COMPATIBILITY AND BIODEGRADABILITY OF BIOPOLYESTER-BASED BLENDS WITH POLY(3-HYDROXYBUTYRATE-*CO*-3-HYDROXYHEXANOATE) (PHBH) AS A BLEND MATRIX

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2024

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2024

ACKNOWLEDGEMENT

This PhD has been a long journey of myself both as a budding young researcher and as a person, and I would like to honour here those who have, in one way or another, nourished me and eased my struggles. Truly, this is as much their achievement as it is mine.

First, I owe my heartiest gratitude to my advisors Prof. Sudesh and Abe-sensei from whom I have learnt so much on just about everything. Thank you, Prof., for instilling my interest in this field, for sharing your boundless optimism and your constant cheerleading, for giving me the chance to keep going and finish this journey. But perhaps most of all, thank you for providing me with the opportunity to take a leap further on the path that led me to meet Abe-sensei. To Abe-sensei, thank you for guiding me in treading the world of polymer chemistry with steady hands and fearless enthusiasm in equal measure. Your friendliness and approachability make it easy to forget how daunting it is letting myself dive into this field, for which I can never thank you enough. Thank you also for providing me space to grow independently and yet keeping me grounded in my work in RIKEN: anywhere else and I think I would have been the lesser for it. Much more than just putting food on my table, your work and insights have never ceased to fill me with hope and give me reason to look forward to another day. For these, and a whole lot more, I am very grateful and honoured to have you both as my advisors: I would not have had it any other way.

Amid this adventurous journey, it is the valuable insights from my colleagues that have continually drawn me back. For this, I extend my gratitude to Goto-san, Hayashi-san, Honda-san, Omura-san, and Tachibana-san. I must also express my appreciation to Nose-san, who has been an excellent companion for engaging in daily conversations and providing invaluable assistance during my stay in Japan. Deepest thanks to Chon-san, Takenaka-san, Kato-san, Imada-san, Hiraishi-san, and Nakata-san as well. To my fellow Ecobiomaterial Lab and Lab 414 lab mates, I am incredibly thankful for the wonderful moments, the delightful meals we enjoyed together, and the laughter we shared, even during the briefest of moments, throughout my time in the lab. I am also indebted to the Public Service Department of Malaysia for the scholarship scheme, RIKEN International Program Associate (IPA) and RIKEN Center for Sustainable Resource Science (CSRS) for funding this research.

But even before RIKEN, I have been blessed with a strong and supportive network of family and old friends. While being some of the best years of my life, the PhD also showed me the deepest depths of despairs, and I would not have survived to tell the tale without my friends. I owe a big thank you for standing by my side when things got rough. Last but not least, to Zaim, Ifan, Ifad, ibu, mak, ayah and abah: if I have managed to achieve anything in life, it is because I had your loving and unconditional support every step of the way. Trite as it may sound, words can do no justice to my gratitude. However, I believe that deeds begin where words end, and so I dedicate this thesis to you all.

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LIST OF SYMBOLS AND ABBREVIATIONS

α	Alpha
β	Beta
β-BL	β-butyrolactone
ε	Epsilon
ε-CL	ε-caprolactone
µg	Microgram
µg/mL	Microgram per milliliter
µL	Microliter
µm	Micrometer
ΔH_{c}	Enthalpy change of crystallization
ΔH_{m}	Enthalpy change of fusion
(*D*)	*Dexter*-isomer
(*L*)	*Laevus*-isomer
(*R*,*S*)	Atactic
(*R*)	*Rectus*-isomer
(*S*)	*Sinister*-isomer
%	Percentage
°C	Degree Celsius
±	Plus-minus
×	Times
× *g*	Times gravity
^{1}H	Proton
3HB	3-hydroxybutyrate

3HH	3-hydroxyhexanoate
3HV	3-hydroxyvalerate
4HB	4-hydroxybutyrate
ABS	Acrylonitrile butadiene styrene
AFM	Atomic force microscopy
AN	Acrylonitrile
ASTM	American Society for Testing and Materials
AU	Absorbance unit
b	block
BCP	Block copolymer
BOD	Biochemical oxygen demand
CSRS	Center for Sustainable Resource Science
CTA	Cellulose triacetate
d	Doublet
D	Average pore or granule size
D_2O	Deuterated water
DART-MS	Direct analysis in real-time mass spectrometry
DSC	Differential scanning calorimetry
EM	Electron microscopy
EO	Ethylene oxide
EPDM	Ethylene propylene diene monomer
g	Gram
GPC	Gel permeation chromatography
h	Hour
HDPE	High-density polyethylene
HPLC	High-performance liquid chromatography

Hz	Hertz
IPS	Interpenetrating spherulites
J	Joule
kg	Kilogram
L	Liter
M	Molar
m/z	Mass-to-charge ratio
mb	multiblock
mcl	Medium-chain-length
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mol%	Mole percent
M_n	Number-average molecular weight
M_w	Molecular weight
$M_{w/}$ M_n	Polydispersity index
NMR	Nuclear magnetic resonance
OM	Optical microscopy
PαMSAN	poly(α-methyl styrene-*co*-acrylonitrile)
P4HB	Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
P5HV	Poly(5-hydroxyvalerate)
PAN	Polyacrylonitrile
PBAT	Polybutylene adipate terephthalate
PBD	Polybutadiene
PBS	Polybutylene succinate

PC	Polycarbonate
PCL	Polycaprolactone
PE	Polyethylene
PEG	Polyethylene glycol
PEO	Polyethylene oxide
PET	Polyethylene terephthalate
pH	Scale of basicity and acidity
PHA	Polyhydroxyalkanoate
PHB	Poly(3-hydroxybutyrate)
PHBH	Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)
PHBV	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PHD	Poly(3-hydroxydecanote)
PHDD	Poly(3-hydroxydodecanoate)
PHH	Poly(3-hydroxyhexanoate)
PHO	Poly(3-hydroxyoctanoate)
PHP	Poly(3-hydroxypropionate)
PHV	Poly(3-hydroxyvalerate)
PLA	Polylactic acid
PLLA	poly(*L* -lactide)
PMMA	Polymethyl methacrylate
PMP	Polymethylene pentene
POM	Polarized optical microscopy
PP	Polypropylene
PS	Polystyrene
psi	Pounds per square inch
PTT	Polytrimethylene terephthalate

