PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) FROM BIOMASS OF *Scenedesmus* sp. BY *Priestia megaterium*

AZIEYATI HANI BINTI HUSSAIN

UNIVERSITI SAINS MALAYSIA

2025

PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) FROM BIOMASS OF *Scenedesmus* sp. BY *Priestia megaterium*

by

AZIEYATI HANI BINTI HUSSAIN

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

August 2025

ACKNOWLEDGEMENT

All praises are to Allah S.W.T the Almighty, by whose Grace and Blessings have enabled me to complete my master study. Firstly, I would like to express my deepest gratitude to my supervisor, Associate Professor Ir. Dr. Husnul Azan Tajarudin, for his guidance, feedback and invaluable patience throughout the process of my study. I am also thankful to my co-supervisors, Dr. Muaz and Dr. Aziah for sharing the knowledge of their expertise. Additionally, I would like to extend my sincere thanks to Kuok Foundation for financing my research through MSM Scholarship Award. I am extremely grateful to my family, especially my parents and siblings for the unconditional love and support they have given to me. To my mother, Mrs. Asniarti, and my father, Mr. Hussain, your prayers had kept me strong, pushing me through all the hardships I faced in this journey. To my siblings, thank you for being very supportive of my dream and helping me out whenever I'm in need. I am also thankful to my aunty, Dr. Nurhidayaty, for the helps she have provided me. To my best friends, Ramizah and Haffizah, thank you for the unwavering supports you both have given me during my ups and downs in this journey. Not to forget, the lab staffs of Bioprocess Technology division, Mr. Azmaizan and Mrs. Najmah, who have facilitated me with resources and equipment needed throughout the conducts of my experiments. Thank you for all who have involved directly or indirectly, and have helped me along the way including my labmates in Lab A407 and PPTI's MSM committees.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

^{o}C	Degree Celcius
%	Percentage
±	Plus minus
T_m	Melting temperature
X_c	Degree of crystallinity
μ	Specific growth rate
ΔH_m	Melting enthalpy
$Y_{x/s}$	Yield coefficient for biomass on carbon substrate
$Y_{PHB/s}$	Yield coefficient for PHB on carbon substrate
P_x	Volumetric productivity for biomass
P_{PHB}	Volumetric productivity for PHB
$Y_{PHB/x}$	Yield coefficient of PHB per biomass
t_d	Doubling time

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BBM	Bold's Basal Medium
CDW	Cell dry weight
dH_2O	Distilled water
DNS	3,5-dinitrosalicylic acid
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared
h	Hours
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
LB	Luria-Bertani medium
min	Minutes
ml	Milliliters
NaClO	Sodium hypochlorite
NaOH	Sodium hydroxide
nm	Nanometer
OD	Optical density
P. megaterium	*Priestia megaterium*
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PTFE	Polytetrafluoroethylene

LIST OF APPENDICES

PENGHASILAN POLIHIDROKSIBUTIRAT (PHB) DARIPADA BIOJISIM *Scenedesmus* sp. OLEH *Priestia megaterium*

ABSTRAK

Kos bahan mentah karbon yang tinggi dalam penghasilan polihidroksibutirat (PHB) daripada mikrob merupakan antara masalah yang masih dihadapi oleh industri, dimana ia secara tidak lansung telah melambatkan peralihan penggunaan plastik konvensional kepada bioplastik. Untuk menyelesaikannya, penggunaan biojisim *Scenedesmus* sp. telah dikaji sebagai sumber alternatif bagi penghasilan PHB oleh *Priestia megaterium*. Pertama, kandungan karbohidrat dari *Scenedesmus* sp. telah diperolehi menggunakan teknik prarawatan asid cair hidroterma. Setelah kajian dijalankan menggunakan kepekatan H_2SO_4, suhu dan tempoh rawatan yang berbeza, telah didapati bahawa prarawatan menggunakan 4% (v/v) H_2SO_4 pada suhu 105°C selama 30 min telah memberikan penghasilan karbohidrat dan gula yang paling tinggi (10.66% (w/w) ± 0.05 karbohidrat, 8.11% (w/w) ± 0.08 gula). Kemudian, tumbesaran *P. megaterium* dan penghasilan PHB di dalam media yang mengandungi gula daripada *Scenedesmus* sp. (PM-MH) telah dinilai dan dibandingkan dengan media yang mengandungi glukosa (PM-GLU). CDW maksimum (3.41 g/L) dan penggunaan gula (83.78%) *P. megaterium* di dalam PM-MH setanding dengan PM-GLU, walaupun PHB yang dihasilkan dari PM-MH (0.74 g/L) lebih rendah berbanding PM-GLU (1.51 g/L). Akhir sekali, pencirian sifat PHB dari PM-MH (PHB-MH) telah dilaksanakan dan dibandingkan dengan sifat PHB komersial (PHB-C). Analisis FTIR bagi PHB-MH mengesahkan kewujudan kelompok berfungsi yang turut ada pada PHB-C. Sementara itu, analisis DSC menunjukkan takat lebur (T_m) bagi PHB-MH lebih rendah berbanding PHB-C, iaitu pada suhu 153.45°C. Kesimpulannya, segala maklumat yang

diperolehi daripada kajian ini telah menunjukkan potensi penggunaan biojisim mikroalga sebagai sumber karbon untuk penghasilan PHB oleh *P. megaterium*.

