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WASTE MATERIALS FOR BACTERIAL POLYHYDROXYALKANOATES (PHA) PLASTIC BIOSYNTHESIS

Abubakar Mayana Bello[1], Safina Abdullahi Yarima[2], Khadija Abdullahi Yarima[3], and Zubaidat A. Binji[4]

[1]*College of Health Science and Technology Tsafe, Zamfara State, Nigeria*

[2]*Department of Environmental Education, Shehu Shagari University of Education, Sokoto, Sokoto State, Nigeria*

[3]*Usmanu Danfodiyo University Sokoto, Nigeria*

[4]*Department of Basic Science, Shehu Shagari University of Education, Sokoto, Sokoto State, Nigeria*

Corresponding email: *superoxidedismutase594@gmail.com.*

Abstract:

Polyhydroxyalkanoates (PHA) are a family of biodegradable polyesters synthesized by bacteria as intracellular carbon and energy storage materials under conditions of limited nutrients such as nitrogen, phosphorus, sulphur and oxygen, and excess carbon source. This paper reviews waste materials for PHA synthesis, biosynthesis of PHA, good features of PHA, and petroleum-based plastics and its environmental impacts. Waste materials serve as cheap carbon sources for sustainable PHA production. Accordingly, four basic sources are utilized which include carbohydrates (starch, lactose, cellulose), alcohols (methanol from biodiesel production), lipids (plant and animal waste oils), and organic acids (dairy waste and lactic acid). Examples of waste include agricultural waste such as palm oil effluent, cheese whey, food waste, wood waste, silage waste; waste water sludge, garden waste, paper waste and biodiesel effluents. The burning of plastic waste as a management strategy releases harmful pollutants such as smoke (particulate matter), dioxin, formaldehyde, hexachlorobenzene (HCB), heavy metals, volatile organic compounds (VOC) and PVC gases which cause asthma, cancer, kidney and liver damage, hormonal disruption and cardiovascular problems. PHA is biosynthesized through biological pathway by culturing microbes such as Bacillus megatherium and Alcaligenes eutrophus in carbon sources at 30-37°C. Glucose from waste is metabolized though Tricarboxylic Acid Cycle (TCA) to give acetyl-CoA, which is condensed by ketothiolase to acetoacetyl-CoA, reduced by acetoacetyl-CoA reductase to R-(-)-3-hydroxybutyryl-CoA and polymerized by PHB synthase to form PHB. Addition of propionic

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or valeric acid leads to formation of copolymer P(HB-HV). Good features of PHA include impermeability to gas (resistant to oxygen attack) and biodegradability. PHA is hydrophobic and is degraded by microbes through depolymerase enzymes into monomers which are used as carbon source, and can be biodegraded in aerobic or anaerobic environments such as landfill, waste treatment facilities or oceans, unlike petroleum-based plastics. Petroleum-based plastics are polymers moulded under temperature and pressure, classified as thermoplastics and thermosetting plastics. They contain monomers of concern such as styrene (in polystyrene) and vinyl chloride (in PVC) which cause immunological changes, respiratory irritation, oxidative stress, liver cancer and hepatotoxicity. Plastics pollution contaminates the food chain. Humans consume plastics through food, especially biota (animal and plant based foods). At molecular level, plastics stimulate protein and gene alterations; at tissue level they cause inflammation; at individual level they trigger behavioral, reproductive and metabolic disorders through oxidative stress, intestine damage and necrosis. The review concludes that PHA is an eco-friendly alternative to petrochemical plastic.

Keywords: *Polyhydroxyalkanoates, Biodegradable plastics, Waste materials, Biosynthesis, Petroleum plastics, Food chain*

1. Introduction

Petroleum-based plastics have become one of the most pressing environmental and public health challenges of the 21st century due to their non-biodegradability, persistence in the environment, and ability to transfer along the food chain (Halden, 2010; Lusher et al., 2017; FAO, 2017). The common practice of open burning of plastic waste as a management strategy further compounds the problem by releasing toxic pollutants such as dioxins, particulate matter, formaldehyde, hexachlorobenzene, heavy metals and volatile organic compounds which cause cancer, asthma, kidney and liver damage, and hormonal disruption (Park et al., 2013; Verma et al., 2016). Furthermore, toxic monomers like styrene and vinyl chloride leached from polystyrene and PVC cause immunological changes, oxidative stress and hepatotoxicity (Gamarro & Costanzo, 2022; Havern, 2022).

