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Biological Significance and Advances in Application of Polyhydroxyalkanoate

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ABSTRACT

In our daily life for various purposes we use plastics. Even though its properties like low cost, stability, and durability make them more convenient for use, they are nondegradable and leads to greenhouse gas emission or ecological damage. These plastics are being originated from nonrenewable sources and its disposal can cause risk to human health, environment, marine, and wildlife. So that there emanate the necessity for an alternate material that could able to eliminate the problems associated with plastics. Thus more attention of researchers was on biopolymers since it is having lots of properties similar to plastics and is essentially biodegradable. Recently, it is well known that Polyhydroxyalkanoates (PHA) which comes under the family of intracellular biopolymers can replace conventional plastics. They are being produced by various types of bacteria as carbon and energy storage granules inside the cell. As well it's biodegradable and is considered to be biocompatible in contact with living tissues. PHA haven can be used for various purposes such as food packaging, controlled drug delivery, drug encapsulation, tissue engineering etc. Thus this review focuses on various applications of PHA.

Keywords: Biopolymers, Polyhydroxyalkanoates, Plastics, Biodegradable, Biocompatible.

1. INTRODUCTION

The plastics have been widely used across the world. These organic polymeric materials possess various properties which make them convenient for use [1]. Plastics can be converted into any shape according to our need by means of any one of the processes such as extrusion, molding, casting, or spinning. They also possess characteristics such as high strength to weight ratio, excellent thermal properties, electrical insulation and resistance to acids, alkali, and solvents. The electrical insulating properties like strength, stress resistance, flexibility, and durability enables its use in electronics. Advances in synthetic polymers lead to the production of plastic such as polyethylene, polypropylene, polyesters, and polyamides. Consecutively, there was a dramatic increase in the types of material for packaging, types of containers and materials for carrying bags [2]. There was an incredible increase in the production and use of plastics in the last two decades across the world. But the fact is that a good number of these plastics are being produced from petrochemicals, which is a nonrenewable resource. According to Malathi *et al.*, around 40% of the produced plastics are being thrown into the landfill in a year and more than 50 billion plastic bags circulated in which less than 3% are being recycled [3]. Plastics have high molecular weight, bonded closely together and thus they are nondegradable. For this reason, its disposal becomes a hard task and at the same time, it causes negative impacts on the environment. Beyond this, a significant point about plastic is that they are being produced from petroleum resources, which is getting limited nowadays. Also, these plastics are unfit for temporary usage [4]. Further, these plastics are seemed to emit greenhouse gases and cause ecological damage. The marine and other habitats are being polluted by these plastics in a great deal. As a result, lots of wildlife are getting entwined in plastics, which in turn injury or impaired movement. Sadly in some situations, this can become a cause of death [2]. Usually, the polymer resins mixed with different types of additives are being used to get better performance. But most of these chemicals are potentially toxic. Examples of such additives are lead and tributyltin in polyvinyl chloride [5]. At present landfill, incineration and energy recovery, downgauging, reusing and recycling are adopted for plastic waste management.

But these methods are inapt for getting rid of from plastic waste. Thus there come up the necessary for an alternate for plastic materials.

2. BIOPOLYMERS

Polymers comprise of repeating macromolecules known as mer units. They are the solid, nonmetallic compounds with high molecular weight. The varying characteristics of different polymers will depend on their composition. If the polymers are being produced from natural renewable resources, they are known as biopolymers. Biological systems like microorganisms, plants, and animals are capable of producing biopolymers. Also, it can be chemically synthesized from biological starting materials like sugars, starch, natural fats, and oils. More significantly they degrade biologically and nontoxic. It can thus treat as an alternative to petroleum-based products. There are mainly three types of biopolymers based on their origin and production. The classification biopolymers are shown in Figure.1. Examples of polymers synthesized classically from bio-derived monomers are polylactic acid (PLA) and other polyesters, whereas the Polyhydroxyalkanoates (PHA), bacterial cellulose, xanthan, curdlan, pullulan etc. comes under the category of polymers directly produced by microorganism [4].

3. POLYHYDROXY ALAKANOATES

Polyhydroxyalkanoates are being produced by lots of bacteria as intracellular carbon and energy storage granules. In fact, a great number of prokaryotic organisms accumulate PHA from 30 – 80% of their cellular dry weight. More often the PHA can be degraded by abiotic degradation, which is nothing but the simple nonenzymatic hydrolysis of ester bonds. Whereas in biotic degradation the enzymes will degrade the leftover products till final mineralization [6]. The general chemical structure of PHA is given Figure.2. From the figure, ‘X' will be 1 or higher and R can be either hydrogen or hydrocarbon chains of around C16 in length. The main characteristics of PHA include water insoluble, resistant to hydrolytic degradation, UV resistance, soluble in chloroform and other chlorinated hydrocarbons, biocompatible, nontoxic, less sticky when melted, biodegradable [7]. On the basis of a total number of carbon atoms within the PHA monomer, it can be classified into three as shown in Figure.3. The main biopolymers come under the family of PHA are given in the following Figure.4 [6]. PHA can be produced from renewable sources, which is an advantage over the synthetic plastics. But the fact is that the high production cost makes PHA more expensive than synthetic plastics. However, if we produce PHA from locally obtainable and renewable sources of carbon like horticultural agricultural wastes, corn, cassava etc. it will become economic by taking into account of the environmental benefits [8]. The microorganisms that inhabit diverse ecological niches, accumulates PHA as a response to the experienced stress. The ecological niche like estuarine sediments, marine microbial mats, rhizopheres, groundwater sediments, engineered ecosystems with fluctuating nutrient contents etc. are well known for the occurrence of microorganisms that are keenly involved in PHA accumulation. In these situations, they supposed to go for PHA accumulation so that they can meet metabolic energy requirements at the time of starvation. This conception can be applied in industry in order to cut down the biopolymer's cost commercially with sustainable production processes. To treat as best PHA producers, the microorganisms should possess some essential characteristics like fast-growing population, ability to utilize cheap carbon and high rate of production.