PRODUCTION OF POLYHYDROXYBUTYRATE (PHB) FROM BIOMASS OF *Scenedesmus* sp. BY *Priestia megaterium*

ABSTRACT

The high cost of carbon substrate for microbial production of polyhydroxybutyrate (PHB) currently remained as one of the obstacles in the industry, hampering the transition of conventional plastic to bioplastics. To address this situation, the biomass of *Scenedesmus* sp. was investigated as an alternative carbon substrate for PHB production by *Priestia megaterium*. First, carbohydrate content from biomass of *Scenedesmus* sp. was extracted using hydrothermal dilute-acid pretreatment. Among different H_2SO_4 concentration, temperature and treatment duration that were studied, it was found that pretreatment with 4% (v/v) H_2SO_4 at temperature of 105°C for 30 min has released maximum total carbohydrate and total sugar of 10.66% (w/w) ± 0.05 and 8.11% (w/w) ± 0.08, respectively. Then, the growth of *P. megaterium* and the production of PHB in medium using sugar hydrolysate from *Scenedesmus* sp. (PM-MH) was evaluated for 120 h and compared to medium using glucose (PM-GLU). The maximum CDW of *P. megaterium* in PM-MH was 3.41 g/L, and total sugar consumed was 83.78%, which were comparable to PM-GLU (3.56 g/L, 75.7%). However, PHB yield in PM-MH was lower than PM-GLU, with value of 0.74 g/L and 1.51 g/L, respectively. Lastly, the properties of PHB produced from PM-MH (PHB-MH) was characterized and compared to the commercial PHB (PHB-C). FTIR analysis of PHB-MH confirmed the presence of functional groups unique to PHB, meanwhile DSC analysis result revealed the melting point (T_m) of PHB-MH to be 153.45°C, which is lower compared to PHB-C. All these findings demonstrate the potential use of microalgae as alternative carbon source in the production of PHB.

CHAPTER 1

INTRODUCTION

1.1 Research background

In the latest report published by Plastics Europe (2024) global production of plastics in 2023 amounted to 413.8 million metric tons (Mt), significantly higher than the total production reported in 2021 (394.2 Mt) and 2022 (400 Mt). If this trend is to continue, it is predicted that by 2050, the production volume of plastics will reach up to 589 Mt (Statista, 2020). Continuous demand for plastics is largely contributed by its affordability and outstanding properties that allow it to be used in diverse applications. This might be a good sign for economic growth, but the widespread use of conventional plastics is linked to many problems, notably the plastic pollution crisis.

Besides the fact that its main raw material comes from non-renewable resources, conventional plastics are also deemed as non-biodegradable because they undergo a very slow decomposition process in natural settings due to the complexity of their chemical structure (Chamas et al., 2020). Nowadays, discarded plastics can be found everywhere as a result of inefficient management of the waste. They accumulated and persisted in the environment, particularly the marine habitats, and caused the death of many wildlife species due to plastic ingestion and entanglement (Thompson et al., 2009). Concerns regarding the use of conventional plastics also centered around the formation of microplastics and the leaching of toxic plastic additives, which can be exposed to human through ingestion, inhalation, and dermal contact (Veidis et al., 2022). In fact, they have been detected in human body systems and pose potential health consequences like hormonal disruption, obesity, cancer, organ damage, and abnormal fetal growth (Veidis et al., 2022).

Bioplastic is one of the viable solutions to resolve the issues surrounding the use of conventional plastics. This polymeric material is bio-based and biodegradable, and has been developed to attain similar functionalities as conventional plastics (Mushtaq et al., 2024). A variety of bioplastics are already available in the market and have begun to substitute their synthetic counterparts in various applications (Wellenreuther & Wolf, 2020). Among the diverse selection of bioplastics is polyhydroxybutyrate (PHB), a thermoplastic polyester that is predominantly produced by bacteria from renewable feedstock. There is growing interest in PHB as it displays almost similar attributes to polypropylene (PP), one of the most produced plastic polymers (Lackner, 2015; Plastics Europe, 2024). General characteristics of PHB include insolubility in water, biodegradable, biocompatible, non-toxic, and display good barrier properties (Bugnicourt et al., 2014). PHB also can be processed under a wide range of temperatures and can undergo similar processing as conventional thermoplastics (e.g. extrusion, injection molding) (Alves et al., 2017). These properties allow applications of PHB especially in packaging, biomedical, and drug delivery (Naser et al., 2021).

PHB is synthesized as intracellular granules for carbon and energy storage functions. This is in response to physiological stress conditions such as limited access to some essential nutrients (e.g. nitrogen, phosphorus) while under excess of carbon source (Koch & Forchhammer, 2021). Other than bacteria, there are also reported studies on the of PHB by microalgae and higher plants (Al-Khairy et al., 2022). In fermentative production of PHB, the amount of PHB accumulated can vary among species. Higher PHB yield was usually observed in gram-negative bacteria like *Cupriavidus necator*, *Pseudomonas* spp., and recombinant *Escherichia coli* (Rajan et al., 2018). Even though so, the potential use of gram-positive bacteria is equally

promising as evidenced by *Priestia megaterium*, which has been reported to accumulate PHB up to 70% of its cell dry weight (Valappil et al., 2007; Faccin et al., 2009). Also, for biomedical applications, PHB produced by gram-positive bacteria has an advantage over gram-negative bacteria since the former lacks lipopolysaccharides (LPS) endotoxins that can trigger a strong immunogenic response (Valappil et al., 2007).

P. megaterium is a non-pathogenic species and can be found in diverse environments, prominently soil (Bunk et al., 2010). Other than the production of PHB, *P. megaterium* is also notably known for its applications in the production of recombinant proteins and vitamins (Biedendieck et al., 2021), and as a plant growth-promoting agent (Lopez-Bucio et al., 2007). Production and recovery of PHB from *P. megaterium* has been extensively studied and well-established since it was one of the earliest PHB producers discovered (Biedendieck et al., 2021). In addition, *P. megaterium* is recognized for its ability to utilize a wide range of carbon sources, either pure substrates (e.g. glucose, sucrose, fructose) or alternative feedstocks (e.g. sugarcane molasses, glycerol, cheese whey). There have been significant efforts to explore the potential use of alternative feedstocks with the aim of reducing the production cost of PHB (Lopez et al., 2012). Pure substrates obtained from carbohydrate-rich feedstocks like sugarcane, corn, sugar beet, and cassava are literally expensive compared to the cost of raw material for conventional plastics and conflicting with the use of arable lands for food crop production (Wellenreuther & Wolf, 2020). On the contrary, alternative feedstocks are cheaper because they originate from non-food biomass crops, agricultural residues, and industrial waste streams (Lovett et al., 2022).