In response to these challenges, attention has shifted to eco-friendly biodegradable alternatives. Polyhydroxyalkanoates (PHA) are a family of biodegradable polyesters synthesized intracellularly by bacteria such as *Bacillus megatherium* and *Alcaligenes eutrophus* (now *Cupriavidus necator*) as carbon and energy storage materials under conditions of excess carbon and limited nitrogen, phosphorus and oxygen at 30-37°C (Ojumu et al., 2003; Chen, 2010; Chen, 2014). PHA can be produced cheaply and sustainably from waste materials including agricultural waste, waste water sludge, garden waste, paper waste and biodiesel effluents, which also contributes to waste management (Wolf et al., 2005). Importantly, PHA is impermeable to gases and completely biodegradable by microbial depolymerases in landfills, oceans and waste treatment facilities, unlike petroleum plastics (Poirier et al., 1995; Shah & Kumar, 2020). Therefore, this paper provides an insight on plastic polymer pollution and the biosynthesis of PHA from waste materials as a sustainable solution for environmental sustainability.

2.Categories of plastics

Plastic is defined as lightweight, resistant material that has the capacity of being molded in different shapes and are utilized for several ways (applications) (UNEP, 2018). Mostly, plastics don't biodegrade, but photodegrade (slowly breakdown to smaller fragments) dubbed as microplastics. SUPs are types of plastics disposable in nature, and they are intended to be used once before been thrown away or recycled; for instance, packaging, grocery bags, bottles, straws, cups, and cutlery (Avio et al., 2016). Single use plastic means any plastic used once, before being discarded or recycled. Thermoplastics are belonging to the family of plastic materials that is capable of been melted when heated and tend to hardened upon cooling. Thermoplastics melt when heated, and hardened when cooled (Eyerer, 2010; Pizzorno, 2024). Thermoplastics are reversible form of plastics, abled to be reheated and reshaped and cooled repeatedly. Examples of thermoplastics include polypropylene (PE), polyethylene terephtalate (PET), low density polyethylene (LDPE), polystyrene (PS), polyvinyl chloride (PVC), polylactic acid (PLA), polycarbonate (PC), polypropylene (PP), and polyhydroxyalkanoates (PHA). Thermosets are categories of plastics that follows a chemical change when heated to make a three dimensional structure. After heating thermosets plastics and forming, they cannot be remelted and reformed, for instance, in the case of urea-formaldehyde resins (UF), epoxy resins, acrylic resins, polyurethane (PUR), and phenolic resins (UNEP, 2018).

3.Plastics pollution and food chain

Mostly, humans consume plastics through food. And many among the major classes of foods are contaminated by plastic pollution or mismanagement, more especially the biota (animal or plant based foods) are contaminated and affect humans (Valentine, 2019). At molecular levels, ingested plastics can stimulate protein changes, and gene alterations; at tissue level, plastics can cause inflammation and histological problems; and on individuals, plastics tend to trigger behavioral, reproductive, and metabolic changes or disorders (GESAMP, 2015; Curlej et al., 2023). Many of the problems cause by plastics are due to their ability to elicit oxidative stress, intestine damage, necrosis, and alterations in energy reserves and metabolism (Rist et al., 2018).

3.1 Contamination of soil by plastics

Soil is an important aspect of agricultural resources that is utilized for production of plant based foods, and animal keeping (that are searching for foods or grazing), and poultry farming. Therefore, presence of plastics in soils is a recipe that may stimulate contamination of either animal -based food or plant based foods (FAO, 2017; Galloway, 2015). Several studies pointed at the possible ways of soil contamination through plastic waste disposal, manure addition, mulching, fertilizer application, etc. Plants (at varying degrees) are able to take in plastics through leaves and upto the leaves, flowers, and fruits therein (FAO, 2018; Galloway, 2015). On many occasions plastics tend to be deposited on plant surfaces due to deliberate manuring (sludge addition, fertilizer spread, household discharges, biosolids, etc), mulching or pollution or agricultural activities (Gamarro & Costanzo, 2022). Additionally, soils are often contaminated by plastics through polluted water, because some of the water supplies of farms are deliberately polluted or

unable to screen plastics. Alternatively, plastics from water bodies travel to the farms to cause pollution (Xu et al., 2025).

