PRODUCTIO N OF BIOPLASTIC (PHB) BY SOIL MICROBIAL ISOLATES: A REVIEW

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Abstract:
Polyhydroxybutyrate (PHB) is a very important polymer of polyhydroxyalkanoates. PHB accumulates inside numerous bacteria under nutrient-limiting conditions with excess carbon, and accumulates as distinct white granules during unbalanced growth in the cell. Soil microorganisms such as Pseudomonas, Bacillus, Alcaligenes, Halomonas etc, are capable of producing this intracellular lipid granule through the action of enzymes. These bioplastics are important in the food industries, agriculture, building and in medicine, due to their good aroma barrier, oxygen permeability, tensile strength and biomoldability. The main limitations for the bulk production of bioplastics are its high production and recovery costs. However, the use of agro waste, industrial by-products, genetic and metabolic engineering has allowed their biosynthesis in several indigenous and recombinant organisms by improving the yields of production and reducing overall costs. The future market for bioplastics will be increasing owing to its sustainability. Also, new innovations of bioplastics in the future can raise the efficiency of production and build new job opportunities most especially, in developing countries.

Keywords*: Soil, PHB, plastics, microorganisms, applications.*

Introduction

Plastic materials that have been used worldwide are now causing serious environmental problems (Nabila and Veenagayathri, 2017; Shakya and Shakya, 2021), with millions of tons of these non-degradable plastics accumulate in the environment annually. This shows that the long term harmful environmental impacts caused by plastics were overlooked and this in turn poses greater difficulties for plastic waste accumulation. There is significant interest to decrease the reliance on petroleum-based plastic products, which causes global environmental pollution (Senem *et al*., 2020).

Eight million tons or more of plastic waste leaks into oceans every year, on which it can be mitigated through innovative redesigns of packaging materials (Senem *et al*., 2020). A number of biodegradable polyesters are emerging so as to overcome the problem of plastic waste accumulation (Nabila and Veenagayathri, 2017). The polyhydroxyalkanoates (PHAs), is a family of biodegradable intracellular polyesters produced by several bacteria (Tehina *et al*., 2019). The main member of the Polyhydroxyalkanoates family is Polyhydroxybutyrate (PHB) (Jehan *et al*., 2016; Li *et al*., 2019; Asiri *et al*., 2020).

PHB degrade naturally and completely to CO_2 and H_2O under natural environment by different microorganisms. Due to its moldability, biodegradability, and thermoresistance, PHB is a promising substitute for conventional petroleum-derived plastics (Asiri *et al*., 2019; Tehina *et al*., 2019). Several bacteria such as *Alcaligenes eutrophus*, *Azotobacter beijierinckia*, *Pseudomonas oleovorans*, *Rhizobium* species, *Staphylococcus*, *Bacillus*, *Micrococcus*, *Rhodococcus*, etc., produce PHBs as reserve food material (Asiri *et al*., 2019, Maity *et al*., 2020; Mourao *et al*., 2021). Because of its biocompatibility, PHB is also used in many medical applications such as drug delivery, reconstructive surgery and bone tissue scaffolding (Kalia *et al*., 2000, Tehina *et al*., 2019). PHBs have also been used, in wrapping, building materials, packaging films, toys household, shopping and garbage bags. More than 250 different species of bacteria possess the ability to biosynthesize polyhydroxybutyrates (PHBs) as intracellular compounds, when the carbon source is available in excess (Irina *et al*., 2017). Depending on the culture conditions that favour PHB accumulation, bacteria that are used for the production of PHB can be classified into two groups (Hadiqa *et al*., 2020). The first group of bacteria requires limitation of essential nutrients such as nitrogen, oxygen and presence of excess carbon source for the efficient synthesis of PHB. The representative bacteria belonging to this group include *Protomonas extorquens* and *Protomonas oleovorans* (Hadiqa *et al*., 2020). On the other hand, the second group of bacteria does not require nutrient limitation for PHB synthesis and can accumulate PHB during exponential growth phase.