In that context, microalgae also have the potential to be utilized as an alternative feedstock for the production of PHB. Microalgae biomass has long been recognized as a valuable feedstock in biorefinery, particularly for the production of bioenergy (e.g. biodiesel, bioethanol) and other value-added products including bioplastics (Ezhumalai et al., 2024). This is because, microalgae biomass is a rich source of biomolecules, especially protein, carbohydrate, and lipid (Parakh et al., 2023). Cultivation of microalgae is relatively simple and flexible compared to conventional plants. It doesn't need arable land, can be grown year-round, and biomass can be harvested in a short period of time due to its short life cycle (Barsanti & Gualtieri, 2018). *Scenedesmus* sp. is one of the most commonly studied microalgae species and is seen as a promising prospect to be used as feedstock for the production of PHB. This species is resilient to harsh environments, has high productivity, and can accumulate high carbohydrate content (Ishaq et al., 2016). Production of *Scenedesmus* sp. biomass also can be integrated with the bioremediation of wastewater effluents or obtained as residual biomass from other biotechnological applications (Moreno-Garcia et al., 2017).

In this study, the potential use of sugar hydrolysate from *Scenedesmus* sp. as the main carbon source for *Priestia megaterium* to produce polyhydroxybutyrate (PHB) was evaluated. Hydrothermal dilute-acid pretreatment was employed to obtain the maximum release of carbohydrate content from *Scenedesmus* sp. biomass, and the obtained microalgae hydrolysate was used to feed *P. megaterium* for the production of PHB. Lastly, the properties of the PHB produced by *P. megaterium* were characterized.

1.2 Problem statement

PHB is being produced at industrial scale, but still not comparable to the production capacity of conventional plastics (Alves et al., 2017). Despite the growing demand and promising future for PHB, the commercial production of PHB is considered as small-scale with capacity of around 1,000 to 20,000 metric tons annually (Akkoyunlu et al., 2024). This is because, it is restricted by high production cost due to the use of pure substrate as feedstock, which makes up about 50-60% of the total production cost (Akkoyunlu et al., 2024). Therefore, there is a need to find inexpensive feedstock to ensure production of PHB can be scaled up and become economically feasible to compete with conventional plastics.

The potential use of carbon substrates from *Scenedesmus* sp. should be explored since the information on the exploitation of microalgae biomass for production of PHB is scarce even though there are wide variety of microalgae species available to be studied. Suitable pretreatment strategy for extraction of carbohydrate content from *Scenedesmus* sp. should be determined too because response of microalgae towards pretreatment are varies depending on species. Though by using *P. megaterium* as PHB producer will guarantee a promising result, there is no reported studies yet on the utilization of microalgae hydrolysate by *P. megaterium* for production of PHB. Most importantly, the selection of producing microorganisms and the choice of carbon sources are both important factors as they can influence the yield and properties of PHB to be obtained.

1.3 Research objectives

1. To determine the pretreatment strategy for maximum release of carbohydrate content from *Scenedesmus* sp. biomass using hydrothermal dilute-acid pretreatment.
2. To evaluate the production of PHB by *Priestia megaterium* fed with sugar hydrolysate extracted from biomass of *Scenedesmus* sp.
3. To characterize the properties of PHB produced by *Priestia megaterium* fed with sugar hydrolysate extracted from biomass of *Scenedesmus* sp.

1.4 Scope of study

1. This study focused on the use of *Scenedesmus* sp. biomass as main carbon source for *P. megaterium* to produce PHB. The amount of total carbohydrate content can be released from lyophilized biomass of *Scenedesmus* sp. through hydrothermal dilute-acid pretreatment using H_2SO_4 as hydrolytic agent was evaluated, by studying the effect of acid concentration, temperature and treatment duration.
2. The effect of glucose and sugar content from microalgae hydrolysate on production of PHB by *P. megaterium* were compared by assessing the value of CDW, carbon source consumption, PHB concentration and stoichiometric parameters. The experiment was conducted for 120 h in batch fermentation using shake flask.
3. The PHB extracted from dried biomass of *P. megaterium* was further characterized by conducting three different analyses (i.e. Spectroscopic, FTIR, DSC) to determine the purity of the extracted PHB by comparing with commercial PHB. Results from these analyses were also used to detect any changes in the PHB properties when using microalgae hydrolysate as main carbon source.

1.5 Thesis outline

In this thesis, all works and data from the present study were organized into five separate chapters. Chapter 1 (Introduction) focused on the research background, and further supported with problem statement, research objectives and scope of the study. In Chapter 2 (Literature Review), in depth review of information related to the components of the study were provided. This includes fundamental knowledge on conventional plastics and problems associated with it. Bioplastics, specifically polyhydroxybutyrate (PHB) was highlighted as a substitute to conventional plastics. The use of *Scenedesmus* sp. as alternative feedstock for production of PHB were investigated by focusing on cultivation, harvesting and pretreatment of microalgae biomass. Also, production of PHB by *P. megaterium* was assessed from various aspects such as the synthesis pathway, carbon sources preferences and recovery method. Then, the overall research flow of this study was presented in Chapter 3 (Materials and Methods). All the experimental works conducted were described in detail, mainly the procedure, chemicals and equipment involved. In Chapter 4 (Results and Discussion), all results regarding potential use of *Scenedesmus* sp. biomass as feedstock for *P. megaterium* to produce PHB were compiled and discussed. This includes effect of pretreatment on the release carbohydrate content from *Scenedesmus* sp. biomass, comparison between the use of pure substrate and sugar hydrolysate of *Scenedesmus* sp. on production of PHB, and characterization of the extracted PHB. Lastly, all the works were summarized in Chapter 5 (Conclusions and Future recommendations). Suggestions for future study and aspects that can be improved were also included in the chapter.