3.2 Contamination of processed foods (FFs)

Plastics are very persistent in the environment, therefore leads to implications after exposure. A water bottle persists for 450 years before complete degradation, plastics bags take 20 years, fishing lines take 6000 years, cups take 50 years, and straws take 200 years. In the course of processing or storage of foods at home or industries, plastics from pipes, water, containers, engines, and machines travel to the foods and lead to contamination. This situation is seen in foods like garri, sugar, honey, meat, beverages, mineral water, sugar, etc (Earth Watch Institute, 2018; Sarkingobir et al., 2020).

3.3 Contamination of aquatic food sources (systems)

Aljaibachi & Callaghan (2018) demonstrated in the study of effects of polystyrene on *Daphnia magna*, that exposure increases mortality on young species. Intentional and unintentional dumping of waste materials in water bodies is a key way that ensured the translocation or transport of plastics to the water bodies (fresh and aquatic) and in turn entering the food system or food chain. Aquatic foods ranging from zooplankton to invertebrates (and small fishes) and predators (large fishes, birds, etc) get contaminated with plastic particles and the ultimate effect falls on human nutrition (Botterell et al., 2019; Sabilillah et al., 2023). Plastics are primarily taken (ingested through by oral exposure) directly (by taking the plastic as first line) and in terms of secondary ingestion (by consuming an organism that had already hid the plastic particles. The color of plastics makes it more attractive to animals for ingestion and incorporation in the food chain). Every consumed plastic can be said to possibly be handled in many ways, such as retention, translocation, and egestion. Translocation is a process of moving plastics to target tissues (Dris et al., 2015). Translocation is evident in fishes, crabs, mussels, etc. For instance, gills and ovaries of crabs contain 250 um translocate plastics and in fishes about 600 um particles travel to the liver. Retention of plastics may occur and it is a process of prolonged stay of particles in the affected organism. Egestion is a process of elimination of particles such as through urine, faeces, sweet, etc (Asmonaite & Bethanie, 2019). Nevertheless, many aquatic animals in fresh or marine water take in plastic and in turn implicating the food chain (Lyon, 2004; Xu et al., 2025).

4.Petroleum-based Plastics and components

According to Lusher et al. (2017) plastics are elucidated this way “Plastic is a catch all term used to describe a range of polymer materials that are moulded under specific temperature and pressure, and have different properties depending on the requirements of the end product.” Gamarro & Costanzo, (2022) defined plastic as a broad group of all synthetic compounds made through polymerization reactions (of monomers) derived typically from fossil fuels or renewable materials (such as starch, and castor oil). The term plastic refers to various categories of polymers to which additives are incorporated to yield desirable properties and shapes (Galloway, 2015; Pelley, 2018). Thermoplastics tend to soften upon heating, and can be remoulded (reshaped). Thermosetting plastics cannot be resoftened and reshaped (remoulded). Thermosetting plastics include polyurethane, silicone, and epoxy resins (FAO, 2018; Gamarro & Costanzo, 2022). Plastics have

several components but major ideas would be unveiled here. Polymers are generally macromolecules of plastics obtained through a process of polymerization. Some polymers are homopolymers because they possessed only one type of monomer united together, for instance, cellulose; some polymers are however denoted as copolymers for having a mixture of monomers such as in some polyhydroxyalkanoates. There are some plastic monomers that are harmful or of great concern to biological beings. Monomers are the building blocks of polymers that are usually utilized (reacted) to make long chain polymers (Valentine, 2019; Pizzorno, 2024; Stancey et al., 2025). Some typical monomers toxic to humans are shown in Table 1.

Table 1Some typical monomers toxic to humans

S/N	Monomer	Health concern
1	Styrene	Styrene is folded in polystyrene plastics. Styrene has been reported for having particles that cause immunological changes at cellular levels (Hwang et al., 2020). Exposure to styrene spurs effects such as irritation of respiratory system, and abdominal pain (Gamarro & Costanzo, 2022) and some suggested for its carcinogenicity.
2	Vinyl chloride	Vinyl chloride is folded in PVC plastics resins and it is a mutagenic material. It causes oxidative stress (such as lipid peroxidation), liver cancer, and hepatoxicity (Gamarro & Costanzo, 2022).

