BIOSYNTHESIS AND OPTIMIZATION OF P(3HB-*co*-3HV-*co*-4HB) TERPOLYMER USING GLYCERINE PITCH AS CARBON SOURCE

ABBAS MUSA IBN

UNIVERSITI SAINS MALAYSIA

2024

BIOSYNTHESIS AND OPTIMIZATION OF P(3HB-*co*-3HV-*co*-4HB) TERPOLYMER USING GLYCERINE PITCH AS CARBON SOURCE

by

ABBAS MUSA IBN

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

November 2024

ACKNOWLEDGEMENT

I am grateful to Almighty Allah (S.W.T) for helping and providing all I need to make this research work possible. I would also like to thank my supervisor, Professor Dato.(Dr.) Amirul Al-Ashraf Abdullah, for the excellent guidance throughout my Ph. D. program, who gave his enormous support, help, and patience during this research work and whose editorial assistance and enthusiasm kept me modified from hour to hour, especially in terms of paper presentations, laboratory work and academic inspirations. I will forever remain grateful for your kindness. Similarly, I am also thankful for the assistance rendered by a senior colleague, Dr Shehu Idris, and other lab mates, including Noor Aida Omar, Aiman Hakimi, Rozina Kakar, NurHanani, Atikah and Intan. You people have made my stay in the U.S.M. very memorable.

I want to express my unreserved gratitude to Prof. Aliyu Shugaba, Prof. Abubakar Gidado, Prof. M. A. Milala, and Prof. B.H. Kabura at the University of Maiduguri, who proffered educational and moral support that spiced this research work to be what it is. May Allah reward you all. I am highly indebted to my parents, who always considered me like an infant. Your spiritual, moral, and academic support is beyond measure. May Allah continue to increase us in faith, health, wealth, and everlasting peace and reward you far beyond measure. My special thanks go to my wife, Halima Bukar Kabura, for her love, understanding, prayers, patience, and support throughout the program; you are a motivator. May Allah fulfill the goodness of your heart's desire and increase your faith and knowledge. To my lovely wife and children Abubakar (Haneef), Zaynab (Ummulkhair), and Hafsat, who endured the absence of their father for quite a long time, I love you all. You people have brought great joy into my life. On this note, I would like to express my gratitude to my siblings; they all have been excellent throughout this period. I wish you prosperous life in this world and hereafter.

Moreover, the effort and contributions of my senior colleagues are enormous, and I sincerely appreciate the likes of Dr M. A. Isa, Dr H.Y. Ismail, Dr Ibrahim Allamin Alkali, Dr Adam Mustapha, and others that I could not mention. Thank you.

My sincere appreciation goes to the Government of the Federal Republic of Nigeria, specifically to the University of Maiduguri Management, for providing sponsorship through TETFund AST&D intervention.

Thank you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF SYMBOLS

α	Alpha
β	Beta
^{13}C	Carbon 13
°C	Degree Celsius
θ	Theta
~	Approximately
C_L	Dissolved oxygen concentration
γ	Gamma
ΔH_m	Heat of Fusion
J/g	Joule per gram
K/g	Kilogram
µg	Microgram
µL	Microliter
µm	Micrometer
Mol%	Mol Percent
%	Percent
M_w/M_n	Polydispersity index
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
wt %	Weight Percent

LIST OF ABBREVIATIONS

ABI	Application binary interface
Abs	Absorbance
AR	Analytical grade
ANOVA	Analysis of variance
ASTM	American society for testing and materials
bp	Base pair
C	Carbon
CEAP	Circular economy action plan
C/N	Carbon/nitrogen ratio
CoA	Coenzyme A
CDW	Cell dry weight
CCD	Central composite design
CME	Caprylic methyl ester
CFU	Colony-forming unit
CoA	Coenzyme A
Da	Dalton
dH_2O	Distilled water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acids
DSC	Differential scanning calorimeter
EDTA	Ethylene diamine tetra acetic acid
FFAs	Free fatty acids
FAME	Fatty acid methyl esters
FID	Flame ionization detector
GC	Gas chromatography

G	Gram
GC-MS	Gas chromatography with mass spectrometry
GPa	Gigapascal
GP	Glycerine pitch
GPC	Gel permeation chromatography
H	Hydrogen
Hr	Hour
HMDS	Hexamethyldisilazane
3HB	3-Hydroxybutyrate
3HV	3-Hydroxvalerate
IS	Internal Standard
kbp	Kilo base pairs
kDa	Kilo Dalton
Kg	Kilo gram
L	Litre
Ltd.	Limited
scl-PHA	Short-chain length polyhydroxyalkanoates
min	Minutes
mM	Millimolar
mm	Millimetre
*M*n	Number-average molecular weight
Molar	Molar
MPa	Megapascal
Mcl-PHA	Medium-chain length polyhydroxyalkanoates
MSM	Mineral salt medium
MT	Metric tonne
M_w	Average molecular weight

NA	Nutrient agar
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate reduced.
NaOH	Sodium hydroxide
NB	Nutrient broth
NCBI	National centre for biotechnology information
Ng	Nanogram
NR	Nutrient-rich
NMR	Nuclear magnetic resonance
OA	Oleic acid
OD	Optical density
PHA	Polyhydroxyalkanoate
PHAs	Polyhydroxyalkanoates
phaA	gene encoding *β*-ketothiolase
PhaB	gene encoding acetoacetyl-CoA dehydrogenase
PhaC	gene encoding PHA synthetase
PLA	Polylactic acid
PE	Polyethylene
POME	Palm oil mill effluent
PP	Polypropylene
PVC	Polyvinylchloride
PS	Polystyrene
PET	Polyethylene terephthalate
PUR	Polyurethane
PTFE	Polytetrafluoroethylene
ppb	Part per billion

ppm	Part per millions
psi	Pound per square inch
PTFE	Polytetrafluoroethylene
rpm	Revolutions Per Minute
RCDW	Residual cell dry weight
rcf (*xg*)	Rotation centrifugation force
RID	refractive index detector
RSM	Response surface methodology
sec	Seconds
SD	Standard deviation
SDGs	Sustainable development goals
SEM	Scanning electron microscope
Sp.	Species
TAE	Tris-acetate EDTA
THF	Tetrahydrofuran
TMS	Tetramethylsilane
T_d	Decomposition temperature
Tg	Glass Transition temperature
*TG*A	Thermal gravimetry analysis
TE	Trace element
Tc	Crystallization temperature
Tm	Melting temperature
TEM	Transmission electron microscope
UV	Ultraviolet
vvm	Volume per volume per minute
XRD	X-ray diffraction

