biopolymers improved along with other properties. Chain-scission was noticed due to melt-processing however, long chain branching and broadened molar mass distribution contributed in improving the melt strength of those biopolymers [32]. Similar conclusion was made by Fei et al. using DCP as crosslinking agent. DCP was found to have positive influence on the melt strength of PHBV because of increased molecular weight distribution which reduced the negative effect of thermal degradation happening during the processing [33]. Complex viscosity of PHBV (3% HV) pellet was recorded 1,300 Pa. s at 180 °C and was 3,000 Pa. s at 145 °C at 1 rad/s [28]. However, in a different research done by Duangphet et al., they reported a viscosity of 525 Pa. s at 1 rad/s whereas use of 1% of epoxy-functionalized chain extender increased the viscosity to 630 Pa. s which was due to the macromolecular chain extension occurring during the extrusion compounding process [31]. PHB processed at 175 °C for 5 mins had very low complex viscosity, 30 Pa. s which increased to 1000 Pa. s at 0.1 rad/s when DCP was used as crosslinking agent. Processing temperature of 160 °C processing time less than 10 mins and

0.5-1% DCP as crosslinking agent was suggested by Fei et al. for crosslinking PHBV (HV 5%) with DCP [33].

1.6.2. Thermal properties of PHBV:

PHBV exhibits one or two melting peaks and a range of melting temperature are reported in various literatures [18][34] and are shown in table below. Double endothermic melting peak was at 129 °C and 153 °C observed by Wei et al. for PHBV with HV content 22% [35] whereas single melting peak at 170 °C was observed for PHBV with HV content 8% [36]. The double endothermic melting peak was due to the isodimorphism phenomenon. During heating of polymer, first the thinner crystal which have higher HV content in PHB crystal lattice- higher amorphous phase ratio- will be melted (T_{m1}), then the lower HV content crystals which have higher crystallinity ratio will need higher amount of heat to get melt, so we will see the second melting temperature (T_{m2})[27][34].

Table 1.2: Thermal properties of PHB and PHBV

| Material | $M_w (\times 10^5)$ g/mol | % HV | T_g (°C) | T_{m1} (°C) | T_{m2} (°C) | T_c (°C) | X_c (%) |
|-----------------------|------------------------------|------|------------|---------------|---------------|------------|-----------|
| PHB-L ^[37] | 3.09 | 0 | 2.8 | 158-171 | - | - | 62-66 |
| PHB-C ^[37] | 4.37 | 0 | 5.1 | 170 | - | - | 62 |
| PHB-O ^[37] | 1.44 | 0 | 3.2 | 141-158 | - | - | 53-57 |
| PHBV ^[38] | 4.43 | 8 | 4.5 | 170 | - | 91 | 64.3 |
| PHBV ^[32] | - | 0 | 5.5 | 174 | - | 93.9 | 40.9 |
| PHBV ^[39] | 7.9 | 34.1 | -3.2 | 147 | - | - | 2.1 |
| PHBV ^[39] | 7.7 | 32.4 | -2.1 | 148 | - | - | 1.9 |
| PHBV ^[39] | 3.0 | 6.4 | 5.5 | 160 | - | - | 44.3 |
| PHBV ^[39] | 4.2 | 18.9 | 0.9 | 156 | - | - | 36.2 |
| PHB ^[35] | 2.9 | 0 | 4.9 | 159 | 169 | 85 | 53.4 |
| PHBV ^[35] | 4.0 | 22 | -4.0 | 129 | 153 | 67 | 17.8 |
| PHBV ^[18] | - | 4.6 | 3.3 | 172 | - | 61.5 | 23.58 |
| PHBV ^[18] | - | 9.5 | 2.7 | 171 | - | 49.0 | 45.08 |
| PHBV ^[18] | - | 20.7 | 1.8 | 171 | - | 73.4 | 19.3 |
| PHBV ^[40] | 1.58 | 2.57 | - | 157.1 | - | - | - |

In the study of laboratory synthesized PHBV by Shang et al., its crystallinity of the polymer decreased from 45% to 19% with increasing HV content from 4.6-20.7% which was a decrease in area under the crystallization peak decreased with an increase in HV content. However, glass transition temperature (T_g) and melting temperature (T_m) decreased by a small amount [18]. At the same time the thermal degradation temperature was between 240-290 °C, which was higher than that of PHB suggesting an increase in the melt processing window for this material [18]. Commercial PHBV with varying HV content (0-22%) was shown to decrease in T_m from 176 °C (HV-0%) to 158 °C (HV-22%). In addition, the crystallinity of PHBV also decreased from 68.5% to 17% with an increase in HV content. Decrease in amorphous density and inhomogeneous distribution of HV sequences in the crystalline and amorphous phases is the main reason behind the decrease in T_m and crystallinity with increase in HV content[41].

It was also observed that, PHBV having higher HV content has low % X_c and relatively lower melting point and lower T_g (Table: 1.2). Hu et al. compared the thermal properties of PHB and the data indicated that there was no co-relation on molecular weight and thermal properties of PHB but at the same time PHB-O extracted after oven drying biomass was found to has low X_c and lower melting point which is desired for processing applications [37]. However, Wei et al. observed that the PHBV with high HV content has higher molecular weight and thermal properties been influenced. Lower T_g , lower T_m and decreased % X_c was observed for PHBV with high HV [39].

1.6.3. Tensile Properties

Tensile strength, Young's modulus, elongation at break, and energy at break (EAB) are key material properties that are used to compare different materials. These properties are related to monomer composition, molecular weight, microstructure and crystallinity of the materials [42][38].

Commercially produced PHB are reported with higher tensile properties compared to lab produced PHB and similar findings were reported for PHBV (Table:1.3) [37][38] [39]. Elongation to break of PHBV was higher than that of PHB suggesting that PHBV was more flexible and ductile. Although having higher HV content lab-extracted PHBV had lower

tensile strength and Young's modulus values however, elongation to break increased more than 10-50% (Table:1.3) [39]. The increase in elongation break is due to the increase in molecular weight of the polymer [37]. Toughness of material is measured by EAB values and it was found that PHBV was tougher than PHB [35].

Table 1.3: Tensile Properties of PHB and PHBV

| Material | $M_w (\times 10^5)$ g/mol | % HV | tensile strength (MPa) | Young's modulus (GPa) | Elongati on % | EAB (J) |
|-------------------------|------------------------------|------|------------------------------|-----------------------------|------------------|---------|
| PHB-L ^[37] | 3.09 | 0 | 14 | 1.8 | - | - |
| PHB-C ^[37] | 4.37 | 0 | 20 | 2.3 | 1.3 | - |
| PHB-O ^[37] | 1.44 | 0 | 14.1 | 2.5 | 0.6 | - |
| PHB ^[38] | - | 0 | 40 | 3.5 | 5 | - |
| PHBV ^[38] | - | 20 | 20 | 0.8 | 50 | - |
| PHB ^[35] | 2.90 | 0 | 23.1 | 2.2 | 13.6 | 0.33 |
| PHBV ^[35] | 4.00 | 22 | 11.8 | 0.9 | 19.6 | 0.45 |
| PHBV ^[36] | 4.43 | 8 | 31.6 | 0.25 | 4.7 | - |
| PHB-C ^[39] | 4.1 | 0 | 22.5 | 1.31 | 2.80 | - |
| PHBV-C1 ^[39] | 3.0 | 6.4 | 17.0 | 0.99 | 10.7 | - |
| PHBV-C2 ^[39] | 4.2 | 18.9 | 20.6 | 1.07 | 8.7 | - |
| PHBV-1 ^[39] | 7.9 | 34.1 | 15.0 | 0.299 | 58.8 | - |
| PHBV-2 ^[39] | 7.7 | 32.4 | 14.8 | 0.289 | 57.6 | - |

1.7. Methods of PHBV properties modification:

PHBV properties are limited because of the difficulties that are encountered during the processing mainly due to its poor melt strength creating problem during stretching and blowing. Therefore, conversion of PHBV into marketable goods by conventional melt processing routes is seen as a challenge. Also, the post process property of PHAs is limited by applicability due to slow crystallization leading brittleness and lower flexibility [1].

A wide range of PHA can be synthesized from variety of carbon sources and their monomeric composition can be varied during their synthesis providing the flexibility of controlling the

properties of the polymer [21][43][44]. PHBV is also highly crystalline in nature like other aliphatic polyester however, due to the presence of HV monomeric units it is less crystalline [22]. The amount of HV composition is seen as the major technique in making PHBV flexible and with better mechanical properties. Melting temperature of PHBV decreases with increase in HV content due to the increased amorphous content in polymer [45][18]. Therefore, degradation rate of polymer also increases with increasing HV content [18]. Since the HV content in PHBV is very crucial in determining physical and mechanical properties of polymer, the PHBV with desired HV content can be selected for specific application [22].

Grafting of PHBV with cellulose fibers [35], nano-particles [46][47][48], lignin [36] has been done to improve the properties of resulting composite products. Peroxide induced crosslinking has been used to couple of researchers to study the mechanism of modification of PHBV [33], [36]. However, properties of PHBV have not yet been accepted for commercial purpose [15]. Wei et al. added DCP to PHB to improve the grafting with cellulose fiber. Reaction times between 5 and 15 min and DCP loadings of 2-5% were used to see the grafting efficiency. The best grafting efficiency was achieved at 5 min and 2% DCP. The experiment was successful in improving the thermal stability and suggested that possibility of increased the flexibility of the composite product that could be formed [49]. Following this conclusion further investigation was done with the same materials and mechanical testing were done in the PHBV reinforced with cellulose fibers and the mechanical properties were found to be enhanced by the improvement in interfacial bonding occurred. The crystallinity of PHB, PHBV were reduced thus increasing the flexibility of the bio-composite [35]. Using 0.5% DCP introduced crosslinking and improved the thermal stability of PHBV [36]. Peroxide radicals abstract hydrogen atom from carbon in carbon-hydrogen backbone in polymer and carbon chain and OH side group in cellulose fiber which leaves carbon and oxygen radicals which later forms C-C crosslinks [49][35] similar mechanism is seen in grafting PHBV with lignin using DCP [36]. Wei et al. also reported the same mechanism for crosslinking PHBV and PLLA individually to improve their melt processing [32].

Blending two polymers is also the widely experimented approach for improving PHBV properties. Blending is basically mixing of two or more polymer to make a new polymer with desired properties. It is done with various reasons either reduce the cost of final product by

using one of the cheaper polymer with similar properties, increasing the performance of polymer, targeted applications [6]. The miscibility of two or more different polymers is critical for the properties of the blend. Not only the miscibility of polymer blend depends upon the ratio of polymers, but it also depends upon the structure of polymers used like molecular weight and size of polymers in the dispersed phase. Zhao et al. has studied the blending characteristics of PLA and PHBV on the injection molded samples [50]. They found that for PHBV-PLA blends a low weight ratio of PHBV, <30%, was recommended. However, with an increase in amount of PHBV had a better influence in properties modification in terms of decreased cell size and increased cell density in foams [50]. Gerard et al. also studied the blending characteristics of PLA/PHBV blend and found that the lower amount of PHBV was able to produce blends with better properties in terms of elongation at break (or ductility) [51].

1.8. Crosslinking of PHBV:

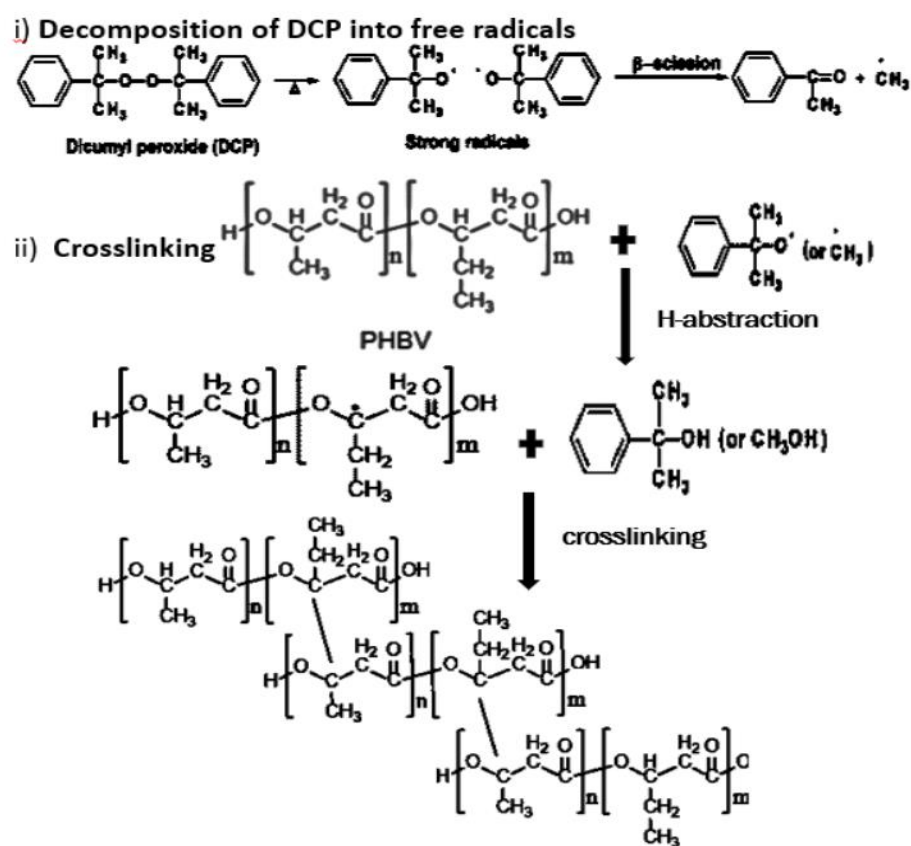


Figure 1.2: Crosslinking mechanism in PHBV

Crosslinking of linear polymers such as natural rubber, polyethylene and hydrogels have been examined to improve properties [33]. Several studies have investigated the crosslinking of PHBV, biopolymer blends and bio-composites however mostly focused on those produced commercially by pure cultures and having low HV content [33][28][31][27].

Work by Wei et al. has shown that PHB, PLA and PHBV can be crosslinked using DCP and this occurs in two stages [32]. In the first step: DCP in contact to heat (during melt processing) decomposes into a free radical. This radical is capable of extracting H atom from tertiary carbon in the PHBV chain therefore leaving free radical in the PHBV chain which can form C-C linkage therefore producing crosslinked structure [36][33].

1.9. Crosslinking PHBV using DCP:

Reactive extrusion is one of the process methods commonly used for crosslinking of polymers (e.g., PHB and PHBV). However, reactive extrusion was found to have negative effects on the mechanical properties and molecular weight of polymer if not controlled. Compared to all the other methods, the mechanism of crosslinking of polymer chain could be controlled to a great extent in the extruder. In the reactive extrusion process, the negative effect of heat on molecular weight and melt viscosity of PHB and PHBV could be reversed and the better mechanical properties, melt viscosity could be added to the polymers [33]. The modification of these parameters is very important for applications like foaming to produce the porous materials [33]. Wei et al. used DCP to improve the properties of PHBV reinforced with α -cellulose fibers [35]. The initiation of the DCP free radical at high temperature was found to be an effective approach to graft PHBV to cellulose. The separate effect of DCP in PHBV was not studied but grafting of PHBV to cellulose induced by DCP showed an increased interfacial bonding and compatibility between the cellulose and polymer matrix [35]. The effect of crosslinking and grafting using DCP for improving the interfacial properties of PHBV and lignin polymer alloys was also investigated by in-situ reactive extrusion [36]. DCP influenced the T_g , thermal stability, and melt strength of these biopolymer alloys. Also, the use of DCP has noticed effect on the reduction of spherulite size and increased nucleation density of PHBV crystals. The intermolecular interaction of the biopolymer alloy was improved using DCP as crosslinking agent [36].

1.10. Effect of Dicumyl peroxide on material properties

Campuzano et al. used DCP (0-1%) as crosslinking agent to make blends of PLA and PBS (40/60% ratio) to improve their compatibility and foaming properties [52]. Low amounts of DCP (0.1%) resulted in an increased impact strength like that of neat, foamed PBS. However, the higher amount of peroxide decreased the mechanical properties of blend due to very high amount of crosslinking. Material properties like melt strength, melt viscosity, tensile strength were also improved for PBS modified for foaming application using DCP [53].

Mechanical properties and rheological behavior of PLLA/PBS blends were investigated to study the effectiveness of in situ compatibilization by the addition of 0.05-0.2% DCP. A concentration of 0.1% DCP was found effective in improving the blend properties, the reduction in spherulite size and better interfacial adhesion was seen as well as the reduction in crystallinity and increased light transmittance [54].