PVA	Polyvinyl alcohol
q	Quartet
rpm	Revolutions per minute
s	Second
s (NMR)	Singlet
SAN	Styrene acrylonitrile
scl	Short-chain-length
SDG	Sustainable development goal
SEBS	Styrene-ethylene-butylene-styrene
SEM	Scanning electron microscopy
t	Triplet
T_c	Crystallization temperature
T_g	Glass transition temperature
T_m	Melting temperature
TEM	Transmission electron microscopy
ThOD	Theoretical oxygen demand
t_R	Retention time
UV	Ultraviolet
w	Weight fraction
w/v	Weight per volume
w/w	Weight per weight
wt%	Weight percent

LIST OF APPENDICES

KESERASIAN DAN KEUPAYAAN BIOTEROSOT CAMPURAN BERASASKAN BIOPOLIESTER DENGAN POLI(3-HIDROKSIBUTIRAT-*KO*-3-HIDROKSIHEKSANOAT) (PHBH) SEBAGAI MATRIK ADUNAN

ABSTRAK

Poli(3-hidroksibutirat-*ko*-3-hidroksihexanoat) (PHBH) telah dinilai mempunyai keupayaan terosot yang sangat baik dalam persekitaran marin. Walau bagaimanapun, penggunaannya masih terhad. Penambahbaikan sifat-sifatnya dianggap perlu bagi meningkatkan potensi PHBH sebagai bahan berprestasi tinggi dan mudah terosot. Dalam kajian ini, PHBH yang mengandungi 5 dan 17 mol% 3-hidroksiheksanoat dicampurkan dengan poli(*R,S*)-3-hidroksibutirat (PHB ataktik), polikaprolakton (PCL), dan polibutilen suksinat (PBS) pada nisbah yang berbeza menggunakan kaedah penyediaan dengan pelarut. PHBH-5%/PHB ataktik dan PHBH-17%/PHB ataktik menunjukkan satu suhu peralihan kaca tunggal (T_g), dan menunjukkan kelarutcampuran dalam keadaan amorfus. Ketaklarutcampuran diperhatikan dalam campuran PHBH-5%/PCL dan PHBH-17%/PCL berdasarkan T_g yang tidak berubah bagi komponen PHBH, kehadiran dua nilai T_g yang berbeza bagi beberapa filem campuran, dan corak penghabluran individu. Sifat terma dan morfologi sferulit filem campuran PHBH-5%/PBS menunjukkan kelarutcampuran separa. Penguraian enzimatik bagi ketiga-tiga jenis campuran oleh depolimerase PHB *Ralstonia pickettii* T1 dan lipase *Burkholderia cepacia* telah disiasat. Bergantung kepada pengkhususan enzim-substrat, kelarutcampuran, dan struktur fasa campuran, kadar penguraian enzimatik adalah berbeza di antara ketiga-tiga jenis campuran. Analisis kuantitatif produk larut air yang terbebas semasa penguraian menunjukkan bahawa cara penguraian campuran oleh enzim dan variasi produk tersebut bergantung kepada kelarutcampuran polimer. Semua filem campuran PHBH/PHB ataktik, PHBH/PCL, dan PHBH/PBS menunjukkan keupayaan terosot di dalam air sungai dan air laut. Campuran PHBH/PCL telah diproses dengan meningkatkan keserasian antara komponen adunan dengan menggunakan kopoliester blok seperti diblok PHB ataktik-*b*-PCL dan diblok PCL/polietilena glikol (PCL-*b*-PEG). Campuran PHBH/PBS pula telah dicampurkan dengan diblok PHB ataktik-*b*-PEG dan triblok PHB ataktik-*b*-PEG-*b*-PHB ataktik sebagai komponen ketiga. Penambahan kopoliester blok telah mengubah keserasian campuran dari aspek sifat morfologi dan mekanikal. Antara empat kopoliester blok ini, penambahan diblok PHB ataktik-*b*-PCL ke dalam PHBH/PCL telah meningkatkan sifat mekanikal adunan paling baik dan oleh itu dipilih sebagai komponen utama dalam adunan bersama PHBH. Ini adalah untuk memaksimakan kebolehan PHBH sebagai polimer berprestasi tinggi dengan mengubah cara diblok ataktik-*b*-PCL diadun dengan PHBH. Prestasi campuran PHBH/PHB ataktik-*b*-PCL telah meningkat lebih baik daripada campuran PHBH/PCL/PHB ataktik-*b*-PCL. Campuran PHBH-5%/PHB ataktik-*b*-PCL menunjukkan peningkatan nilai regangan hingga 900% dan kekuatan regangan paling banyak sehingga 20 MPa sementara campuran PHBH-17%/PHB ataktik-*b*-PCL menunjukkan keanjalan yang lebih tinggi tetapi kekuatan regangan yang lebih rendah. Kajian ini mendapati bahawa dengan meningkatkan prestasi mekanikal campuran juga akan mengurangkan kebolehan terosot campuran. Penilaian produk larut air campuran PHBH/PHB ataktik-*b*-PCL hanya menghasilkan asid hidroksialkanoat oligomerik bukan toksik. Kajian ini telah berjaya menghasilkan adunan berdasarkan PHBH yang mempunyai prestasi mekanikal yang lebih baik dan mempunyai kebolehan bioterosot yang menggalakkan.

COMPATIBILITY AND BIODEGRADABILITY OF BIOPOLYESTER-BASED BLENDS WITH POLY(3-HYDROXYBUTYRATE-*CO*-3-HYDROXYHEXANOATE) (PHBH) AS BLEND MATRIX