Some of the bacteria in this group are *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii* and recombinant *Escherichia coli* (Jiang *et al*., 2018). Currently, the main limitations for the bulk production of bioplastics are its high production and recovery costs. However, genetic and metabolic engineering has allowed their biosynthesis in several recombinant organisms (bacteria, yeasts or transgenic plants) by improving the yields of production and reducing overall costs (Luengo *et al*., 2003). In 1929, Lemoigne a French scientist, first described a bioplastic polyhydroxybutyrate (PHB) in *Bacillus megaterium* (Luengo *et al*., 2003).

This observation was almost forgotten until the mid-1970s when, because of the petroleum crisis, a scientific movement aimed at discovering alternative sources of fossil fuel reserves was undertaken. However, the structure, biosynthetic pathways and applications of many bioplastics have now been established. Microbes belonging to more than 90 genera including aerobes, anaerobes, photosynthetic bacteria, archaea bacteria and lower eukaryotes are able to accumulate and catabolize these polyesters (Luengo *et al*., 2003). The cultivation conditions for PHB biosynthesis are essential requirements for the development of cultivation techniques for large-scale production of PHB (Aragosa *et al*., 2021). In Nigeria, the Federal Ministry of Environment through its enforcement arm – the National Environmental Standards and Regulations Enforcement Agency (NESREA), has adopted Environmental Protection and Regulation model due to the economic, health and environmental implications (Adum *et al*., 2019). This is in line with the National Policy on Environment (1999) (Section 4.12 and 4.19) which stipulates that sustainable industrial development can be achieved through policy initiatives that seek, among others, to ensure production processes incorporate realistic programs for waste minimization through material recovery and recycling. Operational Guidelines for the Environmental Protection and Regulation had been developed and published, delineating responsibilities for the key sector players – the producers, consumers, collectors, recyclers, the Producer Responsibility Organizations (PROs) and the government.

Furthermore, NESREA has established the Alliance on Sustainable Consumption and Production (ASCP), which promotes green and circular economy. To kick start the move, NESREA aligned with the Food and Beverages Recycling Alliance (FBRA) and other manufacturers to fight the plastic menace. National Environmental Standards and Regulation Enforcement Agency (2018) advocated for single use of plastic, by this, they mean that if someone goes to the supermarket to buy some things, they should put them in a plastic bag and should not throw away the plastic bag but use them another day. This paper highlights the production of PHB by soil microorganisms, the advantages of microbial plastics over petroleum based plastics are stressed. The future prospects of microbial plastics are also highlighted.

PHB Producing Bacteria in Soil

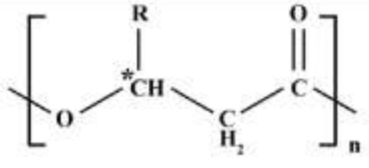
Soil is the topmost layer of the earth crust where man resides. The soil has a lot of human and natural functional roles (Nwaugo, 2008). Microorganisms, also called microbes, are very minute (small) forms of life that can, sometimes, live as single cells, though many also form colonies of cells. Much more microbes exist in topsoil (0 – 5 cm), where food sources are plenty, than in subsoil (deeper than 5 cm). The microbes are especially even more abundant in the area immediately next to plant roots (called the rhizosphere), where sloughed-off cells and chemicals released by roots provide ready food sources (Panigrahi and Badveli, 2013).

Microorganisms are most famously known, by most people, for their negative impacts in terms of disease causing agents. Microorganisms have been an important integral unit of soil from the antiquities of earth formation. It is a verified fact that, although soil organisms comprise < 1 % of the total mass of soil, a hand full of soil is composed of millions of microorganisms which assist in boosting soil fertility and, hence, plant growth. The number of a given species of microorganism, however, directly depends on physical and chemical properties of the soil. Yasser *et al*. (2020) reported that PHB-producing bacteria isolated from the soil of mangrove rhizospheres were not quite the same as those recorded in other marine sources.

