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Review

Bacterial polyhydroxyalkanoates: Opportunities, challenges, and prospects

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ABSTRACT

Excessive utilization of synthetic plastics has led to a major detrimental impact on the environment. Plastic pollution and accumulation in water bodies have threatened the survival of marine life. Plastic pollution can be prevented by using biopolymers that are eco-friendly and can be naturally produced by certain living organisms. The biopolymers have environmental advantages over synthetic plastics, such as biodegradability and biocompatibility. In comparison to plants and other microbial systems, bacteria can accumulate a high amount of polyhydroxyalkanoates (PHAs). However, the major stumbling block in the production of bacterial PHAs is its low cost-effectiveness due to costs associated with fermentaion and down-stream processing. In consideration with the above properties, opportunities and challeges associated with bacterial PHAs, this review focuses on structural diversity of PHAs, biosynthesis mechanism in bacteria, biodegradation, life cycle analysis, and environmental impact of bioplastic production. It further enumerates the advanced tools and techniques for bacterial PHA production, along with various factors affecting the commercialization of bioplastics. Extraction methods, down-stream processing, and biomedical applications of PHAs are also discussed. The opportunities and challenges in the commercialization of bacterial PHAs along with future scenario and environmental sustainability are presented for the purpose of fostering sustainable development.

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1. Introduction

Plastics derived from petroleum-based industries are very much popular and in demand, due to their diversified properties such as toughness, resistance to degradation, lightness, flexibility, and resilience (Alavi et al., 2014). Because of their usefulness, diversity, and wider application in household, pharmaceutical, and commercial sector, plastics have become an essential commodity in modern society (Yadav et al., 2018). The annual synthesis of petroleum-derived plastics was reported as more than 300 million tons, and around 150 million tons of synthetic plastics and plastic-derived materials were consumed worldwide every year (Marichelvam et al., 2019; Anjum et al., 2016). More than 1000 million tons of petroleum-derived plastics were predisposed, and they might take over 100 years to be mineralized (Marichelvam et al., 2019). It has been estimated that 10 million tons of synthetic plastics leached into the ocean every year, which imposed detrimental effect on the oceanic ecosystem (Boucher and Billard, 2019). The demands for plastics and plastic-derived products have been increasing continuously due to modernization and population boom (Kumar et al., 2016b) and this has led to serious environmental problems (Amaro et al., 2019; Kishna et al., 2017). Due to its slow degradation rate and production of toxic byproducts, plastic recycling is the most appropriate way of waste management but the process is tremendously slow and hindered by the diverse properties of different plastics. Plastic materials have a wide range of applications, so the sorting of discarded plastics is problematic. Besides, the presence of heterogeneous materials and additive substances such as coverings, fillers, and coloring materials limit the recycling of synthetic plastics (Anjum et al., 2016).

Thus, to reduce the environmental impact resulting from the excessive use and accumulation, synthetic plastics can be replaced with biopolymers, including polylactide (PLA), polysaccharides (carbohydrates polymers), aliphatic polyesters (Yadav et al., 2018), and polyhydroxyalkanoates (PHAs) (Castilho et al., 2009), which exhibit comparable physiochemical and mechanical properties as synthetic plastics (Amaro et al., 2019; Dietrich et al., 2017). Due to their inherent biodegradability and biocompatibility (Morya et al., 2018), eco-friendly production process, and wide range of applications (e.g., consumable materials and medical sector), these biopolymers are becoming popular and have emerged as an important replacement of synthetic petroleum-derived plastics (Kwan et al., 2018; Mukheem et al., 2018). It is projected that the global bioplastics production volume will be approximately 2.44 million tons in 2022 (Marichelvam et al., 2019).

It is well known that the PHAs are produced by microorganisms to overcome unfavorable environmental conditions (Singh Saharan et al., 2014; Sukan et al., 2015). PHAs are widely recognized as biopolymers because of their high biodegradability, biocompatibility, and sustainability (Kumar et al., 2016a,b). Bioplastics belong to a category of thermoplastic polyesters that have several R-hydroxyalkanoic acids groups. They are produced by several groups of microbes when there are excess substrates (carbon source) under conditions of limited oxygen, nitrogen, phosphorus, or even fluctuating pH of the growth media. Bioplastics are stored as carbon and energy reserves (Kumar and Thakur, 2018) or as the reducing equivalent of energy (Mukheem et al., 2018). When limited nutrients are supplemented in the growth media, the microbes start to degrade the stored PHAs as a carbon source (Kumar et al., 2018b).

The first PHA, polyhydroxybutyrate (PHB), was identified by Maurice Lemoige in 1926 as intracellular granules in bacterium *Bacillus megaterium* (Lemoigne, 1926). Of all the PHA family compounds, the PHB is extensively studied and well characterized, and is utilized as a reserve material in bacteria amounting up to 80% of the dry bacterial biomass (Keshavarz and Roy, 2010). Wastewater sludge contains mixed microbial consortia that can also produce PHA monomers in the form of 3-hydroxybutrate (3HB) as well as 3-hydroxyvalerate (3HV) as prime constituents and 3-hydroxyhexanoate (3HHx) as a minor constituent (Kumar et al., 2016b). Currently, more than 150 diverse monomer constituents of PHAs are known based on their carbon chain length and their linking structure such as straight, branched, saturated, unsaturated, and aromatic. Over 90 microbial genera that are able to accumulate PHAs molecules intracellularly have been investigated (Thakur et al., 2018; Tan et al., 2014). Based on the requirement of nutrients, nutrient stress, and their growth pattern, PHA accumulating

bacteria have been classified into two groups. The bacterial sp., such as *Ralstonia eutropha*, *Pseudomonas oleovorans*, and *Pseudomonas putida* belongs to the prime group as they require limited nutrients such as phosphorous (P), nitrogen (N), oxygen (O), and magnesium (Mg) to store PHAs and are not able biosynthesize PHAs during their growth periods (Guzik et al., 2014). In contrast, accumulation of PHAs by the second group of bacteria (e.g., *Alcaligenes latus*, mutant strain of *Azotobacter vinelandii*, and recombinant *Escherichia coli*) is not affected by nutrient limitation, and it can store PHAs during its growth phase (Muhammadi et al., 2015; Nitschke et al., 2011).

The properties such as elasticity, biodegradability, and renewability of PHA polymers are highly dependent on their synthesis pathways (Masood et al., 2014), monomeric composition (Luzi et al., 2019), chemical structure (Bugnicourt et al., 2014), etc. Life cycle analysis of bioplastics production have revealed that, in general, the production and application of PHAs are more sustainable than synthetic polymers in consideration of the energy consumption and greenhouse gas emissions (Ali and Jamil, 2016), yet they may pose more environmental impact in some environmental indicators (Kourmentza et al., 2017). Furthermore, the production of PHAs is currently not economical in comparison to that of synthetic plastics. To make bacterial PHAs production cost-effective, a few critical factors need to be addressed, such as screening and selection of potential bacterial strains (Kumar et al., 2016a), synthesis pathways, advanced tools and technologies (Możejko-Ciesielska and Mostek, 2019a), carbon and nitrogen source (Zahari et al., 2014), and cost-efficient down-stream processes (Koller and Braunegg, 2018). The purposeful application of the PHAs is also a crucial factor that determines its importance and economy (Cao et al., 2019; Chen et al., 2017).

Based on the above discussion, the objective of the current review is to evaluate the importance of biodegradable polymers produced by diverse groups of bacteria with regards to environmental sustainability, commercialization, and potential applications. The latest applications of advanced tools & technologies, extraction processes along with final production of bacterial PHAs and its biomedical application are discussed. This review also addresses the opportunities and challenges in economical production of bacterial bioplastics, its future scenarios and potential roles in achieving the Sustainable Development Goals (United Nations, 2019).