ABSTRACT

Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH) has been evaluated to have excellent degradability in marine environment. However, its performance for application is still limited. A series of properties enhancement is deemed necessary to improve the potential of PHBH being both a high-performance material and readily biodegradable. In this study, PHBH containing 5 and 17 mol% 3-hydroxyhexanoate (3HH) were blended with atactic poly(3-hydroxybutyrate) (PHB), polycaprolactone (PCL), and polybutylene succinate (PBS) at different ratios by the solvent-casting method. PHBH-5%/atactic PHB and PHBH-17%/atactic PHB showed a single glass-transition temperature (T_g), indicating miscibility in the amorphous state. Immiscibility was observed in the PHBH-5%/PCL and PHBH-17%/PCL blends based on the non-transitioning T_g of the PHBH component, presence of two distinct T_g values for some of the blend films, and individual crystallization manner. The thermal properties and spherulitic morphologies of the PHBH-5%/PBS blend films suggested partial miscibility. The enzymatic degradability of the three types of blends by *Ralstonia pickettii* T1 PHB depolymerase and *Burkholderia cepacia* lipase was investigated. Depending on the enzyme–substrate specificity, miscibility, and phase structure of the blend, the enzymatic erosion rate was different among the three types of blends. The quantitative analysis of the water-soluble products showed that the enzymatic degradation manners and the variation of products liberated depended on the miscibility of the polymer blends. PHBH/atactic PHB, PHBH/PCL and PHBH/PBS blend films also showed a wide range of biodegradability in river water and seawater. Selected immiscible PHBH/PCL blends were then subjected to compatibilization with atactic PHB-*b*-PCL diblock and PCL/polyethylene glycol (PCL-*b*-PEG) diblock, while PHBH/PBS blends were mixed with either atactic PHB-*b*-PEG diblock or atactic PHB-*b*-PEG-*b*-atactic PHB triblock as compatibilizers. This altered the compatibility of the blend films in terms of morphological and mechanical properties. Addition of atactic PHB-*b*-PCL diblock into PHBH/PCL has enhanced the mechanical properties of the blends the most and thus was chosen as the main component in a PHBH-based blend system. This was to maximize the performance of PHBH as a potential high-performance polymer by utilizing the diblock in a different approach. The performance of the PHBH/atactic PHB-*b*-PCL blends has improved better than the compatibilized ternary blends of PHBH/PCL/atactic PHB-*b*-PCL. The blends of PHBH-5%/atactic PHB-*b*-PCL blends exhibited an increase in strain value up to about 900% and a tensile strength of at most 20 MPa, while PHBH-17% /atactic PHB-*b*-PCL blends demonstrated higher flexibility but lower tensile strength. This study has also discovered that enhancing the mechanical performance of the blends would also slightly decrease the blends degradability. The evaluation of water-soluble products of the PHBH/atactic PHB-*b*-PCL blends only yielded non-toxic oligomeric hydroxyalkanoic acids. This study has successfully constructed a series of PHBH-based blends with enhanced properties with promising biodegradability.

CHAPTER 1

INTRODUCTION

1.1 Study background

Plastic pollution was first caught by attention back in the late 1960s, about a decade after its pioneer production. About 6.3 out of 9.2 billion tons plastics were not successfully recycled as of 2018 since the 1950s (Parker, 2019). In this deteriorating menace, the most susceptible habitat is the marine environment. Since ocean is the downstream of every terrestrial site, majority of plastic wastes end up in the ocean, if not on land or air. It is predicted that 10 years from now, plastic wastes ranging in between 22 to 58 million tons will enter waterways reaching for the ocean (Parker, 2020). Once this big volume of plastics enters the ocean, they are impossible to be retrieved. “Where do ocean plastics come from ?”, one might wonder. Plastic wastes can enter ocean either directly or indirectly. Shipping crews, beach tourists and fisheries industry often dump garbage into the sea which give direct contribution to mounted amount of ocean plastics. Approximately 80% of ocean plastics come from lands and not from what are directly thrown into the ocean (Li *et al.*, 2016).

In result of bad ocean health, the survival of living beings within the habitat itself which provides plethora of our own food and other daily necessities is challenged. The infamous photograph of a sea turtle had its nose stuck with what appeared to be non-degradable plastic straw made the world community realized that even the smallest threat can lead to peril. The actual damage accumulation of plastics does to marine wildlife is far beyond that. Plastic pollution increases the chance of intoxication when animals and humans are exposed to toxin-embedded plastic wastes especially those come from the ocean since marine pollution also suffer chemical contaminants. Apart from the deterioration of human health, plastic pollution harms the tourism industry as well. The ocean venues ideal for tourism are filled with plastic wastes which eventually cost extra for beach clean-up. The most efficient way is to avoid using single-used plastics every way possible. However, modern community is so used to the convenience and practicality of petroleum-based plastics that having to avoid using them is somewhat burdening. This leads to the scouting of bioplastics which can offer similar benefits as that of petrochemical plastics. While one cannot eliminate the total dependency on petroleum-based plastics, best alternatives to them might be helpful in reducing its usage.

Bio-based plastics or bioplastics are a group of plastics made from renewable biological sources. Biodegradable plastics can be metabolized by microorganisms and converted into water, carbon dioxide and biomass. However, not all bioplastics can biodegrade in natural environment. One example of a non-biodegradable bioplastic is bio-poly(ethylene terephthalate) (Bio-PET) which can be fully and partially bio-based. It can either be produced from 100% bio-based ethylene glycol (EG) and petroleum-based terephthalic acid or both monomers produced from bio-based materials (Siracusa & Blanco, 2020). Biodegradation process is strongly dependent on the environmental parameters such as the humidity, temperature, oxygen availability and presence of degrading microorganisms. Understanding the terminology used for labelling types of bioplastics is crucial to ensure people are using them properly.

In recent years, the term oxo-degradable has been used in some plastic products that leads to misunderstanding amongst general consumers. Owing to the name ‘degradable’, people find it safe to use the oxo-degradable plastic bags as an effort to go ‘green’. Oxo-degradable plastics are conventional petroleum-based plastics that have been modified with some additives so that they can mimic biodegradation. There is a growing concern about defining plastics as oxo-degradable and interpreting them as biodegradable. Oxo-degradable plastics undergo physical degradation via oxidation, thermal and ultraviolet processes which eventually decrease the molecular weight and ease the bio-assimilation of monomer units. However, the degradation of oxo-degradable plastics is not always a rapid process. Using oxo-degradable packaging can be a threat as they physically degrade into microplastics and resistant to biodegradation. The particularly small-sized particles can enter the waterways or being ingested by marine animals which will eventually end up in the human food chain.

Poly(lactic acid) (PLA) and poly(hydroxyalkanoate) (PHA) are considered as the two most promising biodegradable polymers. However, the debatable ability of PLA to biodegrade and the high production cost of PHA become a big challenge for commercialization. PLA degradation was initially focused on hydrolytic or enzymatic degradation and subsequently by some soil microorganisms. Although PLA claims to be biodegradable, it biodegrades best in selective conditions, such as in industrial compost, high temperature environment and microorganisms-enriched soil. Nevertheless, it can take up to 6 months to observe visible cracks in PLA samples which proves tedious and time-consuming biodegradability. In room temperature and normal pressure, PLA takes years to degrade. On the other hand, PHA is known for its biodegradability. While PLA cannot be degraded in marine environment, PHA is well-known for its excellent biodegradability in various natural conditions including in marine environment. To date, various PHA monomers have been characterised and it is reported that the composition of monomeric unit greatly influenced the properties.