LIST OF APPENDICES

BIOSINTESIS DAN PENGOPTIMUMAN TERPOLIMER P(3HB-*ko*-3HV-*ko*-4HB) MENGGUNAKAN SISA GLISERIN SEBAGAI SUMBER KARBON

ABSTRAK

Plastik konvensional telah menjadi komoditi penting yang memberikan keselesaan melalui aplikasinya dalam industri pembungkusan, perubatan, pertanian dan farmaseutikal. Walau bagaimanapun, plastik tradisional adalah keras terhadap degradasi mikrob. Bioplastik seperti polihidrolisialkanoat (PHAs) adalah bahan biodegradasi dan bioserasi terbukti sebagai alternatif kepada plastik sintetik. Salah satu masalah penting biosintesis PHA ialah kos sumber karbon, yang menyumbang kira-kira 40% daripada jumlah pengeluaran. Penggunaan minyak gred makanan utama adalah tidak ekonomi dan tidak mampan kerana ia mungkin mengehadkan ketersediaan minyak masak dan mencetuskan peningkatan dalam kos minyak, yang membawa kepada persaingan makanan. Sisa gliserin menghasilkan PHA dalam *Cupriavidus malaysiensis* melalui pengkulturan satu peringkat dan dioptimumkan dalam bioreaktor 13 L. Dua monomer lain telah digabungkan dengan penambahan 1,4 butanediol dan 1-pentanol, menghasilkan terpolimer yang terdiri daripada monomer 3HB, 3HV dan 4HB [P(3HB-*co*-3HV-*co*-4HB)]. Kesan kepekatan berbeza sisa gliserin, 1-pentanol, 1,4 butanediol, dan asid oleik telah dikaji. Keputusan awal eksperimen menunjukkan keupayaan bakteria untuk menggunakan dan menghasilkan terpolimer pada kepekatan sisa gliserin, 1-pentanol, 1,4 butanediol dan asid oleik yang berbeza. Kandungan PHA tertinggi diperhatikan dari sisa gliserin 15 g/L, 0.06 wt % C 1-pentanol, 0.25 wt % C daripada 1,4 butanediol dan asid oleik menghasilkan kandungan PHA tertinggi iaitu 79 wt % dan 5.82 g/L daripada berat kering sel (CDW). Kajian pengoptimuman menggunakan kaedah permukaan sambutan telah menghasilkan kepekatan CDW (10.44 g/L) dan PHA yang tinggi (8.93 g/L), yang meningkat dengan ketara masing-masing kepada 45 dan 43%, berbanding dengan kultur sebelum pengoptimuman. Polimer yang terhasil daripada eksperimen dalam bioreaktor mengandungi julat 0-10 mol% 3HV dan 13-19 mol% 4HB. Komposisi monomer polimer ditentukan menggunakan kromatografi gas dan disahkan menggunakan analisis ^{13}C-NMR dan ^{1}H-NMR. Keputusan kromatografi peresapan gel menunjukkan berat molekul (*Mw*) antara 41 hingga 88 kDa dan indeks polidispersiti 2.3-3.5. Terpolimer telah tertakluk kepada analisis terma pengimbasan pembezaan kalorimetri (DSC) dan termogravimetri (*TG*A). Perubahan yang bergantung kepada masa dalam penurunan berat filem PHA telah dipantau. Kadar degradasi P(3HB) dan terpolimer yang disintesis telah dikaji di tasik. Selepas empat minggu, degradasi tertinggi kira-kira 48% b/b direkodkan dalam filem terpolimer dengan komposisi 4HB mol% tertinggi. Semua sampel PHA mempunyai corak degradasi yang sama. Hasil kerja ini menunjukkan keupayaan *C. malaysiensis* untuk menukar sisa gliserin kepada P(3HB-*co*-3HV-*co*-4HB), yang juga menyediakan substrat karbon alternatif yang berjaya mensintesis bioplastik dan salah satu strategi yang berdaya maju untuk pengeluaran polimer berskala industri dan kawalan pencemaran alam sekitar.

BIOSYNTHESIS AND OPTIMIZATION OF P(3HB-*co*-3HV-*co*-4HB) TERPOLYMER USING GLYCERINE PITCH AS CARBON SOURCE

ABSTRACT

Conventional plastics have become an essential commodity that provides comfort through their applications in the packaging, medical, agricultural, and pharmaceutical industries. However, traditional plastic is recalcitrant to microbial degradation. Bioplastics like polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible materials that have proven to be an alternative to synthetic plastics. One of the significant problems of PHA biosynthesis is the cost of carbon sources, which accounts for about 40% of production costs. The use of major food-grade oils is uneconomical and unsustainable as it may limit the availability of cooking oil and trigger an increase in the price of edible oil, leading to food-feed competition. Therefore, glycerine pitch produced PHA in *Cupriavidus malaysiensis* USMAA1020 via single-stage cultivation and optimized in a 13 L bioreactor. Two other monomers were incorporated by the addition of 1,4 butanediol and 1-pentanol, resulting in the synthesis of a terpolymer consisting of 3HB, 3HV and 4HB monomers [P(3HB-*co*-3HV-*co*-4HB)]. Effects of different concentrations of glycerine pitch, 1-pentanol, 1,4 butanediol, and oleic acid were studied. Preliminary experimental results showed the ability of the bacteria to utilize and produce the terpolymer at different concentrations of glycerine pitch, 1-pentanol, 1,4 butanediol, and oleic acid. The highest PHA content was observed in 15 g/L glycerine pitch, 0.06 wt % C of 1-pentanol, 0.25 wt % C of 1,4 butanediol and oleic acid yielded the highest PHA content of 79 wt % and 5.82 g/L of cell dry weight (CDW). An optimization study using Response surface methodology has resulted in high CDW (10.44 g/L) and PHA concentration (8.93 g/L), which