2. Methods

The present review has been intended to recapitulate the current status, recent technologies, and scientific development in the field of bacterial PHA production and application. Moreover, opportunities and challenges and the cost-effectiveness of PHAs in terms of sustainable production and uses have been reviewed. Peer-reviewed journal articles and thoroughly vetted information from commercial sites were studied. The literature search was performed using the most imperative and accessible databases, including Scopus (19), ScienceDirect (76), PubMed (89), and other websites (www.semanticscholar.org (27) https://www.tandfonline.com/(6), https://www.ptonline.com/). Keywords selected for the literature search included bioplastics production; sustainable biorefinery; biodegradable polymers; waste management; biomedical applications; biomass valorization. After that, the abstracts were reviewed with respect to the objectives of the current review (Fig. 1). The reviewed articles highlighted: (1) structural diversity of bioplastic based on carbon chain length, production/enzymatic pathways, and carbon source; (2) unique properties of PHAs molecules, which make them biodegradable and biocompatible; (3) Potential PHA producing bacterial strains and diverse range of carbon sources; (4) strategies to make PHA production cost-effective and environmentally sustainable using waste materials as carbon source along with most appropriate PHA extraction methods, further its biomedical application; (5) advancements in the field of PHA production challenges, opportunities, and future prospects to make them into sustainable bioproducts.

3. Structural classification, diversity, and properties of PHAs

In general, PHAs have a common formula in which various R-hydroxyalkanoic acid groups are attached (Table S1). On the basis of the structure and number of carbon atoms in the chain, along with its branching, PHAs are categorized into three groups: short chain length (scl), medium chain length (mcl), and long chain length (lcl) PHAs (Kourmentza et al., 2017; Kunasundari and Sudesh, 2011). Depending upon the monomeric configuration of polymer, PHAs can also be classified as homopolymer or heteropolymer (Kalia, 2016). Scl PHAs comprise of 3–5 carbon atoms, and include poly(3-hydroxybutyrate) P(3HB), poly(4-hydroxybutyrate) P(4HB), poly(3-hydroxyvalerate) P(3HV), and the hetropolymer including poly (3-hydroxybutrate-copolymer-3-hydroxyvalerate) P(3HB-co-3HV). Mcl PHA polymers consist of 6–14 carbon atoms. Mcl polymers comprise of both homopolymers such as poly (3-hydroxyhexanoate) P(3HHx) and poly(3-hydroxyoctanoate) P(3HO), and heteropolymers such poly (3-hydroxyhexanoate-copolymer-3-hydroxyoctanoate) P(3HHx-co-3HO) (Basnett et al., 2017). More than 14 carbon atoms in the polymeric chain of PHAs come under the category of lcl (Kourmentza et al., 2017; Kunasundari and Sudesh, 2011).

The substrate selectivity and specificity of PHA synthase are crucial for the synthesis of scl and mcl PHAs. PHA synthase can only accept a fixed array of carbon chain length of 3HAs. In *Alcaligene eutrophus*, PHA synthase polymerizes 3HAs having 3–5 carbon atoms, while in *P. oleovorans,* it can polymerize 6–14 C-atoms (Khanna and Srivastava, 2005). Along with scl and mcl, hybrid polymers also exist, which are composed of both scl and mcl monomers such as poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (Castilho et al., 2009). In general, all monomeric unit of PHAs is R-configured, due to stereo-specific nature of the biosynthetic enzymes. The various functional groups in mcl PHAs have also been investigated, such as hydroxy, epoxy, halogen, cyano, carboxyl, and esterified carboxyl (Ciesielski et al., 2015). Biosynthesis of PHAs polymers is highly dependent on the utilization of the type of n-alkanotes (even or odd) as starting materials, for example, biosynthesis of PHB require even n-alkanotes whereas copolymers of 3HB and 3HV will be synthesized only if odd n-alkanotes are utilized as starting materials (Anjum et al., 2016). By using odd carbon alkanoic acid as the substrate, biosynthesis of terpolymers belonging to poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyheptanoate) has been performed by engineered *A. eutrophus*, carrying the PHA synthase gene of *Aeromona caviae* (Fukui et al., 1997). Typically, the size of PHA granules lies in the range of 0.2 ± 0.5 μm; it is synthesized and stored by microbes in the cytoplasm (Raza et al., 2018). The typical molecular mass of PHA molecules is around 2×10^5–3×10^6 Da, and depends upon the microbial species, type of carbon source and its concentration, culture condition (temperature, pH), and fermentation mode (batch, fed-batch, continuous) (Aditi et al., 2015; Możejko-Ciesielska and Kiewisz, 2016). Due to higher refractivity of PHA molecules, it can be visualized by phase contrast light microscope, as well as by using various lipophilic staining dyes, which include Sudan Black B and the oxazine dye (Nile Blue A or Nile red) (Kumar et al., 2016a; Morya et al., 2018). The other importants fetures of PHAs have been discussed comprehensively below.

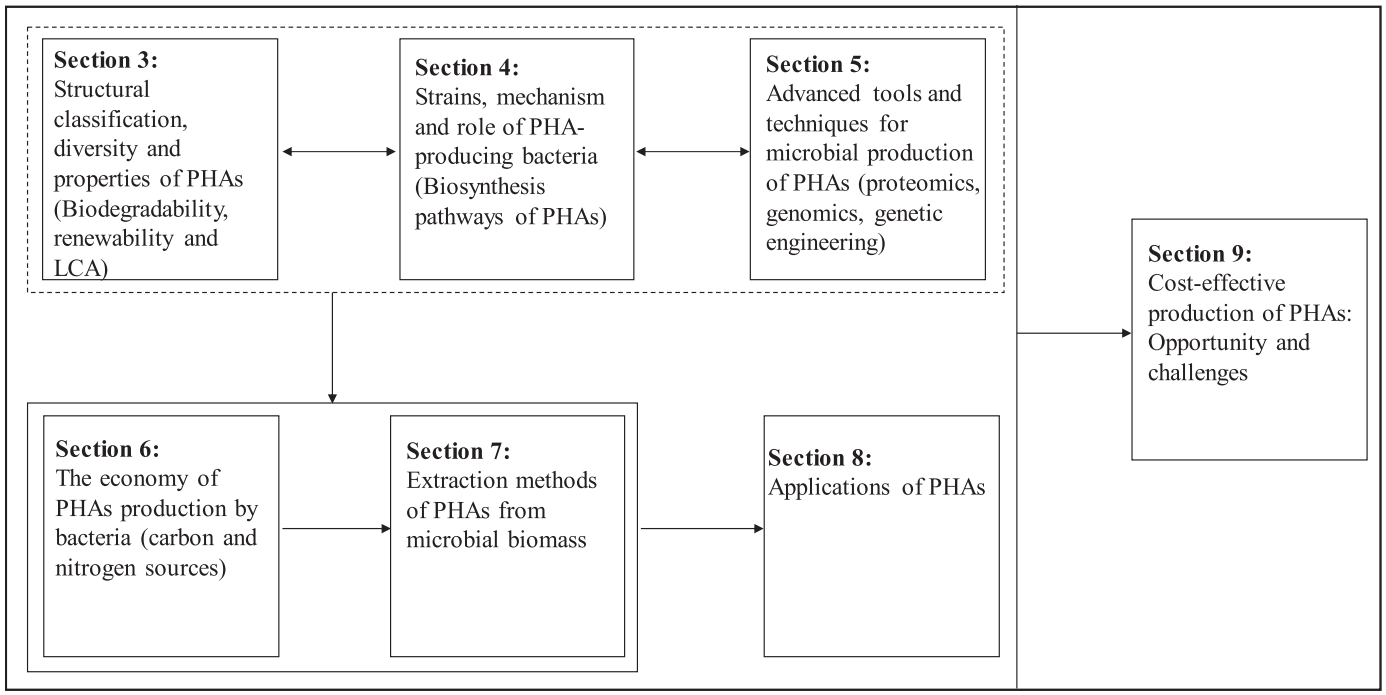

Fig. 1. Schematic representation of workflow and contents of the review.