Despite having a number of advantages over petrochemical-based plastics and other synthetic polymers, PHA still bears some limitations like poor physical and mechanical properties, low compatibility with regular processing techniques, susceptible to thermal degradation and high production cost (Sharma *et al*., 2021). The use of PHA as a direct replacement of conventional plastics hence still remains a major challenge. Polymer blending is an effective and low-cost approach to enhance the poor properties of PHA, lower down cost and increase production to achieve specific standards for suitable applications. This method can also produce PHA blends with desirable mechanical properties, crystallinity, surface features, amphiphilicity and adjustable degradation rates. Generally, blends consist of two or more polymer components that are combined to form a single-phase system. The physical and mechanical properties of PHA blends can be improved via blending by adjusting the blend compositions and preparation conditions. Usually, PHA is blended with other biodegradable synthetic polyesters like polycaprolactone (PCL), polybutylene succinate (PBS) and poly(butylene adipate terephthalate) (PBAT) to retain its biodegradability. As PCL is also one of the promising biodegradable synthetic polymers, blending PHA with PCL has shown to exhibit interesting blend properties. Nishida and colleagues (Nishida *et al*., 2016) studied the effects of mixing ratio of PHA and PCL blends on their mechanical properties. It was observed that the correct ratio of PHA and PCL can balance the brittleness and elasticity of the blends. Other example of PHA blend includes PHB/PBS and PHBV/PBS blends which were found to be partially miscible and enhanced crystallization behavior when prepared via melt compounding (Ma *et al*., 2014). Another study also stated that PHA and PBS blend showed improved mechanical properties particularly its flexibility (Jordá-Reolid *et al*., 2022). Although PHA has the potential to replace the petroleum-based plastics in the market, it is still far off from being as versatile as the latter. The properties of PHA can be efficiently improved with various approaches including blending and subsequent compatibilization.

1.2 Problem statement

PHBH has gained popularity due to its excellence in biodegradability. It is easily consumed by microorganisms, particularly in marine environments where carbon sources are limited. PHBH is also known for its more ductile nature compared to other PHA copolymers. Due to its low crystallinity, PHBH exhibits excellent flexibility and elongation at break, but possesses a low Young’s modulus and tensile strength. Despite being the most promising PHA variant, PHBH has yet to make its way onto the market, for several reasons.

Although PHBH was discovered many years ago and is renowned for its excellent biodegradability, studies focusing on this PHA copolymer continue to be active to this day. The limited availability of PHBH in the market persists due to its restrictive high strain-low strength and slow crystallization qualities, which hinder its industrial processing and applications. Despite PHBH having a wider processing window compared to other PHAs, its production cost remains prohibitively high.

Numerous factors need to be considered prior to the production stage, including different bacterial strains, various precursors, and varying carbon sources. These factors are crucial for producing PHBH with a specific comonomer concentration to achieve different flexibility options. Consequently, commercializing PHBH still requires a laborious, tedious, and costly production process to attain a broader performance window. This is why PHBH has gained notoriety for its high cost, and its commercialization remains a distant prospect, further impeded by the strict confidentiality maintained by polymer manufacturers.

A great deal of effort has been devoted to overcoming the shortcomings of PHBH for plastics applications, highlighting blending with other polymers as a useful way of obtaining new materials with improved properties and compatible with industrial processing. The benefits of polymer blending can be observed from both the perspective of material properties and the economies it can bring to manufacturers. Since polymer blending is a low-cost and efficient approach, it can effectively address the challenge of achieving PHBH with a wider performance window without necessitating extensive production stages. This could result in offering products at the lowest possible price while encompassing a maximum range of desired properties. Additionally, employing a polymer blending process has the potential to minimize the time required for commercialization, thereby further reducing production costs.

Therefore, PHBH was blended with various biodegradable polyesters, and the blends underwent an initial assessment of their miscibility and degradability. Recognizing the likelihood of immiscibility being a challenge in this study, it is imperative to enhance the compatibility of these blends by integrating block copolymers. Furthermore, PHBH performance was further augmented by employing the most efficient block copolymer in a slightly different approach, aiming to achieve superior product performance compared to its neat copolymer counterpart while still maintaining its biodegradability

1.3 Research objectives

The objectives of this study were:

Objective 1: To determine the miscibility of PHBH-based polymer blends.

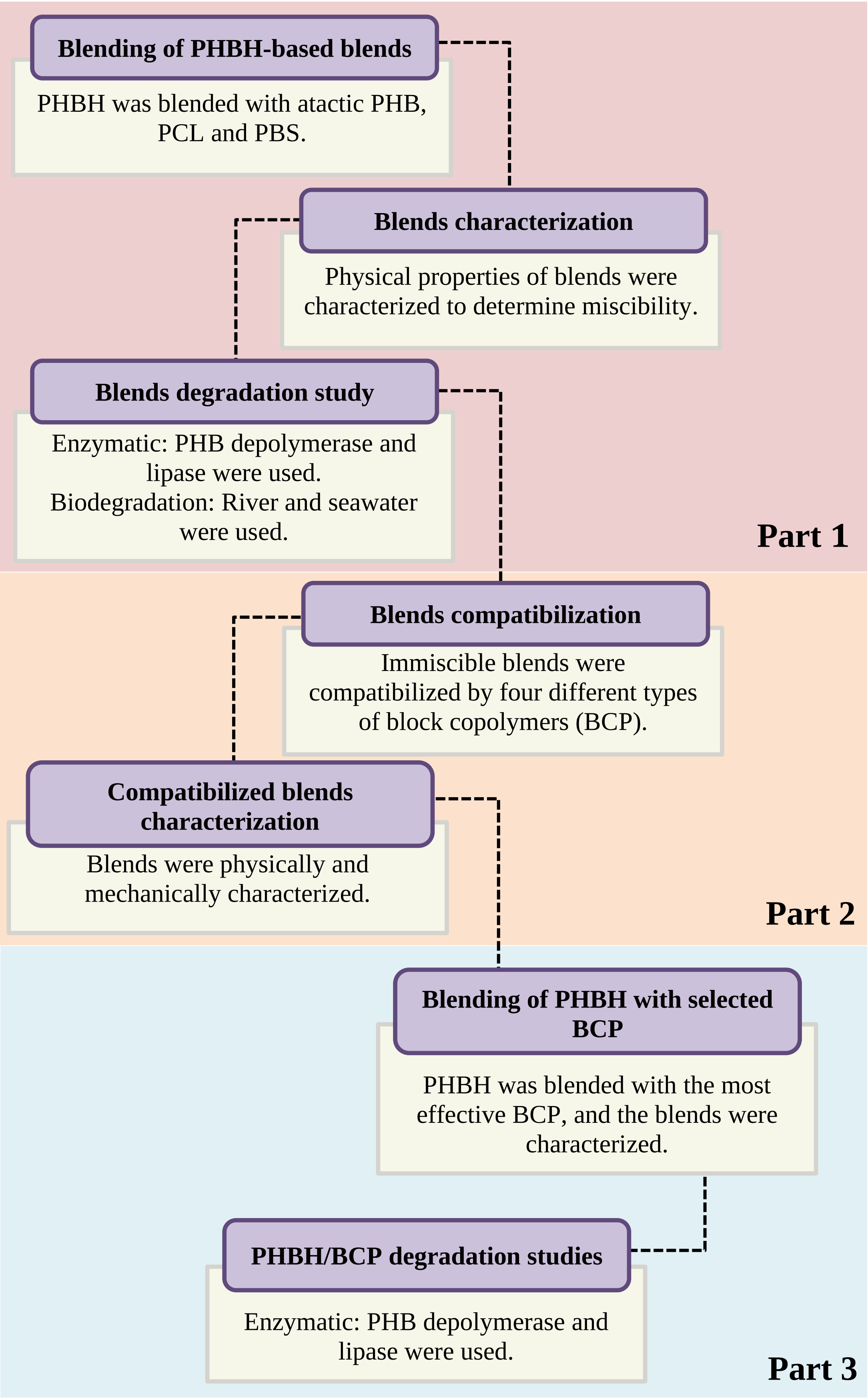
Objective 2: To evaluate the miscibility-degradability relationship of PHBH-based polymer blends.