increased significantly to 45 and 43%, respectively, compared to pre-optimized culture. The resulting polymers from the bioreactor contained a range of 0-10 mol% of 3HV and 13-19 mol% of 4HB. The monomer compositions of the polymer were determined using gas chromatography and confirmed using ^{13}C-NMR and ^{1}H-NMR analysis. The gel permeation chromatography (GPC) result showed molecular weight (M_w) ranging from 41 to 88 kDa and a polydispersity index of 2.3 - 3.5. The terpolymers were subjected to thermal analysis differential scanning calorimetry (DSC) and thermogravimetry (*TG*A). Time-dependent changes in the weight loss of the PHA films were monitored. The rate of degradation of the P(3HB) and synthesized terpolymers was studied. After four weeks, the highest degradation of about 48% w/w was recorded in the terpolymer films with the highest 4HB mol% compositions. All the PHA samples had similar patterns of degradation. The results of this work showed the ability of *C. malaysiensis* USMAA1020 to convert glycerine pitch to P(3HB-*co*-3HV-*co*-4HB). Therefore, its provides an alternative carbon substrate that successfully synthesizes bioplastic and one of the viable strategies for industrial-scale production of polymers and environmental pollution controls.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Conventional plastics have become necessary in our lives, resulting in an extensive range of products that combine comfort and quality. Synthetic plastics are unique because they are lightweight, thermostable, crystalline, and easy to mould. The use of plastic materials has grown in the domestic and industrial sectors, outpacing global production by 400 Mt/year, posing serious problems for disposal, ecosystem toxicity, environmental contamination, and human health (Ganesh et al., 2021; Rihayat et al., 2021). The most commonly utilized synthetic plastics today are petroleum-based, such as polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), polyurethane (PU), and polystyrene (PS) have unique, lightweight, thermostable, crystalline, and easy-to-mould properties and are utilized in packaging, medical, agricultural, and pharmaceutical industries (Mohanan et al., 2020; Shah et al., 2008). However, some of these plastics contain toxic residues (e.g., phthalates as plasticizers) that persist in nature, releasing poisons such as vinyl chloride. Microplastics severely harm aquatic organisms, and they end up in human nutrition. Excessive utilization of petroleum-derived plastics leads to environmental contamination and the release of greenhouse gases, contributing to the degradation of the planet's temperature and overall ecological conditions. As a result, there is a growing need for alternative, renewable energy sources and chemical replacements. The utilization of fossil fuel based plastics, which is reducing the world's oil reserves and contributing to plastic pollution due to its problematic waste disposal, is another issue that is currently a problem (Yadav et al., 2020a).

The "linear economy" concept is based on the assumption that resources are plentiful, easily obtainable, and readily accessible, leading to a straightforward approach to waste disposal (Fernandez-Dacosta, 2018). From the 322 million metric tons (MT) of plastics produced worldwide in 2015, as reported by Ryberg et al. (2019), it was found that 6.2 million MT of large plastics (larger than 5 mm) and 3 million MT of small plastics (smaller than 3 mm) were discharged into the environment. This could lead to various detrimental consequences, including pollution, food chain contamination, energy wastage, financial losses, and other devastating effects.

Bioplastics are unique biomaterials. They're polyesters made by various microorganisms grown under multiple nutritional and environmental conditions (Madison & Huisman, 1999). Biomaterials are natural compounds produced and catabolized by various microorganisms and have a wide range of biotechnological uses. They may be absorbed by a wide range of species (biodegradable) and do not harm the host (biocompatible). Bioplastics are bio-based polymers made from organic, renewable resources with a low environmental impact since they are almost all biodegradable. Polyhydroxyalkanoates (PHA) are a particularly intriguing family of bio-based biodegradable polymers made from a wide range of complex organic substrates, including those found in waste streams such as agroindustry effluent and sewage sludge, via bacterial fermentation. As a result, resource recovery from wastewater treatment procedures can play a part in the circular economy of plastics (Mannina et al., 2019). Several microorganisms, mainly bacteria, produce polyhydroxyalkanoates (PHAs) as internal carbon and energy reserve materials (Anderson & Dawes, 1990; Reddy et al., 2003). During bacterial development, these biodegradable polyesters accumulate due to the lack of necessary nutrients like oxygen, nitrogen, phosphorous, potassium or magnesium in surplus carbon conditions (Brandl et al., 1988).

PHA is a kind of polyester made from renewable, biodegradable, bio-based polymers and biocompatible polymers that occur naturally. Microbes such as fungi, algae, and bacteria produce polyhydroxyalkanoate as an energy-storage molecule in the cytoplasm of their cells (Rashid et al., 2021). PHA, polylactic acid (PLA) and polybutylene succinate (PBS) are considered the green polymers of the future because they are expected to gradually replace conventional plastics with similar physicochemical, thermal, and mechanical properties, such as polypropylene (PP) and low-density polyethylene (LDPE) (Kourmentza et al., 2017). PLA and PBS are made by polymerizing lactic and succinic acids, respectively.

The biosynthesis of PHA polymers relies on the category of n-alkanoates used as raw materials (even or odd). For example, biosynthesis of PHB requires even n-alkanoates, but copolymers can only be produced using odd n-alkanoates (Anjum et al., 2016; Kumar et al., 2020). For growth and PHA synthesis, *Cupriavidus necator* utilizes a variety of carbon substrates to yield high cell density culture. The wild-type *C. necator* PHA synthase has a robust polymerizing affinity for Short Chain length (SCL) 3-5 carbon monomers (Bhubalan et al., 2010). PHA have various physicochemical characteristics, ranging from flexible thermoplastic elastomers to stiff thermoplastics, depending on the monomer content and chain length. For example, medium Chain length (Mcl-PHA) is thermo-elastomeric polyester that is soft and flexible with little or no crystallinity, whereas scl-PHAs are highly crystalline, rigid, and brittle. Mcl-PHA exhibits lower glass transition and melting temperatures, lower tensile strength and modulus, and more excellent elongation at break than scl-

PHAs (Muthuraj et al., 2021). Because they exhibit higher refractivity, PHA granules can be visualized using a phase-contrast light microscope, as well as several lipophilic staining dyes like Sudan Black B and lipophilic dyes such as Nile Blue A or Nile Red (Kumar et al., 2020).

Microorganisms can accumulate homopolyesters, copolyesters, or polyester PHA forms mixes (Steinbüchel, 2001). The polyester chain is reported to include more than 150 distinct monomer units (Steinbüchel & Lütke-Eversloh, 2003). As a result, when bacteria grow on a mixture of different precursors, various copolyesters are predicted (Sudesh et al., 2000). PHAs (Manna & Paul, 2000) such as P(3HB) have gained commercial uses because they are biodegradable in the natural environment due to microbial enzyme activity (Anderson & Dawes, 1990). These microbial polyesters are thermoplastics with biodegradable characteristics that can have their physical properties controlled by changing the monomer makeup (Fay et al., 1967; Tyagi et al., 2022).

PHA biopolymers have been garnering increased attention owing to their potential to replace nonbiodegradable polymers and achieve sustainable development by comparing traditional petrochemical polymers with biopolymers made from PHA. Even though petrochemical polymer manufacturing methods are well-established and commercialized at market-competitive costs, they are energy-intensive due to the severe working conditions of high pressure and temperature and organic solvents (Shahid et al., 2021).