3.1. Inherent properties of PHAs

Due to variation in the chemical and structural composition of the monomeric unit of PHAs, the physical and chemical properties of PHA polymers differ from each other. The behavior of PHB is similar to polypropylene, which shows excellent gas entrapment characteristics and good moisture resistance (Luzi et al., 2019). PHA polymers are water insoluble, which helps to withstand hydrolytic attack, and its sinking properties in water accelerate biodegradation in the absence of oxygen in sedimentary soil (Shah et al., 2008). Moreover, they are very much biocompatible, biodegradable, and piezoelectric in nature (Bugnicourt et al., 2014). The chemical structure of PHA molecules is asymmetrical (chiral), and the biodegradability of polymers is highly dependent on the nature and chemical composition of their constituents, prevailing environmental conditions, and type of microbes (every microbe produces different PHA-degrading depolymerases and hydrolases) (Masood et al., 2014; Boyandin et al., 2013). The solubility of PHA polymers is more in chlorinated solvents such as, chloroform and dichloromethane (DCM), and is insoluble in non-chlorinated solvents such as hexane. The glass transition temperature and melting temperature of PHA polymers are in the range of −50 to 4 °C and 40–180 °C, respectively (Czerniecka-Kubicka et al., 2017). There are several other physico-chemical properties of PHA polymers along with these, such as thermal degradation, breaking strength, modulus of elasticity, and vapor content, which are highly dependent upon the polymeric and monomeric composition of the biopolymers (Bugnicourt et al., 2014).

3.1.1. Biodegradability of PHAs

The higher biodegradability of PHAs in comparision to synthetic polymers is one of the important property that makes this polymer an eco-friendly biological material (Johnston et al., 2018; Emadian et al., 2017). PHA hydrolases and depolymerases are two well-known enzymes, produced by microbes, which assist the degradation of PHA polymers (Choi et al., 2004). The key factors that govern the biodegradability of PHAs in the environment include chemical composition, the polymeric chain length, crystallinity, and the complexity of polymer. Commonly, scl, lower crystallinity, and less complicated structure are more vulnerable to enzymatic degradation (Emadian et al., 2017). Furthermore, the environmental conditions such as temperature, pH, moisture, and the oxygen content are the most important factors that play crucial role in biodegradation of polymers. Madison and Huisman (1999) reported that the biodegradation of PHA polymers in anaerobic sewage sludge could occur in a few months and might take years in saline water; ultraviolet light might speed up the rate of degradation (Shangguan et al., 2006). Due to the biocompatible nature of PHA polymers, no or negligible toxic effects are seen in living systems (Volova et al., 2003). In the mammalian system, the hydrolysis and degradation are very slow. It has been reported by Pouton and Akhtar, 1996 that only less than 1.6% (w/w) mass of polymer was lost in 6 months after implantation in mice.

3.1.2. Life cycle and renewability of PHAs

The production of PHAs is biological, and it is based upon the availability of renewable resources (Licciardello et al., 2019). Mostly, the fermentation process involved in the production of PHAs is dependent on agricultural feedstock as carbon and energy sources, and their biodegradation is compatible to the biological carbon cycle (Ali and Jamil, 2016; Kadouri et al., 2005) (Fig. 2). Therefore, PHA production is important and popular as it is produced from renewable sources instead of non-renewable petroleum-based sources (Ren, 2003).

Life cycle analysis (LCA) from cradle to grave of bioplastics and synthetic plastics production from renewable sources and non-renewable sources, respectively, have been described by Patel (2002, 2005). Comparative studies of energy intensiveness and global warming as equivalent to CO_2 emissions in the production of synthetic plastics and bioplastics are summarized in Table 1 (Gironi and Piemonte, 2011). To improve the properties of bioplastics, synthetic co-polymers are mixed with bioplastics, which

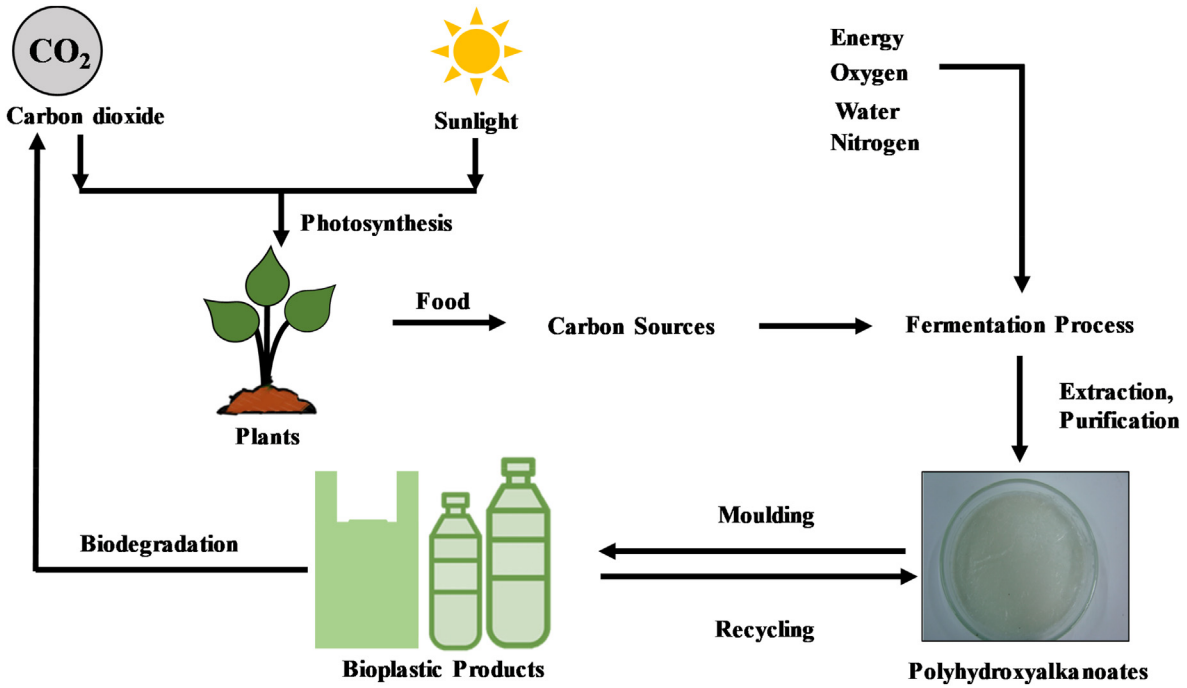

Fig. 2. Representation of synthesis and biodegradation of PHAs polymers (plants utilize carbon dioxide and sunlight for production of food via process of photosynthesis).

significantly increase the CO_2 emission and energy demands of the process. In general, the production and utilization of bioplastics are more beneficial in comparison with synthetic plastics considering the energy demand and greenhouse gas emissions.

Various LCA studies related to PHA production from renewable sources show that the production of these polymers has a higher environmental impact as compared to the production of fossil fuel-based polymers (Kourmentza et al., 2017). The interpretation of LCA data reflects the lesser environmental impact of bioplastics production as compared to synthetic plastics in some environmental indicators. However, some environmental indices are in favor of synthetic plastics production (Gironi and Piemonte, 2011). Therefore, it is necessary to establish an index by which all the environmental indices can be merged and effectively weighed. However, it should also be recognized that the biological production process of PHAs is underdeveloped, while the production process of fossil fuels-based polymers has been optimized and fully developed (Kim and Dale, 2005).