Objective 3: To improve the compatibility of PHBH-based blends by incorporating biodegradable block copolymers as compatibilizers.

Objective 4: To construct PHBH-based blends with enhanced properties.

1.4 Flow of study

All experiments and machine handlings were conducted in the RIKEN Center for Sustainable Resource Science (CSRS), Bioplastic Research Team laboratory, Japan excluding the preparation of poly(3-hydroxybutyrate-*co*-17 mol% 3-hydroxyhexanoate) (PHBH-17%) which was produced in the Ecobiomaterial Lab, School of Biological Sciences, Universiti Sains Malaysia. The study flow in this dissertation is structured into three parts (Figure 1.1). The first part of the study includes the study of the relationship between PHBH-based blends' miscibility and degradability. The initial assessment of the blend's miscibility led to the construction of miscible, immiscible and partially miscible blends. Since the interest of this study included the immiscible and partially miscible blends, it was necessary to subject the samples of interest onto the second part of this thesis. Part 2 of this thesis includes the incorporation of block copolymers to reduce the blends incompatibility. The blends compatibility was reflected on their thermal, morphological and mechanical properties. In the third part of the study, the most effective compatibilizer was selected and blended with neat PHBH to maximize the polymer performance. The block copolymer utilized in this section shares similar components with those in Part 2. It is particularly interesting that the block copolymer is integrated not only as a minor component but also as one of the main blend components. These specific aspects will be further elaborated upon throughout the dissertation, in the introduction of each chapter.



Blending of PHBH-based blends

PHBH was blended with atactic PHB, PCL and PBS.

Blends characterization

Physical properties of blends were characterized to determine miscibility.

Blends degradation study

Enzymatic: PHB depolymerase and lipase were used.
Biodegradation: River and seawater were used.

Part 1

Blends compatibilization

Immiscible blends were compatibilized by four different types of block copolymers (BCP).

Compatibilized blends characterization

Blends were physically and mechanically characterized.

Part 2

Blending of PHBH with selected BCP

PHBH was blended with the most effective BCP, and the blends were characterized.

PHBH/BCP degradation studies

Enzymatic: PHB depolymerase and lipase were used.

Part 3

Figure 1.1 Flow of study

CHAPTER 2

LITERATURE REVIEW

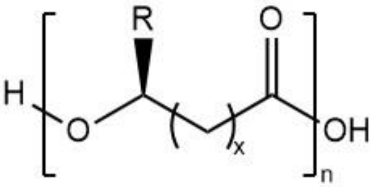
2.1 Biodegradable aliphatic polyesters

Aliphatic polyesters are known to be the most readily biodegradable type of polymers. Chemical structure, certain degree of hydrophilicity and crystallinity are among the factors contributing to the biodegradability of biodegradable polyesters. Most aliphatic polyesters provide the hydrophilic/hydrophobic balance which is important for the enzyme active site to bind with the polymer substrate. This is the main reason why aliphatic polyesters have a much better degradability compared to the more complex and rigid structure of aromatic polyesters (Fakirov, 2015). The most common method to chemically synthesize aliphatic polyester is by the ring-opening polymerization. Biodegradable polyesters are commonly able to be degraded by lipase, one of the members in the esterase group. However, the hydrolyzing ability of lipase is limited to polyesters without optically active carbon. Polymers consisting of an optically active carbon such as poly(hydroxybutyrate) (PHB) and poly(*L*-lactide) (PLLA) cannot be hydrolyzed by lipase (Mukai *et al.*, 1993b; Tokiwa *et al.*, 1986; Tokiwa & Suzuki, 1978).

2.1.1 Polyhydroxyalkanoates (PHA)

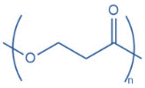
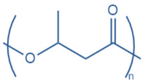
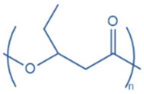
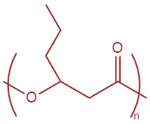
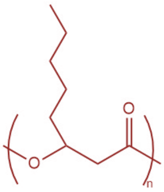
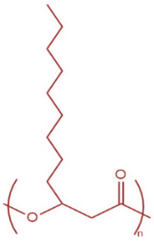
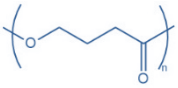
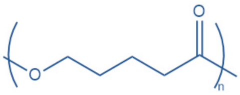
Polyhydroxyalkanoates (PHA) are also a member of biodegradable aliphatic polyesters group. PHA are synthesized as an energy source by PHA-producing bacteria in the presence of excess carbon supply, and they are consequently used under unfavorable growth conditions. Poly(3-hydroxybutyrate) (PHB) is the predominant naturally occurring type of PHA. Despite the identification of different monomers as potential PHA components, the range of naturally synthesized PHA is still quite limited. This limitation arises from the fact that bacterial cells lack the natural ability to produce the majority of these monomers. Thus, in recent bacterial PHA production, monomers are supplied as precursor carbon sources to produce other types of PHA (Sudesh & Abe, 2010). PHA can be classified into two classes of short-chain-length (scl-PHA) and medium-chain-length (mcl-PHA) according to the number of carbons in the side chains. The scl-PHA have less than 5 carbon atoms while mcl-PHA have 5-14 carbon atoms. PHB, poly(3-hydroxyvalerate) (PHV) and their copolymer, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) are the common scl-PHA whereas mcl-PHA are poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH), poly(3-hydroxyoctanoate) (PHO) and poly(3-hydroxydecanoate) (PHD) (Kim *et al.*, 2007; Madison & Huisman, 1999).