Several bacterial strains were found to be associated with PHB accumulation among Gram positive, Gram negative bacteria, and photosynthetic bacteria, including cyanobacteria (Simó-Cabrera *et al*., 2021). As example of PHB synthesizing bacteria, the following genera can be highlighted: *Pseudomonas, Bacillus, Citrobacter, Enterobacter, Klebsiella,* and *Escherichia* (Pradeek, *et al*., 2021). In addition, PHB production is widespread in nitrogen-fixing species, such as *Rhizobium leguminosarum*, *R. galegae*, *R. meliloti*, *Azotobacter beijerinckii*, *A. macrocytogens*, and *A. vineandii* (Simó-Cabrera *et al*., 2021).

Polyhydroxyalkanoates

The carbon number and nomenclature of PHA compounds is influenced by the functional alkyl *R* group, asterisk implies the chiral center of the PHA-building block.

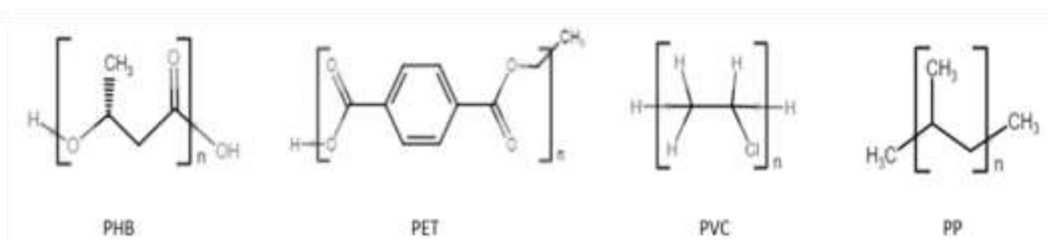


Poly(3-hydroxyalkanoate)

R group	Carbon no.	PHA polymer
methyl	C_4	Poly(3-hydroxybutyrate)
ethyl	C_5	Poly(3-hydroxyvalerate)
propyl	C_6	Poly(3-hydroxyhexanoate)
butyl	C_7	Poly(3-hydroxyheptanoate)
pentyl	C_8	Poly(3-hydroxyoctanoate)
hexyl	C_9	Poly(3-hydroxynonanoate)
heptyl	C_{10}	Poly(3-hydroxydecanoate)
octyl	C_{11}	Poly(3-hydroxyundecanoate)
nonyl	C_{12}	Poly(3-hydroxydodecanoate)
decyl	C_{13}	Poly(3-hydroxytridecanoate)
undecyl	C_{14}	Poly(3-hydroxytetradecanoate)
dodecyl	C_{15}	Poly(3-hydroxypentadecanoate)
tridecyl	C_{16}	Poly(3-hydroxyhexadecanoate)

Figure 1. Nomenclature of polyhydroxyalkanoates (PHA) (McAdam *et al*., 2020)

The different variables of the polymerization process will determine the distribution of the monomers, length, and branching of the polymer chain. Like other thermoplastic polymers, biodegradable bioplastics are semi crystalline polymers characterized by their molecular weight, distribution of branches, melting temperature (Tm), and/or glass transition temperature (Tg). They are water insoluble, and their sinking properties in water facilitate biodegradation in the absence of oxygen in sedimentary soil (Turco *et al*., 2021).



Chemical Structures Among Bioplastics

Wala'a *et al.* (2017) reported that PHBs are predominantly recognized as aliphatic/linear complexes that are optically active biopolyesters and composed of monomers known as (R)-3-hydroxy fatty acid monomer units, with a side chain R group. The latest is usually a saturated alkyl group, although it can also undertake fewer common forms, such as unsaturated alkyl groups, branched alkyl groups, or substituted alkyl group. PHBs have drawn much attention due to their similarities to petrochemical polymers such as polypropylene and polystyrene (Figure 2) which make them a sustainable alternative to replace a high number of petroleum-based plastics that are used in diverse applications (McAdam *et al*., 2020). PHBs are bio-polymers, synthesized inside the cellular structure of microorganisms in the form of granules with diameters ranging between 0.2m and 0.5m and can also be dissolved in chlorinated solvents (Khatami *et al*., 2021).