4. Strains, mechanism, and roles of PHA-producing bacteria

The production of PHAs from bacterial culture is more economical as compared to production from other living organisms, especially plants, due to their higher accumulation capacity (Verlinden et al., 2007). *Cupriavidus necator*, *R. eutropha,* or *A. eutropha* are the commonly studied bacterial sp. for the biosynthesis and generation of PHAs (Vandamme and Coenye, 2004; Vaneechoutte et al., 2004). These bacterial strains were first used by a company called Imperial Chemical Industries for production of a PHBV copolymer, Biopol. The production of PHAs using *C. necator* fermentation process is more economical than other processes. Recently, new processes and technologies have emerged for the production of economical PHAs, but these also utilize PHA synthase genes of *C. necator*. Other potential bacterial strains have been recently investigated for PHA production based on the yield, such as *Bacillus* sp., *Pseudomonas* sp., *Aeromonas hydrophila*, *Rhodopseudomonas palustris*, *Burkholderia sacchari*, and *Halomonas boliviensis* (Table 2).

Table 1
Comparative studies of energy requirement and CO_2 emissions in the production of synthetic plastics and bioplastics from renewable and non-renewable feedstocks (adapted from Gironi and Piemonte, 2011).

Type of plastic	Energy Requirement (MJ/kg)	Global Warming (CO_2 eq/kg)
Non-renewable sources		
Nylon 6	120	7.64
Polyethylene terephthalate (PET)	77.0	4.93
Polyvinyl alcohol (PVA)	102	2.70
High density polyethylene (HDPE)	80.0	4.84
Low density polyethylene (LDPE)	80.6	5.04
Polystyrene (PS)	87.0	5.98
Polycaprolactone (PCL)	83.0	3.10
Renewable sources		
Polylactic acid (PLA)	57.0	3.84
Thermoplastic starch (TPS)	25.4	1.14
TPS + 15% PVA	24.9	1.73
TPS + 60% PCL	52.3	3.60
PHA	57.0	—

Many groups of living organisms can synthesize PHAs and accumulate them inside as granules. The production of PHAs at an industrial scale has been performed using the plant and bacterial systems. Due to the physiological limitation of the plant system, it can only store PHAs up to 10% of its dry biomass, and more than that adversely affects the growth of the plant (Verlinden et al., 2007). Till date, this problem persists in the plant system, and hence, production of PHAs from the plant is not cost-effective. Accumulation of PHAs in the bacterial system is a natural process to cope with the changing environment and imbalance in nutrients (Kumar and Thakur, 2018). These biological molecules accumulate in the bacterial system when an excess of carbon source is available in the media, and it is deprived of nitrogen, phosphorous, and oxygen (Możejko-Ciesielska and Kiewisz, 2016). Due to insoluble nature of PHAs in water, bacterial cells accumulate it intracellularly, which is also advantageous for the cell as it is a stored carbon source and it can provide energy during starvation conditions (Raza et al., 2018). Polymerization of soluble PHA intermediates to non-soluble PHA polymers occurs inside the bacterial system and does not affect the osmotic state of the bacterial cells. This phenomenon is advantageous to bacteria system, as it can prevent the leakage of PHAs from inside to outside the cells while the carbon and energy source remains available at a low maintenance cost (Potter et al., 2002). Phasin is a class of protein, which is composed of phospholipids and protein. This class of protein is coated on PHA molecules, and these are the molecules present on the granular interface. The phasins stimulate and control the granular size and number of PHA molecules (Możejko-Ciesielska and Kiewisz, 2016).

The synthesis of PHAs in microorganism occurs through three well-known pathways (Fig. 3); among these three, the pathway I is well studied in *C. necator*. This pathway starts with the generation of 3HB monomers by the Krebs cycle, and the enzyme β−ketothiolase catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. Formation of 3-hydroxybutyryl-CoA occurs as a result of the action of acetoacetyl-CoA reductase on acetoacetyl-CoA. In the end, P(3HB) synthesis is catalyzed by PHA synthase through polymerizing the 3 hydroxybutyryl-CoA into P(3HB) via esterification (Verlinden et al.,

Table 2
Bacterial strains involved in the production of PHAs using a diverse range of carbon sources.

Bacterial strain	Carbon source	PHAs CDW% (w/w)	PHAs (g/L)	Polymer type	Reference
Burkholderiacepacia	Xylose	58.4	—	P3HB	Pan et al. (2012)
Pseudomonas resinovorans NRRLB-2649	Triglycerides	45.0	1.10 −2.10	mcl PHA	Ashby and Foglia (1998)
Acetate	Activated sludge	59.0	—	scl PHA	Wen et al. (2010)
*Cupriavidusnecator*H16	CO_2	88.9	—	P3HB	Sonnleitner et al. (1979)
Cupriavidusnecator DSM 545	Commercial glycerol	62.0	51.2	scl	Cavalheiro et al. (2009)
Cupriavidusnecator DSM 545	Waste glycerol	52.0	38.1	scl	Cavalheiro et al. (2009)
Cupriavidusnecator	Molasses	31.0–44.0	—	P3HB	Beaulieu et al. (1995)
P. putida Bet001	Fatty acids	49.7–68.9	10.1 −15.45	mcl	Gumel et al. (2014)
P. putida GO16	Terephthalic acid	27.0	0.250	mcl	Kenny et al., 2008
Bacillus sp. ISTC1	Glucose	47.0	0.810	PHV	Kumar et al. (2016b)
*Methylocystis*sp. GB25	Methane	51.0	—	P3HB	Wendlandt et al. (1998)
Pseudomonas aeruginosa PAO1	Oil and wax products from polyethylene (PE) pyrolysis	25.0	—	mcl-PHA	Guzik et al. (2014)
*Pseudomonas putida*KT2440	4-Hydroxyhexanoic acid, Glucose	25.3–29.8, 32.1	—	mcl-PHA	Sun et al. (2007); Davis et al. (2013)
*Pseudomonas putida*mt-2	Toluene, *p*-xylene	22.0–26.0	—	mcl-PHA	Nikodinovic et al. (2008)
Bacillus megaterium	Citric acid, glucose, glycerol, succinic acid	9.0–50.0	—	P3HB	Shahid et al. (2013)
Serratia sp. ISTVKR1	Waste water and glucose	—	0.337	PHV	Gupta et al. (2017)
Serratia sp. ISTD04	$NaHCO_3$ and Glucose	45.5	0.820	PHV	Kumar et al. (2016a)
Methylobacteriumextorquens	Methanol	40.0–46.0	—	P3HB	Bourque et al. (1995)
Pseudomonas sp. PS1	Waste cooking oil	—	2.30	—	Prasad and Seth (2013)
Hydrogenophagapseudoflava	Hydrolyzed whey and valerate	40.0	—	P3HB3HV	Koller et al. (2007)
Azotobacterchroococcum H23	Wastewater from olive oil mill	70.0	—	PHA	Martinez-Toledo et al. (1995)
*Thermusthermophilus*HB8	Whey	35.6	—	scl-mcl-PHA	Pantazaki et al. (2009)
Aeromonascaviae	Unsaponifed olive oil	96.0	—	mcl-PHA	Cromwick et al. (1996)
Cupriavidusnecator NCIM 5149	Potato starch, saccharified waste	55.0	—	P3HB	Haas et al. (2008)
P. oleovorans ATCC 29347	n-alkanes and 1-alkenes	25.0	—	mcl	Lageveen et al. (1988)
*Alcaligeneseutrophus*TF93	4-Hydroxyhexanoic acid, CO_2	67.2, 60.0	—	P3HB	Valentin and Dennis (1997); Ishizaki and Tanaka (1991)
P. fluorescence A2a5	Sugarcane liquor	70.0	22.0	scl	Jiang et al. (2008)
Azohydromonasaustralica	Malt waste	70.1	—	P3HB	Yu et al. (1998)
P. aeruginosa	Pigeon pea waste	41.0	—	—	Khandpur et al. (2012)
P. aeruginosa	Sugarcane bagasse	60.0	—	—	Khandpur et al. (2012)
*Halomonasboliviensis*LC1	Hydrolyzed starch	56.0	—	P3HB	Quillaguamán et al. (2005)
P. aeruginosa	Rice bran	48.0	—	—	Khandpur et al. (2012)
Halomonasboliviensis LC1	Wheat bran hydrolysate	4.00	—	PHB	Van-Thuoc et al. (2008)
Pseudomonas putida	Lard and coconut oil	0.9–1.6	—	PHA	Solaiman et al. (2001)
Pseudomonas saccharophila	Coconut oil and tallow	0.800	—	mcl-PHA	Solaiman et al. (1999)
Burkholderiacepacia IPT 048 and *B. sacchari* IPT 101	Xylose and glucose from sugar cane bagasse	34.8	—	PHB	Silva et al. (2004)
P. putida CA-3	Styrene	25.4	—	Hetropolymer (R-3-hydroxyphenylvalerate	Wang et al. (2005)
B. cereus SPV	Carbohydrates	37.0	—	scl	Valappil et al. (2007)
Azotobacterbeijerinckii	Glucose	24.8	—	P3HB	Lasemi et al. (2012)
Bacillus sp. ISTVK1	Pure glycerol	85.2	4.44	PHV	Morya et al. (2018)