1.11. Recent Studies of PHBV:

Wei et al. obtained PHBV (yields 36-42%) from biosynthesis of fermented dairy manure VFA using a MMC from a wastewater treatment activated sludge seed [39]. The research focused on PHBV properties based on the use of various composition of odd and even numbered VFAs (e.g.: acetic, propanoic, butyric, and valeric acids). The purified PHBV had a high molecular weight (7.9 g/mol) and had a HV content of 33%. The PHBV was highly flexible compared with commercial PHBV (8% HV). The PHBV also had a lower T_m (149 °C) than PHB and commercial PHBV[39].

Subsequent work by Hanson et al. produced PHBV from fermented dairy manure at a yield of 40% and having HV contents between 59 and 75% [55]. Coats et al. using a similar process obtained PHBV at yield between 22.5 to 90.7% with an HV content as high as 73% [56]. For this purpose, PHBV with high HV content (15- 24 % HV) has been produced at pilot-scale on fermented dairy manure liquor [57]. However, the PHBV cell content was approaching commercial scale. PHBV (HV content 32-34%) properties required for industrial processing like melt strength, tensile strength, thermal properties has been assessed [39]. Tensile strength and Young's modulus were comparable and significantly lower, respectively, than

commercially produced PHBV [39]. Crosslinking is expected to increase the strength of polymers and is a viable strategy for property improvement. Since most of the research studies on PHBV modification have been on commercially sourced material, this study will explore the properties of high HV PHBV modified by reactive melt processing using DCP.

2. Research Goals

The objective of the study was to improve pilot scale produced PHBV polymer from fermented dairy manure and commercial PHBV properties by DCP induced crosslinking. Different loadings of DCP 0-1% and reaction times and temperatures were examined and the properties of the modified PHBV evaluated.

The change in melt viscosity of the crosslinked polymer will be determined by dynamic rheology. Crosslinking of polymer will be assessed by determining gel content after the Soxhlet extraction. The properties of crosslinked polymer will be determined using DSC, FTIR spectroscopy and size exclusion chromatography (SEC). Tensile testing will be done on the DCP added samples to see the change in properties due to DCP addition.

3. Materials and methods

Figure 3.1 shows a flow diagram of the experiments performed in this study starting from pre-production phase to extraction, crosslinking of the PHBV and techniques used for materials testing for the research goals.

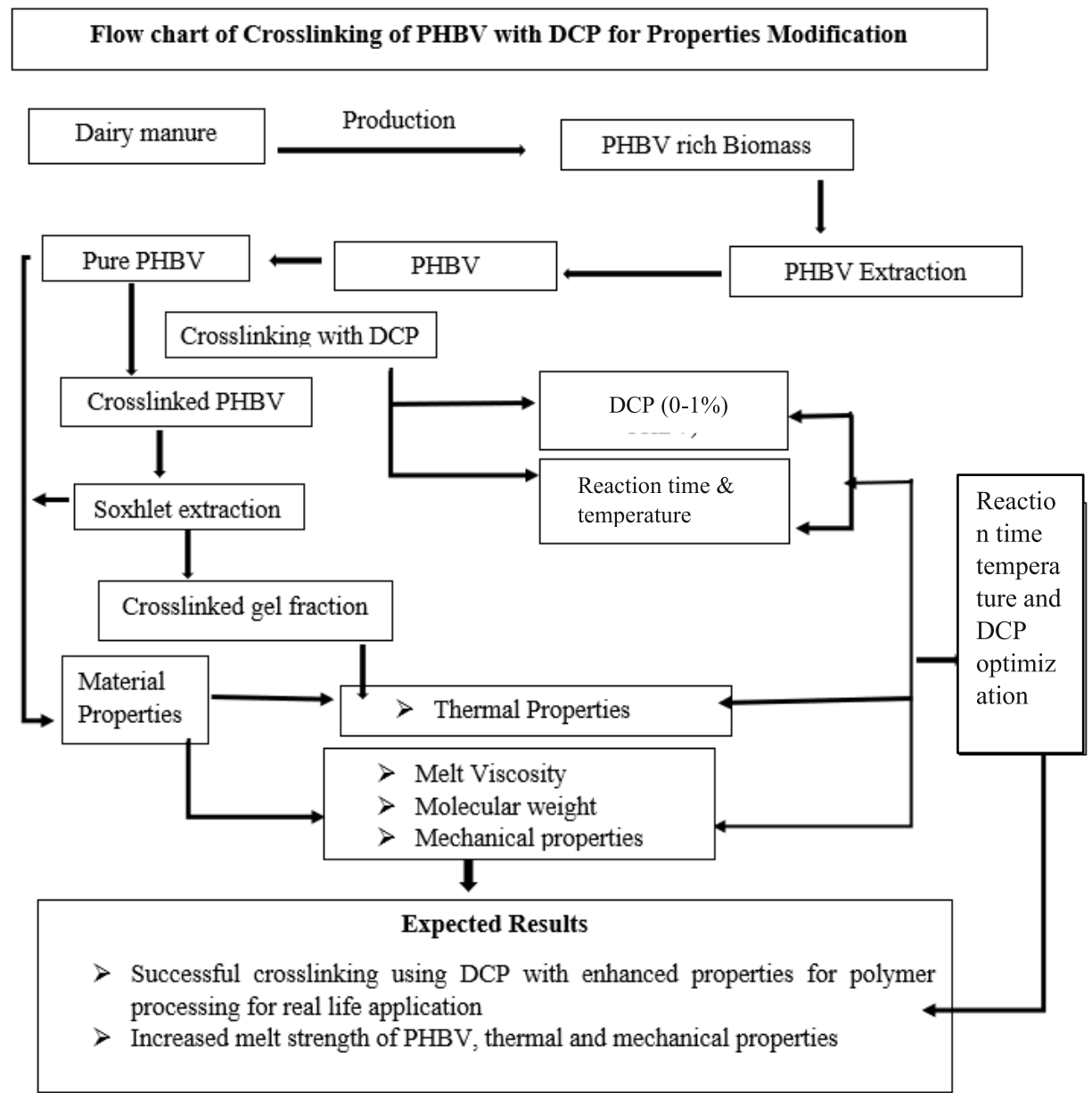


Figure 3.1: Flow chart of crosslinking PHBV with DCP for properties modification

3.1. Materials

3.1.1. PHBV production and extraction

Production of PHBV rich biomass

PHBV- rich biomass was produced from dairy manure in the PHBV pilot facility. Three stage process: fermentation, enrichment, and production) was followed targeted for the optimization of HV content in the polymer. In the first step of the PHBV production was the fermentation of dairy manure. The dairy manure was fermented and passed through wire mess to remove suspended materials, centrifuged, and filtered to obtain fermenter liquor. The fermented liquor was diluted with tap water and passed to the enrichment reactor which contained the activated sludge from the City of Moscow Water Reclamation and Reuse Facility (Moscow, ID, USA). The mixture of the activated sludge and fermenter liquor was now enriched mixed microbial consortia (MMC) which was sent to the production reactor. Fermenter liquor from the fermenter reactor was added to the MMC in production reactor. In the next step, the liquor in production reactor was centrifuged to obtain PHBV-rich biomass which was processed for obtaining pure PHBV in labroatory- PHBV-L [57].

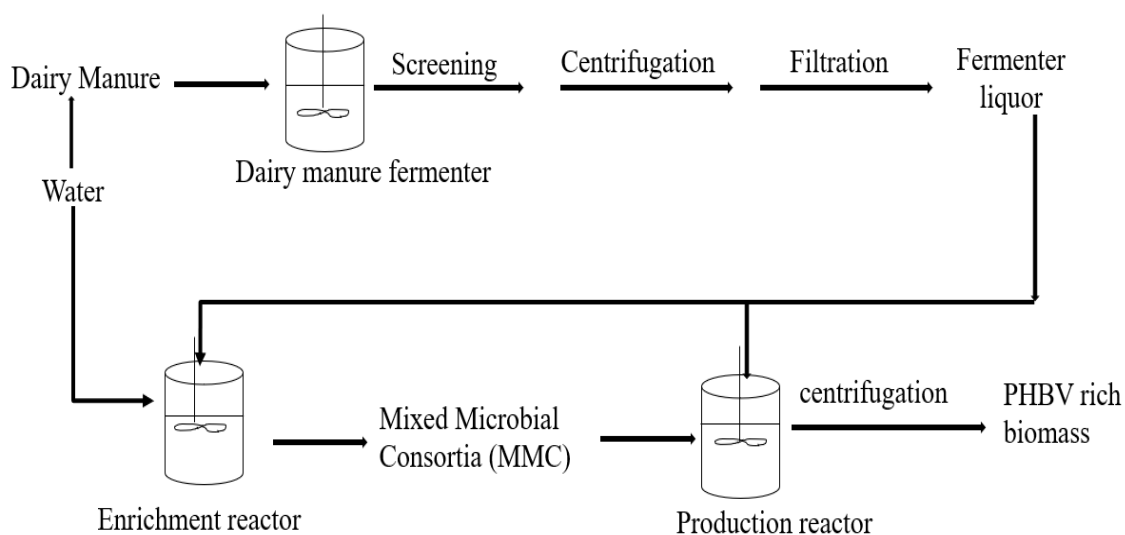


Figure 3.2: Production of PHBV from fermented dairy manure

Extraction and purification of PHBV-L

PHBV-L was recovered from the PHBV-rich biomass produced at the PHBV pilot facility following extraction and purification. The PHBV rich biomass (851g) was first washed with acetone (4 L for the first, 1 L thereafter) for 24 h with continuous stirring to remove lipids. The acetone washed biomass was recovered by vacuum filtration after each wash. After the final washing, the acetone-washed PHBV-rich biomass was air dried. Lipid free-PHBV rich biomass was extracted with chloroform (3.5 L) using a Soxhlet extractor for 24 h which was recovered by concentration to dryness in rotatory evaporator to obtain crude PHBV-L. Crude PHBV-L in 28 ± 1 wt.% of freeze-dried biomass was obtained. A two-step purification process was done using two non-solvent (petroleum ether and methanol) precipitations. First, the crude PHBV was redissolved in chloroform (150 mL) and then added dropwise to cold, constantly stirred petroleum ether (4 °C, 1.5 L) to precipitate the PHBV. The same method was used while the solvent used was methanol in the second step. Finally, 25 wt.% purified PHBV-L (ash free biomass) was recovered by filtration and dried under vacuum.

Commercial PHBV (PHBV-C) pellets (HV-8%) was obtained from Tianan Biopolymer Inc. (China) as a reference. Dicumyl peroxide (DCP) (98%) was purchased from Sigma-Aldrich Co. LLC (USA).

3.2. Material Characterization

3.2.1. Determination of HB/HV content:

HV/HB content were determined by gas chromatography-mass spectrometry (GCMS). Acidified methanol (3% H₂SO₄, v/v, 2 mL) and chloroform (2 mL, containing 0.5 mg/mL benzoic acid as internal standard) were added to freeze-dried biomass (20 mg) in a digestion test tube and the mixture heated at 100 °C for 4 h. Then 1 ml water was added to the cooled mixture, vigorously shaken, the chloroform layer was separated and dried through a small column of anhydrous sodium sulfate and transferred to a GC vial. The 3HV/3HB ratio as their methyl ester derivatives were analyzed by GC-MS (ISQ7000-Trace1300, Thermo Scientific). Separation was performed on a ZB-1 capillary column (Phenomenex, 30 m x 0.25 mm) with helium carrier gas and a temperature profile of 40 °C ramped to 200 °C at 5 °C/min. Xcalibur v4.1.31.9 software was used to analyze the data.

The molar 3-hydroxyvalerate (HV) content in the purified PHBV-L samples (~5 mg in CDCl₃) was determined by ¹H-NMR spectroscopy (Bruker 300 MHz spectrometer). The methyl group signals for HV (δ 0.92 ppm) and HB (δ 1.29 ppm) were integrated to obtain the HV molar ratios.

3.2.2. Molecular Weight determination

Size exclusion chromatography (SEC) was used to determine number and weight-average molecular weights (M_n and M_w) of PHBV were determined. A Jordi DVB linear mixed bed column (7.8 mm x 300 mm) column was used for the separation of PHBV at 30 °C. PHBV samples (100 μL, 2 mg/mL) were filtered (0.45 μm, PTFE), injected on elution with CHCl₃ at 0.5 mL/min and detected with a Waters model 2414 refractive index detector and a PostNova PN3609 (658 nm) multi-angle light scattering (MALS) detector. A refractive index increment (d_n/d_c) value of 0.034 for PHB in CHCl₃ was used (Negulescu et al. 2008). Data were analyzed using the NovaSEC v1.5.0 software. Calibration of the system was done using a narrow polystyrene standard (Malvern Panalytical Ltd, Malvern, UK) having $M_w = 105,268$ g/mol.

3.2.3. Thermal Properties

Differential scanning calorimetry

Glass transition temperature and melting points were determined for as PHBV-L and PHBV-C samples using a TA Instruments model Q200 DSC with refrigerated cooling. All samples were first rapidly heated to 200 °C (100 °C/min) to remove thermal history followed by a cooling scan (-20 °C/min) to -50 °C. The data were analyzed using TA Universal Analysis v4.4A software. The cold crystallization temperature (T_c) was determined from the cooling scan curve. From the heat capacity data curve, T_g , T_m and enthalpy of fusion (ΔH_m) were determined. The degree of crystallinity (X_c %) of pure and crosslinked PHBV were calculated based on

$$X_c \% = (\Delta H_m) / \Delta H_0 \times 100$$

where ΔH_m is the melting enthalpy (reversing curve), and ΔH_0 is melting enthalpy in J/g of 100% crystalline PHBV (146 J/g).

3.3. Sample Preparation

Commercial PHBV (PHBV-C) pellets and laboratory extracted PHBV (PHBV-L) was vacuum dried for at least one week prior to use. Dried PHBV (2 g) was placed in a Dynisco laboratory mixing extruder/molder operating at 175 °C for PHBV-C and PHBV-L more samples prepared at 155 °C for PHBV-L. Polymer was melted for 2 min and mixed (100 rpm) for 3-5 min with addition of DCP (0, 0.25, 0.5, 1, 2 wt.%) and injected into a mold (circular discs, rectangular bars, or dog-bone specimens). Crosslinked PHBV-C and PHBV-L with DCP 0-2% were coded PHBV-C₀₋₂ and PHBV-L₀₋₂.

3.3. Mechanical analysis

3.3.1 Tensile testing:

Crosslinked PHBV-C and PHBV-L (175 °C and 155 °C) with DCP 0-2% were coded PHBV-C₀₋₂ and PHBV-L₀₋₂. Injection molded dog-bone specimens (6 replicates) were tested according to ASTM D1708 for their tensile properties- tensile strength, Young's modulus, and energy at break using an Instron 5500R-1132 universal test machine equipped with a 1 kN load cell. Tests were conducted using a crosshead speed of 1 mm/min and a micro-extensometer (model 3542, Epsilon Technology Corp.) was used to measure strain.

3.4. Melt Viscosity

Complex Viscosity (η^*) was determined using a Bohlin Instruments CVO 100 rheometer, parallel plate (25 mm \emptyset), in oscillating shear mode with an ETC module on molded discs (2 mm x 25 mm \emptyset) for PHBV-C₀₋₂ and PHBV-L₀₋₂. Experiments were performed in the linear viscoelastic region. Viscosity measurement was conducted in the frequency range of 0.01 to 100 Hz at an applied strain of 0.5%. Data was analyzed using the Bohlin rheology v6.51 software.

3.5. Properties of crosslinked PHBV

3.5.1. Gel content of crosslinked PHBV

Cross-linked polymer forms networks which are not easily dissolved in organic solvent and forms swollen gel. The gel fraction was determined on extruded strands that were Soxhlet extracted in CHCl₃ for 24 h to obtain a soluble “sol” and insoluble “gel” fractions and vacuum

dried for 48 h to determine gravimetric yield. The sol fractions obtained after extraction from 0-2 wt. % DCP treatment were coded as PHBV-L_{0-1sol} and PHBV-C_{0-2sol} and gels as PHBV-L_{0-1gel} and PHBV-C_{0-2gel}. Gel content was measured by gravimetric method.

$$\text{Gel content (\%)} = (W_{\text{gel}}/W_0) \times 100$$

where W_0 is the dry weight of the cross-linked polymer, W_{gel} is the weight of dry gel fraction.

3.5.2. Thermal analysis

Differential scanning calorimetry

Glass transition temperature and melting points were determined for as PHBV-L_{0-1gel} and PHBV-C_{0-2gel} using the same process mentioned in 3.2.3.