CHAPTER 2

LITERATURE REVIEW

In this chapter, background information on conventional plastics and bioplastics will be first elaborated to shed more lights on the relevance of this study. Polyhydroxybutyrate (PHB) was highlighted as biopolymer of interests to be pursued. Then, microalgae were introduced as potential feedstock to be used in the microbial production of PHB. Production of PHB was discussed in terms of using *Priestia megaterium* as the producer, the cultivation conditions, recovery of PHB and characterization of the PHB produced. By end of this chapter, the direction of this present study can be seen in bigger picture with much better understanding.

2.1 Conventional plastic

Plastic is one of the widely used materials that makes almost everything we find around us, from household items and food packaging to electrical appliances and building materials. Plastics has a great reputation as a versatile material and even dubbed as the material of the future (Chalmin, 2019). This is owing to its excellent properties which are lightweight and durable, poor conductor of heat and electricity, works in wide range of temperature, ductile and can be easily molded, and resistance to chemical. Besides, production cost of plastics is also lower compared to other type of materials (e.g. steels, woods, rubber, glass), thus make it cheaper and more accessible to consumers (Andrady & Neal, 2009). In the following section of this subtopic, the term 'conventional plastics' will be used in reference to plastic products that are used nowadays, which typically made from fossil fuels and resist degradation.

Conventional plastic is a synthetic polymer which are produced under highly controlled conditions instead of occurring naturally. Industrial production of conventional plastics can be divided into two main stages: production of raw material and transformation into finished products (Larson, 2015). During the first stage, raw materials like crude oil, natural gas and coal will be subjected to series of refining process (e.g. distillation, steam cracking) to obtain olefins (e.g. ethylene, propylene, butylene) (Haribal et al., 2018; Almuqati et al., 2024). Olefins molecules are small unsaturated hydrocarbons which will serve as building blocks (i.e. monomers) that make up the backbone and side chains of the plastic polymers. Main components of the monomer units are carbon and hydrogen atoms, but other elements also can be introduced in the monomer unit such as oxygen, nitrogen, sulfur, fluorine or chlorine (Klein, 2012).

The monomer units will be chemically joined to form hydrocarbon chains known as polymer molecules. These polymer molecules can either be linear or branched. For the polymerization reaction to occur (i.e. addition, condensation), it must be conducted under high temperature and pressure with addition of catalysts and additives (Bittner et al., 2014). The length of individual polymer molecules are varies depending on the degree of polymerization (Balani et al., 2015). Final product that will be obtained from this process are resins, which made up of polymer molecules mixtures that are bonded together by intermolecular forces. Molecular weight of resins are determined from average molecular weight of the polymer chains it consisted of. Resin with higher molecular weights has greater strength, toughness and viscosity due to increase entanglement of the polymer chains. Resin is the core ingredient for all plastic products, and usually, additives and fillers (e.g. flame retardants, antioxidants, dyes and pigments) are added to the base resin to improve and impart specific

properties desirable for the final products (Pathak et al., 2023). Sometimes, different type of plastic resins is also mixed together (refer Figure 2.1). Lastly, resin will be transported to the plastic manufacturing plants in the form of semi-solid or pellets for conversion into finished products.

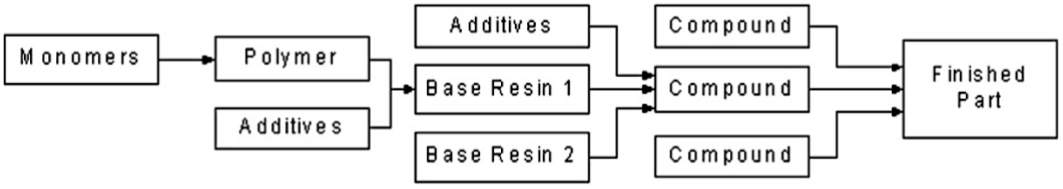

Figure 2.1 General process flow of plastic manufacturing (Bittner et al., 2014).

The first synthetic plastic to be produced dated back in 1907, when bakelite was created by polycondensation of phenol with formaldehyde. Then, more type of resins was invented in the following decades, with more sophisticated attributes and functionalities. However, mass production of plastic only happened around 1950s, after the Second World War (Chalmin, 2019). Since then, the global production of plastics accelerates significantly from 1.7 Mt in 1950, to 413 Mt in 2023 (Plastics Europe, 2011; 2024), with market valuation at USD 712 billion (Statista, 2025). The plastics industry, including both production sector (i.e. raw materials to resins) and conversion sector (i.e. transformation into finished products), has become one of the world's biggest industries. Currently, China is the largest producer of plastics, accounting 31% of global production, followed by other Asia countries, Europe and North America (Burelo et al., 2023). Almost 60% of plastic produced are used in packaging, building and construction, and automotive sectors (Recalde Salas, 2019). Main factors that contribute to the increasing demand including increase in global

population size, rapid urbanization and modernization, and advancement of industrial technology (Kibria et al., 2023).

2.1.1 Properties, classification and applications

There are many types of plastic resins which are differentiated according to their monomers make up and processing method. Each portrays unique characteristics and have distinct properties. Regardless of that, all type of plastic resins are able to flow into a desired shape when heat and pressure are applied to it and to retain the shape when they are withdrawn. Plastics can be extruded as sheets or pipes, painted on surfaces, or moulded to form countless objects (Larson, 2015). Theoretically, all conventional plastics are generally degradable, but they have a very slow breakdown, hence considered non-biodegradable (Coppola et al., 2021). In general, plastics are categorized into two main groups, namely thermoplastics and thermosets. The categorization is based on the differences in chemical bonding between the polymer chains resulting in different properties. It is important to be able to distinguish these two types of plastics as it determines their processing, handling and applications.