2007). During availability of carbon source as well as growth-limiting nutrients in the media, bacteria grow normally and, in that case, inhibition of the 3-ketothiolase takes place due to the availability of free coenzyme-A from the TCA cycle. However, during non-availability of growth-limiting nutrients, the access of acetyl-CoA into the TCA cycle is controlled, and the excess acetyl-CoA is directed towards the synthesis of PHB molecules (Ratledge and Kristiansen, 2001). The generation of various monomeric units of PHAs has been reported through the pathways governing the fatty acid metabolism. β-oxidation of fatty acids plays a crucial role in the control of PHA biosynthesis pathway II. It produces substrates which can be further polymerized by PHA synthase of *Pseudomonads* having its place in the ribosomal RNA-homology group I such as *P aeruginosa*. The intermediate of the β-oxidation pathway such as trans-2-enoyl-CoA is further converted into (R)-hydroxyacyl-CoA by the action of (R)-specific enoyl-CoA hydratase in *A. caviae* (Anjum et al., 2016; Tsuge, 2002).

Pathway III of PHA biosynthesis uses simple and structurally related substrates such as glucose, sucrose, and fructose for the biosynthesis of PHAs monomer (Tsuge, 2002). The enzyme encoded by *phaG* gene (acyl-ACP-CoA transacylase) converts the intermediates generated in fatty acid biosynthesis pathways such as (R)-3-hydroxyacyl into their acyl carrier protein (ACP) through the CoA. The various enzymes involved in overall PHA biosynthesis pathways are presented in Table 3.

5. Advanced tools and techniques for microbial production of PHAs

Contemporary advancements in proteomics, genomics, genetic engineering and synthetic biological tools have provided a common platform to apply all the multidisciplinary technologies for prediction, identification of proteins, genes and modification of the biochemical pathways (Tables 4 and 5) (Kumar et al., 2017, 2018c). Proteomic study of *P. putida* KT2440 by Możejko-Ciesielska and Mostek (2019a) revealed that this strain could be helpful for increasing efficiency of the PHAs production and making it more cost-effective. Similarly, simultaneous proteomic and genomic analysis of lignin degrader and PHAs producer, *Pandoraea* sp. ISTKB, revealed that this strain could serve as a potential candidate for valorization of lignin and economical production of PHAs (Kumar et al., 2018c). Currently, genetic engineering approaches are increasingly applied in the field of polymer production as it can modify the pathways and regulate microbial metabolism (Favaro et al., 2019; Kumar et al., 2019; Thakur et al., 2018) (Table 5).

Genetic modification generally involves modification of biochemical pathways at the genomics level of the biological system, whereas synthetic biology approaches deal with computation algorithms and mathematics (Hu and Dhar, 2015). To make the extraction process of PHA from bacterial biomass cost-effective, synthetic biological approaches are emerging as an indisposable tool, by which PHAs are extracted from the biomass without upsetting the cells by applying novel low molecular mass proteins such as "Phasins" (Rahman et al., 2013). The function of phasins is to reduce the granular size of PHB, which facilitates the secretion of PHB from the bacterial cells. Investigations on the bacterial strain *Herbaspirillum seropedicae* showed that SmR1 caused removal of PhaP1 protein and decrease in PHB accretion ≤50% (Alves et al., 2016). *Methylobacterium extorquens*, which can utilize methanol as carbon source, was modified with the PHA synthesis gene of bacterial strain *P. fluorescens* GK13, and therefore, it could produce functional PHB polymer (Hofer et al., 2010). Commercial PHA production from yeast *Yarrowiali lipolytica* was initially not viable, but now genetically engineered *Y. lipolytica* could produce 1.11 g/L of PHAs using oleic acid as carbon source (Gao et al., 2015). In *E. coli*, after reversing the β-oxidation pathway, the production of mcl-PHAs was increased to 6.62% of CDM (Zhuang et al., 2014). Furthermore, PHA synthase obtained from *Pseudomonas stutzeri* 1317 shows low-substrate-specificity was indroduced to *E. coli*. The recombinant *E. coli* synthesized 12 wt% of CDW scl and mcl PHA or scl-mcl PHA copolymers, in which 21 mol% contributed by 3-HB and 79 mol% by mcl-monomers (Chen et al., 2016).