3.6. Surface Chemistry

Chemical structure of PHBV-L and its crystallinity was assessed with the FTIR analysis also. Absorbance bands corresponds to the specific chemical bonds. As there are three specific types of bonding in PHBV-L, C-O-C, C-C and C=O, intensity of absorbance at 1226 cm⁻¹ corresponds to C-O-C bond, whereas 980 cm⁻¹ corresponds to C-C bond. These absorption bands are sensitive to the crystalline region in the PHBV. The absorbance band at 1720 cm⁻¹ corresponds to C=O bonding in the PHBV-L, this is sensitive to the crystalline region in the polymer however, small shoulder appearing at 1740 cm⁻¹ is due to the amorphous region and is insensitive to crystalline structure. Likewise, bands at 1226 cm⁻¹ and 1455 cm⁻¹ represents C-O bonding. In the FTIR spectra absorbance bands corresponding to 1226 cm⁻¹ is sensitive to crystallization and 1455 cm⁻¹ is insensitive to crystallization. The ratio of wavenumbers 1720/1740 ($I_{\text{C=O}}$), 1226/1455 ($I_{\text{C-O}}$) was calculated to find the crystallinity index.

4. Results and Discussion

4.1. Extraction and analysis of PHBV-L:

The total of 25% of the freeze-dried biomass was recovered from the PHBV rich biomass. HV content of the polymer was calculated from GCMS was 21% and that from NMR analysis was found to be 21%. The M_w of the PHBV-L was $8.90 \pm 0.33 \times 10^5$ g/mol and polydispersity index PDI of 1.07 showing a narrow distribution. Two endothermic melting peaks were observed, T_{m1} at 126.7 °C and T_{m2} at 150 °C (Figure 4.1). Crystallization temperature (T_c) was observed at 79.8 °C, however a double cold crystallization peak (T_{cc}) was also observed during the second heating cycle at 65 °C. Crystallinity of the PHBV-L was 25%. The M_w of PHBV-C was 2.2×10^5 g/mol and only one melting peak was observed at 171 °C (Figure 4.1) and T_c was observed at 120°C and no cold crystallization peak was observed during the second heating scan of the sample. Crystallinity of PHBV-C was 69.7% which is very high as compared to the PHBV-L.

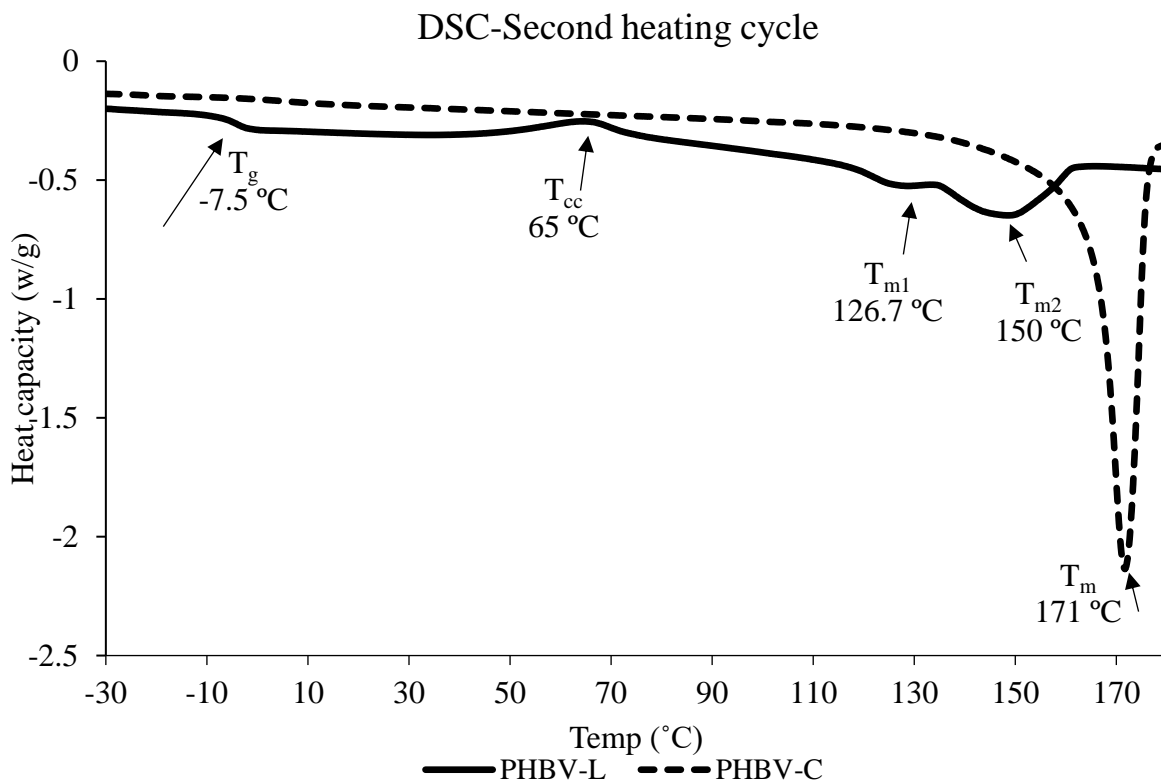


Figure 4.1 : DSC second heating curve for PHBV-C and PHBV-L

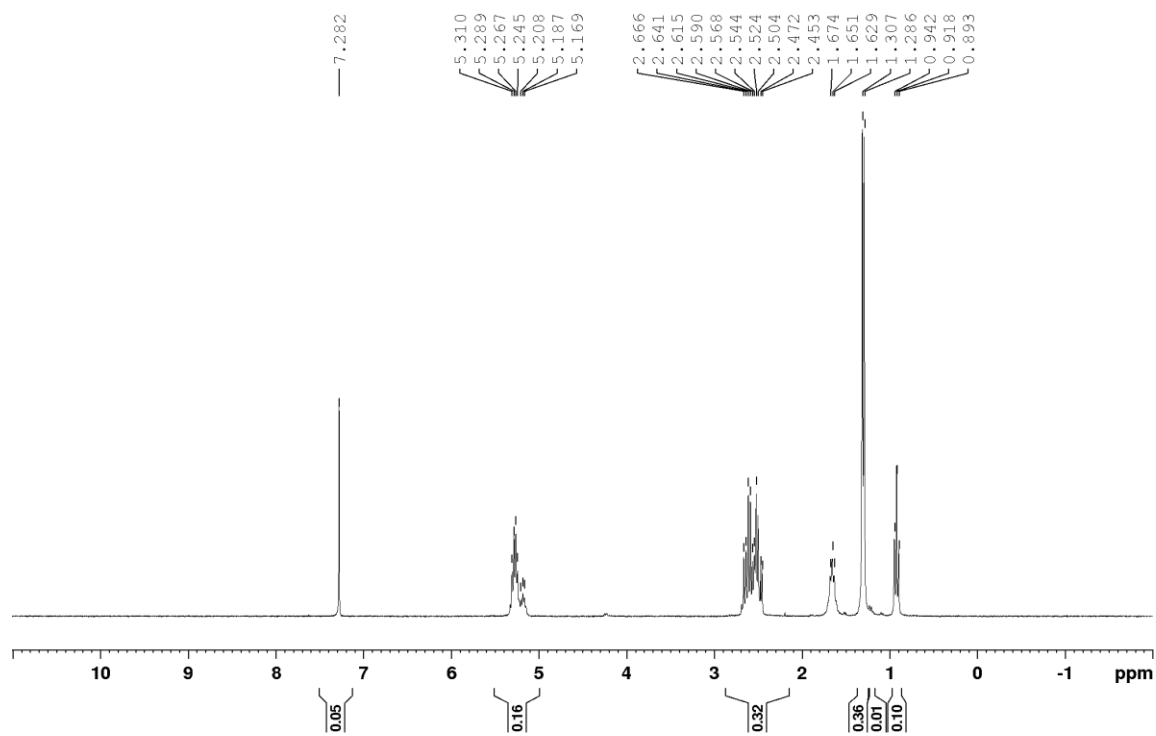


Figure 4.2: NMR spectra of PHBV-L

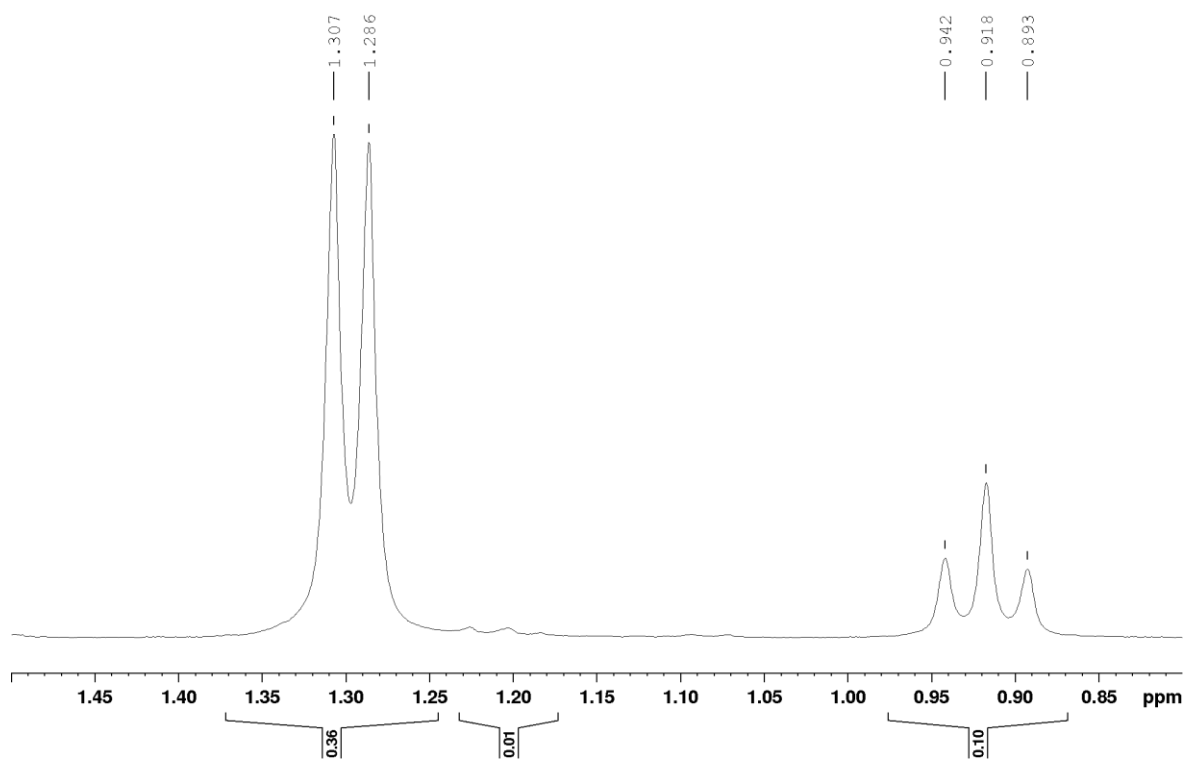


Figure 4.3: NMR spectra of PHBV-L showing HB/HV peaks

4.2. Crosslinking by DCP:

Addition of DCP as a crosslinking agent at high temperature resulted in decomposition of the peroxide into cumyloxy radicals, 60% of which forms methyl radicals by β -scission limits the rate of crosslinking [33][58]. When DCP is added to PHBV and processed in high temperature, there is thermal degradation of PHBV that occurs simultaneously to crosslinking. Therefore, the processing time must be shortened to maximize cross-linking and minimize degradation. A decrease in cross-linking rate is due to the reduction in peroxide content due to decomposition [33]. Also during the decomposition, these free radicals tend to abstract H-atoms from tertiary carbon site of the polymer chain which initiates the crosslinking phenomenon [36]. Therefore, shorter reaction time and optimum amount of DCP is required for increasing the crosslinking efficiency.

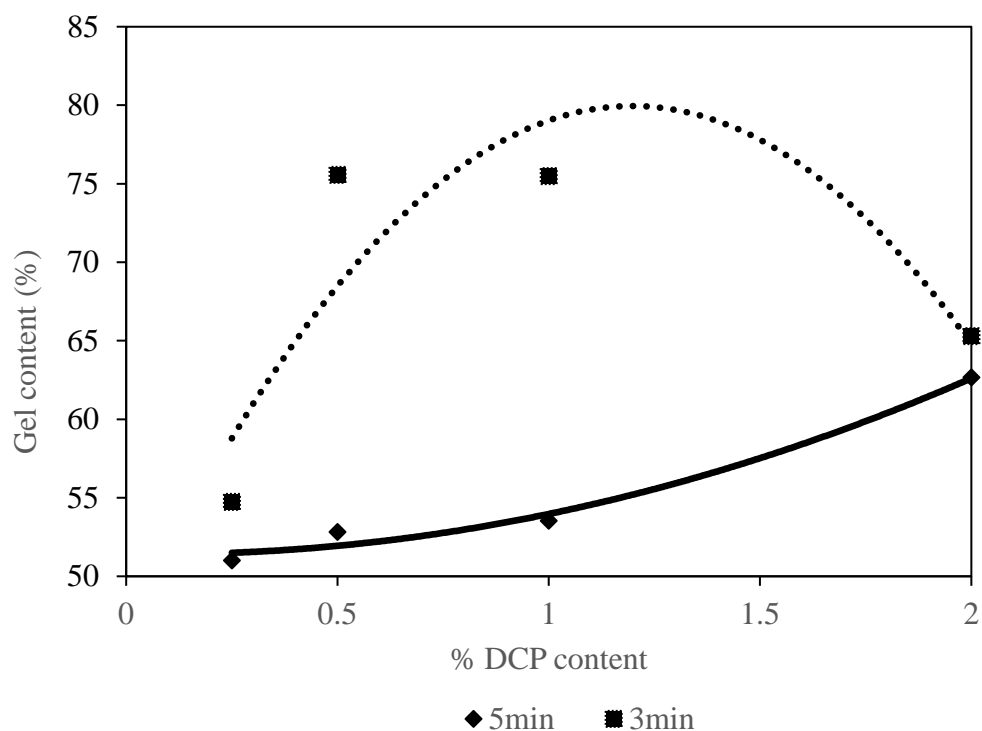


Figure 4.4: Graph of gel content versus DCP concentration for cross-linked PHBV-C

PHBV-C (0-2% DCP) that reacted for 3 min had a higher gel % content. Gel content increased from 18.7 ± 0.6 to $75.5 \pm 0.9\%$ for 0-1% DCP and later decreased to $65.3 \pm 0.3\%$ for a 2% DCP content (Figure 4.4). This result is in agreement with the results obtained by Fei et al. [33], which suggested optimum amount of DCP for effective crosslinking was 0.5-1%

DCP, and gel % decreased when % DCP was increased to 3 % and 5%. When higher amount of DCP is used, the polymer chain starts to degrade due to the abstraction of H-atoms from the tertiary carbon sites due to excessive free radicals [35]. When PHBV-C reacted for 5 min a decrease in gel content decreased (Figure 4.2). Crosslinked gel fraction increased from increasing DCP content for DCP content 0.25-2%. The results of cross-linked gel % of PHBV-C using DCP at 5 mins is comparable to the results obtained by Fei et al. and Luo et al. [36][33]. However, these two different experiments were done with 5% and 8% DCP and processing time 5 and 8 min respectively, the results in terms of gel content obtained were very similar i.e., ~55% whereas, in this experiment, gel content was 52-53% at 0.5-1% DCP. However, material used in both experiments had similar molecular weight ($\sim 4.4 \times 10^5$ g/mol) and low HV content of 5-8%.

Addition of DCP 0.25 and 0.5 %, to PHBV-L resulted no measurable gel content however, at 1% DCP a gel content of 1% was obtained. Since no/low gel fraction was obtained this does not mean that cross-linking in PHBV-L did not occur. However, Fei et al. [33] was able to get 1% gel content using 0.5% DCP and gel content increased up to 55% for 1% DCP. The increased amorphous nature of PHBV-L due to its higher HV content (20-30%) may contribute to its increased solubility and low gel content. The lower cross-linking efficiency might be due to the higher molecular weight of the PHBV [36] which in turn is related to increased HV %.

4.3. Thermal Properties:

4.3.1. Thermal properties of PHBV-C-175 °C

Thermal properties such as T_m and X_c determined by DSC (Figure 4.5 and Table 4.1) are the important properties for cross-linked polymers. Cross-linking of PHBV-C-175 °C, the amount of amorphous content increased and a concomitant decrease in X_c . This leads to a decrease in the T_m of PHBV-C with an HV content of 8%. DSC analysis on crosslinked polymer shows that T_m decreased with higher DCP content. For PHBV-C, the T_m of PHBV-C, 3 min was lower than that of PHBV-C, 5min and the strong correlation was observed between T_m and DCP content (Figure 4.6).