All types of PHA are water-insoluble and present in the bacterial cells in a form of granules. The granules can be observed in bacterial cells by using the Nile blue and Nile red staining method. Light fluorescent staining is also practical since the stain has good affinity towards PHA granules and can exhibit a strong orange fluorescence under fluorescent microscope. In addition, the granules also have an electron transparent appearance allowing their sizes, numbers and localization evaluated under transmission electron microscopy (TEM) (Wahl *et al.*, 2012). PHA differ in wide range of properties and chemical composition as homopolymers or copolymers depending on their structural variations. Probably the most interesting properties PHA possess is their excellent biodegradability. Since PHA is a type of polymer produced by bacterial cells for an emergency carbon supply, they can be readily degraded in natural ambient.



General structure of PHA

Table 2.1 Chemical structures of scl-PHA and mcl-PHA.

Number of repeating units, x	Alkyl group, R	Polymer type	Polymer structure
1	Hydrogen	Poly(3-hydroxypropionate) (PHP)	
	Methyl	Poly(3-hydroxybutyrate) (PHB)	
	Ethyl	Poly(3-hydroxyvalerate) (PHV)	
	Propyl	Poly(3-hydroxyhexanoate) (PHH)	
	Pentyl	Poly(3-hydroxyoctanoate) (PHO)	
	Nonyl	Poly(3-hydroxydodecanoate) (PHDD)	
2	Hydrogen	Poly(4-hydroxybutyrate) (P4HB)	
3	Hydrogen	Poly(5-hydroxyvalerate) (P5HV)	

n refers to number of repeating units

2.1.1(a) Poly(3-hydroxybutyrate) (PHB)

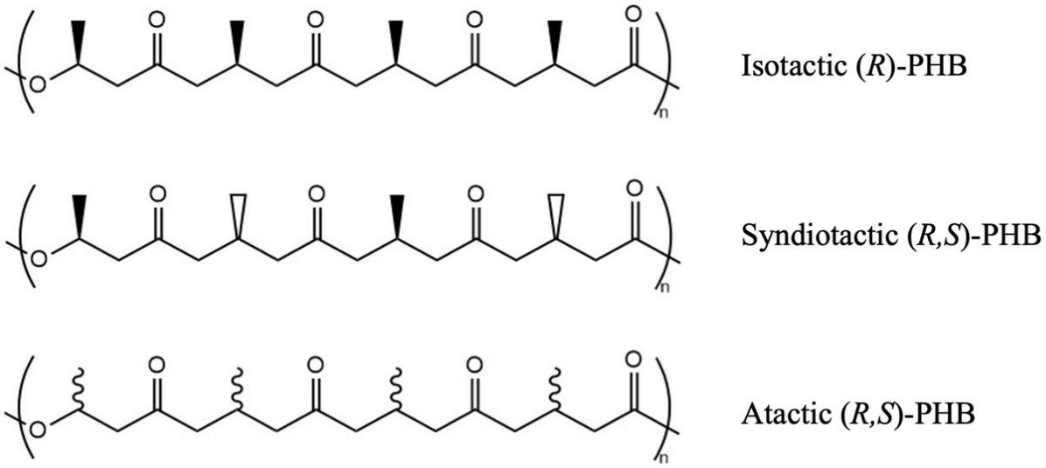


Figure 2.1 Tacticity of PHB

PHB is the most common homopolymer ever since PHA was first discovered. Natural bacterial PHB is strictly produced as an isotactic polyester of the monomer 3-(*R*)-hydroxybutyric acid structure (Hocking & Marchessault, 1993; Cornibert & Marchessault, 1975). Since PHB has only one optically active form and the configuration is absolute, its crystallinity can be more than 95%. High degree of crystallinity contributes to its brittleness which becomes a major disadvantage for product application (Hill, 2005). Crystalline fractions in a polymer can be controlled via tacticity which aims to alter its crystal structure. Changes in its configuration can significantly influence the properties. Unfortunately, the biosynthesis approach in PHA production does not offer tacticity control since microorganisms can only produce isotactic PHB. Therefore, new synthetic routes have come into focus in modifying polymer properties.

Over the years, PHB of different tacticity such as isotactic, syndiotactic and atactic PHB have been widely synthesized. Figure 2.1 shows the molecular configuration between PHB of different tacticity (Hillmyer & Tolman, 2014; Anastas & Kirchhoff, 2002). Previous studies found that when the isotacticity decreased to 70-80%, the crystallization was significantly influenced and the melting point decreased to 100-130 °C (Abe *et al*., 1994b; Tanahashi & Doi, 1991). Syndiotactic PHB was not synthesized until late 1990s. Its properties have shown that Young's modulus strongly depends on the syndiotacticity, and the melting transition increases linearly as the degree of syndiotacticity rises (Ajellal *et al*., 2009; Kricheldorf & Eggerstedt, 1997).

These differences in chemical structures also influence their biodegradability. For example, isotactic PHB has a high melting point owing to its uniform crystalline ordering and thus makes it less susceptible to enzyme hydrolysis. On the other hand, atactic PHB with random chiral carbons configuration is completely amorphous without melting point and cannot be hydrolyzed by PHB depolymerase.

2.1.1(b) Atactic poly[(*R*,*S*) 3-hydroxybutyrate] (atactic PHB)

The readily made bacterial PHB composed of *R* configuration chiral carbons, a perfectly isotactic polymer with a left-handed helical structure (Hocking & Marchessault, 1993; Cornibert & Marchessault, 1975). PHB with different chiral carbons configuration can be synthetically synthesized using racemic monomer with various catalysts yielding synthetic PHB of different tacticity. Atactic PHB, characterized by a random arrangement of chiral carbons, lacks a melting point and exists in a completely amorphous state. The atactic PHB displays a liquid-like appearance, which is typically transparent and clear due to its complete amorphous nature. Handling the atactic PHB sample proves challenging as it is very sticky and difficult to pick up with a spatula, posing difficulties in measuring its weight. Moreover, it is worth noting that atactic PHB does not undergo autonomous hydrolysis when exposed to PHB depolymerase. Interestingly, the degradation of atactic PHB can only be induced through crystallinity, requiring its mixture with a polymer of any level of crystallinity to be susceptible to hydrolysis by PHB depolymerase (Focarete *et al*., 1998; Abe *et al*., 1995b).