There exist mainly two ways to get new efficient PHA producing bacteria's. Firstly, strains that are isolated from the natural environment and the second way is to design genetic recombination strains [9]. In an inapt condition, various genera of eubacteria are known to produce PHA based on cultivation strategy, nature of bacterial strains, and type of polymerase gene present. Also, the composition of these polymers may vary at the monomer level [10]. Both gram- negative and gram- positive bacteria are known to produce PHA. Among gram-negative bacteria, *Alcaligenes*, *Ralstonia*, and *Pseudomonas* are well known as PHA producers. Of these *Alcaligenes* and *Ralstonia* are renowned to have the ability to use up the pure substrates, agricultural wastes, oily wastes, dairy products and CO_2 in order to produce PHA. On the other hand, *Pseudomonas* usually produces mcl – PHA on various aliphatic alkanes or fatty acids, agricultural and oily wastes. Also, they can produce scl – mcl PHA. Gram-positive bacteria's are not widely studied for PHA production and only a few genera's like *Bacillus*, *Clostridium*, *Corynebacterium*, *Nocardia*, *Rhodococcus*, *Streptomyces* and *Staphylococcus* are known to produce PHB and certain copolymers [11].

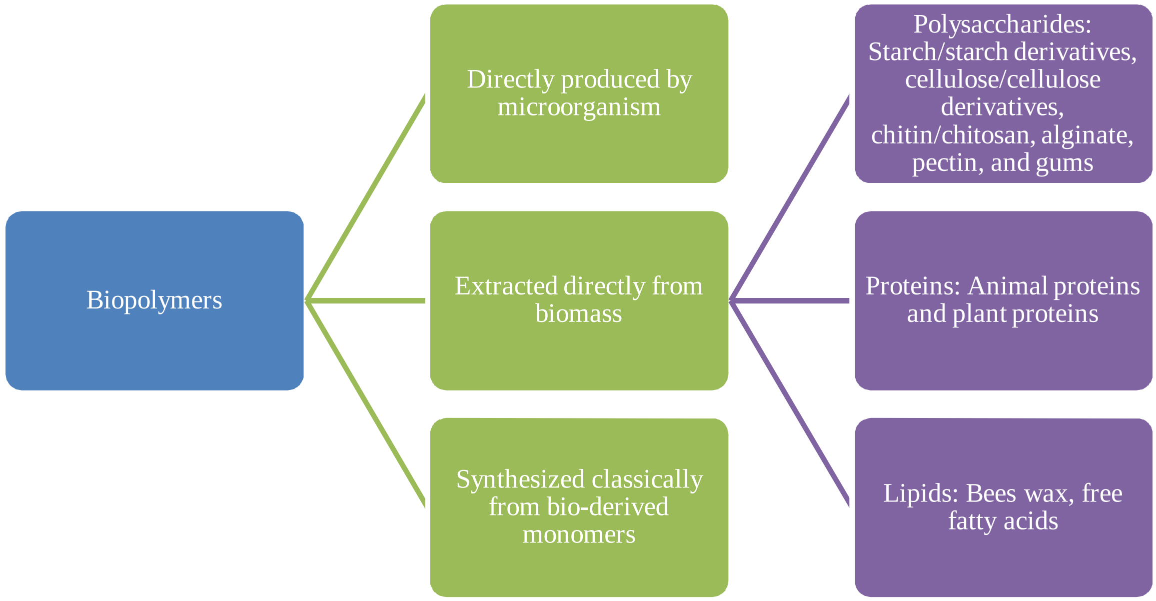

Figure 1. Classification of biopolymers

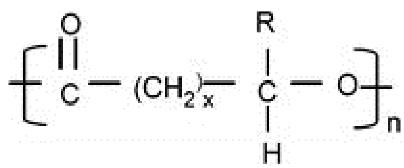

Figure 2. General Chemical structure of PHA

4. PHA PRODUCTION

Rhodobacter sphaeroides can be grown at 37ºC in GM medium containing (g/l) L-glutamic acid 3.8, D,L-malate 2.7, yeast extract 2.0, KH_2PO_4 0.5, K_2HPO_4 0.5, $(NH_4)_2SO_4$ 0.8, $MgSO_4.7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.053, $MnSO_4.5H_2O$ 0.0012 and basal medium with the addition of 30 g/l NaCl. Basal medium contained (mg/l) nicotinic acid 1.0, thiamine 1.0 and biotin 0.01. The pH was adjusted to 7.0 [12].In case of *Methylobacteriumextorquens*a mineral salt medium consisting of : $(NH4)_2SO_4$ 1.0 g/l; KH_2PO_4 1.305 g/l; $Na_2HPO_4.7H_2O$ 4.02 g/l; $MgSO_4.7H_2O$ 0.45 g/l; $FeSO_4.7H_2O$ 1.3 mg/l; $CaCl_2.2H_2O$ 3.3 mg/l; $MnSO_4.7H_2O$ 100 µg/l; $ZnSO_4.7H_2O$ 130 µg/l; $Na_2MoO_4.2H_2O$ 40 µg/l; $CuSO_4.5H_2O$ 40 µg/l; $CoCl_2.6H_2O$ 40 µg/l; H_3BO_3 30 µg/l and 1 %(v/v) methanol was used for inoculum development [13].According to Alejandra *et al.*, the medium used for the isolation of the bacteria was HM composed of (g/l): NaCl, 44.5; $MgSO_4$. $7H_2O$, 0.25; $CaCl_2$. $2H_2O$. The conventional medium used for PHB production was the minimal mineral medium which contained (g/l): NaO, 0.09; KCl, 0.5; NaBr, 0.06; peptone, 5; yeast extract, 10; glucose, 1; and granulated agar, 20; adjusted to the pH value of 7.0. $2HPO_4.2H_2O$, 4.5; KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.2; NaCl, 1; $(NH_4)_2SO_4$, 2; $CaCl_2.2H_2O$, 0.02; $NH_4Fe(III)$ citrate, 0.05; agar, 15; trace element solution SL6, 1 ml; glucose, 10 g; adjusted to the pH value of 7.0. SL6 was composed by (mg/l): $ZnSO_4.7H_2O$, 100; H_3BO_3, 300; $CoCl_2.6$ H_2O, 200; $CuSO_4$,6; $NiCl_2.6H_2O$, 20; Na_2MoO_4. $2H_2O$, 30; $MnCl_2.2$ H_2O, 25. [14]. Even though the researchers could able to isolate efficient PHA producers, the high cost of production remained as a problem. Thus more focus comes on the production of PHA from inexpensive carbon sources [9].Lignocelluloses, corn steep liquor, plant oils, molasses, starch, whey are the few carbon sources used for PHA production. The use of these materials not only reduces the cost of production but also reduced the environmental pollution caused by these materials [1].