The expensive cost of manufacturing PHAs is one of the primary obstacles to their commercial adoption. The price of commonly used polymers, such as PHB, ranges from US$4000/MT to US$15000/MT, while traditionally used polymers cost roughly US$1000–1500/MT (Kosseva & Rusbandi, 2018). The expenses associated with the carbon source, typically the substrate and the culture phase, are factors that likely make the most significant contributions to the overall production expenses of PHA from an economic standpoint (Lee and Choi, 1999). These biopolymers are becoming popular and have emerged as an essential replacement for synthetic petroleum-derived plastics due to their inherent biodegradability and biocompatibility (Morya et al., 2018), eco-friendly manufacturing process, and wide range of applications (e.g., consumable materials and medical sector) (Kwan et al., 2018). The worldwide bioplastics production volume is projected to reach 2.44 MT in 2022, according to projections (Kumar et al., 2020). Microorganisms produces PHA to cope with adverse environmental circumstances, as is widely known (Singh Saharan et al., 2014). PHA is commonly considered biopolymers due to their excellent biodegradability, biocompatibility, and sustainability (Kumar et al., 2020). Biopolymers have been able to mimic the qualities of synthetic polymers, such as moisture resistance, long shelf life, and high tensile strength (Kumar et al., 2020).

PHA-accumulating bacteria are classified into nutritional requirements, nutrient stress, and growth patterns. The primary category includes bacteria like *Ralstonia eutropha, Pseudomonas oleovorans*, and *Pseudomonas putida,* which require restricted resources, including phosphorous (P), nitrogen (N), oxygen (O), and magnesium (Mg) to store PHAs and are unable to biosynthesize PHAs throughout their development phases (Guzik et al., 2014). The buildup of PHAs by the second group of bacteria (*Acaligenes latus,* a mutant strain of *Azotobacter vinelandii*, and recombinant *Escherichia coli*, for example) is unaffected by nutritional constraints. During its development phase, it can accumulate PHAs (Afzal & Hameed, 2015).

PHA fermentation is the biological process of generating PHA. Because PHA accumulates intracellularly, effective PHA manufacturing for high productivity and cheap production cost has been investigated extensively (Huang et al., 2006; Khanna & Srivastava, 2005). Various types of PHA can be generated based on a variety of parameters, such as the carbon sources and precursors utilized, the feeding strategy used during fermentation, and the genetic alteration of bacterial cells (Amirul et al., 2008; Steinbüchel and Füchtenbusch, 1998; Yamane et al., 1996). As a result, depending on the type of PHA generated, it has a wide range of uses.

1.2 Problem Statement

Disposal of combustible wastes like glycerine pitch has been a major problem to the community. Burning the waste can mean converting it into acrolein, a highly volatile compound well-known for its toxicity and very hazardous to life (Hazimah et al., 2003). Conventionally, the most utilized plastics are petroleum-based and are hazardous to ecological habitats; their characteristics are responsible for their extensive use. One of society's most pressing environmental concerns today is the ever-increasing prevalence of plastics, which produces worrisome global waste in the environment. Recently, there has been a shift to biobased plastics such as PHA. Polyhydroxyalkanoates (PHAs) have gotten a lot of interest as a source of biodegradable plastic. However, using carbon feedstock such as sugars, vegetable oils, and fatty acids for PHA production accounts for about 40% of the total production cost of PHA, making it an expensive commodity. However, the biological conversion of glycerine pitch as a potential carbon substrate into microbial polyester would positively impact economic and environmental aspects. Production of P(3HB-*co*-3HV-*co*-4HB) terpolymer using a combination of waste glycerine with the addition of carbon precursor is still limited. It is imperative to study the variable factors affecting the P(3HB-*co*-3HV-*co*-4HB) terpolymer accumulation using glycerine pitch. Therefore, this research aimed to utilize glycerine pitch derived from biodiesel for PHA production. Glycerine pitch is an inedible waste material; its utilization as a carbon substrate may lower PHA production costs.

1.3 Objectives of the study

i- To synthesize terpolymer P(3HB-*co*-3HV-*co*-4HB) through a one-stage cultivation process by *Cupriavidus malaysiensis* USMAA1020 using glycerin pitch under various conditions.

ii- To optimize the production of terpolymer P(3HB-*co*-3HV-*co*-4HB) through response surface methodology.

iii- To characterize the terpolymer produced and determine the terpolymer P(3HB-*co*-3HV-*co*-4HB) environmental degradation.

CHAPTER 2

LITERATURE REVIEW

2.1 Synthetic Plastics

Global plastics production has increased fourfold during the past forty years, and by 2050, it is anticipated to contribute 15% of the world's carbon budget (Geyer et al., 2017; Jambeck et al., 2015). In 2021, approximately 8.4 million tons of plastic waste was generated, with 25.9 ± 3.8 thousand tons released into the oceans (Peng et al., 2021). Plastic pollution is widespread and persistent (Karami et al., 2017; Rochman et al., 2016), especially in the garbage patches of oceanic gyres, and is thought to have an indirect effect on human health, food security, and biodiversity (Rochman et al., 2016). Local restrictions on microbeads and single-use plastic bags, MARPOL, Rio+20, the Honolulu Strategy, and the United Nations Environmental Program's (UNEP) Clean Seas campaign are a few examples of national and international initiatives to minimize plastic pollution (United Nations, 2016; UNEP, 2012). Subsequently, one of the strategies to lessen the environmental impact of the plastics industry is to replace fossil-based plastics with bio-based plastics (Hillmyer, 2017; Weiss et al., 2012; Yates & Barlow, 2013).

The manufacture of plastics, which are necessary for modern economies, is mainly done with fossil as raw materials, which also have negative consequences on the environment apart from reducing fossil fuel (Nicholson et al., 2021). Moreover, worldwide plastic production is projected to continue escalating (Shams et al., 2021). Plastic manufacture has led to environmental worries due to its non-degradability and ends in different environments, such as oceans and landfills (Law & Narayan, 2022). Additionally, despite the significant efforts to raise recycling rates, where recycling capacity has grown to 79% from 2006 to 2016 in Europe, only 31% of total plastics were recycled in Europe in 2016. 27.3% of the plastics are in landfills, while 41.6% are used for energy recovery in industrial processes or electricity production (European bioplastics). Therefore, biodegradable plastics or biologically produced plastics, referred to as bioplastics, will replace synthetic plastics (Kalia et al., 2021; Kumar et al., 2021).

However, about 85% of plastic products can be replaced with bioplastics, but bioplastics produced worldwide only account for 1% (European bioplastics). The manufacture of bioplastics is projected to expand to approximately 2.44×10^6 tons in 2022, out of which only 1.09×10^6 tons are biodegradable (European bioplastics). The expected increase in bioplastic is inadequate compared to the ever-growing demand for plastics worldwide. The manipulation of growth conditions or requirements determines the structure and molecular weights of the PHA; the most utilized types of PHA are PHB and PHBV, among the different kinds of carbon numbers, which form the basis of their classification.