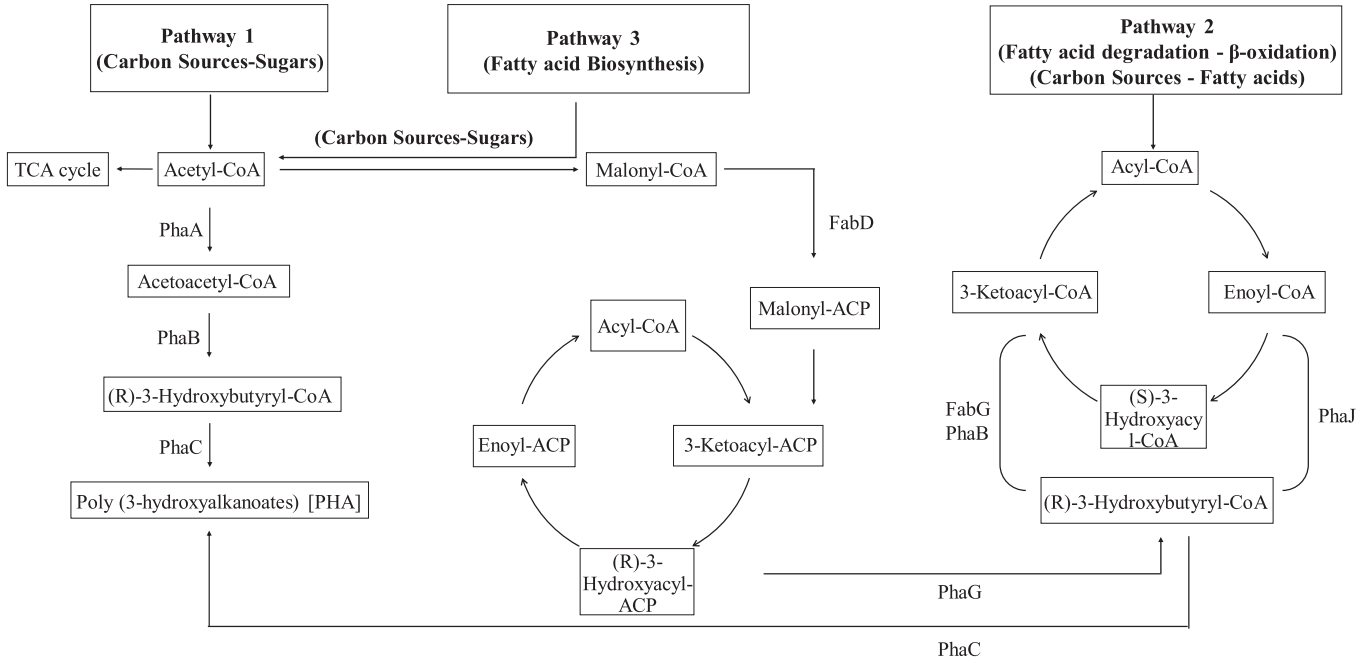

Fig. 3. Representation of microbial biosynthesis pathways of PHAs (PhaA = 3-Ketothiolase, PhaB = Acetoacetyl-CoA reductase, PhaC = PHA synthase, FabG = 3-oxoacyl-[acyl-carrier-protein] reductase, PhaJ = (R)-specific enoyl-CoA hydratase, PhaG = (R)-3-hydroxydecanoyl-ACP:CoA transacylase, FabD = malonyl-CoA-ACP transacylase).

Table 3
Enzymes involved in the biosynthesis of PHAs.

Enzyme	Representation	Bacterial strain	Reference
3-Ketothiolase	PhaA	*Cupriavidus necator*	Peoples and Sinskey (1989)
NADPH-dependent acetoacetyl-CoA reductase	PhaB	*Cupriavidus necator*	Peoples and Sinskey (1989)
PHA synthase	PhaC	*Cupriavidus necator* and many	(Peoples and Sinskey, 1989; Kadouri et al., 2005)
4-Hydroxybutyrate dehydrogenase	4HbD	*Clostridium kluyveri*	Valentin and Dennis (1997)
4-Hydroxybutyrate-CoA: CoAtransferase	OrfZ	*Clostridium kluyveri*	Valentin and Dennis (1997)
NADPH-dependent 3-Ketoacylreductase	FabG	*Pseudomonas aeruginosa*	Ren et al. (2000)
Succinic semialdehyde dehydrogenase	SucD	*Clostridium kluyveri*	Valentin and Dennis (1997)
Acetyl-CoA carboxylase	ACC	*Escherichia coli* K-12 MG1655	Lee et al. (2011)
Malonyl-CoA:ACP transacylase	FabD	*Escherichia coli* K-12 MG1655	Lee et al. (2011)
3-Ketoacyl carrier protein synthase	FabH	*Escherichia coli* K-12 MG1655	(Lee et al., 2011)
Methylmalonyl-CoA mutase	Sbm	*Escherichia coli* W3110	Aldor et al. (2002)
Methylmalonyl-CoA decarboxylase	YgfG	*Escherichia coli* W3110	Aldor et al. (2002)
3-Ketothiolase	BktB	*Cupriavidus necator*	Slater et al. (1998)
Acyl-CoA synthetase	FadD	*Pseudomonas putida*CA-3	Hume et al. (2009)
(*R*)-Enoyl-CoA hydratase	PhaJ	*Pseudomonas putida*KT2440	Sato et al. (2011)
3-Ketoacyl-CoA thiolase	FadA	*Pseudomonas putida*KT2442	Ouyang et al. (2007)
Caprolactone hydrolase	ChnC	*Acinetobacter* sp. SE19	Brzostowicz et al. (2002)
Cyclohexanol dehydrogenase	ChnA	*Acinetobacter* sp. SE19	Brzostowicz et al. (2002)
3-Hydroxyacyl-ACP:CoA transacylase	PhaG	*Pseudomonas mendocina*	Zheng et al. (2005)
6-Hydroxyhexanoate dehydrogenase	ChnD	*Acinetobacter*sp. SE19	Brzostowicz et al. (2002)
6-Oxohexanoate dehydrogenase	ChnE	*Acinetobacter*sp. SE19	Brzostowicz et al. (2002)
Glyceraldehyde-3-phosphate dehydrogenase	—	*Cupriavidus necator*	Raberg et al. (2011)
Pyruvate dehydrogenase complex	—	*Burkholderiacepacia*	Raberg et al. (2011)
Alcohol dehydrogenase, putative	—	*Aeromonas hydrophila*4AK4	Xie and Chen (2008)
Hydroxyacyl-CoA synthase, putative	—	Mutants and recombinants of *Cupriavidus necator*	Valentin et al. (1995)
Methylmalonyl-CoA racemase	—	*Nocardiacorallina*	Valentin and Dennis (1997)
Lactonase, putative	—	Mutants and recombinants of *Cupriavidus necator*	Valentin et al. (1995)
NADPH-dependent acetoacetyl-CoA reductase	—	*Rhizobium* (*Cicer*) sp. CC 1192	Chohan and Copeland (1998)

In the group of biodegradable plastics, PLA can apply in the field of food packaging, biomedical, drug carrier etc due to its cost-effectivness. Nevertheless, due to its poor mechanical and thermal characteristics, its large scale application is vulnerable (Chen et al., 2016). Therefore, to improve the properties of PLA copolymerization of LA with HA monomers is considered as potential technology. Various PHA synthases were genetically modified, which can be able to use lactyl-CoA (LA-CoA) and 3-hydroxybutyryl-CoA (3HB-CoA) as substrates (Yang et al., 2011; Taguchi et al., 2008). Single step biosynthesis of LA-incorporated PHA co-polyester, P(6 mol% LA-co-94 mol% 3HB) has been reported by Taguchi et al. (2008), without hampering the inherent properties of PHB and PLA. Shozui et al. (2011) introduced the monomers including 3HV, 3HHx into the LA copolymers, to achieve the better desirable properties. Production of a novel glycolate-containing biopolymers poly(glycolate-co-lactate-co-3-hydroxybutyrate) using glucose as carbon source by recombinant *E. coli* strain was reported by Li et al. (2016). Wang et al. (2014), reported production of novel terpolymer by the recombinant *E. coli* strain and the yield was 3.90 g/L at shake flask level. The

Table 4
Application of proteomic and genomic tools for screening of potential bacterial strains for the production of PHAs.

Bacteria strain	Carbon source	Type of PHAs	Yield	Reference
Proteomic analysis of PHAs producing bacteria strains				
Pseudomonas putida KT2440	Citrate	mcl-PHAs	24% of CDW	Możejko-Ciesielska and Mostek (2019a)
Pseudomonas putida KT2440	Oleic acid	mcl-PHAs	48.8% of CDW	Możejko-Ciesielska and Mostek (2019b)
Pandoraea sp. ISTKB	Kraft lignin	—	—	Kumar et al. (2018c)
Pandoraea sp. ISTKB	Vanillic acid	—	—	Kumar et al. (2018c)
Mixed microbial cultures (MMCs).	Dairy manure	—	40% of CDW	Hanson et al. (2016)
Novosphingobium nitrogenifigens Y88	Glucose	PHB	81% of CDW	Smit et al. (2012)
Genomic analysis of PHAs producing bacteria strains				
Pseudomonas sp. MPC6	Sugars, decanoate, ethylene glycol, organic acids	copolymers of scl and mcl	2.1–30% of CDW	Orellana-Saez et al. (2019)
Pseudomonas corrugata (Pco)	—	mcl-PHA	—	Licciardello et al. (2019)
Pseudomonas mediterranea (Pme)	—	mcl-PHA	—	Licciardello et al. (2019)
Zobellella denitrificans ZD1	Crude glycerol with ammonia and nitrate	PHB	9.5 g/L and 16.3 g/L of fermentation media	Wu et al. (2019)
Pandoraea sp. ISTKB	Kraft lignin	—	—	Kumar et al. (2018c)
Pandoraea sp. ISTKB	Vanillic acid	—	—	Kumar et al. (2018c)
Mixed microbial cultures (MMCs).	Dairy manure	—	40% of CDW	Hanson et al. (2016)
Pseudomonas extremaustralis 14-3b	Sodium octanoate	PHB	35.80% of CDW	Catone et al. (2014)

Table 5
Genetically modified bacteria strains for the production of PHAs.