PHBV- C_0 exhibited a single endothermic melting peak at 171.7 °C. Upon the addition of DCP to PHBV-C the endothermic peak decreased. For PHBV- $C_{2\text{gel}}$, 5min, T_m decreased to 163 °C by 7 °C whereas it decreased by 10 °C to 161 °C for PHBV- $C_{2\text{gel}}$, 3min. T_m decreased by less than 2 °C for PHBV- $C_{0.25\text{gel}}$, 3-5 min. A similar phenomenon was observed for the cooling cycle of the DSC curve in that T_c decreased from ~122 °C for PHBV- $C_{0.25\text{gel}}$ to ~118 °C PHBV- $C_{2\text{gel}}$ for 5 min, whereas it decreased to ~116 °C PHBV- $C_{2\text{gel}}$ for 3 min reaction time. In comparison, the T_c for PHBV-C was 91 °C and increased to 109 °C with the addition of 0.5% DCP [36] implying an increase in crystallization rate of polymer. However, the results presented (Table 4.1) here are in contrast with Fei et al. [36], but are in agreement with [33] where T_m and T_c both decreased with DCP content. The decrease in T_m and T_c is due to the decrease in X_c of the crosslinked polymer [33]. The X_c of PHBV- C_0 was very high i.e., 69.7 % which decreased to 57.6% for PHBV- $C_{2\text{gel}}$, 5min and 55.8 % for PHBV- $C_{2\text{gel}}$, 3min. This result is comparable with X_c of PHBV (8%HV) at 64.5% which decreased to 60.8% due to addition of 0.5% DCP [36]. The results are also in line with the conclusions made by [33], where bioplastic crystallinity decreased from 53% to 33% with the for DCP content 0-5%.

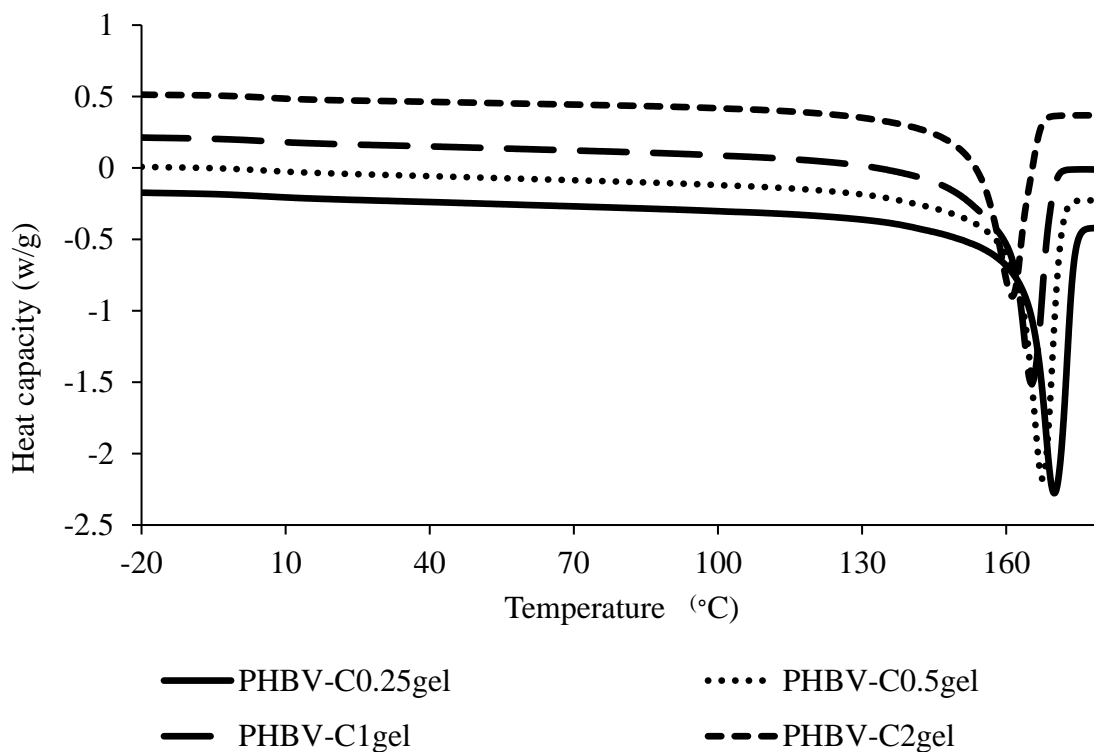
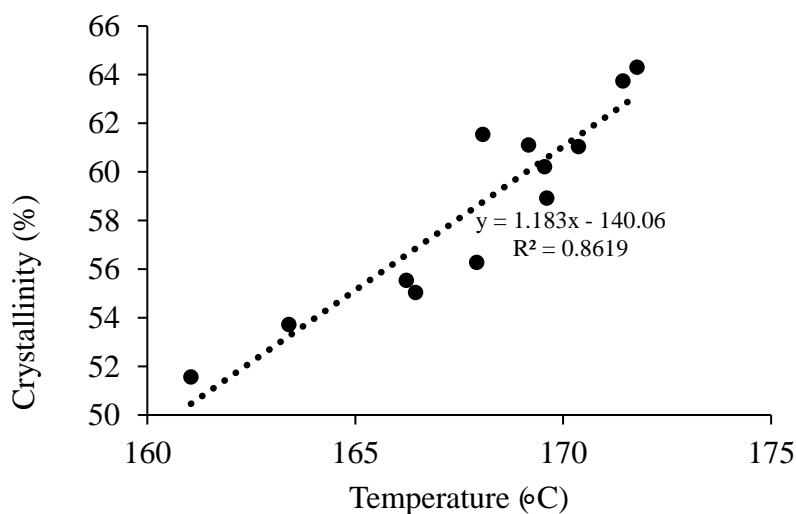


Figure 4.5: DSC thermograms for second heating cycle for crosslinked PHBV-C processed at 175 °C, DCP 0-2% at 3 min reaction time

Table 4.1: Thermal properties of crosslinked PHBV-C processed at 175 °C

| Polymer type | Time (min) | Gel content (%) | T_g (°C) | T_m (°C) | T_c (°C) | X_c (%) |
|---------------------------|------------|-----------------|------------|------------|------------|-----------|
| PHBV-C _{0gel} | 0 | 18.71±0.6 | -8.0±0.7 | 171.7±0.1 | 121.8±0.1 | 69.7±2.5 |
| PHBV-C _{0.25gel} | 3 | 54.9±1.6 | -1.4±0.5 | 170.3±0.3 | 122.4±0.1 | 66.4±0.7 |
| PHBV-C _{0.5gel} | 3 | 75.6±9.6 | -1.61±1.3 | 168.1±0.6 | 121.7±0.1 | 65.9±0.8 |
| PHBV-C _{1gel} | 3 | 75.5±0.9 | -2.9±0.5 | 166.2±0.9 | 120.94±0.8 | 60.1±1.1 |
| PHBV-C _{2gel} | 3 | 65.3±0.3 | -2.0±1.1 | 161.1±0.2 | 116.4±0.3 | 55.8±0.7 |
| PHBV-C _{0.25gel} | 5 | 50.6±2.9 | -1.6±1.4 | 171.4±0.3 | 122.9±0.2 | 68.7±0.0 |
| PHBV-C _{0.5gel} | 5 | 52.8±0.0 | -2.9±0.5 | 169.6±0.5 | 121.8±0.6 | 63.8±3.3 |
| PHBV-C _{1gel} | 5 | 53.5±3.3 | -1.8±0.0 | 166.4±0.4 | 120.2±0.3 | 55.2±0.4 |
| PHBV-C _{2gel} | 5 | 62.7±0.2 | -0.7±0.3 | 163.4±0.4 | 118.3±0.4 | 57.6±0.6 |

The T_g increased with % DCP content (Table 4.1). The T_g of PHBV-C_{0gel} was -8 °C which increased to -2.0 °C for PHBV-C_{2gel}, 3min and -0.7 °C for PHBV-C_{2gel}, 5min. A similar phenomenon was observed by Fei et al. for PHBV, where T_g increased from 4.5 °C to 5.7 °C with addition of 0.5% DCP [36]. This was because of the higher thermal energy required to initiate the segmental motion of the polymer in amorphous phase and also due to the branching of polymer due to the addition of DCP [36].

Figure 4.6: Relationship between X_c and T_m for crosslinked PHBV-C-175 °C

4.3.2 Thermal properties of PHBV-L:

The thermal properties of as extracted (PHBV-L) and processed PHBV-L-155 °C and PHBV-L-175 °C were determined by DSC (Figures 4.7– 4.10, and Table 4.2) PHBV-L (with an HV content of 21%) exhibited a noticeable T_g at -7.5 °C and two endothermic melting peaks T_{m1} (126.7 °C) and T_{m2} (150 °C). In addition, a cold crystallization peak (T_{cc}) at 65 °C was observed [35]. The cooling curve showed a T_c at 79.8 °C. Enthalpy of heat during cold crystallization is 27.3 J/g which is very high compared to the enthalpy during crystallization 6.5 J/g. Similar results were obtained by [35] with PHBV (22% HV), where T_{m1} , T_{m2} , T_g and T_{cc} were 129 °C , 153 °C, -4.0 °C and 67 °C, respectively with non-defined (ND) crystallization temperature in the cooling cycle (Figure 4.7). The X_c was 25.3%. The crystallinity of PHBV-L processed at 155 °C was low compared to PHBV-C and reported earlier and by the other researchers [39][36][33], where the difference is attributed to the difference in HV content. The addition of DCP to the PHBV-L processed at 155 °C did not induce gel formation for PHBV_{0.25-0.5} and negligible gel content was obtained for PHBV₁ at this temperature. However, the thermal properties of the gel fraction of crosslinked polymer PHBV-L_{1gel}, exhibited the predicted change in T_m and X_c . T_{m1} remained almost unchanged however T_{m2} decreased from 150 to 144 °C. Similar trend was observed for PHBV-L processed at 175 °C in terms of T_{m1} , T_{m2} , T_g and T_{cc} . No gel was induced for DCP addition 0-0.5% and negligible gel content was obtained for PHBV-L₁.

T_{cc} shifted towards higher temperature to 83 °C. T_c (Figure 4.7) was not observed during the cooling cycle. Similar phenomenon was observed by Wei et al. [35] during the grafting of α -cellulose with PHBV using DCP as initiator. The disappearance of T_c and an increase of T_{cc} is due to cross-linking that causes an increased rate of crystallization which does not allow enough time for the polymer chains to arrange themselves in a crystalline structure during cooling which is later exhibited during the heating cycle [35]. The X_c of PHBV-L_{1gel} decreased to 9.6% compared to that of PHBV-L and the sol fraction of the PHBV-L₁ (Figure 4.6).

However, the properties of the sol fractions for PHBV-L were determined. The sol and gel fraction had clear difference in their thermal properties (Figure 4.7). Both the exothermic and endothermic peaks were broader and less steep for PHBV-L_{1gel} compared to PHBV-L_{1sol},

meaning decrease in X_c . Also, the T_{cc} increased for PHBV-L_{1gel} which means crystallization starts at lower temperature thus allowing more time required for complete crystallization [59]. It is also explained as the effect of slower nucleation and crystal growth in the crosslinked structure [35].

For PHBV-L processed at 155 °C and 175 °C, no significant difference in melting temperature was obtained. Crystallinity of the material remained similar with no regards to processing temperature and % of DCP added for the soluble fraction. Difference was observed in crystallinity, melting points and in the gel fraction which was very lower.

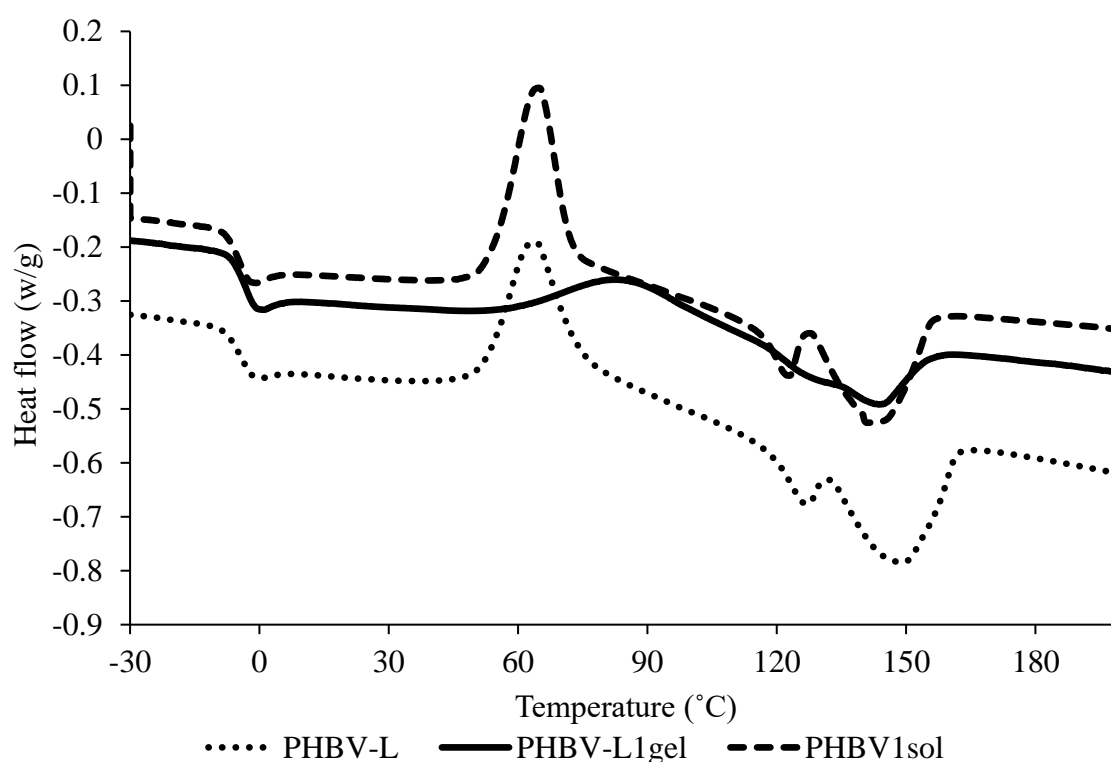


Figure 4.7: DSC thermograms of the cooling cycle for PHBV-L sol and gel fractions processed at 155 °C

Table 4.2: Thermal Properties of cross-linked PHBV-L reacted for 3 min processed at 155 °C and 175 °C

| Sample | Temperature (°C) | Gel content (%) | T _g (°C) | T _{m1} (°C) | T _{m2} (°C) | T _c (°C) | T _{cc} (°C) | X _c (%) |
|-------------------------|---------------------|--------------------|---------------------|----------------------|----------------------|---------------------|----------------------|--------------------|
| PHBV | | - | -7.5±0.4 | 126.7±0.5 | 150±0.3 | 79.8±5.3 | 65.0±1.0 | 25.3±0.6 |
| PHBV _{0gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{0.25gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{0.5gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{1 gel} | 155 | 1.0±0.2 | -6.6±0.0 | 126.7±0.2 | 144.4±0.5 | ND | 83.4±0.4 | 9.6±0.3 |
| PHBV _{0sol} | | 100 | -10.6±2.3 | 124.5±1.1 | 148.8±0.8 | 68.7±3.4 | 67.3±5.0 | 23.5±0.2 |
| PHBV _{0.25sol} | | 100 | -7.7±0.2 | 124.3±0.1 | 148.3±0.0 | 72.3±0.1 | 63.3±0.3 | 24.1±0.8 |
| PHBV _{0.5sol} | | 100 | -9.7 ±1.6 | 119.8±3.2 | 143.8±2.9 | ND | 66.2±2.3 | 22.2±1.1 |
| PHBV _{1sol} | | 99.0±0.2 | -8.0 ±0.1 | 122.1±0.0 | 144.2±0.4 | ND | 66.9±2.2 | 21.5±0.2 |
| PHBV _{0gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{0.25gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{0.5gel} | | 0 | ND | ND | ND | ND | ND | ND |
| PHBV _{1 gel} | 175 | 1.3±0.3 | -6.7±0.5 | 126.9±0.3 | 144.7±0.4 | 71.7±0.9 | ND | 17.5±1.7 |
| PHBV _{0sol} | | 100 | -7.4±0.2 | 126.2±0.6 | 149.6±0.1 | 77.9±0.0 | 63.7±0.2 | 23.8±3.6 |
| PHBV _{0.25sol} | | 100 | -7.2±0.9 | 123.5±0.6 | 145.2±2.3 | ND | 65.7±1.3 | 27.3±2.4 |
| PHBV _{0.5sol} | | 100 | -7.5±0.3 | 124.2±0.5 | 147.5±0.8 | 75.2±0.0 | 63.0±0.7 | 25.4±0.4 |
| PHBV _{1sol} | | 98.7±0.7 | -8.9 ±1.6 | 121.4±1.0 | 142.8±0.8 | ND | 66.7±0.3 | 25.2±0.7 |

Figure- 4.8 shows the second heating cycle DSC thermograms for the PHBV-L₀₋₁ sol and gel fractions at 155 °C. It is evident that all the heating curves look similar with small shifts in the peaks and their sharpness. However, the heating cycle for the crosslinked gel looked different with relatively flat T_{cc} at higher temperature and less noticeable T_{m1} and the smaller area under the curve spread over broader range clearly showing a decreased X_c compared to PHBV-L and PHBV-L_{0-1sol} 155 °C. Cross-linked material properties are less affected by the sol fraction and more by the cross-linking density. In the polymer matrix of both the sol and gel fractions are combined intricately and thus the sol fraction will have little effect in T_m and T_c in poorly cross-linked polymer, whereas highly cross-linked structures will show an increased effect [33]. This can be explained by the heating and cooling cycles of PHBV-L_{1sol} and PHBV-L_{1gel}.