Global challenges like the problem of managing plastic waste, environmental pollution, and resource depletion have recently attracted worldwide attention to sustainable strategies like zero-waste practices, climate-neutral models, and a sustainable circular economy (Gatto & Re, 2021). International initiatives have been put in place to support a sustainable economy and environment, including the European Union Circular Economy Action Plan (CEAP), the Association of Southeast Asian Nations (ASEAN) Framework of Action on Marine Debris, and the United Nations Sustainable Development Goals (SDGs) (Amadie et al., 2022; Walker et al., 2021). The transition from non-renewable, fossil-based or materials chemically synthesized to environmentally friendly or bio-based products, like biofuel, biogas and bioplastic, has been sped up by the appearance and shifting of apparent macro-trends (Vigneswari et al., 2021a; Mahari et al., 2022). Thus, research on the creation of bioplastics and the breakdown of synthetic plastics is expanding quickly. This directly supports the 12th UN SDG for responsible consumption and production while indirectly influencing the 13th, 14th, and 15th UN SDGs regarding weather change and livelihood on land and in the ocean (Amadie et al., 2022; Gatto & Re, 2021; Walker et al., 2021).

Plastics have excellent mechanical and thermal qualities and are lightweight, moldable, moisture-resistant, and generally inert (Al Hosni et al., 2019; Haider et al., 2019). They are also very durable and have other desired properties. However, because of these beneficial characteristics, plastic production, consumption, and trash production have increased at rates that are faster than the rate of plastic deterioration (Kasar et al., 2020; Park & Koo, 2017). Construction, electronics, clothes, automotive, toys, utensils, packaging and throwaway plastic items (such as hotel amenities and takeaway packing) are just a few of the many everyday uses for plastics in the modern world (Filho et al., 2019). According to statistics, PP manufacturing has increased by 18%, LDPE by 17%, HDPE by 14%, PVC by 10%, PET by 9%, and Others by 6%. Journal In 2020, there will be around 370 million tonnes of pre-proof, up from 2 million tonnes in 1950 (Plastics Europe, 2022).

Because of the non-biodegradable qualities and diminishing petroleum resources of plastics, there is an increasing need for research on plastic degradation (Shabbir et al., 2020). For instance, due to their resistance to disintegration, traditional polypropylene (PP) and polyethylene (PE), which are made from fossil hydrocarbons and derived from petroleum, typically take several centuries to decompose naturally (Geyer et al., 2017; Norliyana et al., 2023). Thus, the practically indestructible petroleum-based plastics dominate other materials, which cause long-term pollution and severely threaten the environment, involving species and natural ecosystems (Shabbir et al., 2020; Norliyana et al., 2023).

Apart from the changes observed in the biosphere, such as altered microbial diversity in plastic-contaminated regions, the accumulation of discarded and stray plastics in landfills, ocean garbage patches and incinerators has been exacerbated by the overproduction and slow degradation of plastics (Al Hosni et al., 2019; Arrieta et al., 2014; Giacomucci et al., 2020). The subsequent innovative advancements of bioplastics as one of the alternatives have been driven by global issues and participation of national efforts to tackle and abate plastic pollution (Fauziah et al., 2021; Goh, 2021; Steger, 2021; US Plastics Pact, 2022; Waste and Resources Action Programme 2018). Plastic biodegradation involves the enzymatic breakdown of plastics through the activity of crucial plastic-degrading microorganisms like bacteria, fungi, and algae, which utilize them as a source of energy and carbon assimilation (Norliyana et al., 2023; Waheed et al., 2021). Bioplastics, on the other hand, are plastics derived from biological materials, and some of them are biodegradable, meaning they can be broken down into H_2O and CO_2 or even CH_4 and inorganic mixtures, resulting in additional by-products.

Plastics are classified into different categories based on the materials used in their manufacture. Small fragments of the primary polymer material, monomers, are the first step in manufacturing plastics. Then, the monomers are combined to form large polymer particles through the polymerization process, which is applied to the small molecules (Arrieta et al., 2014). The polymerization process consolidates the monomers to give the polymers their desired physicochemical properties. Flexibility, material strength, colour, durability, erosion protection, modulus-to-weight ratio, conductivity, hardness, processing ease, and cost are the vital physicochemical characteristics considered during plastics manufacture (Ahmed et al., 2018). More than 300 different types of plastic exist, with 60 being the more popular and well-known variants (Evode et al., 2021). Some of the most well-known plastics are high-density polyethylene (HDPE), polyethylene terephthalate (PET), polystyrene (PS), polyvinyl chloride (PVC), extended polystyrene (EPS), polyurethane (PUR), low-density polyethylene (LDPE), PE, PP, and phenolic resin.

2.1.1 Bioplastics

According to international union of pure and applied chemistry (IUPAC), bioplastic can be shaped by flow at some point during the processing of the material into finished products (Lackner, 2015). Bioplastics can also be made from biomass or monomers. According to Nandakumar and colleagues (2002), bioplastics are either biodegradable, biobased polymers, or both. According to (Alshehrei, 2019; Gatea et al., 2018; Manali Shah et al., 2021) and others, they are produced from renewable resources such as potato starch, maize starch, pineapple, jute, hemp, and banana stem fibres. They are also made from microbes and numerous agricultural wastes. Biodegradability and renewability are the two main criteria for categorizing polymers into bioplastics regarding environmental sustainability(Rahman & Bhoi, 2021). Unlike conventional plastics, bioplastics biodegrade in the environment to CO_2 and H_2O, preventing litter and harm to marine life. Although bioplastics currently hold less than 1% of the market, they are expanding at 20–30% yearly rates (Narancic et al., 2020). Bioplastics are extremely important for boosting sustainability, a balance between environmental, economic, and social business factors that may be applied to various areas. Economic, ethical, environmental, and engineering factors must be considered sustainably producing PHAs (Koller et al., 2017; Yadav et al., 2020).

The evaluation of the process' impact on the environment is a crucial prerequisite for sustainable industrial development. Environmental assessment tools like ecological footprint and life cycle assessment (LCA) demonstrated that PHA production could help reduce waste and incorporate bioeconomy principles while also reducing greenhouse gas emissions (GHGs) by about 200 per cent and fossil energy use by about 95 per cent, respectively (Dietrich et al., 2017).

Biomass is converted into bioplastics through hydrolysis, pretreatment, and fermentation to create bioethanol. After that, bio-based plastics are created utilizing bioethanol as substrate (Rahman & Bhoi, 2021). The main non-biodegradable bioplastics include bio-polytrimethylene terephthalate (bio-PTT), bio-polyethylene (bio-PE), bio-polypropylene (bio-PP), bio-polyethylene-terephthalate (bio-PET), and bio-polyamide (bio-PA). In contrast, the Polyhydroxyalkanoate (PHA), polylactide (PLA), poly(butylene adipate-*co*-terephthalate (PBAT), polyglycerol sebacate (PGS) and polycaprolactone (PCL), are some examples of biodegradable plastics (Lackner, 2015; Niaounakis, 2013).