Features of PHB can differ from brittle thermoplastics to gummy elastomers depending on the type of the utilized carbon source, bacterial host and fermentation conditions (Khatami *et al*., 2021; Majerczak *et al*., 2022). The categorization of PHBs, is according to the number of the present carbon atoms in their constituent monomer blocks. Short chain length (scl-PHBs) and medium chain length (mcl-PHBs), comprising 3 to 5 and 6 to 14 carbon atoms, respectively (Sadaat and Jamil, 2020).

Different chain lengths originate from the substrate specificity of the PHA synthase enzyme that is capable of accepting a 3-hydroxyalkanoic acid containing a particular number of carbons (Li *et al*., 2019).

Figure 2. Chemical structures of PHB in comparison to commonly used petroleum-based polymers (polyethylene terephthalate (PET), polyvinylchloride (PVC), and Polypropylene (PP). (McAdam *et al*., 2020)

As a member of the PHA family, PHB is characterized by having a methyl functional group (CH_3) and an ester linkage group (−COOR), it is these functional groups that are responsible for the materials thermoplastic, hydrophobic, high crystallinity, and brittle characteristics.

Properties of PHB

PHB is a linear homopolymer of D-3-hydroxybutyric acid (Aragosa *et al*., 2021; Boura *et al*., 2022). Bioplastics as represented by PHB are environmentally friendly due to their lower persistence when abandoned in the environment (Carpine *et al*., 2020). Another property of the bioplastics is that they are non-toxic and compostable, which means that they are not harmful to living organisms (Boura *et al*., 2022). Bioplastic materials used in packaging applications work by protecting products from the environment and preserving the quality of products (Ibrahim *et al*., 2021).

In bioplastic packaging applications such as food and beverage, healthcare, cosmetics, etc., most packaging is produced by the food processing sector. Food packaging is a combination of the art, science and technology of containment of a commodity in order to ensure the supply of the products to customers in good condition (Ibrahim *et al*., 2021). PHBs are more useful for cosmetic packaging, such as the fact that it has no harmful effect when it is applied in contact with skin, it emits less greenhouse gases, and has very high biodegradability as well as excellent biocompatibility in different environments (Ibrahim *et al*., 2021).

Biosynthesis of Bacterial PHB

The biosynthesis of bacterial PHB is catalyzed by chemical reactions that converts the carbon source, generally a sugar, into the PHB biopolymer by the action of three main enzymes as shown in Figure 3; The α -ketothiolase that converts the Acetyl-CoA into Acetoacetyl-CoA, acetoacetyl-CoA reductase which transforms the Acetoacetyl-CoA into the (R)-3-hydroxybutyryl-CoA (PhaB), and the PHB synthase which finally triggers the reaction to the synthesis of PHB. During unbalanced growth conditions, the level of acetyl-CoA increases while the level of Co-A decreases (Aragosa *et al*., 2021). This triggers the enzyme α -ketothiolase to start the three-step process for the synthesis of PHB. On the other hand, when the level of Co-A increases the first enzyme, α ketothiolase, is inhibited and the synthesis of the biopolymer stops. The formation of PhaB is controlled by the intracellular mediation of acetyl-CoA (Kavitha *et al*., 2018).

Under growth conditions without nutrient restrictions during exponential growth phase, the pool of free CoASH is enhanced. However, when growth is nutrient limited, CoASH level is condensed by phosphate or ammonium where PHB synthesis is favoured. An increased PHB content was found under oxygen limitation. This activation and deactivation process of the PHB synthesis is regulated by the enzyme α - ketothiolase which is a key catalyst of the synthesis as illustrated in Figure 3 (McAdam *et al*., 2020; Aragosa *et al*., 2021).