Recombinant strain	PHA genes source	Carbon sources	PHA type	Fermentation type	Yield (g/L)	Reference
Cupriavidus necator	*Chromobacterium* sp. USM2	Sodium valerate	P(3HB-co-3HVco-3HHx)	Fed-batch	8.10	Bhubalan et al. (2010)
Cupriavidus necator	*Aeromonas caviae*	Palm kernel oil	P(3HB-co-3HVco-3HHx)	Batch	6.20	Bhubalan et al. (2008)
Shimwellia blatae	*Ralstonia eutropha*	Glycerol	P(3HB-co-3HP)	Two step fed-batch	7.10	Sato et al. (2015)
Cupriavidus necator	*Burkholderia* sp. USM (JCM 15050)	Crude palm kernel oil	P(3HB-co-4HB)	Fed-batch	1.60	Lau and Sudesh (2012)
Burkholderia sp. USM (JCM 15050)	*Aeromonas caviae*	Crude palm kernel oil	P(3HB-co-3HHx)	Fed-batch	1.10	Chee et al. (2012)
Escherichia coli JM109	*Comamonas* sp. EB172	Glucose	P(3HB-co-3HV)	Batch	0.700	Yee et al. (2012)
Escherichia coli	*Pseudomonas* sp.LDC-5	Molasses	mcl-PHA	Batch	3.06	Saranya and Shenbagarathai (2011)
Escherichia coli	*Ralstonia eutropha*	Glucose	P(3HP	Batch	1.00	Meng et al. (2015)
Escherichia coli K24KP	*Azotobacter* sp. (FA8)	Glycerol	P(3HB)	Batch	3.30	Almeida et al. (2011)
Escherichia coli K24KL	*Cupriavidus necator*	Glycerol	P(3HB)	Fed-batch	26.4	Nikel et al. (2010)
Escherichia coli K24KP	*Azotobacter* sp. (FA8)	Glucose	P(3HB	Aerobic batch	3.50	Almeida et al. (2010)

mechanical and thermal properties analysis of this hybride terpolymer, showed better toughness and reduced crystanallity as compare to PHB homopolymer.

Accumulation of PHAs in Gram-negative bacteria is well known, while Gram-positive bacterial species are known for the non-production of endotoxins, without PHA synthesizing genes (Raza et al., 2018). Therefore, by using genetic engineering and synthetic biology tools, the genes responsible for PHA synthesis could be inserted in Gram-positive bacteria, generating endotoxin free products. Currently, few studies have confirmed the production of PHAs by the ideas mentioned above (Song et al., 2012; Valappil et al., 2006a,b). Production of PHAs by Gram-positive bacteria using the waste stream as a carbon source has also been reported (Bhuwal et al., 2013). The extensive study of Gram-positive *Bacillus* sp. for the production of biopolymers (homo and co-polymers) using soft carbon source and waste material has confirmed the production up to 70–80% of its CDW without any endotoxins (Sonakya et al., 2001). The bacterial accumulation of PHAs up to 80% of CDW could be adopted at the industrial level, which can be further enhanced by using synthetic biology tools. Other potential Gram-positive bacterial strains need to be screened and altered for the better yield.

6. The economy of PHAs production by bacteria

Despite several advantages of microbial bioplastics over synthetic plastics, the main stumbling block in its production is its non-cost-effectiveness (Koller and Braunegg, 2018). The major costs involved in its production are the fermentation process, carbon source, PHAs yield on a particular carbon and other energy sources, productivity of the process, and down-stream processing (Koller and Braunegg, 2018; Zahari et al., 2014). Because of these, commercialization of PHAs is still struggling in comparasion to synthetic plastics. The market price of synthetic polymers such as polypropylene and polyethylene is US$0.60–0.87/lb, PHA cost is approximetly 3–4 times higher, ranging from 2.25 to 2.75US$/lb (Kourmentza et al., 2017; Plastics Technology, 2017). Various industries have commercialized the production of PHAs, as presented in Table 6. The final yields and cost of PHA are dependent on PHA accumulation capacity of bacteria and thus, the production processes. For making the PHA production economical, screening, and selection of a potential microbe are equally important along with carbon substrate.

6.1. Influence of substrates on PHA yield

The chemical composition and yield of the polymers are dependent on the source of carbon and its availability in the growth media. Such physiochemical properties and yield of PHAs are directly influenced by the functional and biochemical features of the microorganisms, biosynthesis pathway, together with adaptability to growth media (Volova, 2004). Incorporation of different phenoxy, olefin, esters, phenyl, halogens, alkyls, and chemical groups with the substrate in the polymers was performed using *P. putida* (Kim, 2000). PHAs with functional carbon-carbon triplex bond, phenoxy, methylphenoxy, nitrophenoxy, and cyanophenoxy were also produced by *P. putida*. Kim (2000) used 36 different carbon substrates for the biosynthesis of diverse ranges of polymers by *P. putida* KCTC2407. The carbon metabolic pathway of *P. putida* is not much efficient to utilize lower molecular weight organic acids as carbon source with organic and inorganic functional groups such as cyclohexyl, bromine, ethoxy, etc. for production of PHAs. The higher total production cost of the polymers is the major challenge that restricts its commercialization. Diverse range of substrates have been used to produce economically viable polymers. The production of PHAs by bacterial strain *Pseudomonas* sp. DR2 was done using different carbon sources such as citrate, glucose, acetate, glucose, palmitate, corn oil, butyrate, and waste fried oil; in all the sources utilized, the maximum yield was 37.3% of CDW with corn oil and trailed by 23.5% of CDW with waste fried oil at 30 °C for 72 h (Song and Jeon, 2008). With the utilization of soybean oil and N-hexadecane as carbon source, approximately 50.3% and 40.7% (w/w) of PHAs are produced, respectively, from *P. aeruginosa*, which is mutated by γ-rays (Abid et al., 2016; Raza et al., 2016). Along with carbon source C:N ratio, also play a key role in microbial production of PHA.

6.2. Influence of carbon and nitrogen sources on PHA production

Various studies showed the increase in microbial biomass along with PHA biosynthesis at different carbon to nitrogen (C:N) ratios (Ahn et al., 2015; Cui et al., 2017). In general, PHA accumulation is directly proportional to C:N ratio and inversely proportional to bacterial growth (Cui et al., 2017). Yao et al. (1999) confirmed the influence of C:N on the composition and accretional potency of *P. nitroreducens*. The reduction in 3-HB content was observed from

Table 6
Pilot and industrial scale PHAs production companies and their production cost (adapted from Kourmentza et al., 2017; Plastics Technology, 2017; Chanprateep, 2010; Chen, 2010).