Thermoplastics are made up of linear or branched polymer chains that are held together by weak intermolecular forces. This type of chemical bonding can be broken down easily when heated, therefore allowing the material to melt. When cooled down, it will solidify back again, and this cycle can be repeated several times. It is the most commonly used type of plastic as statistics has shown that the five major types of plastic resins which accounted the largest share of overall plastics production are thermoplastics (Singh et al., 2022). They are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and acrylonitrile butadiene styrene (ABS) (Singh et al., 2022). Thermoplastics are used for a wide range of applications, especially for packaging due to its moldability. Thermoplastics waste also can be

recovered and refabricated by application of heat and pressure. In simpler words, they can be recycled.

Meanwhile, thermosets are made up of polymer chains that are cross-linked between each other which locked the molecules into a rigid structure. Crosslink is a permanent chemical bond that cannot be broken down by heat, thus thermosets material cannot be melted once it was solidified and take shape. The process is irreversible, and the resin cannot be softened and might be degraded if high heat is applied persistently. Thermosets also insoluble in almost all inorganic solvents. Even though so, it makes thermosets to be tougher and stable compared to thermoplastics. Thermosets are mostly utilized to manufacture products that requires stability for long-term usage such as building materials and automotive components. Examples of thermoset resins are polyurethane (PU), epoxy resins, phenolic resins, and urea and melamine formaldehyde. However, thermosets are more brittle than thermoplastics and cannot be reclaimed from wastes. Table 2.1 compiled the applications for major type of plastic resins for both thermoplastics and thermosets.

Table 2.1 Properties of different type of plastic resins and their respective applications.

	Type of resins	Physical properties		General applications	References
THERMOPLASTICS	Polyethylene (PE)	HDPE:	Opaque, high stiffness	Milk and detergent bottles, fuel tanks, pipes	Burelo et al., 2023
		LDPE:	Translucent, high flexibility	Plastic bags and packaging, flexible toys, thin films, squeeze bottles	Burelo et al., 2023
		LLDPE:	High elasticity, tough, flexible, transparent	Surface coatings, medical appliances, electrical appliances insulators	An et al., 2022
	Polypropylene (PP)	Translucent, high stiffness and melting point, excellent chemical resistance		Rigid packaging, food containers, textiles, medical components, sports equipment	Hossain et al., 2024; Maddah, 2016
	Polyvinyl chloride (PVC)	Good insulators, high chemical resistance, good adhesive properties		Pipes, cabling, flooring, automotive interiors, packaging with printing	Lewandowski & Skórczewska, 2022; Mnyango & Hlangothi, 2024
	Polyethylene terephthalate (PET)	Strong and durable, high chemical resistance, high tensile strength		Packaging for food and beverages, composite fibre materials	Dhaka et al., 2022
	Polystyrene (PS)	High refractive index, easily fabricated		Disposable cutlery, protective packaging	Arfin et al., 2014
	Acrylonitrile–butadiene–styrene (ABS)	High toughness and durability, low to high surface gloss, dimensional stability		Musical instruments, safety helmets, telephone sets, automotive interior	Pious & Thomas, 2016
	Polyamide (Nylons)	Strong, elastic, wear and corrosion resistant		Fibers (e.g. fishing line, carpeting, ropes), toothbrush bristles, light duty gears	Ashby, 2013
	Polycarbonate (PC)	Transparent, stiff, hard, tough		Safety helmets, riot shields, transparent roofing, glazing panels	Ashby, 2013
THERMOSETS	Polyurethane (PUR)	Flexible, high chemical resistance		Coating adhesives, sealants, car seats,	Maurya et al., 2023
	Phenolics	Stiff, resist heat and corrosion		Electronic components, brake pistons	Ashby, 2013
	Epoxy resins	High chemical resistance, strong adhesion		Pultruded rods, furniture, electronics	Dallaev et al., 2023
	Unsaturated polyester (UP)	Depending on additional materials used		Bowling balls, boats, sewer pipe gaskets, surface gel coatings, laminated structures	Ashby, 2013

2.1.2 Downside of conventional plastics

The production of conventional plastics is not sustainable because fossil-fuels are non-renewable resources. Although fossil fuels reserves are increasing due to new exploration and discovery, but it will soon deplete because there is end dates to this energy resources, and it can only be extended every time there is new reserve discovery (Abas et al., 2015). For plastics production, it has been estimated that 20% of total oil consumption need to be allocated for this industry by 2050 (Di Bartolo et al., 2021). Indirectly, this will create a competition for fossil fuels as source of raw materials since it is also used to produce gasoline, which fuels most of light vehicles in the world right now. Manufacturing of plastics from raw material to final products also consumes a bulk share of fossil-based energy (Lackner, 2015). The tendency of fossil fuels price to fluctuate and unstable global political situations are among factors that should not be overlooked too (Bernard, 2014).

The biggest concern on the use conventional plastic is plastic pollution. This global scenario is largely contributed by increasing in plastics production and consumption, especially single-use plastics, which has led to mounts of generated plastic wastes that are known to resist degradation and will eventually persist in the environment. Plastics cannot be degraded by microorganisms due to chemical properties like high molecular weight, high degree of crystallinity and high hydrophobicity, which are too complex for microbial digestion (Atiwesh et al., 2021). As a result, it greatly reduce the degradability of plastics. Although about 60% of plastics waste are disposed to landfill, there are considerable amount have escaped these disposal routes due to mismanaged of waste and littering (Hong et al., 2021; Rosenboom et al., 2022). These wastes are clogging the drainage systems and can caused severe flood during heavy rain. Landfilling also not a sustainable method as

plastic waste can become integrated with the soil matrix and changing the soil structure (Kibria et al., 2023). As a matter of fact, plastic wastes also the major sources for marine pollution. The buoyancy of plastics allows it to float and travel further into the ocean by wind and turbulences (Kibria et al., 2023) and forms garbage patch. Large number of marine wildlife are killed due to plastic waste ingestion and entanglement.