PHA, PLA, and other extracellular secretions like alginate are the polymers that microbes make most frequently, significantly, when grown on carbon-rich sources like glucose or oil (Nandakumar et al., 2021). The two biopolymers are the only bioplastics with all three ideal characteristics of a conventional bioplastic—bio-based, biodegradable, and biologically generated. Despite the doubts that some researchers have regarding the biodegradability of PLA, a recent study has demonstrated that the polymers are more biodegradable than petroleum-based plastics when favourable conditions are present, such as increased warmth and moisture (Nandakumar et al., 2021). Furthermore, biodegradability is rarely characterized in a spatiotemporal context; as a result, a substance becomes biodegradable when it begins to break down within a few months, whereas a material that takes longer to break down becomes durable (Cho, 2017).

2.2 Polyhydroxyalkanoates

Polyhydroxyalkanoate (PHA) are microbial biopolyesters primarily formed by bacteria and archaea as insoluble granules within the cell or cytoplasm measuring 0.2-0.5 μm (Muneer et al., 2020). PHAs are a broad class of biologically manufactured carbon storage polymers in polyesters with mechanical qualities mimicking petrochemical polyesters and are biodegradable (Surendran et al., 2020).

Polyhydroxyalkanoates (PHAs) are members of the polyester family. They are biodegradable, biocompatible, and bio-based polymers. Collectively, polybutylene succinate and polylactic are known as green polymers of the future as they are expected to slowly replace petroleum-based polymers with the same physicochemical, thermal, and mechanical features as LDPE and PP (Alshehrei, 2017; C. Kourmentza & Kornaros, 2016). Bacteria naturally achieve PHA polymerization, whereas polylactic acid and polybutylene succinate are obtained from lactic and succinic acid polymerization. Stored energy in PHA within *Bacillus megaterium* in water acts as a biocontrol agent (Defoirdt et al., 2009). Different groups of bacteria can accumulate PHA as intracellular lipid granules, which are stored as carbon and energy reserve materials. However, PHA production is achieved from an inadequate supply of essential nutrients for growth in the presence of surplus carbon in the growth medium. Many bacterial organisms can synthesize PHAs during active growth without growthsuppressing factors. This carbon storage is employed in bacteria as fatty acid sources are metabolized during stress, thereby serving as the critical survival pathway.

These compounds are known to have various chains of application that cut across fields of medical, marine, and agricultural industries and are stored as granules or energy storage molecules in the cells of bacteria (Amelia et al., 2019; Kehail & Brigham, 2018; Wang, 2017). PHA granules comprised 1.87% proteins, 97.7% polyester, and 0.46% lipids. Structurally, PHAs are divided into the monomer units and the R-group, which differ because of the carbon chain length. Various types of PHA exist, and they vary in central and side chain lengths (Aslan et al., 2016). Polymerization of the monomeric hydroxyalkanoates substrates in PHA biosynthesis to form PHA is achieved with PHA synthase. Regardless of the discovery about 30 years ago, limited structural information about the enzyme is known. The initial structure of the catalytic domain of a PHB synthase is PHA C from *Cupriavidus necator* (Chek et al., 2017). Packing materials and disposable items are produced from (SCL-PHAs). Simultaneously, the (MCL-PHAs) play a significant role in medicinal implants, matrices for surgical sutures, drug delivery, etc. (Chen, 2009). Production of PHA is achieved via various substrates and strategies. Modifying functional groups halogens, epoxy, OH, and -COOH raises the thermal and mechanical features. The difference between SCL-PHA and MCL-PHA monomeric units is that their combination carries more prominent physical and thermal properties, which are accomplished through enzymic modification (Sharma et al., 2017).

Primarily, microorganisms are the producers of stress response strategy due to excess carbon or a limited supply of essential nutrients and oxygen (Prieto et al., 2016). Additionally, PHAs protect microbial cells against osmotic stress and environment-induced imbalances (Obruca et al., 2018). PHAs have been the most investigated of all bio-based and biodegradable polymers, but their high manufacturing costs compared to petro-polyesters restrict their potential for broad application (Yasim-Anuar et al., 2021).

PHA discovery has been one of the most significant advances in environmental sustainability and development since the turn of the century (Hassan et al., 2013). Their function in preserving finite fossil fuel supplies and reducing greenhouse gas emissions has been explored (Surendran et al., 2020). One of the appealing qualities of PHAs among biopolymers that made them attractive as a potential substitute for synthetic plastics is their biodegradability and compostability. Due to its simplicity in converting to various forms with the appropriate qualities, it has successfully displaced petro-polymers in the manufacture of plastic (Bhatia et al., 2021). Due to PHAs' sustainability and efficiency in achieving the Sustainable Development Goals of the United Nations, there is growing interest in manufacturing PHAs in polymeric science research (Muneer et al., 2020). PHAs have many structural variations and are entirely biodegradable, processable, and non-toxic. These characteristics make them very useful in medical applications, including orthopaedics (screws, cartilage tissue engineering scaffolds, bone graft substitutes), cardiovascular system devices (blood vessels, cardiac valves, cardiovascular stents), dressings, and nano- and microspheres for controlled drug release (Grigore et al., 2019).

In addition to addressing the issue of plastic waste pollution and its applications in healthcare, PHAs can potentially contribute to the development of environmentally friendly sustainable biomaterials that are utilized in various agricultural, industrial, and household applications (Kalia et al., 2021). It is undeniable that PHAs hold immense importance in the contemporary world as they can serve as substitutes for synthetic plastics, mainly when effective strategies are implemented to enhance their commercial viability and sustainability (Dietrich et al., 2017).

2.2.1 Structure, Classification and Characteristics of PHAs

Because of their fermentative production, naturally occurring PHAs are perfectly isotactic, solely emphasizing the configuration (R) at the chiral stereo centre in the main chain (Yadav et al., 2020a). About 150 PHA analogues have been identified (Raza et al., 2018). Fig. (2.1) depicts the overall structures of PHAs. When the R group is = CH_3, the resulting polymer is known as PHB, but if R = C_3H_7, this is known as polyhydroxyoctanoate (PHO), and so on.