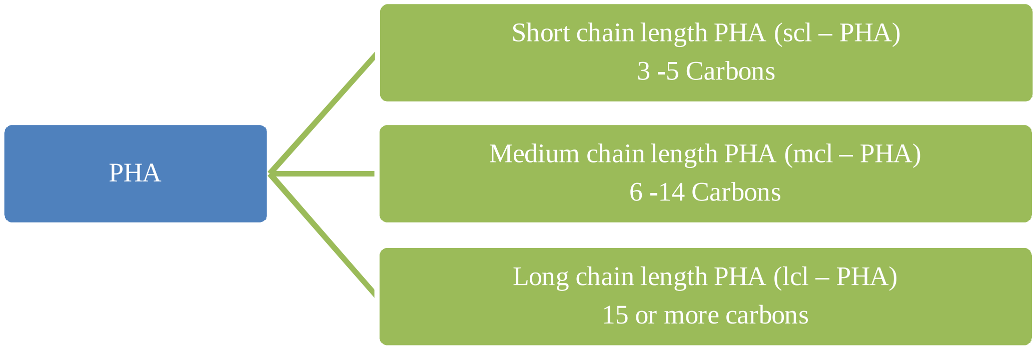

Figure 3. PHA classification [51]

5. EXTRACTION OF PHA

The PHA produced will be stored as granules inside the microorganisms. So in order to access the PHA, we need to lyses the cell followed by precipitation. There are different methods of PHA extraction.

5.1. Dispersion method of sodium hypochlorite and chloroform

First, collect the cells by centrifugation at 10000 rpm for 15 minutes at room temperature. Wash the pellets with phosphate buffer saline (pH 7.4). Air-dry the pellets for 1-2 hours and measure the weight. Add chloroform and 4% sodium hypochlorite to the pellets in a ratio of 1:1. i.e, 12.5 ml per mg of pellet weight. Keep the mixture at 30°C overnight. Then centrifuge the dispersion at 8000 rpm for 10 min at room temperature, which will result in the formation three phases. The bottom phase will be the chloroform which will be containing PHB. Transfer this phase to a fresh tube and measure the volume. To this add 5 x volumes a mixture of methanol and water (7:3 v/v). Then centrifuge at 10000 rpm for 15 minutes, which will yield the PHB in the form of precipitate [19].

5.2. Extraction using the solvent chloroform

Harvest the bacterial culture. Then remove the lipids from the cell pellets using methanol which should be 40 times the volume of cell pellets, after the cells incubated at 95°C for 1 hour. Filter it to completely remove the methanol. Incubate the sediment granules in an oven at 65°C till it dry. Add chloroform to the dried granules and incubate at 95°C for 10 minutes. Gently mix the mixture overnight, after cooling. Then filter the solution to get the debris. Precipitate the PHA using 7:3 (v/v) methanol and water. Wash the precipitated PHA using acetone and dry it [20].

5.3. PHB recovery by ATPS (Aqueous two-phase separation system)

Develop two-phase system using 26% w/v polyethylene glycol (PEG 6000) and 20% (w/v) KH_2PO_4. For partitioning in ATPS made of PEG 6000 and KH2PO4 standard PHB of concentration 3.0 g/l can be used. ATPS should be prepared by taking a point at the center of tie line of the bimodal curve. Using a disposable syringe remove PEG phase and transfer it to another flask. Then the flask should be kept in rotation at 200 rpm for 10 minutes. Separate the PHB on the top and analyze the PHB concentration. Partitioning coefficient K_d can be calculated using the following equation [21].

$$K_d = \frac{PHB\, in\, PEG\ phase \left(g/l \right)}{PHB\, in\, aqueous\ phase \left(g/l \right)} \qquad (1)$$

6. APPLICATIONS OF PHA

Due to the petroleum crisis during 1970, there arises the need for an alternate material which could replace petroleum-based plastic materials. Thus microbial PHA production gained widespread attention. The wide use of plastics resulted in the deposition of these nondegradable plastics which raises lots of environmental as well as health problems. PHA is thermoplastic having properties similar to polyethylene [15]. Different properties of PHA like biocompatibility, biodegradability, negligible cytotoxicity to the cells etc. make it suitable for use in various fields. Thus PHA gained the attention as a replacement for petrochemical-based polymers and can be used in packaging, medical and coating materials [16].The main applications of PHAs are given in Figure 5. Among various PHA, now only poly-3-hydroxybutyrate (PHB) and copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV) have been reported to produce on a commercial scale. According to its applicability, the PHA can be blended, surface modified and/or composite with other polymers, inorganic materials, and growth factors in order to meet the required properties like biocompatibility, mechanical strength, and degradation rates etc [17]. In order to enhance the biological as well as mechanical elongation properties of PHA, chitosan, chitin, and chondroitin sulfate etc. are being used. It is also reported that the bioactivity and cell interaction capacity for bone implants and tissue engineering can be improved by adding some inorganic compounds like nano-HA, bioactive glass, tricalcium phosphate, calcium silicate, zirconium dioxide and herafill to PHB and PHBV. There were studies which used graft copolymerization or in situ polymerization or multi-block copolymerization to impart chemical modification of PHB [18].