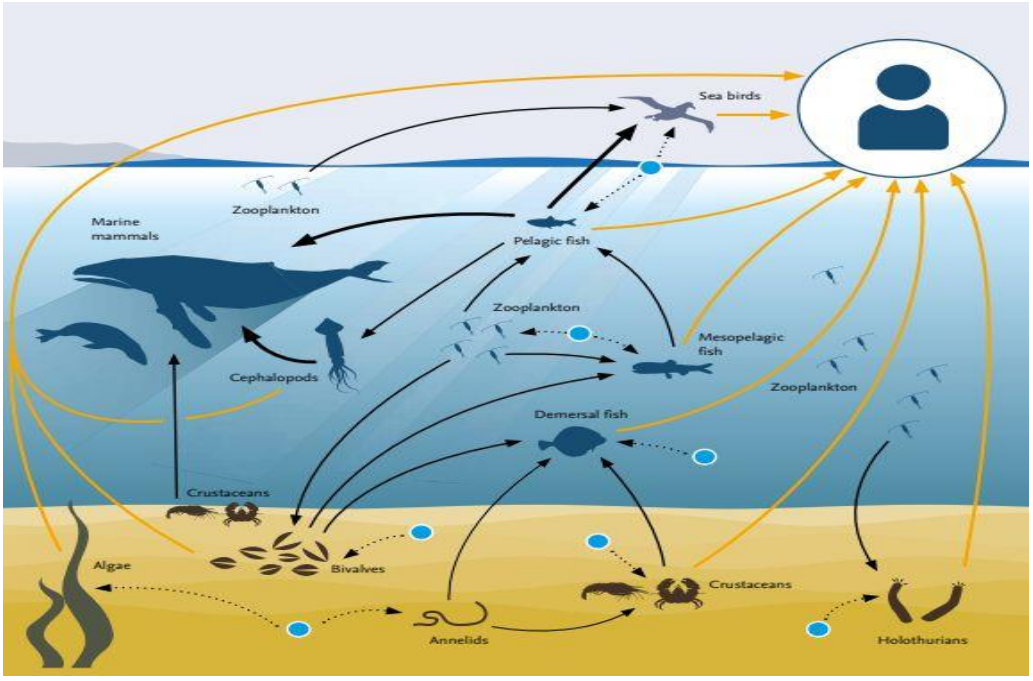


Figure 1Transfer of plastics along food chain; Source: FAO (2017)

5.Waste materials for PHA synthesis

According to Wolf et al. (2005) there are four basics sources that are utilized in cheap. The sources are carbohydrates (such as stretch, lactose, cellulose), alcohols (from biodiesel making eg methanol), lipids (such as plant and animal wasted oils), and organic acid (diary waste and lactic acid) (Koostra et al., 2017; EPA, 2021). Specifically, the waste in PHA synthesis has been exemplified in Table 2.

Table 2Examples of waste products of PHA synthesis

S/N	Type of waste	Examples
1	Agricultural waste	Sludge, palm oil effluent, olive oil effluent, cheese whey, dairy waste, food waste, wood waste, etc, silage waste
2	Waste water sludge	Domestic waste water, industrial waste water
3	Garden waste	Fruits and vegetable waste materials
4	Paper waste	Paper mill waste products
5	Biodiesel pollution effluents	Fatty acid waste, and vegetable waste products

Source: Beaman et al. (2016)

6.Plastic waste mismanagement and concern

6.1 Burning of plastic waste

Many people resorted to burning of waste materials (of any kind) as management strategy nowadays, but there is method confirmed evidence indicating that this method releases particulate matter, acid gases, dioxins, carbon monoxide, carbon dioxide, and other harmful chemicals leading to putting self, family, and the environment at risk (Kirwan et al., 2011; Adeyi et al., 2019; Alam et al., 2019). Some of the risks of waste burning are as contained in table 3.