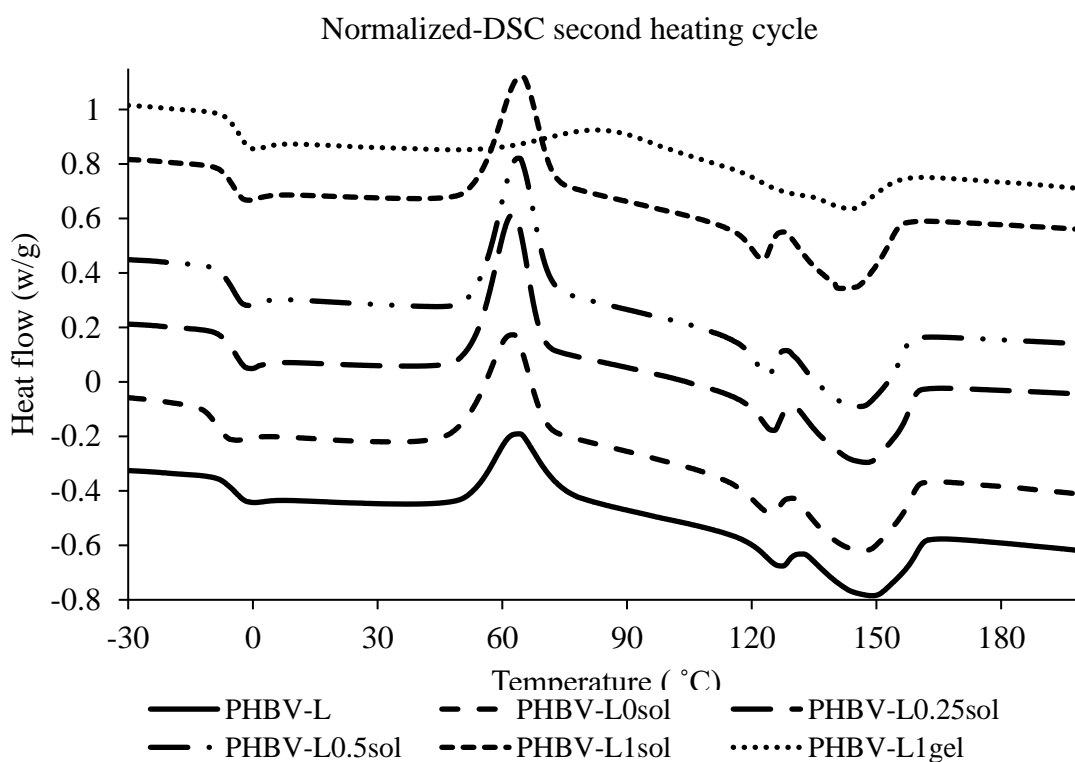


Figure 4.8: DSC thermograms (normalized) of the second heating cycle for PHBV-L and crosslinked polymers- 155 °C

T_c disappeared and T_{cc} was observed for PHBV-L (Figure 4.9), this is due to the high HV content in the polymer and the increased molar mass distribution as a result of DCP addition molar mass distribution as a result of DCP addition [33][36].

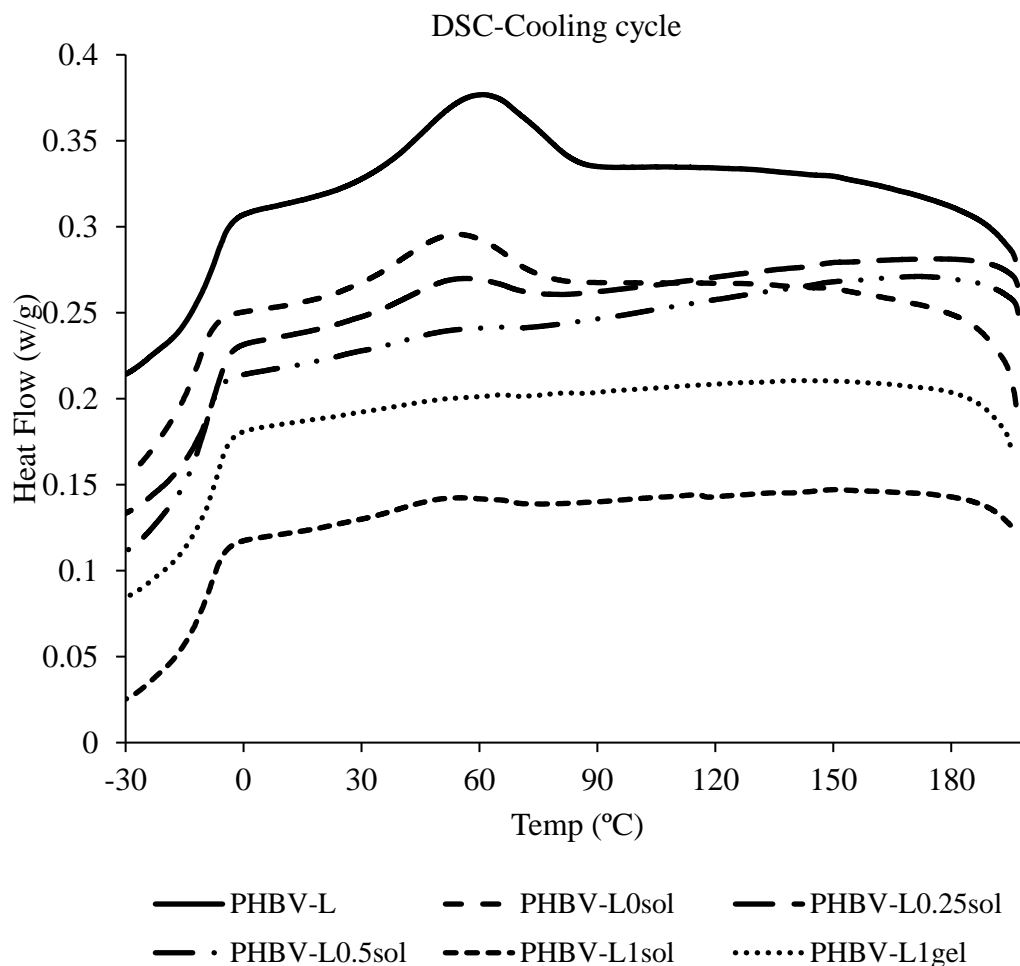


Figure 4.9: DSC thermograms of the cooling cycle for PHBV-L sol and gel fraction- 155 °C

Similar trend was observed for PHBV-L processed at 175 °C. Melting temperature decreased from 149 °C to 142 °C due to addition of DCP 1% with no significant decrease in crystallinity. Also, the gel fraction of PHBV-L-175°C is expected to show the similar trend as that of PHBV-L-155 °C. No significant difference was observed in thermal properties as shown by DSC due to different processing temperature for PHBV-L.

4.4 Tensile properties:

The tensile properties of PHBV-L and PHBV-C and crosslinked polymers were determined to establish the change in properties (Tables 4.3-4.4, 4.10-4.11). Tensile strength of the PHBV-L- 155 °C was half that of the PHBV-C. Tensile strength of PHBV-L₀-155 °C was 13.5±0.7 to 15.2±0.6 MPa where tensile strength of PHBV-C was 26.9±1.8 to 34.4 ± 0.3 MPa (Table 4.4,

Figure 4.10). The difference in tensile properties was attributed to the differences in HV content [33]. There was no significant difference in the tensile strength of PHBV-C in relation to the processing time 3-5 min processing time for PHBV-C.

Table 4.3: Tensile Properties of crosslinked PHBV-C

| Time | Sample | Tensile stress (MPa) | Tensile Modulus (MPa) | Energy at Break (J) |
|------|------------------------|-------------------------|--------------------------|------------------------|
| 3min | PHBV-C ₀ | 31.6±0.7 | 234.4±5.1 | 0.99±0.12 |
| | PHBV-C _{0.25} | 31.1±0.3 | 236.0±13.2 | 0.94±0.13 |
| | PHBV-C _{0.5} | 31.5±0.1 | 256.5±3.9 | 0.77±0.04 |
| | PHBV-C ₁ | 28.3±0.7 | 198.6±4.6 | 0.83±0.10 |
| 5min | PHBV-C ₀ | 34.6±0.3 | 303.8±5.9 | 0.72±0.03 |
| | PHBV-C _{0.25} | 30.3±1.2 | 236.0±17.1 | 0.70±0.09 |
| | PHBV-C _{0.5} | 26.9±1.8 | 224.9±16.6 | 0.55±0.09 |
| | PHBV-C ₁ | 29.9±0.2 | 176.3±5.5 | 1.64±0.11 |

Young's modulus gradually decreased with the increase in % DCP for both PHBV-C and PHBV-L (Figure 4.9). Young's modulus of PHBV₀ was 167.8±6.9 MPa which decreased to 122.5±6.2 MPa for PHBV-L_{1.0}. Similar trend was seen for the commercial sample, where tensile modulus values were lower for 1% DCP content (Table 4.3-4.4, Figure 4.11). The results obtained for PHBV 22% HV by [35] showed tensile strength 11.8±2.0 MPa, Young's modulus 900±10 MPa and EAB 0.45±0.03 J. Compared to the previous results tensile strength is higher whereas Young's modulus lower and energy at break reported in here is lower. Similarly, tensile test the results obtained by Wei et.al [39], tensile strength was 14.8 and 15.0 MPa for HV content 32.4-34.1% respectively. Whereas Young's modulus was 289-299 MPa for PHBV reported by Wei et.al which is higher than reported here. The increased tensile strength and decreased modulus of the PHBV-L is related to the higher HV content and higher M_w [39]. The M_w of PHBV were between 7.7×10⁵ to 7.9×10⁵ g/mol in the results obtained by [39]. However, the M_w was much lower (4.0×10⁵ g/mol) for the PHBV 22% HV [35]. The M_w of

PHBV-L is 8.9×10^5 g/mol, so it can be inferred that higher molecular weight of material was responsible for the increase in the tensile strength of material [32].

Table 4.4: Tensile Properties of crosslinked PHBV-L at 155 °C and 175 °C

| Polymer type | Temperature (°C) | Reaction Time | Tensile stress (MPa) | Tensile Modulus (MPa) | Energy at Break (J) |
|------------------------|------------------|---------------|----------------------|-----------------------|---------------------|
| PHBV-L ₀ | 155 | 3min | 14.8±0.9 | 167.8±6.7 | 0.2±0.1 |
| PHBV-L _{0.25} | | 3min | 13.5±0.7 | 150.1±4.1 | 0.2±0.0 |
| PHBV-L _{0.5} | | 3min | 14.7±0.5 | 138.8±3.5 | 0.3±0.0 |
| PHBV-L ₁ | | 3min | 15.2±0.6 | 122.5±6.3 | 0.5±0.1 |
| PHBV-L ₀ | 175 | 3min | 12.1±0.5 | 78.2±5.5 | 0.9±0.1 |
| PHBV-L _{0.25} | | 3min | 11.6±0.5 | 71.3±3.1 | 1.2±0.2 |
| PHBV-L _{0.5} | | 3min | 9.7±0.5 | 67.3±1.7 | 1.1±0.1 |
| PHBV-L ₁ | | 3min | 8.6±0.6 | 65.9±2.2 | 1.5±0.6 |

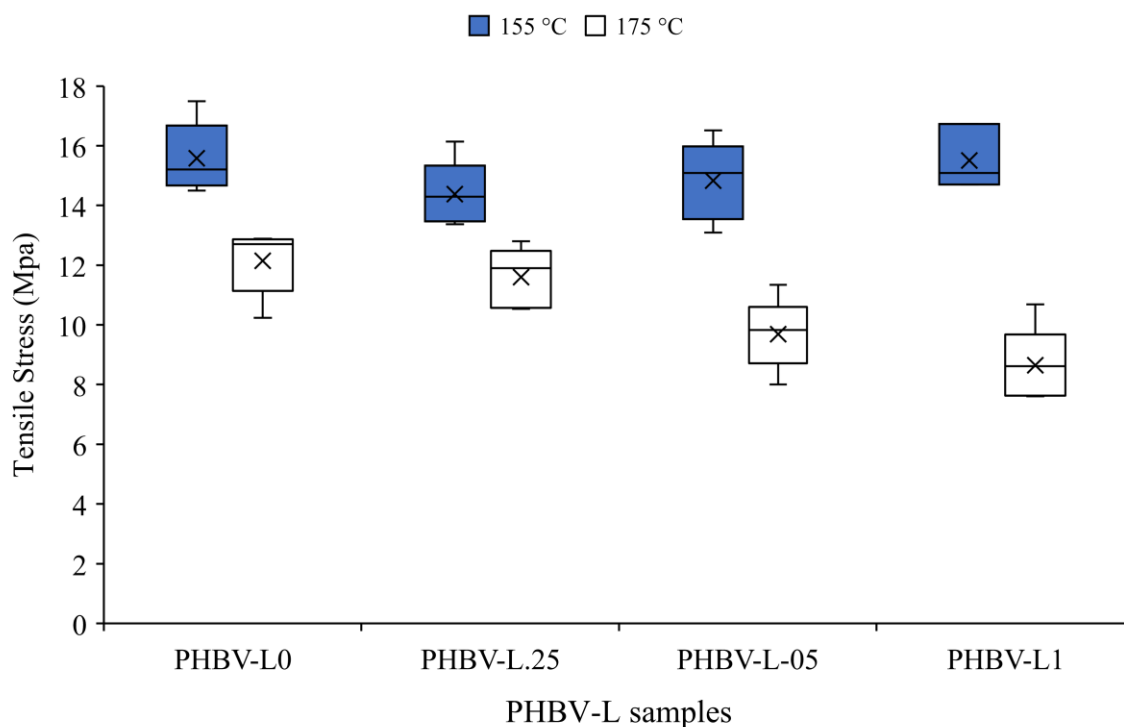


Figure 4.10: Tensile stress (MPa) for PHBV-L processed at 155 °C and 175 °C

Energy at break (EAB) increased with increase in the amount of DCP added to PHBV-L at 155 °C. No significant change in EAB was observed for PHBV-L_{0-0.5}, however EAB increased significantly from 0.21-0.51 J by 55% for PHBV-L_{1.0} compared to PHBV-L₀. However, increase in processing temperature to 175 °C decreased the tensile strength and modulus of PHBV-L while increasing the energy at break significantly. Compared to 0-0.25 % DCP loading, the effect of DCP addition in terms of tensile properties were more evident for 0.5-1% DCP for PHBV-L processed at 175 °C. Therefore, toughness of the polymer was highly increased with increase in processing temperature.