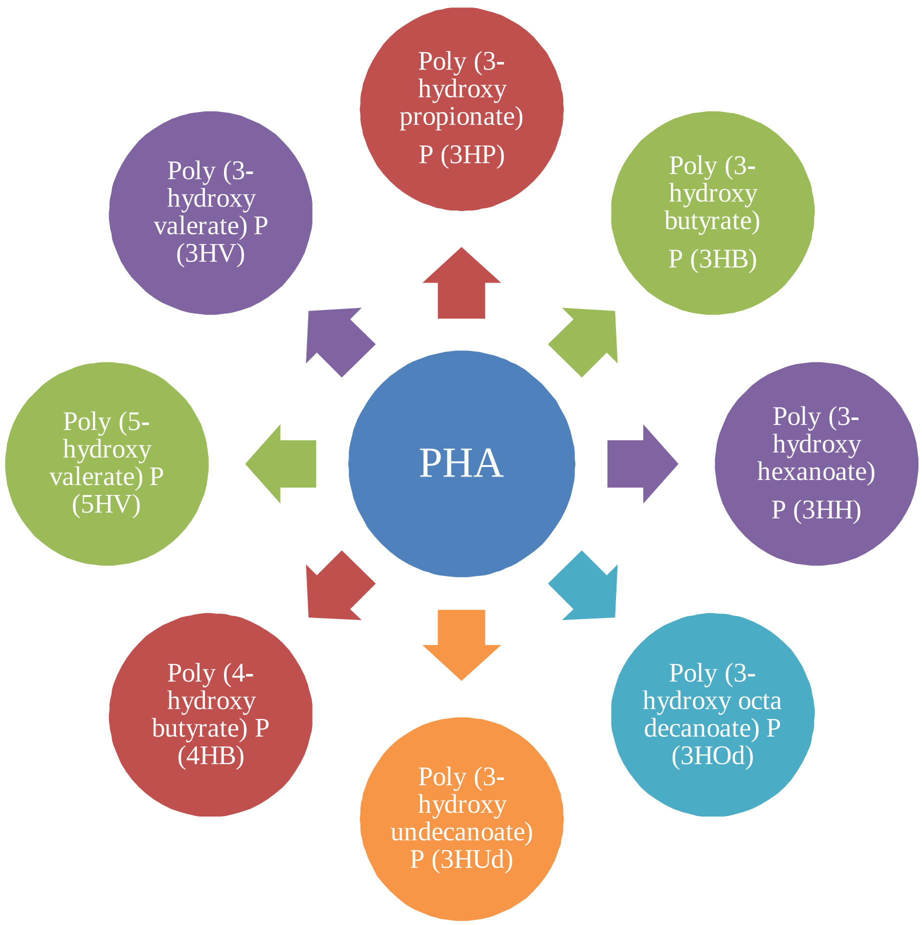

Figure 4. Polymers of PHA family

6.1. PHA in medical industry

In the medical industry, different polymers are used for various purposes like preventive medicine, clinical inspections, and surgical treatments of diseases. When there is direct contact with the living cells of our body polymeric biomaterials, which is an important group of the polymers is being used. In order to act a biomaterial, it must possess some important characteristics. It should be nontoxic, effective, sterilizable as well as biocompatible [22]. In order to apt a material for the medical application, it should be biocompatible. That is when it is being introduced to soft tissues or blood of a host organism it should not leads to severe immune reactions. Interestingly PHA is mostly biocompatible and can thus be used for various medical applications [10]. There are lots of factors which are known to determine the biocompatibility of materials. It mainly includes shape, surface porosity, the chemistry of the material, as well as the environment where it is being introduced [23]. Biodegradability is the important property of PHA which make it compatible with living tissues. According to Ali Iftikhar and Jamil Nazia, by considering various studies P(3HB) showed small resistance towards in vitro degradation and biodegradation in living mammalian cells in comparison with other biopolymers such as PLGA, PGA etc. Whereas the study of resorption of implants of P(3HBco3HV)and P(3HBco3HHx) enabled the researchers to reach in a conclusion that due to its biodegradability the PHA can be used as a biomaterial and it will not give rise to any queries related to its persistence after implantation. One of another important property of PHA is non-carcinogenic behavior. Because they exhibit improvements in cell growth either in vitro or following in vivo implantation. Even though there were lots of studies about the role of PHA on cell proliferation and biocompatibility, there is no clear evidence that they are completely noncarcinogenic for all cell lines [10]. Among PHA, poly 3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly 4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3- hydroxyhexanoate (PHBHHx) and poly 3-hydroxyoctanoate (PHO) and its composites etc. were used to prepare lots of medical devices. The important examples are sutures, repair devices, repair patches, slings, cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/ regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, bone marrow scaffolds, and wound dressings [24]. The main applications of biomaterials in the medical field are given in Figure 6. The degradation of PHA yields the product 3HB. In fact this degradation product can increase cytosolic calcium concentration as well as protect mitochondrion function. Thus in order to treat bone and nerve system diseases, we can use 3HB as a promising drug candidate. As the degradation rate of PHA is quite slow, the effects of 3HB would not be dominant at the early state of cell attachment [25].

Figure 5. Applications of PHA

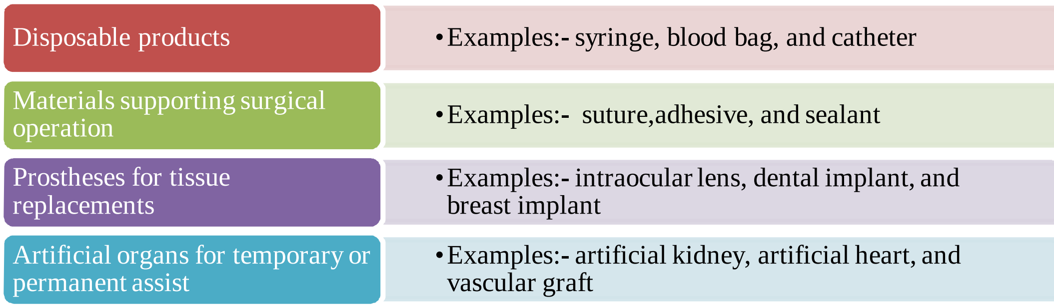

Figure 6. Applications of biomaterials in medical field

6.1.1. PHA in medical scaffolding

As PHA is biodegradable and biocompatible, it can be adapted well for medical application. According to Bringham and Sinskey, there were studies in which bone defects are being treated using PHB and hydroxyapatite (HA) blends. Furthermore, in order to produce pulmonary valve leaflets and pulmonary artery scaffolds in sheep, the copolymer of polyglycolic acid (PGA) and PHB were used in some studies. At the same time, this study leads to the development of PHA based heart valve scaffolds and it was inserted into the sheep surgically. This was the important studies which help to illustrate that it is possible to use biopolymer scaffolds in tissue engineering [26]. Based on the purpose scaffold materials can be synthetic or biologic, degradable or nondegradable. While the composition, structure with the arrangement of their constituent macromolecules will determine the properties of polymers. Considering structural, chemical, and biological characteristics, it can be classified into different types. One such classification is naturally occurring polymers, synthetic biodegradable and synthetic nonbiodegradable polymers. Furthermore, the natural polymers are being considered as the first biodegradable biomaterials used clinically [27]. Tissue engineering deals with reconstitution and regeneration of lost or damaged tissues. In fact, the biomaterials are known to play a major role in this field. The preparation of scaffolds which are biocompatible is being considered as the chief area of research in tissue engineering fields. Actually in order to bring out specific cellular functions, to direct cell-cell interactions both in implants which are initially cell-free and may serve as matrices to conduct tissue regeneration as well as in implants to support cell transplantation biomimetic synthetic polymers were developed. Thus the tissue engineering research must focus on the development of scaffolds which are capable to mimic structure as well as the function of the native extracellular matrix [28]. Ideal surgical sutures should possess some important characteristics. During the wound healing, the sutures should exhibit high in vivo tensile strength. When the critical period is over, it should offer high absorption rate. The other properties of an ideal suture are minimal tissue reactivity, no memory, predictable performance, easy handling as well as knot security [29]. For successful tissue engineering, the scaffolds should have some important properties (Figure 7).