Table 3Some pollutants released upon burning of waste materials and risk

S/N	Pollutant	Possible risk or effect
1	Smoke	Smoke contains tiny microscopic materials (particulate) that tend to go deep down the lung and stay for months or years. The smoke particulate causes asthma attacks, coughing, and shortness of breathing, wheezing, and chest pain. Particulate can cause heart attack, and irregular heartbeat.
2	Dioxin	Dioxin is a known carcinogen and is deposited in water, crops, and soil or food chain at large. Dioxins are persistent, bio accumulative, and dwelled in the food chain, water, air, and vegetation. Dioxins can cause immense disruption of hormones, and may lead to cancer.

3	Formaldehyde	Formaldehyde causes watery eyes, nausea,. skin rashes, and difficulty in breathing
4	Hexachlorobenzene (HCB)	HCB is a persistent organic pollutant that accumulates in fishes, birds, lichen, marine foods, animals, etc. HCB is a carcinogen in human and exposure lead to kidney, liver, damage. It also causes skin irritation, and fatigue.
5	Heavy metals (HMs)	Heavy metals released from plastics are diverse. Chromium causes cancer and impact on respiratory system as well. Mercury causes kidney and nervous system problems. Arsenic causes anemia, liver and kidney damage. lead causes brain damage, and effect on every organ of the human body
6	Volatile organic compounds (VOC)	VOCs are reported for causing difficulty in breathing and triggering asthma attacks
7	PVC	PVC burning disgorges chloride gas and hydrochloric acid

Source: Wagner et al. (2014), Earthday Network (2018); Gazal & Gheewala, (2020); Katsara et al. (2025)

7.Biosynthesis of polyhydroxyalkanoates (PHA)

Bioplastics are manufactured by transforming sugar content of plastics into plastic. Some of the natural things utilized to make plastic include sugarcane, wheat, sugar beets, and potatoes. Therefore, bioplastics are renewable and better to ensure sustainable environment (Washam, 2010). Typical example of major bioplastics made include polylactide or polylactic acid (PLA) and polyhydroxyalkanoates (PHA). Initial material is made by milking the sugar source to provide dextrose that is later acted upon by bacteria or yeast in order to give out lactic acid. Lactic acid is joined into oligomers (through a reaction that involves release of water) and turned to lactide, and polymerization arises to make polylactic acid (PLA) (Washam, 2010; Fesseha & Abebe, 2019). The bacteria or microbes that specialized in converting sugars to plastics are looking for energy source that is what derived them to this process. It is via breakdown of polysaccharides that glucose molecules are released. Therefore, glucose is subjected to a stepwise process of glycolysis to pyruvate and ATP. Pyruvate can be acted by lactate dehydrogenase to form lactic acid. According to Ojumu et al. (2003), Chen (2010), Poirier et al. (1995) some examples of bacteria with good ability to make PHA are:

1. Bacteria:

- *Bacillus megatherium*- First bacteria discovered to accumulate PHA (PHB) as carbon store
- *Alcaligenes eutrophus* (now called *Cupriavidus necator / Ralstonia eutropha*) - Most widely used for PHB synthesis from glucose when nitrogen or phosphate is deficient
- *Alcaligenes latus*- Produces PHB, demonstrated by Lee et al. (1991) to break down R3HB using PHB depolymerase enzyme

- *Pseudomonas* species- Used for hydrolytic degradation and synthesis of chiral (R)-3-hydroxyalkanoic acids (RHA) – Saito & Doi (1993).

2. Other Major PHA Producing Microbes are:

Short-Chain-Length PHA (scl-PHA) producers - Produce PHB, PHBV:

- *Bacillus cereus*
- *Bacillus subtilis*
- *Bacillus thuringiensis*
- *Azotobacter vinelandii*
- *Azotobacter chroococcum*
- *Cupriavidus necator*
- *Ralstonia eutropha*
- *Alcaligenes latus*
- _*Protomonas extorquens*

Medium-Chain-Length PHA (mcl-PHA) producers - Produce elastomeric PHA:

- *Pseudomonas oleovorans*- Produces PHA from n-alkanes and fatty acids
- *Pseudomonas putida*
- *Pseudomonas aeruginosa*
- *Pseudomonas fluorescens*
- *Pseudomonas stutzeri*

Recombinant / Genetically Engineered Microbes for PHA:

- *Escherichia coli*- (recombinant with PHA genes from *Ralstonia eutropha*)

"Many of the species including bacteria (such as *Bacillus megatherium*) accumulate a reasonable amount of PHA as a carbon store when there are limited nutrients. The microbes store the carbon in form of PHA, but sometimes it can be degraded for energy and carbon (Ojumu et al., 2003; Mohamed et al., 2021)." All these microbes synthesize PHA when there is excess carbon source (glucose, starch, fatty acids, waste materials) and shortage of nutrients* such as nitrogen, phosphorus, sulphur, oxygen at 30-37°C.