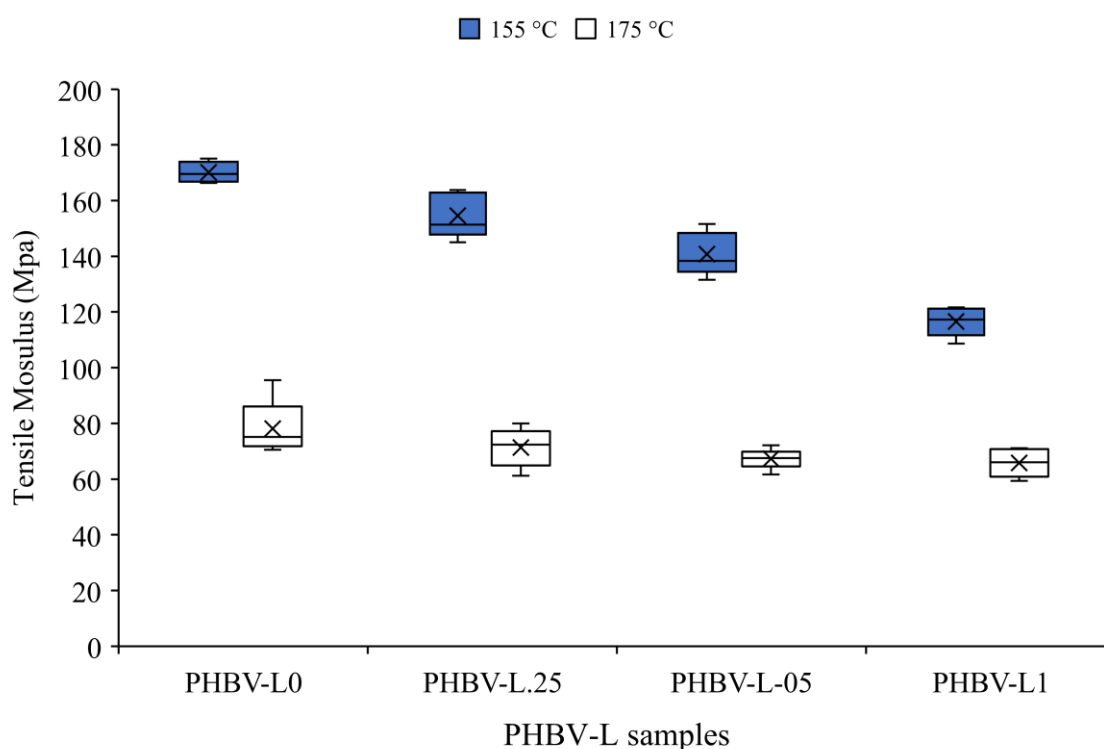


Figure 4.11: Tensile Modulus (MPa) of PHBV-L processed at 155 °C and 175 °C

However, with the comparable HV content (21%) for PHBV-L compared to commercial PHBV (22%) studied by Wei et al. had a lower Young's modulus [35]. However, in the subsequent experiment on the laboratory extracted PHBV by Wei et.al, the values of Young's modulus were relatively closer suggesting that the commercial PHBV and lab extracted PHBV have different properties [39].

4.6. Melt viscosity Molecular weight

Complex viscosity (η^*) of PHBV-C₀ at processing temperature 175 °C was 169 Pa. s. at frequency 0.01 Hz (Figure 4.12). η^* decreased with an increase in frequency and shear thinning was observed which is a very common behavior of polymers. This is due to breaking of molecular chains of polymers due to high temperature exposure during testing [27][28][31]. Crosslinking with DCP 0.25-1% shows gradual increase in the η^* from 210 Pa. s. to 792 Pa. s at 0.01 Hz (Figure 4.13). The increase in η^* is due to the molecular chain entanglement due to the crosslinking. Shear thinning was higher for crosslinked PHBV-C_{0.25-1} [62][62]. The Mw of of crosslinked-PHBV-C was not obtained because it was gel and not soluble.

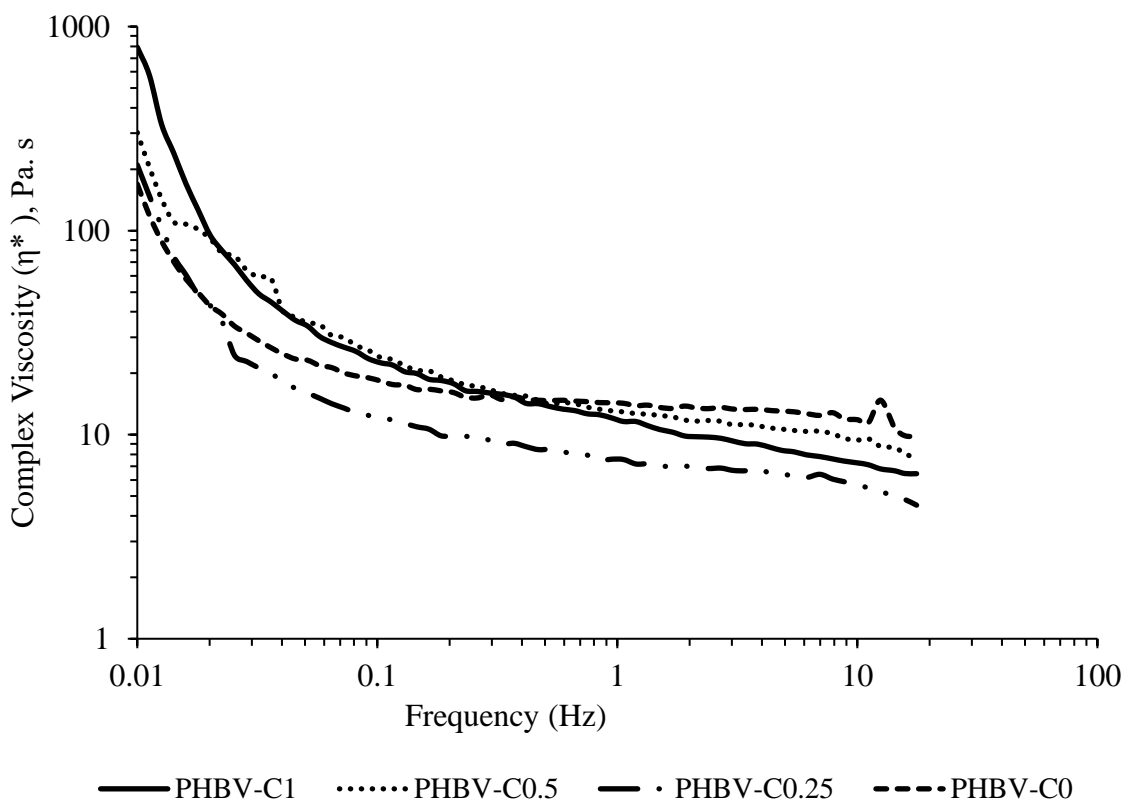


Figure 4.12: Melt flow curve complex viscosity versus frequency of PHBV-C at 175°C

Processing temperature of PHBV-C was set higher at 175 °C where PHBV-L was tested at 175 °C and 155 °C to see the effect of high and low processing temperatures. η^* of PHBV-L-155 °C is very high at 0.01 Hz. η^* of PHBV-L₀ was 42030 Pa. s at 155 °C which decreased to 1880 Pa. s for PHBV-L₁(Figure: 4.9, Table: 4.5) upon crosslinking. Since, PHBV-L has lower melting point i.e., 150 °C, η^* at 175 °C is still higher than that of PHBV-C at same temperature. However, crosslinking increased the viscosity of PHBV-C to a great extent, PHBV-L having higher HV content and higher molecular weight had higher melt strength. Complex viscosity (η^*) was seen decreasing for PHBV-L_{0.25-1} at 155 °C which has also been observed for PHBV-C. η^* of PHBV-L₀ was 42,030 Pa. s at 155 °C at 0.01 Hz.

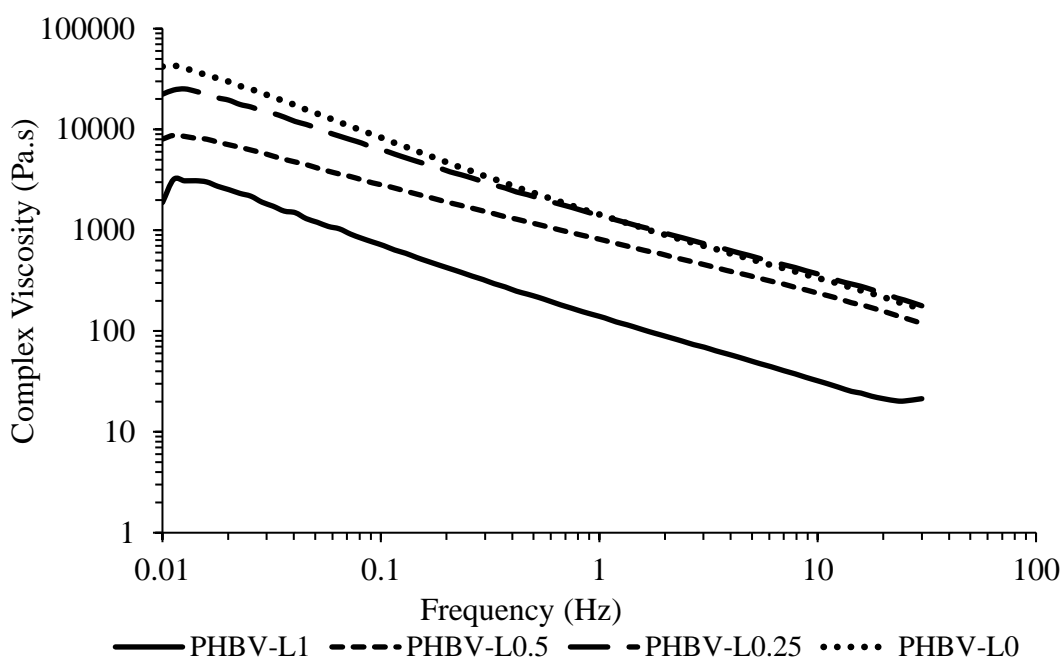


Figure 4.13: Melt flow curve of complex viscosity (η^*) versus frequency for crosslinked PHBV-L processed at 155 °C

PHBV processed at 175 °C exhibited very low melt viscosity (Figure 4.14, Table: 4.5). However, η^* for PHBV-C (Figure 4.12) and PHBV-L (Figure: 4.14) processed at 175 °C were comparable at low shear rate. Addition of DCP 0.25- 0.5 % increased melt viscosity of PHBV-L slightly. However, addition of 1% DCP increased the melt viscosity of PHBV-L and PHBV-C by more than ten times but the increase was higher for PHBV-L. Comparing the η^*

of PHBV-L at 155 °C and 175 °C the initial viscosity at 155 °C is very high however, addition of DCP lowered the η^* and the phenomenon was opposite for PHBV-L-175 °C. η^* was however in the same range at 1% DCP loading for PHBV at both high and low processing temperatures. However, further extrusion parameters like residence time and screw speed has not been taken into consideration here [63].

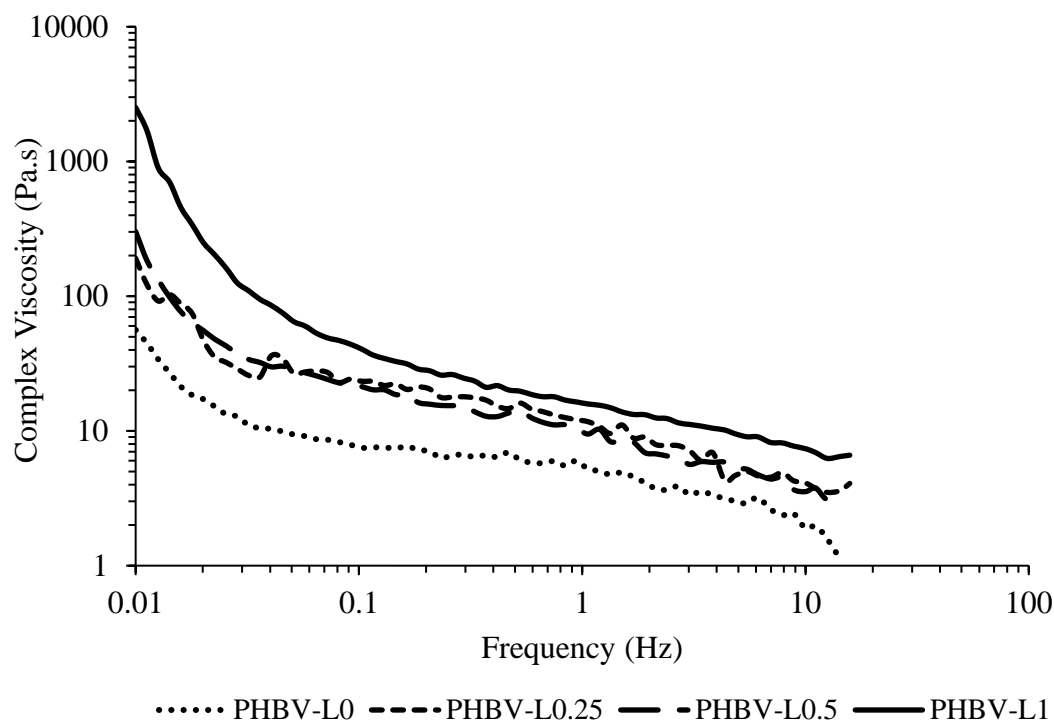


Figure 4.14: Melt flow curve of complex viscosity versus shear rate for crosslinked PHBV-L processed at 175 °C

Weight average molecular weight (M_w) of PHBV is very important as it directly affects its various properties thermal, mechanical and melt flow properties. Crosslinking is done to increase the chain entanglement in the polymer i.e., increasing amorphous content and thus increase melt viscosity in the polymer. The M_w of PHBV-L was 8.90×10^5 g/mol. M_w decreased from 8.33 to 4.57×10^5 g/mol (Table: 4.5) when the polymer was extruded at 155 °C for 3 mins. The M_w decreased by 48.6% of the original weight after injection molding which agrees with the % decrease M_w observed by Bugnicourt et al. at similar temperature range [64]. Addition of DCP 0.25-0.5 % further decreased the M_w to 3.28×10^5 g/mol, however, there was a slight increase in M_w 3.71×10^5 g/mol for PHBV-L₁ and PDI also increased as to

2.10. This indicated the continuous degradation of polymer molecule due to thermal degradation and subsequent recombination due to the crosslinking effect of DCP thus broadening the molecular weight distribution [63]. Slight decrease in molecular weight is expected due to the chain scission [32] but the decrease (Table 4.5) nearly halved. In many instances, the negative effect of thermal degradation due to processing was offset by the crosslinking that increases the M_w and decreasing the reaction time to 3-5 mins [49] but, in this case, the effect of 0.25 and 0.5% of DCP was insignificant in crosslinking PHBV-L. Also, considering the minimal gel fraction (1%) obtained from extruded PHBV-L₁ and information of M_w and η^* in Table 4.5, crosslinking did not occur as extensively as that reported in the literature [33][49].

Table 4.5: Molecular weight of and complex viscosity of crosslinked PHBV-L-155 and 175 °C

| | Temperature (°C) | M_n ($\times 10^5$ g/mol) | M_w ($\times 10^5$ g/mol) | PDI (M_w/M_n) | η^* (Pa.s) at 0.01 Hz |
|---------------------------|---------------------|---------------------------------|---------------------------------|----------------------|-------------------------------|
| PHBV-L | | 8.33±0.28 | 8.90±0.33 | 1.07 | NA |
| PHBV-L _{0-ext} | 155 | 4.24±0.08 | 4.57±0.08 | 1.08 | 42030 |
| PHBV-L _{0.25sol} | | 2.55±1.12 | 3.39±0.14 | 1.33 | 22360 |
| PHBV-L _{0.5sol} | | 2.32±0.08 | 3.28±0.11 | 1.42 | 8020 |
| PHBV-L _{1sol} | | 1.79±1.08 | 3.71±0.15 | 2.10 | 1880 |
| PHBV-L _{0-ext} | 175 | 3.57±0.10 | 4.00±0.30 | 1.12 | 56 |
| PHBV-L _{0.25sol} | | 2.15±0.69 | 2.86±0.56 | 1.33 | 191 |
| PHBV-L _{0.5sol} | | 1.91±0.19 | 2.91±0.61 | 1.52 | 302 |
| PHBV-L _{1sol} | | 2.04±0.93 | 3.72±0.49 | 1.82 | 2519 |

Higher molecular weight material thus high melt viscosity is preferred for sheet extrusion since polymer degrades rapidly under screw rotation accompanied by high temperature and residence time [65] [26]. PLA with molecular weight of 100×10^3 g/mol was used for injection molding and 300×10^3 g/mol was preferred sheet extrusion [63]. Therefore, addition of DCP can be beneficial for injection molding purposes where there is less susceptibility of long exposure to temperature.

4.8. FTIR spectral analysis:

Chemical structure of PHBV-L and PHBV-C and their crystallinity was assessed with the FTIR spectral analysis (Figures 4.15 and 4.16 and Table 4.6). Absorbance bands corresponds to the specific chemical bonds. The absorbance bands at around 3290 cm^{-1} and 2900 cm^{-1} were assigned to OH and C-H group, respectively. As there are three specific types of bonding in PHBV-L, C-O-C, C-C and C=O, intensity of absorbance at 1226 cm^{-1} corresponds to C-O-C bond, whereas 980 cm^{-1} corresponds to C-C bond related to the crystalline phase of PHBV-L and PHBV-C [37] [35]. These absorption bands are sensitive to the crystalline region in the PHBV. The absorbance band at 1720 cm^{-1} corresponds to C=O stretching in the PHBV-L, this is sensitive to the crystalline region in the polymer however, small shoulder appearing at 1740 cm^{-1} is due to the amorphous region and is insensitive to crystalline structure. Likewise, bands at 1226 cm^{-1} and 1455 cm^{-1} represents C-O bonding. In the FTIR spectra absorbance bands corresponding to 1226 cm^{-1} is sensitive to crystallization and 1455 cm^{-1} is insensitive to crystallization. The ratio of area under the bands $1720/1740$ ($I_{\text{C=O}}$) and $1228/1455$ ($I_{\text{C-O}}$) were calculated to find the crystallinity index: Table 4.7 [32].