Conventional plastics contribute to the release of greenhouse gaseous (GHGs) and toxic chemicals throughout its lifecycle, namely production, transportation, usage and disposal (Williams & Rangel-Buitrago, 2022). Additives used in plastics are released to the air during manufacturing which can be hazardous to living organisms near the manufacturing plant. The mode of transportation for the raw materials are very risky too (i.e. spills, collisions, explosions) because they are transported daily by pipelines, ships, trains and tanker trucks (Williams & Rangel-Buitrago, 2022). Plastic additives (e.g. BPA, phthalates) are highly mobile chemicals that can easily migrate out from plastic materials during usage, thus exposing humans to high level of these toxic chemicals without realized (Maddela et al., 2023). Incineration process also releases GHGs and emit harmful fumes (volatile chemicals) like dioxins, polychlorinated biphenyls and furans (Kunwar et al., 2016; Acharjee et al., 2023). Due to lack of awareness, open burning of plastic wastes is still practiced in certain places, which results in the releasing of poisonous gases into the environment (e.g. dioxins, polychlorinated biphenyls) (Pathak et al., 2023).

The discovery of microplastics (MPs) has proven that conventional plastics are posing threats to environments and living things regardless of size. In general, MPs are plastics that are measured smaller than 5 mm (Schmid et al., 2021). The primary source of MPs comes from manufactured products which initially has MPs as part of their ingredients (e.g. personal care products), while secondary source of MPs are the

results of unintentional fragmentation of plastics products or wastes in the environment (S. Ghosh et al., 2023). MPs can act as reservoir to pathogens, pollutants and chemical contaminants (Acharjee et al., 2023). Due to their size and high dispersion capabilities, the ubiquitous presence of MPs goes unnoticed in the aquatic environment, air and soils (Chaudhry & Sachdeva, 2021). As a result, there are high tendency of MPs to be consumed (e.g. food, water source) and inhaled by living organisms. Several health conditions have been linked with presence of MPs in human's body system such as cancer, respiratory issues, cardiovascular disease and reduced fertility (Ghosh et al., 2023).

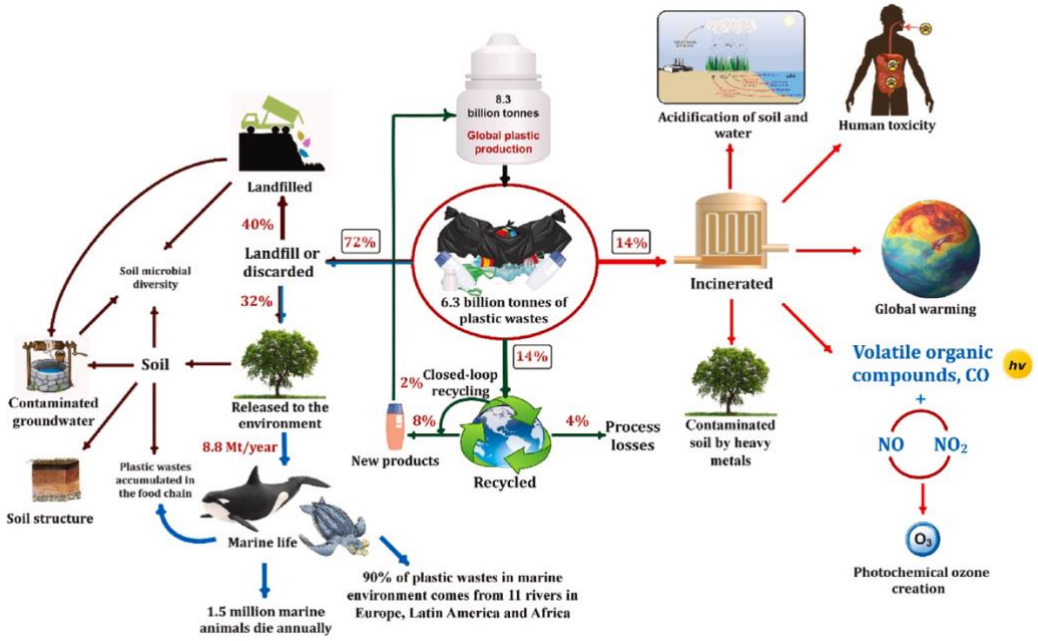

Figure 2.2 Schematic diagram on negative impacts of plastic pollution (Evode et al., 2021)

2.1.3 Strategies in mitigating impacts of conventional plastics

There has not been a single effective solution yet to curb the issues related with conventional plastics. Currently, most of plastic wastes are disposed to municipal solid waste landfills (MSWLFs) together with other domestic wastes. Landfilling is the primary method used worldwide in handling plastic waste due to its cost-effectiveness

and simplicity although linked with several issues (Yaashikaa et al., 2022). However, many landfills are reaching their limit and construction of new landfill sites are becoming more difficult due to land scarcity (Kamaruddin et al., 2017).

Another route where plastic wastes have been directed to is recycling. During recycling, the collected plastic wastes will be sorted, cleaned and undergoes transformation into secondary materials which can be reused to create new products (Pilapitiya & Ratnayake, 2024). There are three major recycling methods, namely, mechanical, chemical and thermal recycling (Pilapitiya & Ratnayake, 2024). Mechanical recycling is the most traditional method and low-cost which plastic wastes will be processed (e.g. shredded, melted) into pellets to be used for production of new plastic products. Chemical recycling involves depolymerization of the plastic waste into their respective monomers or converting the hydrocarbon content into energy (e.g. fuel, gas). Thermal recycling utilized combustion and incineration to completely transform plastic waste into energy (e.g. heat, steam, electricity). Overall, recycling helps to reduce consumption of fossil fuels for plastics production by reusing the old materials (Evode et al., 2021). Besides that, volume of plastic wastes disposed to landfills also can be lessened. Despite the advantages, statistics showed that less than 10% of plastic wastes are actually recycled (Hong et al., 2021).