Meanwhile, PHA can be made either through following the chemical or biological pathways. PHA is synthesized by microbes in a sustainable way by culturing microbes in carbon sources such as glucose, starch, fatty acids, and nutrients present in waste materials at 30-37^{o}C and atmospheric pressure (Ojumu et al., 2003). PHA plastics are sets of polyesters of hydroxyalkanoates with the general structure as in Figure 2. Many of the species including bacteria (such as *Bacillus megatherium*) accumulate a reasonable amount of PHA as a carbon store when there are limited nutrients. The microbes store the carbon in form of PHA, but sometimes it can be degraded for energy and carbon (Ojumu et al., 2003; Mohamed et al., 2021). One very important aspect of synthesis of PHA is through glucose. Glucose is obtained from carbon sources (such as waste) materials and is treated through Tricarboxylic acid cycle (TCA) by a series of enzymes to give acetyl-CoA, 2 molecules of acetyl-COA are made to condensed by action of ketothiolase to give out acetoacetyl CoA, which is subsequently handled by a specific reductase (acetoacetyl-CoA reductase) to make R-(-)3-hydroxybutyrl-CoA and in turn PHB (polyhydroxybutyrate) a typical

PHA category. PHB is made by the action of PHB synthase on the recent product. Typically, *A. eutrophus* can be able to synthesize this polymer if there is glucose, and deficiency of nutrients such as nitrogen or phosphate (Ojumu et al., 2003).

However, in the second aspect of PHA plastics biosynthesis, addition of propionic acid or alternatively valeric acid in the growth medium (to supplement glucose) leads to the formation of a copolymer containing 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). In this format, propionyl-CoA and acetyl-CoA can be converted to 3-ketovaleryl-CoA, and ultimately to HB-HV (Ojumu et al., 2003).

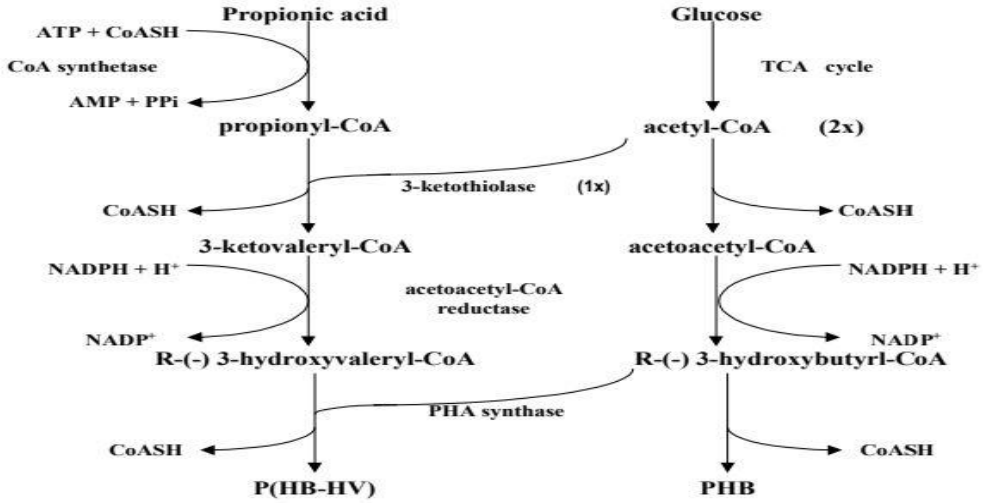


Figure 2Basic structure of biosynthesis of PHA; source: Ojumu et al (2003)