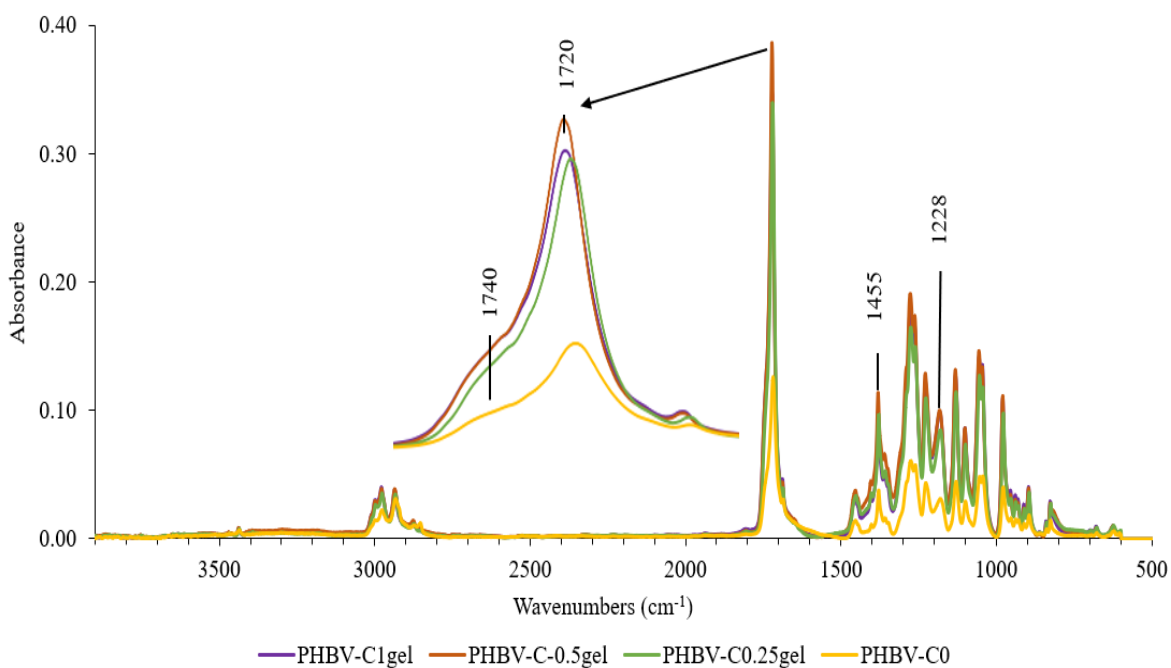


Figure 4.15: FTIR spectra of crosslinked PHBV-C processed at $175\text{ }^{\circ}\text{C}$

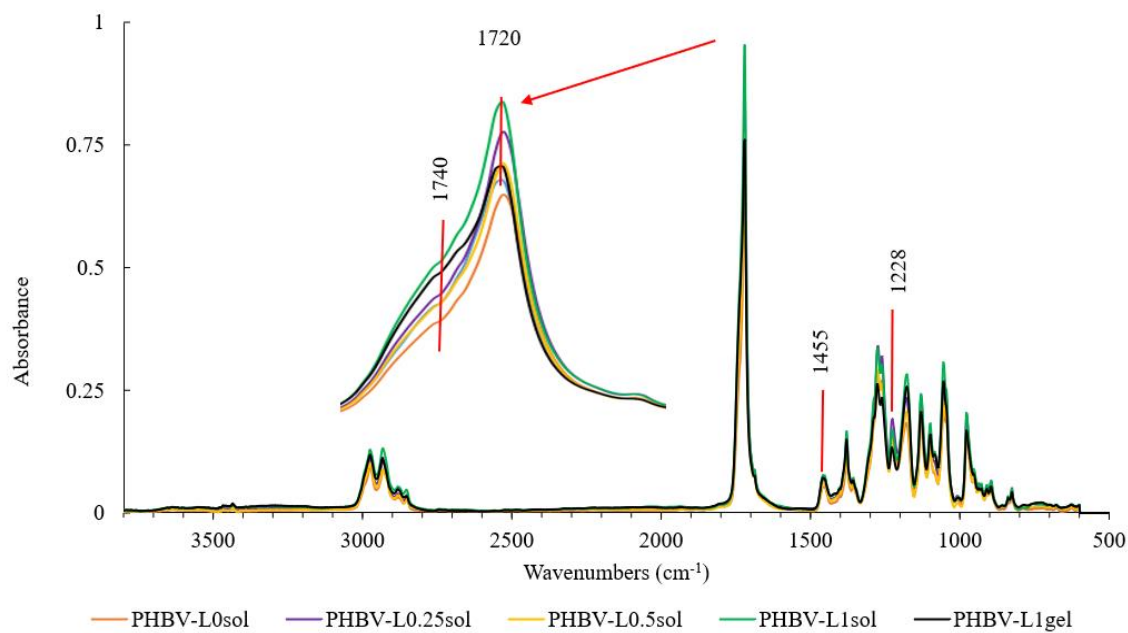


Figure 4.16: FTIR spectra of crosslinked PHBV-L processed at 155 °C

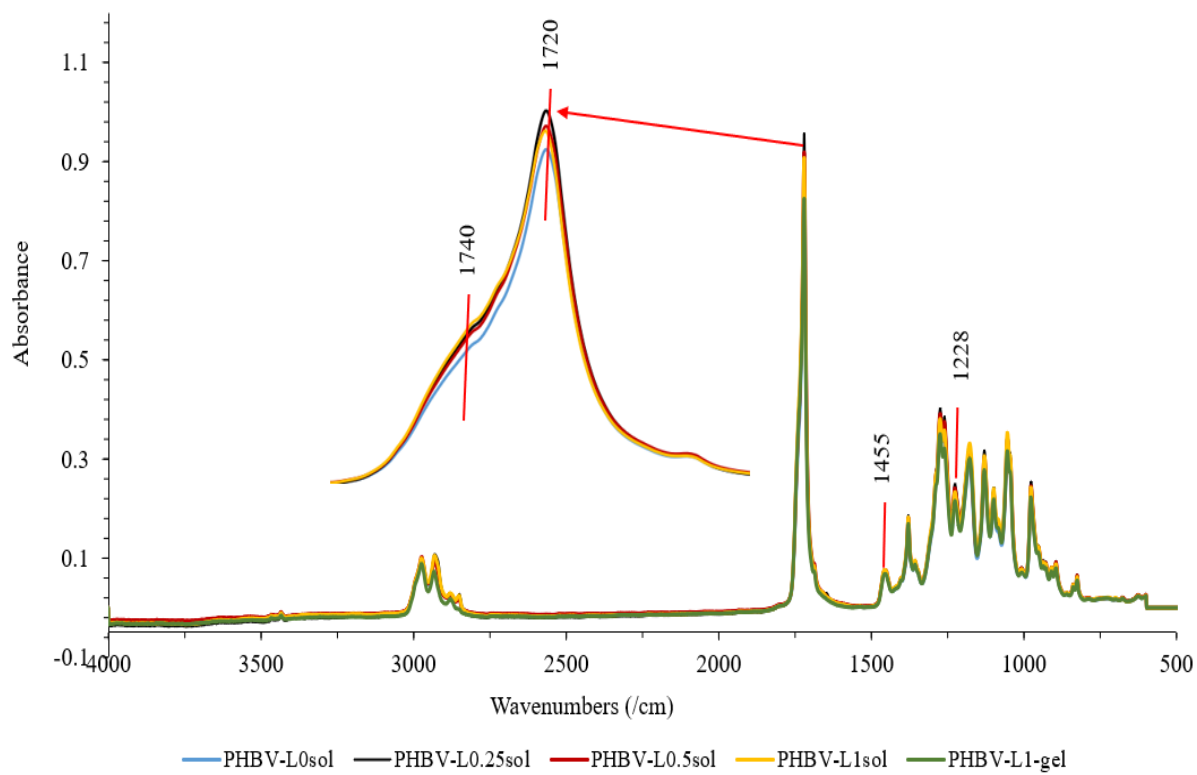


Figure 4.17: FTIR spectra of crosslinked PHBV-L processed at 175 °C

Absorbance band at both 1720 cm^{-1} and 1740 cm^{-1} intensified for PHBV-C₀₋₁ (Figure: 4.14, Table 4.6). The increase in intensity of absorption bands was relatively more at 1740 cm^{-1} compared to 1720 cm^{-1} due to crosslinking [32][35][36]. Crystallinity index $I_{C=O}$, decreased from 11.34 to 3.24 for PHBV-C_{0.25gel} (Table 4.7) which remained almost similar for PHBV-C_{0.5gel} and PHBV-C_{1gel}. However, in crystalline index for C-O stretch, I_{C-O} , increased for PHBV-C_{0-1gel}. Wei et al noticed a noticeable decrease in crystallinity index $I_{C=O}$ and I_{C-O} of PHBV due to crosslinking with 0.5% DCP [36]. Similarly, $I_{C=O}$ index for PHB was found to decrease from 1.30 to 0.39 for increasing DCP content 0-1% [32].

Table 4.6: Crystallinity index of crosslinked PHBV-L and PHBV-C by FTIR analysis

| PHBV-C-175 °C | | | PHBV-L-155 °C | | | PHBV-L-175 °C | |
|---------------------------|-----------|-----------|---------------------------|-----------|-----------|---------------|-----------|
| Sample | $I_{C=O}$ | I_{C-O} | Sample | $I_{C=O}$ | I_{C-O} | $I_{C=O}$ | I_{C-O} |
| PHBV-C ₀ | 11.34 | 1.59 | PHBV-L _{0sol} | 2.68 | 0.75 | 2.37 | 0.82 |
| PHBV-C _{0.25gel} | 3.24 | 2.13 | PHBV-L _{0.25sol} | 2.07 | 0.87 | 2.17 | 0.81 |
| PHBV-L _{0.5gel} | 3.14 | 2.18 | PHBV-L _{0.5sol} | 1.91 | 0.77 | 2.30 | 0.73 |
| PHBV-L _{1gel} | 3.46 | 2.01 | PHBV-L _{1sol} | 1.52 | 0.38 | 2.01 | 0.72 |
| - | - | - | PHBV-L _{1 gel} | 1.31 | 0.44 | 1.63 | 0.57 |

The higher the amount of amorphous content in the polymer, the intensity of absorption band at Compared to PHBV-C, crystallinity index given by FTIR analysis also shows that crystallinity of PHBV-L is lower (Table 4.6). The increase in amount of added to DCP 0-1% in PHBV-L, there was decrease in the crystallinity index of the polymer basically in the determined form the intensity of C=O stretch between 1650 cm^{-1} and 1850 cm^{-1} [32]. It can be observed from the crystallinity index of C=O and C-O of the polymer Table 4.7. The crystalline index $I_{C=O}$ of the pure PHBV-L processed at 155 °C was 2.68 which decreased to 1.31 for PHBV-L_{1gel}. I_{C-O} also decreased from 0.75 to 0.38 for PHBV-L_{0-1sol}. Crystallinity index of PHBV-L processed at 175 °C (figure 4.17 and Table 4.6) was comparable to the PHBV-L processed at 155 °C and showed similar trend, crystallinity index related to amorphous phase decreased due to crosslinking [32]. However, bands related to crystalline phases appeared intense for the crosslinked PHBV-L due to which crystallinity did not decreased very significantly [35]. Also, in the DSC thermograms, the crystallinity of the PHBV-L₁ was relatively lower than that of PHBV-L_{0.0.5}, which corresponds to the results obtained from FTIR.

5. Conclusions

Properties of PHBV are found very encouraging for the industrial processing because of their low crystallinity, good mechanical properties. Increasing HV content in the polymer enhances the properties and using the cheaper carbon sources using MMC will reduce the cost of production. This points towards possibility of PHBV to upscale the production and industrial processing. To be acceptable for commercial production and application, higher melt strength, thermal stability along with mechanical properties are very important. Using small amount of DCP as crosslinking agent for defined time can alter these properties.

Therefore, production of PHBV was done in pilot scale, was extracted, and purified successfully from biomass derived from MMC fed fermented dairy manure having M_w , 8.90×10^5 g/mol and 21% HV content. PHBV-L was successfully crosslinked with DCP (0-1%) and the properties were modified. Thermal and mechanical properties of crosslinked PHBV-L changed noticeably for 0.5-1% DCP. PHBV-L was found to be very viscous above its melting temperature at 155 °C and decreased to processable limit by DCP addition. Processing at 175 °C lowered the melt viscosity significantly however addition of DCP improved the melt strength. Though, extrusion of polymer reduces the M_w of PHBV-L nearly by half of the extracted material and melt strength decreased, thermal stability and mechanical properties were not compromised for PHBV-L₁ due to DCP addition. PHBV-L₁ having lower melt strength had better thermal stability as the complex viscosity did not decrease with increase in temperature due to DCP addition. Therefore, processing temperature be increased to lower the viscosity if higher melt flow is required. Processing PHBV-L at 175 °C, improved the toughness of the polymer and melt viscosity also improved. Therefore, flexibility and toughness can be imparted in polymer without adverse effect on the strength of at DCP 0.5-1% at higher temperature was proved to be more useful for controlling melt viscosity and flexibility of polymer.

Therefore, for crosslinking of PHBV extracted from MMC, DCP is useful to modify its properties that are essential during the blow film molding and extrusion applications.

6. Limitations and Future Recommendations

Crosslinking by DCP with shorter reaction time 1-2 min reaction might decrease the effect of thermal degradation, that has not been covered in this research. It would be important produce blown film and foaming products by crosslinking with DCP and characterize to understand the feasibility of commercial scale production. Though PHBV is totally biodegradable, structural modification of PHBV occurs during crosslinking this might affect the process of biodegradation in polymer and the unreacted DCP during the process may interfere with the process as well.

References:

- [1] J. M. Lagarón, A. López-Rubio, and M. José Fabra, "Bio-based packaging," *J. Appl. Polym. Sci.*, vol. 133, no. 2, 2016, doi: 10.1002/app.42971.
- [2] M. Selvamurugan Muthusamy and S. Pramasivam, "Bioplastics – An Eco-friendly Alternative to Petrochemical Plastics," *Curr. World Environ.*, vol. 14, no. 1, pp. 49–59, 2019, doi: 10.12944/cwe.14.1.07.
- [3] R. P. Babu, K. O'Connor, and R. Seeram, "Current progress on bio-based polymers and their future trends," *Prog. Biomater.*, vol. 2, no. 1, p. 8, 2013, doi: 10.1186/2194-0517-2-8.
- [4] A. Luc and P. Eric, "Environmental Silicate Nano-Biocomposites," *Green Energy Technol. Springer*, 2012.
- [5] K. Van De Velde and P. Kiekens, "Biopolymers: Overview of several properties and consequences on their applications," *Polym. Test.*, vol. 21, no. 4, pp. 433–442, 2002, doi: 10.1016/S0142-9418(01)00107-6.
- [6] M. Avella, E. Martuscelli, and M. Raimo, "Properties of blends and composites based on poly(3-hydroxy)butyrate (PHB) and poly(3-hydroxybutyrate-hydroxyvalerate) (PHBV) copolymers," *J. Mater. Sci.*, vol. 35, no. 3, pp. 523–545, 2000, doi: 10.1023/A:1004740522751.
- [7] V. Ivanov, V. Stabnikov, Z. Ahmed, S. Dobrenko, and A. Saliuk, "Production and applications of crude polyhydroxyalkanoate-containing bioplastic from the organic fraction of municipal solid waste," *Int. J. Environ. Sci. Technol.*, vol. 12, no. 2, pp. 725–738, 2014, doi: 10.1007/s13762-014-0505-3.
- [8] J. Il Choi and S. Y. Lee, "Process analysis and economic evaluation for poly(3-hydroxybutyrate) production by fermentation," *Bioprocess Eng.*, vol. 17, no. 6, pp. 335–342, 1997, doi: 10.1007/s004490050394.