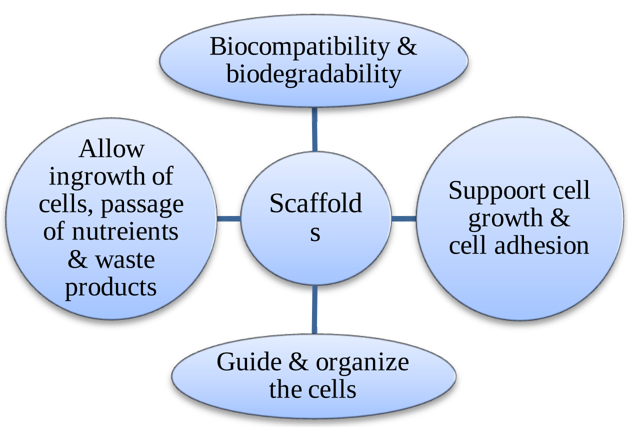

Figure 7. Properties of scaffolds

Apart from this, it is also important to analyze the surface structure. In order to get a porous surface, we can go for leaching in which the PHA is being blended with salts and washed with water [23]. There are mainly three groups of strategies for tissue engineering. They are listed below.

- Implantation of isolated cells or cell substitutes into the organism
- Delivering tissue-inducing substances
- Placing cells on or within different matrices

The macro and microstructural properties of scaffolds can affect modeling of cell shape and gene expressions, both related to cell growth and preservation of native phenotypes. Another important factor for scaffolds is their porosity and pore interconnectivity. Interconnected open pore geometry with spread porosity and a highly porous surface and microstructure is essential for scaffolds. This is an important requirement for in vitro cell adhesion, in growth and reorganization. Also to offer required space for in vivo neo-vascularization it is essential. In diffusion of physiological nutrients and gases to cells, removal of metabolic waste and by-products from cells the pore interconnectivity plays a major role. Furthermore, the surface characteristics of scaffolds also impart role in in-vitro cell adhesion, migration, phenotype maintenance, intracellular signaling, in vivo cell recruitment and healing at the tissue-scaffold interface. Morphology, hydrophilicity, surface energy, as well as charge, are the important surface characteristics. The mechanical strength of scaffolds is another important concern. It should bear adequate mechanical strength during in vitro culturing. This is essential to maintain the spaces required for cell ingrowth and matrix formation. Also, it must offer adequate temporary mechanical support which will match the mechanical properties of the host tissue in order to bear in vivo stresses and loading [30, 31]. Lots of biodegradable polymers were utilized by researchers for the preparation of scaffolds. Collagen sponges and gels, hyaluronic acid matrices, polyglycolide (PGA), polylactide(PLA), poly(lactide-co-glycolide) (PLGA), poly(l-lactide-co-caprolactone) (l-PLCL), poly(glycolide-co-trimethylene carbonate) (PGTMC) etc were mainly used by researchers for the development of scaffolds for cartilage repair [32, 33].Among different polymers available for scaffolds preparation Poly(3-hydroxybutyric acid) (PHB) which is a hydrophobic biopolymer got more attention. Due to its biodegradable surfaces, it can be used as devices that demand a long half-life in-vivo. PHB is also considered as eco – friendly as it will be biodegraded when deposited in the microbially active environment without the formation of any toxic byproducts. Also in most of the higher living organisms, 3-Hydroxybutyric acid is a common metabolite. This provides a proof for the nontoxic behavior of PHB [28]. Other studies were showed that the quantity of PHB in a PHB/P(HB-Co-HHx) blend present on the surface can affect the maturational differentiation of chondrocytes. At the same time, we could effectively use the unblended P(HB-Co-HV) scaffolds for cartilage repair. Also in a comparative study of healing response of matrices of P(HB-Co-HV) and scaffolds of collagen impregnated with calcium phosphate by implanting into cartilage defects in rabbit, matrices of P(HB-Co-HV) showed a better response. While the implantation of PHA copolymers scaffolds in rats exhibited only mild tissue response [26]. PHB implants can exhibit more of tissue response. This is due to the fact that PHB is so rigid. Thus it can exert mechanical stimulus to the tissue surrounding the implant. In correlation with 3 HB content the implants rate of biosorption decreases. In order to use in tissue repair, the P(HB-Co-HV) platforms geometry should be analyzed. Also, the cell proliferation or migration through the microspheres is not affected by laser micro perforation [26]. Among tissue engineering scaffolds, there mainly two type of scaffolds. i.e, natural as well as synthetics scaffolds. Polyglycolic acid and polylactic acid (PLA) are synthetic polymers which are being used as medical sutures, stents, and drug delivery carriers. Natural polymers are biocompatible and can be used as tissue engineering scaffolds. They are expected to give yield promising results. Some of the examples of natural polymers include collagen, fibrin, chitosan, and PHA. From PHA family only PHB, PHBV, and PHBHHx are reported to produce industrially [34, 35]. Some of the examples of tissue engineering applications of PHA are listed in Table 1.

6.1.2. PHA in surgical materials

A polymeric material must possess the excellent tensile strength to apt for its use in sutures so that it will become effectual in wound closures. poly(4-hydroxybutyrate) (P4HB) is normally used for the manufacture of surgical materials. Because it is having certain properties like stronger than polypropylene sutures (410-460 MPa) as well as considerably lower Young's modulus for its sutures when comparing with others. For example, the PHB and P(HB-co-HV) sutures can heal muscle-fascial wounds. After the oral surgery in dogs P(HB-co-HV) films known to heal it [26]. Lots of medical implant devices are being developed using PHA. Certain examples include sutures, suture fasteners, meniscus repair devices, rivets, tacks, staples, screws, bone plates and bone plating systems, surgical mesh, repair patches, slings, cardiovascular patches, orthopedic pins, adhesion barriers, stents, guided tissue repair/regeneration devices, articular cartilage repair devices, nerve guides, tendon repair devices, atrial septal defect repair devices, pericardial patches, bulking and filling agents, vein valves, bone marrow scaffolds, meniscus regeneration devices, ligament and tendon grafts, ocular cell implants, spinal fusion cages, skin substitutes, dural substitutes, bone graft substitutes, bone dowels, wound dressings, and hemostats. Tepha FLEX which is an absorbable suture was prepared using P4HB is considered as the first FDA approved the product for clinical application [17].