Most countries have enforced policies that focused on the bans of single-used plastics (Knoblauch & Mederake, 2021). In addition to that, pricing mechanism, plastic taxing, regulation on plastic waste disposal and management (e.g. segregating plastic waste), and educational campaign also part of the ongoing efforts or in the process of implementation by the governments. There are also initiatives on cleaning the plastic wastes that are polluting freshwater and marine habitat through innovative inventions like interceptor, sea bin and ocean clean up array (Kibria et al., 2023).

Replacement of conventional plastics with bio-based and biodegradable materials like bioplastics, paper, and reinforced natural fibers (e.g. bamboo, kenaf) for several applications have been increasing significantly too (Moshood et al., 2022).

2.2 Bioplastics

Bioplastics can be defined as polymeric materials that are either bio-based or bio-degradable or belong to both parts (Singh et al., 2022), which means fossil-based biodegradable plastic and non-biodegradable bio-based plastics will also fall into the category. Bio-based material is derived from renewable resources, while biodegradable material means that it can be broken down in natural environments by microorganisms such as bacteria and fungi (Singh et al., 2022). The term 'bioplastic' is quite ambiguous and has caused confusion to the general public which tend to assume that all bioplastic materials are bio-based and biodegradable. Therefore, bioplastics that will be referred for the rest of this chapter is those that are produced from renewable resources and capable to biodegrade.

In general, there are three routes in the production of bioplastics (Di Bartolo et al., 2021). First is bioplastics that are produced by polymerization of bio-based monomers (e.g. PLA, PGA, PBS). For example, polylactic acid (PLA) monomer, lactic acid, can be produced through microbial fermentation using sugars derived from agricultural waste (Flieger et al., 2003). Second, production of bioplastics from modification of naturally occurring polymers (e.g. TPS, cellulose, chitin, chitosan). Starch of different sources (e.g. cassava, corn, potatoes, wheat) can be made into thermoplastic starch (TPS) by modification with plasticizers and additives and undergo processing like kneading, heating and extrusion (Zhang et al., 2014; Coppola et al., 2021). Lastly, bioplastics also can be produced by extracting biopolymers from microorganisms fed with feedstock from renewable sources. Polyhydroxyalkanoates

(PHAs) are intracellular storage compounds which can be accumulated by certain microorganisms in large amounts.

There is a great diversity of bioplastics, mainly differentiated by their monomer composition and physicochemical properties (Vicente et al., 2023). Bioplastics like PLA and PHB display comparable mechanical properties to few types of conventional plastics (e.g. PP, PET, PS) in term of tensile strength and flexibility (Boey et al., 2022). Bioplastics also can be developed as a biocomposite by reinforcement with inorganic fillers and natural fibres to improve their mechanical properties without compromising with their biodegradability (Boey et al., 2022). Therefore, it is safe to say that bioplastics are suitable to be used in multiple range of applications currently dominated by its synthetic counterpart. As can be referred to Table 2.2, bioplastics are currently finding their use in the sectors of biomedical applications, packaging and consumer goods, textiles, agricultural, automotive, and transport applications. Currently, bioplastics only account to 1% of the worldwide plastic production. Despite the small production volume, the global market for bioplastics is anticipated for steady growth in the future (Lackner, 2015). The use of bioplastics as packaging materials will positively impact waste management and pollution issues (Vicente et al., 2023). Bioplastics can be a great substitute to conventional plastics in agricultural sectors because of their natural degradation that are safe to surrounding environment and can enhance soil properties (George et al., 2020). Besides, due to its non-toxicity and biocompatibility, presence of bioplastics in human body are literally safe, thus have been used in biomedical (e.g. implants, tissue engineering) and pharmaceuticals (e.g. drug delivery) (George et al., 2020).

Table 2.2 Properties of different type of bioplastics and their respective applications.

Type of bioplastics	Properties	Applications	References
Polyglycolic acid (PGA)	High crystallinity	Surgical sutures, medical materials (e.g. screws, stents, grafts), drug delivery, dental applications, bone and tendon scaffolds	Budak et al., 2020
Polylactic acid (PLA)	Semicrystalline or amorphous structure, excellent processability, great barrier properties	Food packaging, biomedicine and tissue engineering, compost bags, disposable tableware, 3D printing	Ramezani Dana & Ebrahimi, 2022; Hussain et al., 2024
Polybutylene succinate (bio-PBS)	Low melting point (i.e. 115^{o}C), excellent mechanical properties, good thermostability	Beverage cups, textiles, agriculture mulch film	Rafiqah et al., 2021
Thermoplastic starch (TPS)	Has similar properties to PET and PP	Biomedical, mulch films, hygiene products	Carvalho, 2008
Cellulose acetate	Translucent material, good impact resistance, hard and rigid	Textile, photography films, food packaging, cigarette filter tows, membrane materials	Yadav & Hakkarainen, 2021; Syarif et al., 2022
Polyhydroxyalkanoates (PHAs)	Flexible, elastic, non-toxic	Antibacterial packaging films, agricultural nets, tissue engineering, drug carrier, scaffold	Diniz et al., 2023

2.2.1 Raw materials for production of bioplastics

Raw materials used in the production of bioplastics are from renewable sources and have lower carbon footprint than fossil fuels due to the uptake of carbon dioxide during their growth (Di Bartolo et al., 2021). The raw materials can be utilized in two ways; direct conversion into bioplastics or used as feedstock for microorganisms. Wide range of renewable resources have been explored and exploited in bioplastics production. First-generation feedstocks are materials from carbohydrate-rich food crops such as sugarcane, sugar beet, corn, potato, and oily seeds. Second-generation feedstocks are sourced from lignocellulose-rich raw material including wood, forestry residues and agricultural wastes (e.g. wheat straw, bagasse, corn stover, organic waste) (Singh et al., 2022). Third-generation feedstock are derived from algae, municipal waste and industrial waste (e.g. activated sludge, paper mill wastewater, dairy wastewater).