- [9] J. M. B. T. Cavaleiro *et al.*, “Effect of cultivation parameters on the production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) and poly(3-hydroxybutyrate-4-hydroxybutyrate-3-hydroxyvalerate) by *Cupriavidus necator* using waste glycerol,” *Bioresour. Technol.*, vol. 111, pp. 391–397, 2012, doi: 10.1016/j.biortech.2012.01.176.
- [10] Y. Wang *et al.*, “Biosynthesis and thermal properties of PHBV produced from levulinic acid by *Ralstonia eutropha*,” *PLoS One*, vol. 8, no. 4, pp. e60318–e60318, Apr. 2013, doi: 10.1371/journal.pone.0060318.
- [11] C. Kourmentza *et al.*, “Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production,” *Bioengineering*, vol. 4, no. 2, pp. 1–43, 2017, doi: 10.3390/bioengineering4020055.
- [12] I. Taniguchi, K. Kagotani, and Y. Kimura, “Microbial production of poly(hydroxyalkanoate)s from waste edible oils,” *Green Chem.*, vol. 5, no. 5, pp. 545–548, 2003, doi: 10.1039/b304800b.
- [13] P. H. Yu, H. Chua, and P. A. L. Huang, “Conversion of food industrial wastes into bioplastics with municipal activated sludge,” *Macromol. Symp.*, vol. 148, pp. 415–424, 1999, doi: 10.1002/masy.19991480131.
- [14] B. Colombo, T. P. Sciarria, M. Reis, B. Scaglia, and F. Adani, “Polyhydroxyalkanoates (PHAs) production from fermented cheese whey by using a mixed microbial culture,” *Bioresour. Technol.*, vol. 218, no. 2016, pp. 692–699, 2016, doi: 10.1016/j.biortech.2016.07.024.
- [15] W. Jaruwattanayon, *Fabrication and Modification of Compression Molding Poly (hydroxybutyrate-co-valerate) Thin Sheet*. Michigan State University. Packaging, 2014.
- [16] Z. Chen, L. Huang, Q. Wen, and Z. Guo, “Efficient polyhydroxyalkanoate (PHA) accumulation by a new continuous feeding mode in three-stage mixed microbial culture (MMC) PHA production process,” *J. Biotechnol.*, vol. 209, pp. 68–75, 2015, doi: 10.1016/j.jbiotec.2015.06.382.

- [17] M. S. Wong, T. B. Causey, N. Mantzaris, G. N. Bennett, and K. Y. San, "Engineering poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer composition in *E. coli*," *Biotechnol. Bioeng.*, vol. 99, no. 4, pp. 919–928, 2008, doi: 10.1002/bit.21641.
- [18] L. Shang, Q. Fei, Y. H. Zhang, X. Z. Wang, D.-D. Fan, and H. N. Chang, "Thermal properties and biodegradability studies of poly (3-hydroxybutyrate-co-3-hydroxyvalerate)," *J. Polym. Environ.*, vol. 20, no. 1, pp. 23–28, 2012.
- [19] A. Anjum, M. Zuber, K. M. Zia, A. Noreen, M. N. Anjum, and S. Tabasum, "Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: A review of recent advancements.," *Int. J. Biol. Macromol.*, vol. 89, pp. 161–174, Aug. 2016, doi: 10.1016/j.ijbiomac.2016.04.069.
- [20] L. Wei, *Characterization and modification of selected bioplastics*. University of Idaho, 2015.
- [21] W. Zhu, L. Qian, and J. G. Shen, "A dependence of the K factor in the nuclear shadowing region," *Phys. Rev. D*, vol. 44, no. 9, pp. 2762–2767, 1991, doi: 10.1103/PhysRevD.44.2762.
- [22] A. L. Rivera-Briso and Á. Serrano-Aroca, "Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate): Enhancement strategies for advanced applications," *Polymers (Basel)*, vol. 10, no. 7, pp. 1–28, 2018, doi: 10.3390/polym10070732.
- [23] W. Kai, Y. He, and Y. Inoue, "Fast crystallization of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with talc and boron nitride as nucleating agents," *Polym. Int.*, vol. 54, no. 5, pp. 780–789, 2005, doi: 10.1002/pi.1758.
- [24] B. A. Morris, "5 - Rheology of Polymer Melts," in *Plastics Design Library*, B. A. B. T.-T. S. and T. of F. P. Morris, Ed. Oxford: William Andrew Publishing, 2017, pp. 121–147.

- [25] J. Abraham, T. Sharika, R. K. Mishra, and S. Thomas, "14 - Rheological characteristics of nanomaterials and nanocomposites," in *Woodhead Publishing Series in Composites Science and Engineering*, R. K. Mishra, S. Thomas, and N. B. T.-M. and N. F. C. (MFCs and Nfc. from P. B. Kalarikkal, Eds. Woodhead Publishing, 2017, pp. 327–350.
- [26] J. Vera-Sorroche *et al.*, "The effect of melt viscosity on thermal efficiency for single screw extrusion of HDPE," *Chem. Eng. Res. Des.*, vol. 92, no. 11, pp. 2404–2412, 2014, doi: 10.1016/j.cherd.2013.12.025.
- [27] S. J. Modi, K. Cornish, K. Koelling, and Y. Vodovotz, "Fabrication and improved performance of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) for packaging by addition of high molecular weight natural rubber," *J. Appl. Polym. Sci.*, vol. 133, no. 37, Oct. 2016, doi: 10.1002/app.43937.
- [28] D. Szegda, S. Duangphet, J. Song, and K. Tarverdi, "Extrusion foaming of PHBV," *J. Cell. Plast.*, vol. 50, no. 2, pp. 145–162, 2014, doi: 10.1177/0021955X13505249.
- [29] D. H. S. Ramkumar and M. Bhattacharya, "Steady shear and dynamic properties of biodegradable polyesters," *Polym. Eng. Sci.*, vol. 38, no. 9, pp. 1426–1435, 1998, doi: 10.1002/pen.10313.
- [30] M. Cunha, B. Fernandes, J. A. Covas, A. A. Vicente, and L. Hilliou, "Film blowing of PHBV blends and PHBV-based multilayers for the production of biodegradable packages," *J. Appl. Polym. Sci.*, vol. 133, no. 2, p. n/a, 2016, doi: 10.1002/app.42165.
- [31] S. Duangphet, D. Szegda, J. Song, and K. Tarverdi, "The Effect of Chain Extender on Poly(3-hydroxybutyrate-co-3-hydroxyvalerate): Thermal Degradation, Crystallization, and Rheological Behaviours," *J. Polym. Environ.*, vol. 22, no. 1, pp. 1–8, 2014, doi: 10.1007/s10924-012-0568-5.
- [32] L. Wei and A. G. McDonald, "Peroxide induced cross-linking by reactive melt processing of two biopolyesters: Poly(3-hydroxybutyrate) and poly(l -lactic acid) to improve their melting processability," *J. Appl. Polym. Sci.*, vol. 132, no. 13, pp. 1–15, 2015, doi: 10.1002/app.41724.

- [33] B. Fei *et al.*, “Crosslinking of poly[(3-hydroxybutyrate)-co-(3-hydroxyvalerate)] using dicumyl peroxide as initiator,” *Polym. Int.*, vol. 53, pp. 937–943, Jul. 2004, doi: 10.1002/pi.1477.
- [34] Y. Lv, H. Zhu, M. fang An, H. jun Xu, L. Zhang, and Z. bao Wang, “Multiple endothermic peaks resulted from different crystal structures in an isomorphous copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate),” *Chinese J. Polym. Sci. (English Ed.)*, vol. 34, no. 12, pp. 1510–1522, 2016, doi: 10.1007/s10118-016-1866-5.
- [35] L. Wei, N. M. Stark, and A. G. McDonald, “Interfacial improvements in biocomposites based on poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) bioplastics reinforced and grafted with α -cellulose fibers,” *Green Chem.*, vol. 17, no. 10, pp. 4800–4814, 2015, doi: 10.1039/c5gc01568e.
- [36] S. Luo, J. Cao, and A. G. McDonald, “Interfacial Improvements in a Green Biopolymer Alloy of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and Lignin via in Situ Reactive Extrusion,” *ACS Sustain. Chem. Eng.*, vol. 4, no. 6, pp. 3465–3476, 2016, doi: 10.1021/acssuschemeng.6b00495.
- [37] S. Hu, A. G. Mcdonald, and E. R. Coats, “Characterization of Polyhydroxybutyrate Biosynthesized from Crude Glycerol Waste Using Mixed Microbial Consortia,” pp. 1314–1321, 2013, doi: 10.1002/app.38820.
- [38] A. K. Singh, J. K. Srivastava, A. K. Chandel, L. Sharma, N. Mallick, and S. P. Singh, “Biomedical applications of microbially engineered polyhydroxyalkanoates: an insight into recent advances, bottlenecks, and solutions,” *Appl. Microbiol. Biotechnol.*, vol. 103, no. 5, pp. 2007–2032, 2019, doi: 10.1007/s00253-018-09604-y.
- [39] L. Wei, N. M. Guho, E. R. Coats, and A. G. McDonald, “Characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) biosynthesized by mixed microbial consortia fed fermented dairy manure,” *J. Appl. Polym. Sci.*, vol. 131, no. 11, 2014, doi: 10.1002/app.40333.

- [40] Q. Liu, T. Shyr, and C. Tung, "Particular thermal properties of poly (3-hydroxybutyrate- co-3-hydroxyvalerate) oligomers," 2012, doi: 10.1007/s10965-011-9756-6.
- [41] M. G. Silva, H. Vargas, L. H. Poley, R. S. Rodriguez, and G. B. Baptista, "Structural Impact of Hydroxyvalerate in Polyhydroxyalkanoates (PHA scl) Dense Film Monitored by XPS and Photothermal Methods," vol. 16, no. 4, pp. 790–795, 2005.
- [42] I. Zembouai, S. Bruzard, M. Kaci, A. Benhamida, Y. M. Corre, and Y. Grohens, "Mechanical Recycling of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate)/Polylactide Based Blends," *J. Polym. Environ.*, vol. 22, no. 4, pp. 449–459, 2014, doi: 10.1007/s10924-014-0684-5.
- [43] G. Q. Chen and Q. Wu, "Microbial production and applications of chiral hydroxyalkanoates," *Appl. Microbiol. Biotechnol.*, vol. 67, no. 5, pp. 592–599, 2005, doi: 10.1007/s00253-005-1917-2.
- [44] Y. Wang, J. Yin, and G. Q. Chen, "Polyhydroxyalkanoates, challenges and opportunities," *Curr. Opin. Biotechnol.*, vol. 30, pp. 59–65, 2014, doi: 10.1016/j.copbio.2014.06.001.
- [45] S. Serafim, C. Oliveira, M. Eiroa, and M. G. E. Albuquerque, "Recent Advances in Polyhydroxyalkanoate Production by Mixed Aerobic Cultures : From the Substrate to the Final Product," pp. 885–906, 2006, doi: 10.1002/mabi.200600112.
- [46] G. X. Chen, G. J. Hao, T. Y. Guo, M. D. Song, and B. H. Zhang, "Structure and mechanical properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/clay nanocomposites," *J. Mater. Sci. Lett.*, vol. 21, no. 20, pp. 1587–1589, 2002, doi: 10.1023/A:1020309330371.
- [47] S. Wang *et al.*, "Characteristics and biodegradation properties of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/organophilic montmorillonite (PHBV/OMMT) nanocomposite," *Polym. Degrad. Stab. - POLYM Degrad STABIL*, vol. 87, pp. 69–76, Jan. 2005, doi: 10.1016/j.polymdegradstab.2004.07.008.

- [48] Q. Guan, "Fabrication and Characterization of PLA , PHBV and Chitin Nanowhisker Blends , Composites and Foams for High Strength Structural Applications," 2013.
- [49] L. Wei, A. G. McDonald, and N. M. Stark, "Grafting of Bacterial Polyhydroxybutyrate (PHB) onto Cellulose via In Situ Reactive Extrusion with Dicumyl Peroxide," *Biomacromolecules*, vol. 16, no. 3, pp. 1040–1049, 2015, doi: 10.1021/acs.biomac.5b00049.
- [50] H. Zhao, Z. Cui, X. Sun, L. S. Turng, and X. Peng, "Morphology and properties of injection molded solid and microcellular polylactic acid/polyhydroxybutyrate-valerate (PLA/PHBV) blends," *Ind. Eng. Chem. Res.*, vol. 52, no. 7, pp. 2569–2581, 2013, doi: 10.1021/ie301573y.
- [51] T. Gerard, T. Budtova, A. Podshivalov, and S. Bronnikov, "Polylactide/poly(hydroxybutyrate-co-hydroxyvalerate) blends: Morphology and mechanical properties," *Express Polym. Lett.*, vol. 8, no. 8, pp. 609–617, 2014, doi: 10.3144/expresspolymlett.2014.64.
- [52] J. F. Campuzano and I. D. López, "Study of the effect of dicumyl peroxide on morphological and physical properties of foam injection molded poly(Lactic acid)/poly(butylene succinate) blends," *Express Polym. Lett.*, vol. 14, no. 7, pp. 673–684, 2020, doi: 10.3144/expresspolymlett.2020.55.
- [53] Z. Feng *et al.*, "Preparation of Enhanced Poly(butylene succinate) Foams," *Polym. Eng. Sci.*, vol. 56, no. 11, pp. 1275–1282, 2016, doi: 10.1002/pen.24362.
- [54] R. Wang, S. Wang, Y. Zhang, C. Wan, and P. Ma, "Toughening modification of PLLA/PBS blends via in situ compatibilization," *Polym. Eng. Sci.*, vol. 49, no. 1, pp. 26–33, 2009, doi: 10.1002/pen.21210.
- [55] A. J. Hanson, N. M. Guho, A. J. Paszczynski, and E. R. Coats, "Community proteomics provides functional insight into polyhydroxyalkanoate production by a mixed microbial culture cultivated on fermented dairy manure," *Appl. Microbiol. Biotechnol.*, vol. 100, no. 18, pp. 7957–7976, 2016, doi: 10.1007/s00253-016-7576-7.

- [56] E. R. Coats, B. S. Watson, and C. K. Brinkman, "Polyhydroxyalkanoate synthesis by mixed microbial consortia cultured on fermented dairy manure: Effect of aeration on process rates/yields and the associated microbial ecology," *Water Res.*, vol. 106, pp. 26–40, 2016, doi: 10.1016/j.watres.2016.09.039.
- [57] N. M. Guho *et al.*, "Pilot-scale production of poly-3-hydroxybutyrate-co-3-hydroxyvalerate from fermented dairy manure: Process performance, polymer characterization, and scale-up implications," *Bioresour. Technol. Reports*, vol. 12, p. 100588, 2020, doi: <https://doi.org/10.1016/j.biteb.2020.100588>.
- [58] S. Poongavalappil, P. Svoboda, R. Theravalappil, D. Svobodova, T. Ougizawa, and T. Sedlacek, "Influence of branching density on the cross-linkability of ethylene-octene copolymers," *Polym. J.*, vol. 45, no. 6, pp. 651–658, 2013, doi: 10.1038/pj.2012.189.
- [59] N. Peelman, P. Ragaert, K. Ragaert, B. De Meulenaer, F. Devlieghere, and L. Cardon, "Heat resistance of new biobased polymeric materials, focusing on starch, cellulose, PLA, and PHA," *J. Appl. Polym. Sci.*, vol. 132, no. 48, 2015.
- [60] J. P. Mofokeng and A. S. Luyt, "Dynamic mechanical properties of PLA/PHBV, PLA/PCL, PHBV/PCL blends and their nanocomposites with TiO₂ as nanofiller," *Thermochim. Acta*, vol. 613, no. February, pp. 41–53, 2015, doi: 10.1016/j.tca.2015.05.019.
- [61] V. Jašo, M. Cvetinov, S. S. S. Rakič, and Z. S. Petrovič, "Bio-plastics and elastomers from polylactic acid/thermoplastic polyurethane blends," *J. Appl. Polym. Sci.*, vol. 131, no. 22, 2014, doi: 10.1002/app.41104.
- [62] X. Zhao, V. Venoor, K. Koelling, K. Cornish, and Y. Vodovotz, *Bio-based blends from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and natural rubber for packaging applications*, vol. 136, no. 15. 2019.
- [63] D. Plackett, *Biopolymers : New Materials for Sustainable Films and Coatings*. New York, UNITED KINGDOM: John Wiley & Sons, Incorporated, 2011.

- [64] E. Bugnicourt, P. Cinelli, A. Lazzeri, and V. Alvarez, "Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging," *Express Polym. Lett.*, vol. 8, no. 11, pp. 791–808, 2014, doi: 10.3144/expresspolymlett.2014.82.
- [65] K. Formela, Zedler, A. Hejna, and A. Tercjak, "Reactive extrusion of bio-based polymer blends and composites—current trends and future developments," *Express Polym. Lett.*, vol. 12, no. 1, pp. 24–57, 2018, doi: 10.3144/expresspolymlett.2018.4.