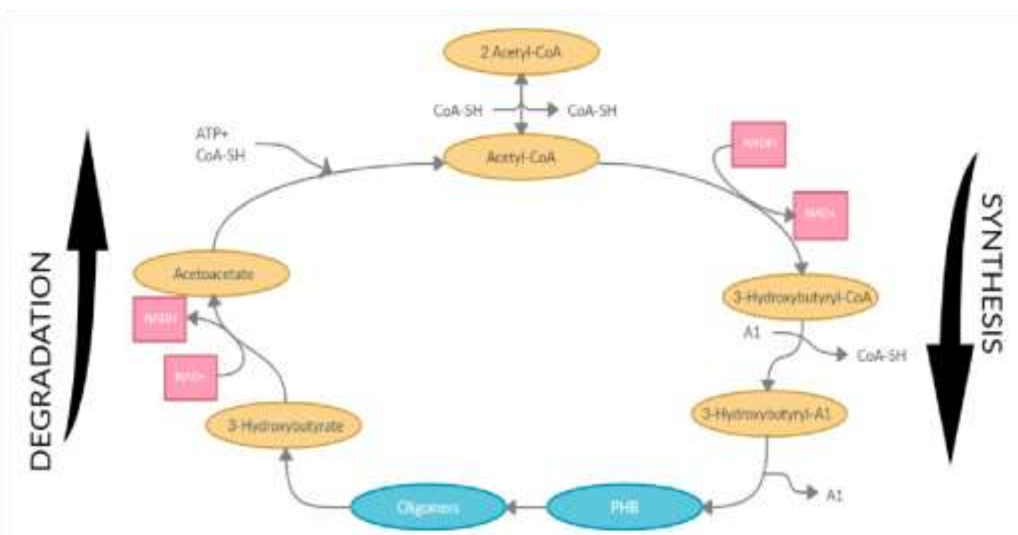


Figure 3. Polyhydroxybutyrate (PHB) synthesis and degradation process (McAdam *et al*., 2020)

Production of PHB

Singh and Parmar (2011), and Yasser *et al*. (2020) reported the following procedure for PHB production in the laboratory. Fifty millilitres (50mL) of minimal salt medium (MSM) (fermentation medium) containing the following salts are used: 0.3g $Na_2HPO_4.7H_20$, 0.1g KH_2PO_4, 0.2g $(NH_4)_2SO_4$, 0.1g $MgSO_4$, 0.1g $CaCl_2$, 4.0g glucose, 0.2g NaCl, and 0.5g nutrient broth. 1.0 mL of 24 hours old isolate was inoculated into cooled sterile MSM and incubated at 37°C for 72 hours. After 72hours of incubation of the MSM at 37^0C, the MSM was centrifuged at 8000 rpm for 15minutes and the supernatant was discarded and the crude PHB is the pellet.

Commercial production of PHB

The commercial production of PHB, is achieved by extraction and purification of the polyester from the bacteria via optimization of the conditions of microbial fermentation of sugar or glucose (Carpine *et al*., 2020). In the 1980s, Imperial Chemical Industries developed poly (3-hydroxybutyrate-co3-hydroxyvalerate) obtained via fermentation that was named 'Biopol'. It was sold under the name 'Biopol' and distributed in the U.S. by Monsanto and later Metabolix. As a raw material for the fermentation, carbohydrates such as glucose and sucrose can be used, but also vegetable oil or glycerine from biodiesel production. Researchers in industry are working on methods with which bacteria can produce PHB as energy storage in their tissues.

Techniques for detecting PHB

i. Sudan black B staining method of screening

This method was reported by Anish *et al*. (2013) and Wala'a *et al.* (2020) where bacterial isolates were cultured for 3 days at 37^0C in nutrient agar medium. A solution of Sudan black B was applied to stain bacterial colonies and the plates kept undisturbed for 30 minutes. The excess dye should be discarded and the plates be rinsed gently by adding 100% ethanol. The appearance of white colonies indicates inability to incorporate the Sudan black B and the appearance of bluish black colouration, indicates the presence of PHB, thus this is a presumptive test (Yasser *et al*., 2020).

ii. Fluorescence staining method

Yasser *et al*. (2020) reported using this method to further confirm PHB accumulation in bacteria isolated from mangrove rhizosphere. The fluorescent dye, acridine orange was used in this method where 50 µL of dye was added to 10 µL of 72 hours old bacterial culture, and incubated at 35^{0}C for 30 minutes. The bacterial culture was centrifuged for 5 minutes at 6000 rpm, after that, a smear was prepared and observed by fluorescent microscope. The appearance of yellow-colored granules inside the cell is a positive indication of PHB accumulation (Yasser *et al*., 2020).