Table.1. Tissue engineering applications of PHA

Applications	Descriptions	Ref.
Heart valve	For the regeneration of functional living heart valve replacement using tissue engineering mainly focuses on the materials that can be configured into the heart valve shape with following cell seeding. In order to afford cell growth and differentiation, many materials have been studied which mainly includes decellularized extracellular matrix, polyglycolic acid (PGA), polylactic acid (PLA), polycaprolactone as well as PHA. As mcl – PHA is known to be more flexible than other materials like PGA and PLA, it is fit to function as leaflets inside a tri-leaflet valve. The studies showed that it is possible to implant a whole trileaflet tissue-engineered heart valve using copolyester of 3-hydroxyhexanoate and 3-hydroxyoctanoate [P(3HHx-co-3HO)] and autologous cells in the pulmonary position in a lamb model.	[17]
Vascular grafts	In order to repair o replace the malfunctioning blood vessels in the arterial or venous system vascular grafting is being employed. Commonly for the replacement of large diameter blood vessels, synthetic grafting materials are being used. But they are inappropriate for the replacement of small diameter grafts. Because it becomes closed quickly. P(3-HB-co-4HB) was studied as a synthetic graft coatings in dogs for about 10 weeks and it was found out that after the 2 weeks of implantation degradation started. Whereas very slow degradation of the polymer was observed when it was used as impregnation substrate in rat models. Interestingly almost all P(3HHX-co- 3HO) – PGA grafts offered unrestricted blood flow.	[29]
Artery augment-tation	When P(3HB) nonwoven patches were studied as trans annular patches, after 3 -24 months the regeneration of neointima and as well as neomedia were observed in test organism are comparable with native arterial tissues. P(4HB) was also seemed to be successful in the preparation of scaffolds for antilogous cardiovascular tissues.	[29]

6.1.3. PHA as drug carrier

It is important to deliver the pharmacologically active materials to cells, tissue, and organs specifically in order to improve the therapeutic benefit while reducing the side effects. Thus lots of research are being carried out to find out an apt material for the controlled delivery of drugs. There were lots of studies using polymers as drug carriers. As polymers are flexible it allows engineer multiple functionalities which are an important requisite for efficient drug delivery as well as maintaining biocompatibility, facile manufacturing, and stable formulation. Also, it is vital that the biopolymer should meet all the necessity of physical properties. At the same time, it should accomplish its biocompatibility test [36]. Due to its various properties, PHA can be utilized as drug carrier system [10]. The drug delivery system based on PHA mainly covers subcutaneous implants, compressed tablets for oral administration and micro-particulate carriers for subcutaneous and intravenous use [37]. There were many studies regarding the applicability of PHA in controlled drug delivery systems. In order to treat extremely resistant infections, it is important to maintain the antibiotic concentration at the infection sites [37]. According to studies, the 3HB monomers released by the PHA polymer can raise the calcium levels inside cells.

This will also help in protecting the mitochondria. These studies lead to the conclusion that, PHA will be apt for the drugs meant for bone and nervous system diseases. Now a day's some current studies reported about the use of PHA nanoparticles for targeted drug delivery. The main area of attention when going for the study of targeted drug delivery will be cancer. Mostly the cancer treatment involves the use of cytotoxic drugs. But these cause lots of side effects. Targeted drug delivery is considered as a solution to this problem. It will be possible to accumulate the particles within the target tissues if we link the targeting ligand to drug delivery particles. Even though there were lots of investigations are there, as PHA possesses significantly more advantages researchers' interest become on it [37]. In future, there will be lots of studies about the applications of PHA as a drug carrier. These will be more specific for cancer therapy and tumor-related. On the other hand, it is necessary to study the appropriate formulation of the microsphere/ microcapsule/ nanoparticles of PHA which contains the desired drugs. Because it is vital to make sure that the properties of a drug as well as the PHA are not affected. Also, the speed of drug release should be close to its target [10].

6.1.4. PHA in nerve regeneration

Recently some of the PHAs like P(3HB), P(3HBV), P(3HBco4HB), and P(3HBco3HHx) known to give hopeful results for improved neural survival, promotion of greater axon dendrite segregation, and for neural stem cells (NSC) growth, proliferation, and viability. If combine the distinctive surface characteristics with required bio functions it will be the most suitable strategy for neural repair or regeneration. It is also expected that if we make use of various PHA blends into this strategy it will give rise a solution to this problem. The alkali treatment of the P(3HBco3HHx)films improved its hydrophilicity and which in turn result in the better attachment of neural stem cells and neural progenitor cells (NPC) in the presence of low quantities of serum. But there is a need for much more works by considering the biocompatibility of biomaterial used as well as the sensitivity of the target material in order to promote the nerve regeneration with in vivo studies. It is inferring that the ideal option for nerve regeneration will be a thin, flexible, and porous PHA biomaterial with suitable superficial characteristics and neuro-inductive and neuro-conductive ability [10].

6.2. PHA in agricultural field

For the agricultural purposes, PHA can be utilized as mulch films. More significantly for the controlled release of insecticides P(3HB-3HV) can be used. This is achieved by incorporating the insecticides into the P (3HB-3HV) pellets and it will be sew together with the farmer's crops. Then the rate of release of insecticide will depend on the level of pest activity. Because the bacteria which are breaking down the polymer would be affected by the similar environmental conditions of the soil pests [38]. Apart from this PHA are used in bacterial inoculants which are used for improving nitrogen fixation in plants. While selecting the bacterial inoculants in order to use in preparation of inoculants for agricultural uses, it is important to note down that whether it is having the ability to survive in the stressful environment. Also, it is necessary to store the bacterial cells for long periods and endure desiccation and hot conditions. Thus the inoculants should be capable to carry on the high survival rates within the carrier. For that reason, the researchers are now focusing on the research to increase the quality of carriers like the addition of elements like nutrients or other synthetic products which will result in prolonged survival. The studies on *Azospirillum brasilense* inoculants showed that the plant growth-promotion result was more constant with *A. brasilense* inoculants which contain high amounts of intracellular PHA while carriers may vary. Further, in Mexico, the field experiments in maize and wheat confirmed this. Increasing crop yield by using peat inoculants prepared with PHA-rich Azospirillum cells gives enhanced consistency. That is intracellular PHA has chief importance for increasing the shelf life, efficiency, and reliability of commercial inoculants [38].