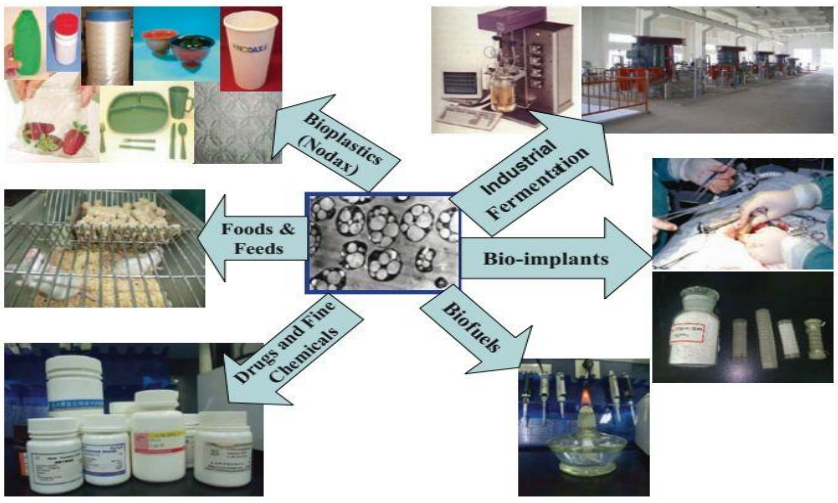


Figure 3Typical uses of polyhydroxyalkanoates polymers; Source: Chen (2010)

7.2 Good features of PHA

When there is shortage of nutrients (such as sulphur, oxygen, nitrogen, and phosphorus) in the environment, some microbes use the presently available carbon to synthesize and store extracellular granules of PHA. PHA is similar to petroleum-based plastics. PHA has been diverse and of wide range of uses according to the embedded properties including the followings:

- Impermeable to gas- Ojumu et al. (2003) disclosed that PHA is resistant to oxygen attack.
- Biodegradable- Since the PHA is hydrophobic, it is therefore attractive to microbes that in turn release enzymes that turn PHA (or PHB) into monomers units, because the microbes (ideally utilize it as carbon source (Poirier et al., 2002; Ojumu et al., 2003; Chen, 2010). Biodegradation of plastics is carried out by microbes by decreasing the molecular weight of plastics involved. Since, the big polymer material of the plastics cannot be taken easily into the fold of the microbe; the organism had to employ its enzymatic assembly to split the polymer into monomer for easy mineralization (Fesseha & Abebe, 2019; Kour et al., 2023). The property of biodegradability entails that the material can be broken-down by a living organism especially the microbes (Chen, 2010). In the case of PHA polymers, it is evident that these can be broken down by microbes through depolymerization (for example). Saito & Doi (1993). had successfully made the chiral (R)-3-hydroxylalkanoic acids (RHA) and its methyl esters by using hydrolytic degradation of PHA acted by the pseudomonas. Lee et al. (1991) in their work demonstrated that, R3HB under environmental settings had successfully broken down by activating PHB depolymerase enzyme in *Alcaligenes latus*. Shah & Kumar (2020) denoted that Polyhydroxyalkanoates (PHAs) are eco-friendly alternatives to petrochemical plastics and are quickly biodegraded in aerobic or anaerobic environments such as a landfill, waste treatment facilities or oceans.

8.Conclusion

Petroleum-based plastics have become a major environmental and public health threat due to their non-biodegradability, release of toxic monomers like styrene and vinyl chloride, additives and heavy metals, and their ability to transfer along the food chain from zooplankton to fish to humans, causing protein changes, inflammation, reproductive and metabolic disorders. The burning of plastic waste worsens the problem by releasing dioxins, particulate matter, heavy metals and acid gases that cause cancer, respiratory diseases, kidney and liver damage. Polyhydroxyalkanoates (PHA) provide a sustainable and eco-friendly alternative. PHA can be produced cheaply from various waste materials such as agricultural waste, waste water sludge, garden waste, paper waste and biodiesel effluents, which helps in waste management. Its biosynthesis by bacteria through TCA cycle to PHB and P(HB-HV) is sustainable at low temperature and pressure. Most importantly, PHA possesses good features of impermeability to gas and complete biodegradability by microbial depolymerases in landfill, oceans and waste treatment facilities, unlike petroleum plastics. Therefore, the adoption of PHA from waste materials should be encouraged as a solution to plastic pollution, food chain contamination and to achieve environmental sustainability.

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