Methods of PHB extraction

Methods of extracting PHB involves cell wall lysis, solubilization and purification of PHB component, and precipitation of PHB polymer. Solvent extraction methods, chemical- and enzyme-based digestion methods are some of the common methods for PHB polymer extraction from microbial biomass. Solvent extraction is the most well-established and commonly used to obtain PHB polymer from biomass due to its high purity. In a study by Folino *et al*. (2020), the following solvents were evaluated for PHB extraction under various conditions methylene chloride, chloroform, and 1,2-dichloroethane.

Cellular debris was removed via filtration after solvent extraction, and rotary evaporation was used to concentrate the solution before the PHB polymer was precipitated by dropwise addition of ice-cold ethanol. The advantage of solvent extraction is due to its high PHB recovery purity, however there are much distress about expensive cost of operation as well as ecological impact caused by the generation of toxic waste. One of the ways to curb this menace, is through the use of waste solvents for PHB extraction (Carpine *et al*., 2020).

Sodium hypochlorite is used in chemical-digestion, so as to solubilize non-PHB biomass there by obtaining separation of PHB content which can be recovered by centrifugation (Zhou *et al*., 2021). While the method is simple and effective, PHB polymers obtained through hypochlorite digestion are of lower molecular masses because of the severe polymer degradation (Zhou *et al*., 2021). To settle this problem, a composite approach was used by dispersion of sodium hypochlorite solution as solubilizer of cell, and chloroform to protect PHB from degrading further after its release from cells, thereby taking advantage of both hypochlorite digestion and solvent extraction (Zhou *et al*., 2021).

Enzymatic digestion requires milder operating conditions while achieving negligible product degradation, compared to solvent extraction and chemical digestion (Abate *et al*., 2022). This is done by suspending the biomass in a specialized buffer and incubating at a specific temperature, which was optimized for enzymatic activity. After enzymatic hydrolysis, the PHB polymer was recovered by centrifugation. A polymer purity of up to 90% could be attained with this method (Abate *et al*., 2022). Enzyme-based PHB recovery methods are safer in terms of operation, pose lower health risks, and has lower environmental footprint.

Advantages of bioplastics over conventional plastics

It is known that plastic is one of the main pollutant resources in the environment which is produced daily (Gonzalez *et al*., 2020). To reduce the environmental pollution, an alternative must be developed by changing the use of conventional plastic. Many environmental issues can be solved by using PHB (Sidek *et al*., 2019). The properties of bioplastics like aroma barrier and biomoldability make bioplastics good alternative compared to conventional plastics. Besides, bioplastics also have unique features such as biodegradable, eco-friendly, energy efficient and compostable (Sidek *et al*., 2019). Moreover, in certain cases bioplastics exhibit better properties compared to conventional plastic such as good in mechanical properties, thermal properties, oxygen permeability, gas barrier and water vapour transmission rate (Sidek *et al*., 2019).

Disadvantages of bioplastics

However, possible problems might come along with the use of bioplastics. Here are the disadvantages of bioplastics:

1. High costs: It is acclaimed that bioplastics costs two times more than conventional plastics. However, the amount of large-scale industrial production of bioplastics which are more common in the future with the implementation of cost reduction is expected;
2. Recycling problems: Bioplastic material might actually contaminate the recycling process if not separated from conventional plastics.
3. Reducing raw materials: Bioplastics produced from renewable sources might reduce raw material reserves. Moreover, in order to reduce energy consumption during the production of bioplastics and potential competition with agricultural resources for foods and also to provide additional raw material sources, the exploitation of food by-products is also the current trend (Simo-cabrera *et al*., 2021).
4. Misconception: The description of bioplastic as compostable can be misleading because not all bioplastics are biodegradable at home like organic food waste but usually demand an industrial composting treatment. Also, bioplastics and related terms are being misapplied by different manufacturers to place their products more captivating on the market. Some

logos used by manufacturers such as "environmental friendly", "non-toxic", "degradable/totally degradable", are a guile with the uninformed and overwhelmed consumer (Filho *et al*., 2021; Bandini *et al*., 2022).