6.3. PHA in food packaging

In the food chain, food packaging plays a vital role. The main intentions of food packaging are mechanical support, transition, an extension of shelf life, and preservation of food. For this purpose, petrochemical-based polymers are being used widely. This is due to the fact that it bears lots of properties like mechanical properties, easy processing, a cost which is the important requisite for packaging materials [39]. It is necessary to restrict the production of synthetic polymers since it can lead to the exhaustion of petroleum resources. Furthermore, these thermoplastics cannot be totally recyclable or biodegradable. This will leads to serious ecological problems. Thus there arises the need for an alternate material [40]. In order to reduce the packaging waste, new bio-based materials are being utilized. While the new type of packaging should increase the shelf life as well as enhance the quality of food. But still, there is the only limited use of edible and biodegradable polymers since there are lots of problems related to performance. It mainly includes brittleness, poor gas, and moisture barrier, processing and cost [41]. In order to use for food packaging, the material has to possess some important characteristics. It should be capable of protecting the food from environmental conditions such as microbial attack, chemical contaminants, dust, UV-radiation, mechanical damage, humidity, and desiccation. Also, it should maintain the sensory food quality as well as stability under extreme conditions [13]. Biodegradable materials such as polyhydroxyalkanoates (PHA), poly(lactic acid) (PLA), and polycaprolactones (PCL), offers processability with conventional plastics machinery [39]. Among various biomaterials available for food packaging PHA gained more attention as a packaging material. Poly(3-hydroxybutyrate) (PHB) is one of the important biodegradable poly(hydroxyalkanoates) (PHA). This natural thermoplastic polyester offer many mechanical properties comparable to synthetically produced degradable polyesters such as the poly-L-lactides. The main challenge in front of food packaging industry is the development of biodegradable primary packaging with matching the durability of the packaging with product shelf life. It is necessary that the biological based packaging material should not undergo any changes in mechanical and barrier properties. Also, it must work from storage to disposal. For this purpose it is should be noticed that the environmental conditions leading to biodegradation must be avoided during storage of the food product. After that, while discarding the optimized conditions for biodegradation must arise. Most of the studies related to applications of PHA are related to replacement of petrochemical plastics. Currently, most of the packaging, as well as coating purposes, are being carried out using plastics. Now a day more interestingly more studies were focusing on PHA. Because it is expected that PHA can partially or fully replace plastics. More precisely most of the researchers concentrate on the packaging like containers and films [42]. PHA is having high attention as a basic material for food packaging due to the following reasons [13].

- Using thermoforming PHA or other compatible synthetic or bio-based polymers can be processed as packaging films.

- PHA can be processed based on their adaptable degree of crystallinity and elasticity towards differently formed parts. For example, thermoforming films can be used as flexible foils for wrapping. Rigid and robust cast components can be made via injection moldings which will act as storage boxes and containers.

- PHA films exhibit a high water vapor barrier due to the hydrophobicity of these types of water-insoluble materials. Also, this can provide high barrier properties against CO_2.

- homopolyesters PHB, poly (4HB) and in dependence on the composition, scl-PHA copolyesters (PHBV) shows a high oxygen barrier or low oxygen transmission rate. This was an important requirement to restrict the growth of aerobic microbes and the oxidative spoilage of unsaturated fatty acids. These barrier properties make the PHA as the basic materials for producing bottles for liquid foods, and also for CO_2-containing liquids.

- PHA is having high UV-barrier which helps to protect especially unsaturated lipid components in food from the formation of radicals, thus accelerating their spoilage.

- PHA-based packaging spoiled with food residues such as blood, tissue liquor, fruit juices, etc. can be conveniently be discarded by composting.

Cost, narrow processability window, stiffness, brittleness properties etc. leads to the limited the use of PHB in food packaging applications [43]. PHB is known to have incomparable stereo-chemical purity, completely isotactic and capable of crystallizing as it is being produced enzymatically. But due to this PHB is very stiff as well as brittle. These limitations arise lots of restrictions towards its applicability. Also, its processing is considered as difficult. Because it seems to be an only poorer difference is there between its melting point (175°C) and the temperature at which it starts to degrade (185 °C) [33]. Upon degradation, PHB chains produce shorter chain fragments with carboxylic terminal groups according to random chain scission mechanisms with crotonic acid as one of the signature by-products [45]. But it is known that blending the PHB with other polymers can improve its properties. Due to its properties like biodegradable nature, its flexibility, toughness, thermal stability etc make Polycaprolactone (PCL) a good option for this purpose. Also, it is possible to enhance the PHB mechanical, gas barrier, and thermal properties using nanofillers such as organo-clays. These could be incorporated at low filler content (less than 5%) and nanoscale distribution [43].The combination of a polymer matrix and fillers which is having not less than one dimension in the nanometer size range is known as polymer nanocomposites. But surprisingly only a small number of nanofillers are known to enhance the thermal, mechanical as well as barrier properties of the polymer [44]. It is expected that metal nanoparticles can act as catalysts in melt processing of polymer blends. In fact, it is already known that metal nanoparticles may enhance or suppress the thermal stability depending on the type of polymer [45].

6.4. Industrial applications of PHA

There are a number of remarkable uses of PHA in industry. Instead of using aluminum to make water-resistant cover paper or cardboard which is nonbiodegradable, PHA latex can be used. As the only little amount of PHA is needed for this, it is also cost-effective.PHA can be used to make foils, films, and diaphragms. There are various companies which deal with the production and use of PHA for various purposes. One such example is biomer, which is a German company having the technology to produce P(3HB) from Alcaligeneslatuson a large scale. This strain can accumulate up to 90% P (3HB) in dry cell weight when the cells are grown in a mineral medium using sucrose as a carbon source. Combs, pens, and bullets are made using this polymer. Commercially they are selling the polymer pellets in order to use in classical transformation processes. As these polymers become low viscous when melted it can be used to make articles with thin walls by injection molding. Even though the end product is very hard it will degrade within two months in the environment. Also, it can be used in a temperature range of 30^{o}C to 120^{o}C [38].