2.1.1(c) Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH)

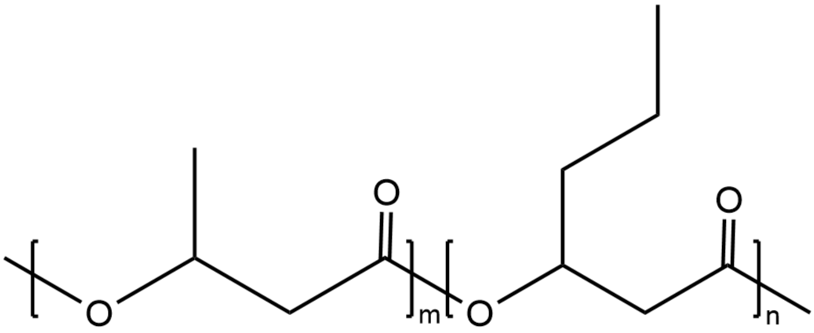


Figure 2.2 Chemical structure of PHBH

Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBH) is a copolymer with the incorporation of 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HH) as the second monomer. The inability of 3HH monomer fraction to co-crystallize with 3HB substantially reduces the degree of crystallization and the crystallization rate of PHB. This consequently improves its processability and physical properties. Intra-plasticization occurred by the addition of a small percentage of 3HH monomer can cause a high depression in melting point of PHB. Therefore, the crystallinity of PHBH decreases steeply with further increase of 3HH monomer fraction (Doi *et al.*, 1995). The reduction in melting temperature and crystallinity leads to a tougher material.

Table 2.2 The properties of PHBH with different 3HH molar fractions

Samples	T_g (°C)	T_m (°C)	Tensile strength (MPa)	Strain (%)	Reference
PHB	4	178 ± 2	43	5	(Doi et al., 1995; Doi, 1990)
P(3HB-*co*-4% 3HH)	−1	164			(Murugan *et al.*, 2017)
P(3HB-*co*-5% 3HH)	0	151			(Doi et al., 1995)
P(3HB-*co*-10% 3HH)	−1	127	21	400	(Chang *et al*., 2014; Doi *et al* 1995)
P(3HB-*co*-12% 3HH)	−9	163			(Murugan *et al.*, 2016)
P(3HB-*co*-15% 3HH)	−6 ± 6	135 ± 20	23	760	(Murugan *et al.*, 2017)
P(3HB-*co*-17% 3HH)	−2	120	20	850	(Miyahara *et al.*, 2021)
P(3HB-*co*-19% 3HH)	−2 ± 2	128 ± 17			(Doi *et al.*, 1995; Murugan *et al.*, 2016)
P(3HB-*co*-25% 3HH)	−4	52			(Doi *et al.*, 1995)

The properties of PHBH copolymers can vary depending on the type of copolymerization, which can occur in the form of random or block copolymers. For example, in a block copolymer of PHBH with 42 mol% of 3HH monomeric unit, two T_g values were observed: approximately –16 °C and 3 °C. These values correspond to the T_g values of PHB and poly(3-hydroxyhexanoate) (PHH), respectively (Tripathi *et al*., 2012). Since the PHH homopolymer is naturally amorphous, the melting point of PHBH typically belongs to the melting crystals of 3HB blocks. In terms of thermal and crystallization behavior, random copolymers of PHBH usually reveal two close melting points. Several studies have indicated that an increase in the 3HH monomer content leads to a decrease in the T_g of the PHBH random copolymer, as well as a significant reduction in both the T_m and final degree of crystallinity. According to these studies, with each 10 mol% increase in the 3HH comonomer content, the T_g can decrease by approximately 4 °C, whereas the reduction of T_m and the degree of crystallinity are approximately 40 °C and 20%, respectively (Miyahara *et al*., 2021; Cheng *et al*., 2008; Alata *et al*., 2007; Abe *et al*., 1998; Doi *et al*., 1995). The observed thermal behaviors are attributed to the inclusion of longer side chains of 3HH comonomers into the side-chained 3HB, which restricts molecular mobility and reduces the packing efficiency of the crystallites (Dong *et al*., 2010). PHBH samples may undergo a secondary crystallization at room temperature due to their slow crystallization rate and low T_g points. This phenomenon is commonly known as cold crystallization and is a result of aging (Kabe *et al*., 2018).

PHBH’s compostable nature under various conditions makes it a suitable candidate for commodity applications, as it can biodegrade in most environments without the need for industrial composting. The presence of ester bonds in the chemical structure of PHBH enables hydrolytic degradation, leading to chain scission and the segregation of PHBH chains into oligomeric or monomeric units. The degradation rate of PHBH can be influenced by its crystallinity. As the content of 3HH comonomer increases, the crystallinity of PHBH decreases, resulting in a faster degradation rate. Nevertheless, studies carried out by Doi *et al*. (1995) and Wang *et al*. (2004) indicated that degradation was impeded in samples containing more than 15 mol% of 3HH. Further increasing the comonomer content of 3HH can decrease the surface roughness of PHBH samples. This enhanced surface smoothness may hinder the adhesion and diffusion of enzymes on the surface, which are crucial for the degradation process.

2.1.2 Poly(caprolactone) (PCL)

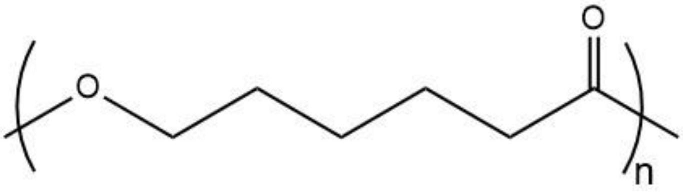


Figure 2.3 Chemical structure of PCL

PCL, one of the pioneering polymers, was initially discovered by the Carothers group during the 1930s (Natta *et al*., 1934). While PCL initially garnered notable research attention, its popularity was soon superseded by other novel and more versatile resorbable polymers such as polylactides and polyglycolides. After nearly two decades of being overlooked, PCL has experienced a resurgence of interest, propelling it back into the spotlight. PCL is a hydrophobic semi-crystalline polyester consists of hexanoate repeating units. It can be synthesized either by the ring-opening polymerization of *ε*-

caprolactone or polycondensation of 6-hydroxyhexanoic acid (Labet & Thielemans, 2009). PCL has a T_g value of –60 °C and a melting point ranging between 59 and 64 °C (McKeen, 2021; Woodruff & Hutmacher, 2010).