Applications of bioplastics

Bioplastics are receiving more attention in various applications in industries. This is because bioplastics are good alternative in order to decrease the capacity of inert materials disposed in landfills and create pollution free environment which is very important to society at large and also industries. Bioplastics have been explored in construction and building industry. However not only builders but home owners are also attracted to use bioplastics for different products such as in fencing, decking, e t c (Bandopadhyay *et al*., 2020).

Furthermore, companies manufacturing electroacoustic devices, use bioplastics as a membrane for high quality sound (Bauer *et al*., 2021). The advantage of this kind of material is providing the same sound velocity as an aluminium or titanium diaphragm and along with the delicate sound. Besides, it also produces the trebles sparkling clear sound and bass notes that are remarkably deep. On the other hand, bioplastics are also applied in membrane for reinforcement for high quality electronic paper (e-paper), combustible cells (hydrogen) and as an ultrafiltration membrane for water treatment (Bauer *et al*., 2021).

The development of bioplastics in packaging industry is slowly being welcomed by grocery store and food service industry for use as film for sandwich wraps, for fresh products packaging such as vegetables, fruits, salads, pasta and other bakery goods (Sidek *et al*., 2019). The potential use of bioplastics for short- and long-term packaging, as well as items that do not require outstanding oxygen or water barrier qualities, necessitates the commercialization of these bio-based packaging materials (Coppola *et al*., 2021). The main features required in food packaging are moisture and oxygen permeability and mechanical properties. Raw meat is highly susceptible to spoilage bacteria and pathogens growth. High oxygen concentration in the packaging is required to preserve the fresh meat's color, so high oxygen permeability is required. So vacuum packaging is often considered a good choice, while adding oxygen adsorbing layers, resulting in active packaging, can better preserve cured meat (Coppola *et al*., 2021). Dairy products need low oxygen permeability materials to avoid oxidation and microbial growth. In addition to that, a good barrier to light can preserve fats' oxidation. Other main features are the water evaporation factor and the avoiding of odour absorption from the exteriors. These features can reside in some forms of polysaccharides as pectins, which are mainly produced by extraction from fruit and vegetable sources and could act as a safety barrier for food products (Coppola *et al*., 2021).

Polyhydroxybutyrate can also be used in construction materials, automotive interior materials, electrical devices, containers, and packaging materials (Kavitha *et al*., 2018). PHB is also compatible with body tissues, which makes their use in medical areas like surgical sutures, wound dressings and ocular devices possible (Kavitha *et al*., 2018). PHB can be used to make heteropolymers by moulding, extruding, spinning into fibres and processing into food packaging films.

As compared to the conventional packaging material, these films were distinguished by excellent oxygen permeability (OP), considerable tensile strength, high antioxidant activity, high water vapour transmission rate (WVTR) and significant antimicrobial action (Varghese *et al*., 2022). The applications of bioplastics in agriculture are quite limited. So far, agricultural uses such as grow bags, farm nets and mulch films have been studied. Agricultural nets made up of biodegradable PHAs are being considered at the moment. The compostability of PHA's biodegradable meshes allows the bioplastic to be disposed of directly into the soil. Different bioplastics such as PHB and their blends with PLA are most widely used for manufacturing agricultural nets due to their high tensile strength (Varghese *et al*., 2022). Production of PHA-based grow bags sequesters nitrogen from the water, does not pollute the surrounding water bodies, prevents root reformation, and avoids pollution concerns (Varghese *et al*., 2022). Agricultural mulch films are critical for increased crop yield and protection, apart from grow bags. Mulch films can retain moisture content, maintain excellent soil structure and prevent contamination by managing nuisance weeds and it is noted that plastic mulch accounts for 40% of agricultural mulch (Varghese *et al*., 2022).

Challenges to Industrial Scale Production of PHBs

PHBs have unique characteristics, which make them suitable for commercial production. However, production of bioplastics become most challenging because the production must not disturb the potential food sources (Bauer *et al*., 2021). This circumstance can be reduced by utilizing the non-food resources for the purpose. The application of PHA as a biodegradable alternative to conventional plastics has been hampered by its high production cost. Global efforts have been made to solve this problem; however, two bottlenecks in the PHA production process need attention.