6.5. Oil absorption by PHA

Kumar Sudesh et al. reported the oil-absorbing property of three different types of PHA films cast from solution. It is expected that this newly found property of PHA will enable its wide applicability in the cosmetics and skin care industry. Most of the oil blotting materials which are being used now a day are made up of special plant fibers or thermoplastics like PP, polyurethane films. In recent times the oil blotting films made up of thermoplastic materials are widely used. This is due to the fact that, they are softer as well as more flexible than plant fiber-based materials. A good cosmetic oil blotting material should satisfy three main criteria [46].

- Rapid oil absorption by gentle blotting
- Ability to retain the absorbed oil
- Ability to indicate visually that the oil has been absorbed.

The ordinary plastic material needs to be undergoing different surface treatments to enable it to absorb oil. The treatment mainly involves the addition of oil-absorbing and oil-retaining chemicals. Also, some colorants were added in order to improve the oil-indicator property. While Kumar Sudesh et al. reported that PHA films which cast from solution naturally possess good oil-absorption, retention, as well as indicator properties. Apart from that they also found out that the surface microstructures such as porosity and smoothness affect the oil-absorption property of PHA [46].

Table 2. Significant polymers used for food packaging applications [39]

Polymer	Application
Polyethylene (PE)	Packaging of cooking oil, milk and water containers
Polyethylene - terephthalate (PET)	Applied in food, beverage, and other liquid containers
Polyvinylchloride (PVC) and Poly-propylene (PP)	For yogurt, spice ice tea, and margarin packing
Polystyrene (PS)	For eggs and mushroom packing
Polyamide (PA)	For flexible packaging of perishable food, such as meat and cheese

6.6. PHA in aquaculture

Poly-β-hydroxybutyrate (PHB) can be used as an alternative anti-infective strategy for aquaculture rearing.PHB is being produced by bacteria as an intracellular energy and carbon reserve. Also, it is insoluble in water as well as biologically degradable into β-hydroxybutyric acid. It shows antimicrobial activity towards certain pathogens and protects sartemia like other short-chain fatty acids (SCFA) do. Thus if PHB is supplemented by the feed it will be degraded in the gastrointestinal tract of aquaculture organisms. Furthermore, the locally released SCFA or PHB oligomers may induce their beneficial effects [47]. Nowadays it has been demonstrated that probiotics and prebiotics can be used as an alternative viable therapy in fish culture. It shows potential as a biological control strategy and thus it is considered as a vital part of the aquaculture practices in order to enhance the growth as well as disease resistance. This strategy has lots of advantages which help to meet the limitations and side effects of antibiotics and other drugs. It can also offer high production. Probiotics are nothing but the applications of whole or component of a micro-organism which are useful to the health of the host. The success of probiotics leads to other concepts like prebiotics. In prebiotics which is an option for manipulating endogenous microbes for better health, selected favorable indigenous microbial populations is being stimulated [48].PHB can be used as prebiotic. It has been already demonstrated that PHB can act usefully for lots of aquatic animals. The studies showed that the PHB provided a better growth rate of larvae of giant freshwater prawn when it was supplied through live food. Also, the same effect happened in the case of juvenile European seabass when it was included in the diet at 2 and 5% (w/w).When included in the diet at 2% (w/w), in case of Siberian sturgeon (*Acipenserbaerii*) fingerlings the growth rate, as well as survival rate, got increased. It was found to provide protection against *Vibrio anguillarum* and *Vibrio harveyi* BB120 infection in case of zoea larvae of the Chinese mitten crab (*Eriocheirsinensis*) and larvae of giant freshwater prawn respectively. Bacteria, as well as fungi, have the ability to produce extracellular PHB depolymerase. The studies showed that if we isolate PHB degrading bacteria from the gastrointestinal tract and evaluate their capacity to improve the effect of PHB in a synergetic approach we can improve the gastrointestinal health of aquatic animals [49].

6.7. PHA in animal feed

Recently it has been reported that PHB can be included as a component in animal feeds in order to enhance the metabolizable energy content. PHA needs to be undergoing pretreatment with sodium hydroxide to increase the digestibility. Also, it was illustrated that if we use animal feed containing whole bacterial cells with PHA we can modulate the gut flora by means of delivering short-chain fatty acids such as 3-hydroxybutyric acids. Actually, monogastric animals have a poor digestibility of PHA. But the excretion of PHB in the fecal pellets is important. Because the main aim is to increase the metabolized energy of the animal feed via conversion of these energy-rich substances within the nutritional chain [50].

7. CONCLUSION AND FUTURE PROSPECTS

Nondegradable plastics are widely used across the globe. It was considered as the major contributor to the solid waste problem. A huge amount of plastics are being deposited on earth each day and now its disposal becomes a big deal. Thus there arises the need for an alternate for these plastics. Biopolymers are now being considered as the best solution to these problems. PHAs are one of the important biopolymers. As its being biodegradable, biocompatible and most importantly being produced by renewable resources it can be used instead of plastics. Also, it possesses lots of properties compared with conventional plastics. Applications of PHA in food packaging, medical scaffolding, tissue engineering, nerve regeneration, drug carrier material etc. are being studied now. As researchers are still concentrating more on these PHA, various applications of these materials will come into existence in future. Even though various applications of PHA have been already studied, most of these studies still restricted to laboratory itself. Scale up as well as the industrial application of these results remains as a question even now. There are lots of challenges are being faced by the researchers when coming to the scale up and industrial application of PHA. The main challenges include the high cost of the substrate, production cost, etc. To meet these obstructions researchers are in constant search to find the ways to reduce the cost of production. It mainly deals with the development of bacterial strain providing high yield of product, more efficient fermentation or recovery process, and selection of cost-effective as well as readily available substrate. It is expecting that in the future world all the conventional plastic materials will be replaced with these biopolymers and the world become free from the solid wastes.

ABBREVIATIONS

PHA	Polyhydroxyalkanoates
PHB	Poly -3- hydroxybutyrate
scl – PHA	Short chain length PHA
mcl – PHA	Medium chain length PHA
lcl – PHA	Long chain length PHA
PCL	Polycaprolactone
PGA	polyglycolic acid
PLA	polylactic acid
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PVC	Polyvinylchloride
PS	Polystyrene
PA	Polyamide

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