PCL has been widely used in the pharmaceutical industry for drug delivery, owing to its high permeability to many commercial drugs, excellent biocompatibility and ability to be fully excreted from the body after absorption (Ruckh *et al*., 2010). On account of its low melting point, blend-compatibility and good solubility in various solvents, PCL has been extensively studied for drug-delivery materials. It can be readily degraded by lipases and cutinases, considering that the structure of PCL is analogous to that of cutin (Nair *et al*., 2017; Tokiwa & Suzuki, 1977). Nevertheless, because of its high crystallinity and hydrophobic nature, PCL can take up to 3-4 years to biodegrade. This does not put PCL as a suitable candidate for meeting the demand of polymer matrices that release encapsulated drugs within a short timeframe of days or weeks. Despite the low degradation rate, PCL still has promising features for various applications in current medical device industry.

PCL can be biodegraded by outdoor living organisms such as bacteria and fungi, but it is not biodegradable in animal or human bodies due to the absence of suitable active enzymes. However, PCL is a bioresorbable polymer that exhibits the ability to be completely eliminated through natural pathway *in vivo* with no residual side effects (Vert *et al*., 1992). *In vivo*, PCL undergoes a two-step degradation process, beginning with the non-enzymatic hydrolytic cleavage of ester groups, followed by the intracellular degradation. The latter occurs when the polymer has higher crystallinity and lower molecular weight. The uptake of PCL fragments by phagosomes of macrophages and giant cells was observed, supporting the theory that PCL is completely resorbed and degraded via an intercellular mechanism once its molecular weight is greatly reduced (Woodward *et al*., 1985). Several studies concerning *in vitro* and *in vivo* degradation of PCL found no differences between the rate of PCL degradation *in vitro* (saline) and *in vivo* (rabbit). It was also noted that the first stage of PCL degradation via intracellular mechanism obeyed first-order kinetics. This stage was essentially identical to the *in vitro* hydrolysis at 40 °C. This concluded that enzymatic involvement in the first stage of PCL degradation was not a significant factor (Lam *et al*., 2009; Sun *et al*., 2006; Pitt *et al*., 1981).

2.1.3 Poly(butylene succinate) (PBS)

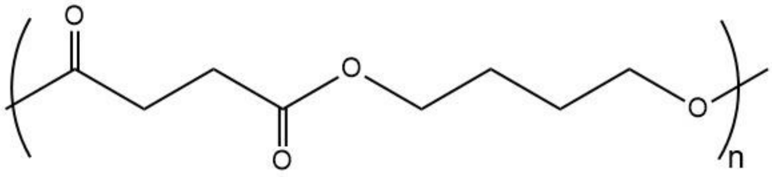


Figure 2.4 Chemical structure of PBS

Apart from PCL, poly(butylene succinate) (PBS) is also one of the most promising polymers in the aliphatic polyester family. PBS is prominently known under the trade name Bionolle which was mass produced by Showa Denko, Japan before the company decided to terminate the production in 2016. It is synthesized by the polycondensation of 1,4-butanediol with succinic acid. Prior to the polycondensation process, the oligomers are produced by esterification of 1,4-butanediol and succinic acid. The starting materials for PBS synthesis are widely available in fossil fuels and can also be derived by the fermentation method from renewable resources (Luyt & Malik, 2019).

PBS is a white crystalline thermoplastic polymer with a T_m that falls within the range of 90 to 120 °C and a T_g of approximately –45 to –10 °C. It exhibits good mechanical properties and can be easily processed into textile filament, injection molds and extruded products. Thermal analysis by differential scanning calorimetry (DSC) on the isothermally crystallized PBS revealed that PBS shows multiple melting behaviors. Wang *et al*. (2007) reported four melting endotherms and a crystallization exotherm peak, each attributed to a different mechanism. The crystallization exothermic peak is particularly caused by the melt recrystallization of the crystallites that have poor thermal stability. Furthermore, the endothermal peaks can be attributed to the three mechanisms: (1) remelting of crystallites formed during recrystallization; (2) the presence of different type of crystals; and (3) an annealing peak marking the transition of the solid-like to liquid-like rigid amorphous fraction (Yoo & Im, 1999). Wang and colleagues (Wang *et al*., 2007) also reported that the annealing peak was observed in all melting curves and its position was higher than the corresponding T_c. The same behavior can be found in literature for other polymeric systems (Xie *et al*., 2021; Schick & Androsch, 2020; Fenni *et al*., 2019). Table 2.3 shows the basic properties of several commercially available PBS.

The degradability of PBS has been extensively studied through hydrolytic, enzymatic and biodegradation under various environmental conditions. Studies have shown that PBS can be effectively degraded in activated sludge, soil burial, as well as in compost. Kanemura *et al.* (2012) evaluated the effect of water contact on the biodegradability of PBS and discovered that temperature significantly affects the process. Additionally, the enzymatic degradation of PBS is largely influenced by its crystallinity and chemical structure. These factors play a significant role in the breakdown of PBS and other aliphatic polyesters. On the other hand, environmental biodegradation depends on both the properties of the polyesters and the biotic and abiotic factors of the environment, including moisture, microorganisms and temperature (Phua *et al*., 2012). The biodegradability of PBS is particularly efficient under composting conditions. Zhao *et al*. (2005) managed to isolate four strains of PBS-degrading microorganisms, and *Aspergillus versicolor* was found to have the highest activity. A lot of good PBS-degrading microorganisms are from the fungi family including *Aspergillus*, *Fusarium* and *Candida* (Hegyesi *et al*., 2019; Jung *et al*., 2018; Hu *et al*., 2016).

Table 2.3 List of commercialized PBS grades and their properties

Grade	Supplier	Melt flow rate (g/10min)	Density (g/cm^3)	T_g (°C)	T_m (°C)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	Ref.
Bionolle 1001 MD	Showa Denko (Tokyo, Japan)	1.3	1.26	–32	114	62	660	470	(Showa Denko, 2013)
Bionolle 3001 MD		3.0	1.23	–45	94	40	780	320	
SOL-POL 5000	Solchemical (Gangwon-do)	3.0 ± 1.0	1.25	–32	115	≤44	≤500	-	(Soltech Solchemical, 2020)
TH803S	Blue Ridge Tunhe Polyester	11.0 ± 4.0 18.0 ± 2.0	1.25	-	110-116	≥40	≥350	-	(Xinjiang Blue Ridge Tunhe Chemical Industry, 2023)
BioPBS FZ71	Mitsubishi Chemical (Tokyo, Japan) & PTT Global Chemical (Bangkok, Thailand)	22.0	1.26	-	115	30	170	630	(PTT MCC Biochem, 2016)
BioPBS FZ91		5.0	1.26	-	115	36	210	650	
BioPBS FD92		4.0	1.24	-	84	24	380	250	