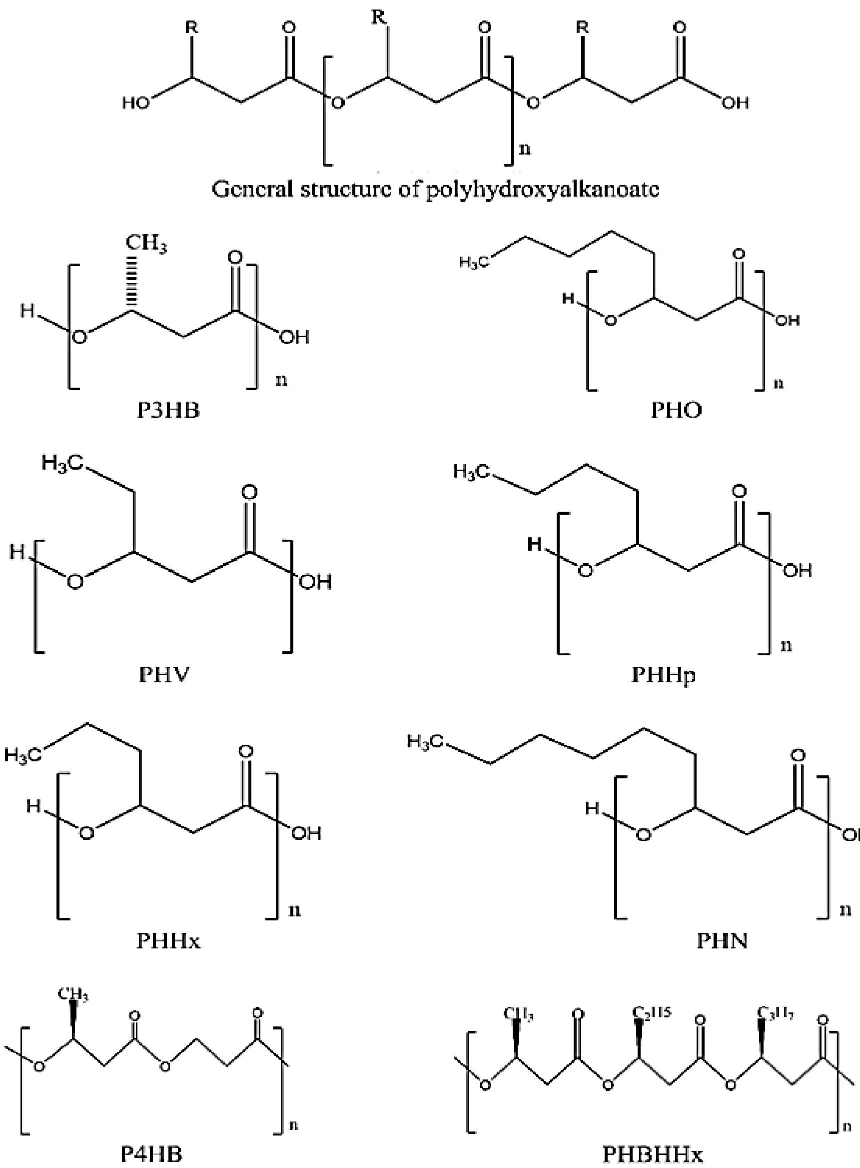

Figure 2.1 Chemical structures of different types of polyhydroxyalkanoate (PHA).

PHAs are a diverse group of biopolyesters with various molecular masses and configurations. The variation in PHAs is attributable to differences in structures and the length of the repeating monomers (Steinbüchel & Füchtenbusch, 1998; Pötter & Steinbüchel, 2006). Poly-3-hydroxybutyrate (P3HB) is the most commonly known PHA monomer bacteria generated. It comprises (R)-3HB repeating monomers that link to create a polymeric chain (Muneer et al., 2020). Most of the other monomers are polymerized similarly, resulting in various PHAs. As a result, they have the same overall structure with minor differences in the main chain of their monomer at position -(CH2)n, where 'n' can vary from 1 to 4 carbon atom (Muneer et al., 2020). In terms of complexity, the number of repeats of a specific monomer to build a chain distinguishes between distinct PHAs. A specific monomer may be repeated up to 30,000 times, providing insight into the many forms of PHAs.

PHAs are divided structurally into three types: short-chain length PHAs (scl-PHAs), Poly(3-hydroxybutyrate) or P(3HB), poly(4-hydroxybutyrate) or P(4HB), poly(3-hydroxyvalerate) or P(3HV), and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) or PHBV copolymer are examples of scl-PHAs with 3-5 carbon atoms. Scl-PHAs are frequently found in food packaging and single-use throwaway items (Kunasundari & Sudesh, 2011).

Medium-chain length PHAs (Mcl-PHAs) PHAs with medium chain lengths have carbon atoms ranging from 6 to 14. Poly(3-hydroxyhexanoate) (P(3HHx)) and poly(3-hydroxyoctanoate) (P(3HO) are famous examples of homopolymers. The MCL-PHAs were initially discovered in *P. oleovorans* in 1983 (Rai et al., 2011). Mcl-PHAs are amorphous macromolecules whose side chain length increases as the glass transition temperature lowers (Mannina et al., 2019). Long-chain length PHAs (lcl-PHAs), are PHAs with long chains have more than 16 carbon atoms. They are scarce and of special importance in bioplastic development. Microorganisms' production of long-chain fatty acids (at least C16) has received little attention, which may explain why Lcl-PHAs are rarely synthesized by bacteria (Grazia Licciardello et al., 2019). SCL-PHAs are rigid, stiff, brittle, and crystalline, whereas MCL-PHAs are flexible and crystalline (Bhardwaj et al., 2014). As a result, scl-PHAs exhibit qualities similar to standard plastics, but Mcl-PHAs are classified as elastomers and rubbers (Grazia Licciardello et al., 2019; Suriyamongkol et al., 2007). Co-polymers of scl-PHAs and

Mcl-PHAs and the transformation of their functional groups were proposed to increase their physicochemical characteristics (Kalia et al., 2021). As a result, functional modification of monomers, such as the insertion of unsaturated and halogenated branched chains, has improved the characteristics of resultant PHAs (Suriyamongkol et al., 2007).

According to Nielsen et al. (2017), there are more than 150 different PHA monomers, each of which plays a crucial part in establishing the characteristic functions of a given PHA. As a result, only a few known PHA types have traits in common, while most are highly diverse (Suriyamongkol et al., 2007). PHAs made from microbial cells, however, resemble traditional polymers like polypropylene in terms of their characteristics. They have a granular nature and are found inside the cytoplasm of many organisms, particularly bacteria. According to (Muneer et al., 2020), the granules feature an amorphous polyester core. Several PHA depolymerase bacteria can break them down within a year into carbon dioxide (Jendrossek, 2001). Many PHAs are also biocompatible and biodegradable. They can be recycled because they are made from renewable resources (Poirier, 1999).