Each source of feedstocks have their own advantages and disadvantages. The second and third generation biomass is more eco-friendly than the first-generation feedstock materials (Singh et al., 2022). First generation feedstocks are raising concerns over their potential competition with food crops for human consumption and the requirement of arable lands for cultivation. They also need to use treated water, fertilizers and pesticide, thus increasing the overall cost of the feedstocks (Singh et al., 2022). Even though so, most of bioplastics products are derived from first-generation feedstock since they have an established and matured technology (Wellenreuther & Wolf, 2020). Meanwhile, lignocellulosic biomass requires suitable cost-effective pre-treatments for decomposition into sugar monomers since they composed of cellulose, hemicellulose, and lignin (Coppola et al., 2021).

2.2.2 Degradation of bioplastics

Disposal of bioplastics are more flexible than conventional plastics because the concern on degradation capacity and toxicity issues have been eliminated (Endres & Siebert-Raths, 2012). Eventually, bioplastics will completely degrade and integrated with the surrounding environment. Degradation of bioplastics mainly involved microbial action (biotic factors), and abiotic factors (e.g. temperature, UV, aerobic or anaerobic). Combination of these two factors and chemical composition of the bioplastics (e.g. molecular weight, crystallinity) will determine the rate of degradation (Pradhan et al., 2020). It can take few weeks, months or years for a complete degradation. Biodeterioration is the first step take place during the process, in which bioplastic waste will be broken down into smaller fractions as a result of biotic and abiotic activity (Di Bartolo et al., 2021). Then, depolymerization will occur as polymer chains are further broken down by enzyme secretion from microorganisms inhabiting the biopolymer. This second step is also influenced by abiotic factors. Last step is assimilation and mineralization in which monomers are consumed by microorganisms, and there will be released of environment gases, organic compounds and salts.

2.2.3 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are a group of linear chain polyesters that can be synthesized by more than 250 bacteria from 92 genera of both gram-positive and gram-negative bacteria (Vicente et al., 2023). PHAs are stored as granule-like inclusions called carbonosomes in the cytoplasmic region, with size about 0.2-0.6 μm diameter. Normally, these intracellular biopolymers are accumulated to serve as energy and carbon storage compounds for the bacteria under stressful condition, when there are nutrients limitation (e.g. nitrogen, phosphorus, oxygen) but in excess of carbon source (Nanda et al., 2022). The use of this polymer by the bacteria is considered a

survival strategy in changing environments. In general, PHAs structure are comprised of hydroxy acid monomers. As can be referred to Figure 2.3, the number of monomers in PHAs polymer chain, x, can be greater than zero and alkyl side chains, *R*, can be varying according to type of PHAs (McAdam et al., 2020). Monomers are linked by an ester bond when the carboxyl group of a monomer unit is reacted to the hydroxyl group of another monomer unit to form PHAs polymer (Surendran et al., 2020). There are over 155 unique PHA monomer subunits, which can be formed into variety of homopolymers, random copolymers and block copolymers (Yanez et al., 2020). The composition, physicochemical properties and thermal properties of PHAs polymer are influenced by the species of bacteria used, the carbon substrates and cultivation condition (Park et al., 2024).

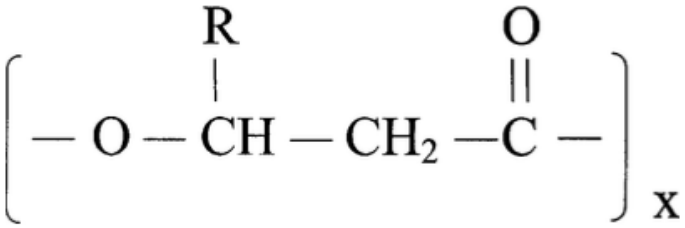

Figure 2.3 General structure of PHAs (Reddy et al., 2003)

PHAs can be further subdivided according to the number of carbon atoms that made up the monomer units. Monomers of short chain length PHAs (scl-PHA) consisted of three to five carbons, monomers of medium chain length PHAs (mcl-PHA) consisted of six to 14 carbons, and monomers of long chain length PHAs (lcl-PHA) consisted of more than 15 carbon atoms (Samrot et al., 2021). However, commonly described PHAs in literature are scl-PHA and mcl-PHA, particularly polyhydroxybutyrate (P(3HB)), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(3HB-co-3HV)), poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-

4HB)), and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(3HB-co-3HHx)). The molecular weight of PHAs are in the range of 50,000–1,000,000 Da and can vary according to the PHAs producer, conditions of growth, and extraction method (Reddy et al. 2003; Rajan et al. 2018).

PHAs have high melting temperature and low permeability to O_2, H_2O, and CO_2 (Rajan et al., 2018). PHAs also soluble in halogenated solvents like chloroform (Nanda et al., 2022). Main advantage of PHAs over other type of bioplastics is its ability to biodegrade in both aerobic and anaerobic condition (Garcia-Depraect et al., 2022). Degrading microorganisms will secrete enzymes (i.e. depolymerase) that break down PHAs into its molecular building blocks called hydroxy acids, which then utilized as their carbon source. Biodegradation of PHA under aerobic conditions results in carbon dioxide and water, whereas in anaerobic conditions the degradation products are carbon dioxide, water and methane (Pradhan et al., 2020).

Other than bacteria, PHAs also can be produced by plants and microalgae but the PHAs yield are very low (Verlinden et al., 2007). As for bacteria, they accumulate PHAs at low concentration (i.e. 1–15%) under normal growth condition, but the value can increase up to 90% of cell dry weight under special growth conditions and fermentation strategies (Alves et al., 2017). Certain PHAs producers are able to biosynthesize copolymers from single or mixed substrates (Surendran et al., 2020). PHA copolymers are composed of more than one type of monomer, for example, P(3HB-co-3HV) which comprised of 3-hydroxybutyrate and 3-hydroxyvalerate monomers (Rivera-Briso & Serrano-Aroca, 2018). Interestingly, PHAs structures also can be tailored by altering the monomers' structure (e.g. side chain) (Al-Khairy et al., 2022).