First, the carbon source as the substrate selection has an important effect on the composition and properties of the final biopolymer (Gonzalez *et al*., 2020). Some

producers use xylose, acetate, or glycerol as the sole carbon source, which are quite expensive and contribute more than 50% of the total production costs (Varghese *et al*., 2022). Nevertheless, the cost associated with the carbon source can be reduced using cheap carbon sources such as wastewater (Varghese *et al*., 2022). Another economic factor involved in bioplastic production is bacterial culture selection, but costs can be reduced using mixed microbial cultures (MMC), which are microbial populations of unknown composition, such as the microbial consortium from active sludge of wastewater treatment plants (Mesquita *et al*., 2015). This may not require extensive microbiological screening and analysis. Thus, the cost of production is reduced; energy is saved. The second bottleneck, the recovery process, is today the biggest challenge in order to achieve high recovery efficiency and a high degree of purity in a cheap and environmentally friendly way while at the same time maintaining its molecular weight and thermal and mechanical properties (Moshood *et al*., 2021). Therefore, the importance of developing an economical and efficient recovery process cannot be overestimated. However, there are still a few challenges, which limit their up-scaling. The physical and chemical properties which demand attention include: lowering of melting point and glass transition temperature, elastic modulus, tensile strength and elongation (Naser *et al*., 2021). These characteristics depend upon the monomeric composition and the molecular weight of the polymer. Copolymers of PHBs with high molecular weights have the potential to overcome these limitations.
In addition, strategies to manipulate feed composition culture conditions such as independence from nutritional imbalance, increased microbial biomass, high expression of polymerase genes and genetic modifications to synchronize termination of PHB biosynthesis with cell lysis will certainly help in economic production of PHBs on an industrial scale (Sakthiselvan *et al*., 2022).

Future Prospects

The environmental impact caused by the large quantity of non-degradable waste materials is promoting research to develop new biodegradable plastics that can be extracted from bacteria and fungi. The developments of bioplastics in the future can raise the efficiency of production, build new applications and new opportunities of bioplastics (Varghese *et al*., 2022). Furthermore, the future market for bioplastics will be increasing owing to its sustainability. Besides, the biotechnology of microorganisms gives an opportunity to bioplastic manufacture because it could significantly be applied and commercialized for various industries such as agriculture, medical, pharmaceutical, and food (Bauer *et al*., 2021). Hence, a new guideline and standard for bioplastics should be developed for production, usage and waste management of bioplastics over the world. Thus, labeling legislation must be enhanced based on product, raw material usage, energy consumption, emissions from manufacture and use. Recent development of technology, continued innovation and global support is important to commercialize and demonstrate the bioplastics. But nevertheless, the bioplastics must be based on an integrated environmentally friendly to increase the sustainability of materials and processes throughout its lifetime (Brizga *et al*., 2022). Many countries around the world have already begun to integrate these materials into their technologies (Ojha and Das, 2022). In America, McDonald is making biodegradable containers for their fast food. Other companies such as Bayer, DuPont, Dow Cargill, Nike, Danone, etc. are also producing biodegradable packaging (Arikan and Ozsoy, 2015).

Based on advantages of bioplastics, there are certainly an abundant amount of materials and resources to create and find more uses for bioplastic. Based on disadvantages of bioplastics, for the sustainability several parameters must be considered, including the raw materials from which the bioplastic is generated, the energy consumed during bioplastic conversion and its life cycle assessment analysis from production to ultimate disposal or recycle (Claudia & André, 2020).

Conclusion

The problem of plastic pollution, has led to intensified research on microbial bioplastics. Bioplastics will replace petroleum based plastics because of their inherent characteristics in the future. Also, the use of less costly substrate will reduce the cost of production. Microorganisms from diverse habitats should be screened for potential to produce microbial plastics. Microorganisms are ubiquitous ad cheap to obtain ad will ease the production process ad cost, bioplastics ensure sustainable environment.

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