P(3HB) is the most widely used and studied PHA. It is a stiff substance with low elasticity and high tensile strength. It is a polymer that is biocompatible, compostable, and biodegradable. It is a desirable choice for eco-friendly packaging due to its high crystallinity, resistance to hydrolytic degradation, and hydrophobicity (Bhardwaj et al., 2014). P(3HB) has a polydispersity of 2 and an average molecular weight of 1×10^4 to 3×10^6 Da (Doi, 1990). Due to the proximity of its melting temperature (T_m) and thermal deterioration temperature (T_{deg}), which are 180 ºC and

185 ^{o}C, respectively, it is not suited for higher-temperature thermal processing (Surendran et al., 2020). In addition, the P(3HB) homopolymer is known to have high crystallinity compared to LDPE, as evidenced by a high *Tg* of 4 ^{o}C (Doi, 1990). X-ray diffraction crystallographic pictures show that P(3HB) crystals typically exist as crystals. However, according to (Lambeek et al., 1995), some bacterial strains can produce a -form paracrystalline structure with a twisted planner zigzag shape. The nature of more complicated PHAs, such as Mcl-PHAs, are often elastomers and sticky (Suriyamongkol et al., 2007). The final polymer is more desired when PHAs are copolymerized with heteropolymers or when scl-PHAs and Mcl-PHAs are formed. PHA copolymers with a majority of HB and a small amount of more extended chain monomers, including HV, HHX, or HO, are more robust and more flexible plastics. The flexibility of P(3HB) can be improved by adding a second monomer to the PHA backbone. Contrary to the amorphous traditional Mcl-PHA, which typically has a high composition of 3-hydroxyoctanoate (3HO) and 3-hydroxydecanoate (3HD), long-chain monomer concentrations favour side-chain crystallization. Surendran et al. (2020) have demonstrated various improvements in the physicochemical properties of PHA copolymers (Surendran et al., 2020).

2.2.2 PHA-Producing Microorganisms

Both eukaryotic and prokaryotic bacteria can generate different kinds of PHAs. PHAs are utilized to manage seizures and metabolic illness and to improve cardiac efficiency. They have also been found in humans' and animals' blood and tissues (Rehm, 2009). PHAs are produced in various ways by microorganisms. As a result of having Mcl-PHA synthases for synthesizing PHAs with 6–14 carbon atoms, *Fluorescent pseudomonas* strains, for instance, are established to accumulate Mcl-PHAs (Kim et al., 2000). PHA production has been linked to more than 300 different bacterial species. *Ralstonia, Burkholderia, Halomonas, Alcaligenes,* and *Pseudomonas sp*. are the most commonly employed microorganisms due to their capacity to tolerate various carbon sources and create several forms (Bhatia et al., 2021; Yadav et al., 2021).

Regarding economic considerations, PHA's bacterial culture production is more similar to the production process of living organisms, particularly plants. This culture is due to its higher capacity for accumulation, making it more cost-effective. Commonly studied bacterial species for PHA biosynthesis included *A. eutropha, R. eutropha,* and *Cupriavidus necator*. Additionally, other bacterial strains such as *Pseudomonas* sp., *Bacillus* sp., *Rhodopseudomonas palustris, Burkholderia sacchari*, *Halomonas boliviensis*, and *Aeromonas hydrophilia* have recently been investigated for their potential in PHA synthesis, focusing on their yield (Vigneswari et al., 2021).

PHA-producing bacteria can be categorized based on nutrient stress, growth pattern, and nutrient need (Shrivastav et al., 2013; Vigneswari et al., 2021). Two types of bacteria have been identified based on this classification. The first category, known as the prime group, is where the bacteria that make up this group store PHA because they cannot biosynthesize PHA while growing and only need a limited amount of oxygen, magnesium, phosphorus, and nitrogen (Shabina et al., 2015). These bacterial species, such as *P. putida, P. oleovorans*, and *R. eutropha*, are examples. The second group, on the other hand, can continue producing PHA throughout its growth stage and is not affected by the availability of nutrients (Vigneswari., 2021). The second category of bacterium species includes mutant strains of *Alcaligenes latus,* recombinant *Escherichia coli,* and *Azotobacter vinelandii* (Vigneswari et al., 2021).

PHB was initially discovered and characterized by French scientist Maurice Lemoigne in a media containing *Bacillus megaterium* as an inoculant in 1926 when PHAs were first discovered (Choi et al., 2020). As a result, it was determined that many kinds of bacteria, fungi, algae, and higher-living organisms can produce PHA in an environment favourable to their growth. The strains of *Burkholderia sacchari, C. necator, R. eutropha, P. putida, and P. oleovorans*, which are the most extensively studied PHAs-producing bacteria, are among the over 300 microbial species currently known to be capable of producing PHAs (Dalton et al., 2022). Other frequently reported eubacterial species include *Staphylococcus epidermidis, Pseudomonas aeruginosa, P. fluorescence, P. jessenii, Bradyrhizobium japonicum, P. stutzeri, Spirulina platensis Rhizobium meliloti, R. viciae, Microlunatus phosphovorus,* and *Rhodopseudomonas palustris* (Yasim-Anuar et al., 2021).

Similar to this, PHA production by individuals belonging to the genera *Agrobacterium, Bacillus, Bordetella, Clostridium, Mycobacterium, Vibrio, Streptomyces, Fusobacterium, Burkholderia, Pseudomonas, Neisseria, Ralstonia, Legionella, Acinetobacter, Rickettsia* and *Sphingomonas,* has also been documented (Kalia et al., 2021). Recent studies have emphasized the importance of Recent research has highlighted the significance of several major PHA-accumulating species, including *Acinetobacter, Calothrix, Dyella, Flavobacterium, Novosphingobium, Qipengyuania*, and *Tsukamurella* (Correa-Galeote et al., 2022). Extremophilic archaea species include (Muneer et al.,2020; Obruča et al., 2022), *Haloferax mediterranei, Haloarcula hispanica* (Wang et al., 2019), and *Aneurinibacillus* sp. (Pernicova et al., 2020), *Geobacillus* sp. (Giedraityte & Kalediene, 2015), *Magnetospirillum gryphiswaldense* was reported to generate PHAs as well. More so, *Chlorogloea fritschii, Synechocystis* sp., *Synechococcus* sp., *Nostoc muscorum* and *Aulosira fertilissima* are among the recognized cyanobacteria that produce PHAs (Gradíssimo et al., 2020).

Although many organisms have the natural potential to produce PHAs, only a tiny percentage of them can fulfil the required production rate due to issues with generation time, ideal growth circumstances, and downstream processing. As a result, engineering of the most suited microbial species has been pursued, with notable results. According to Kalia et al. (2007), some well-known recombinant species include *Escherichia coli, Staphylococcus epidermidis* ATCC 12228, *Brucella suis* 1330, *Streptomyces coelicolor* A3 and *Burkholderia sp.* DSMZ 9242. *Schelegelella thermodepolymerans* DSM 15,344 has been identified by (Kourilova et al., 2020) as a recombinant PHA producer that uses glucose as a carbon source. Transgenic plants like *Arabidopsis thaliana, Camelina sativa,* and *Gossypium sp*. have also produced several forms of PHAs (Muneer et al., 2020).

In a bioreactor, the production of PHA is influenced by several factors, including the microbial strain used, the carbon supply, the operating conditions, the nutrient composition, and the downstream processing. Researchers have been examining these elements for many years to create a PHA fermentation process that is both affordable and sustainable. Along with productivity, the carbon source, polymer