Manufacturers	PHA and Tradename	Microorganism (Biocatalyst)	Production Capacity (ton/year)	Price (kg^{-1})
Bio-On Srl., Italy	PHB, PHBV spheres (minerv®-PHA)	*Cupriavidus necator*	10,000	–
Mitsubishi Gas Chemical Company Inc. (Japan)	Biogreen®, PHB	—	10,000	€2.50 −3.00
Biomatera, Canada	PHA resins (Biomatera)	Non-pathogenic, non-transgenic bacteria isolated from soil	—	—
BluePHA, China	Customized PHBVHHx, PHV, P3HP3HB, P3HP4HB, P3HP, P4HB synthesis	Microbial strains developed via synthetic biology tools	—	—
Telles (US)	PHB Mirel™	*Ralstonia eutropha*	50,000	€1.50
PHB Industrial Company (Brazil)	PHB Biocycle®	—	50	—
Danimer Scientific, USA	mcl-PHA (Nodax® PHA)	—	—	—
Kaneka Corporation, Japan	PHB-PHHx (AONILEX®)	*Ralstonia eutropha*	3500	—
Biomer Inc. (Germany)	PHBV and PHB Biomer®	*Alcaligenes latus*	50	€3.00 −5.00
Newlight Technologies LLC, USA	PHA resins	Newlight's 9X biocatalyst	—	—
PHB Industrial S.A., Brazil	PHB, PHBV (BIOCYCLE®)	*Alcaligenes* sp.	3000	—
Tianan Biologic, Ningbo (China)	PHBV, PHBV + Ecoflex blend Enmat®	*Ralstonia Entropha*	10,000	€3.26
PolyFerm, Canada,	mcl-PHA (VersaMerTM PHA)	Wild microbial strain	—	—
P&G (US)	PHBH Nodax™	—	20,000−50,000	€2.50
Shenzhen EcomannBiotechnology Co. Ltd., China	PHA pellets, resins, microbeads (AmBio®)	—	5000	—
SIRIM Bioplastics Pilot Plant, Malaysia	PHA	—	2000	—
Lianyi Biotech (China)	PHBH Nodax™	—	2000	€3.70
Tianjin GreenBio Material Co., China	P (3, 4HB) films, pellets/foam pellets (Sogreen®)	—	10,000	—

100 to approximately 7% (w/w) along with the decrease in cellular growth, while the accumulation of PHAs content increased from 0.5 to 33.5%. It would be a good strategy if excess carbon source available in growth media could lead to higher accumulation of PHAs (Montie, 2013). In another study, *P. Putida* KT2440 was shown to accumulate PHAs up to 70% of its CDW without any nutrient limitation (Sun et al., 2007).

6.3. Utilize cost-effective carbon source for reducing PHA production cost

Despite the various advantages of bioplastics over petroleum-based plastics, its production is still not much attainable due to 5−10 times higher cost associated with its production as compared to the synthetic plastic such as polyethylene. The major cost involved in its production is related to the carbon source that is more than 50% of the overall cost (Aslan et al., 2016). Therefore, research endeavors are looking to make bioplastic cost-effective by using waste materials such as organic waste (Alvi et al., 2014), waste plant oil (Ciesielsk et al., 2015), glycerol (Morya et al., 2018; Haron et al., 2018), wastewater (Gupta et al., 2017; Morgan-Sagastume et al., 2016), and sewage sludge (Kumar et al., 2018a) as feedstock or carbon source. Prior to use these waste material as carbon source, physicochemical characterization and pretreatment are necessary in production of PHA (Rodriguez-Perez et al., 2018).

The pretreatment might increase the carbon content of the waste materials and its availability for the growth of microorganism (Pittmann and Steinmetz, 2016; Pais et al., 2016). Also this process dilutes the quatity of organic matter (Pittmann and Steinmetz, 2016; Alsafadi and Al- Mashaqbeh, 2017), regulate the temperature (Kourmentza et al., 2015), sterilization of wastes (Oh et al., 2015), control the pH (Amulya et al., 2016), and remove suspended solids from liquid waste (Basset et al., 2016). So far, several pretreatment processes have been applied including anaerobic fermentation (Pittmann and Steinmetz, 2016) and chemical hydrolysis (Oh et al., 2015; Pais et al., 2016) (Table 7). The waste materials used for growth of PHA producers are mostly from either waste treatment plants or food industries (Rodriguez-Perez et al., 2018; Basset et al., 2016). The relationship between hydrolysis process and selected waste is not much explored. This approach resolve the problem of waste disposal and also reduce the feedstock cost associated with PHA. Integrated waste treatment by anaerobic fermentation and PHA production is an emerging technology, techno-economical analysis is required prior to pilot or industrial level application. Several waste materials applied for the production of PHAs are discussed in next section.

6.3.1. Molasses as a substrate for PHA production

Molasses are complex viscous waste material produced as a byproduct of the sugar cane industry. Based on the use of sulphur dioxide (SO_2) as a stabilizing agent in the extraction processes, it can be classified into sulphured and non-sulphured molasses. The main components of molasses include sucrose, glucose, and fructose with a minor concentration of Fe, Mg, Ca, K, and vitamins (B_7), which all act as a supplement for the growth of bacteria (Shasaltaneh et al., 2013). Using molasses as substrate, an increase in the production of PHAs to 6.0 g/L has been reported (Santimano et al., 2009). *Pseudomonas* sp. utilized molasses and produced rhamnolipids and bacterial biomass of 1.45 g/L and 1.67 g/L, respectively (Raza et al., 2007). The liquor media obtained from sugar cane processing industry can make the PHA production cost-effective as the *P. fluorescens* A2a5 produces PHAs $\leq$70% of CDW (Cromwick et al., 1996).

6.3.2. Waste frying oils and plant oils as substrates

The waste frying oil with high biological oxygen demand (BOD) and chemical oxygen demand (COD) is produced from fast food industries, and its proper disposal is a major challenge. There are several reports that confirmed the utility of waste frying oils as a potential substrate for the bacterial biosynthesis of rhamnolipids along with bioplastics, (Haba et al., 2007; Raza et al., 2006). Nevertheless, as compared to plant oil production of biopolymers, the yield is not significant using waste frying oils as substrate (Akaraonye et al., 2010). Bacterium strain *P. aeruginosa* 47T2 produced 7.6 g/L and 10 g/L of PHAs and rhamnolipids, respectively, with waste frying oil as a substrate in growth media (Haba et al.,

Table 7
Pretreatment technologies of waste for the production of PHAs.

Bacterial sp./strain	Substrate	Pre-treatment	Additional supplement	Fermenter size (L)	PHA Yield (g/g substrate)	Reference
Not specified	Activated wastewater sludge	Hydrolysis, Anaerobic fermentation, filtration and dilution	Synthetic medium including VFAs	70 0.17 g PHA/g substrate	0.170	Jia et al. (2014)
Not specified	Urban wastewater + Organic fraction of municipal solid waste	Fermentation, solid/liquid separation	—	0.800	0.220	Basset et al. (2016)
Corynebacterium Hydrocarbooxydansy and Bacillus megaterium	Carboxylic acid (in glycerol fermentation effluent from propanodiol manufacturing)	Anaerobic fermentation	Synthetic medium	10.0	0.020	Pan et al. (2016)
Plasticicumul ansacidivorans	Waste water from candy bar factory	Hydrolysis, Anaerobic fermentation	Waster (dilution) Nutrients solution (including trace elements) HCl and NaOH, Allythiurea	200	0.370	Tamis et al. (2014)
Pandoraea sp.	Crude glycerol	—	Synthetic medium, Propionic acid or Hexanoic acid	—	0.16/ 0.22/ 0.05/ 0.05/0.04	de Paula et al. (2016)
Not specified	Activated wastewater sludge	Hydrolysis, centrifugation and filtration	Urban wastewater	400	0.38	Morgan-Sagastume et al., 2016
Not specified	Composite food waste, boiled rice, cooked vegetables, un-cooked vegetables, waste cooking oil, vegetable peelings, cooked meat, boiled spices	Masticated, filtered, gravity Separator, diluted, pH adjustment, hydrolysis, acidogenic fermentation	Nutrients solution, pH Control, Urban wastewater	200	0.170	Amulya et al., 2016
C necator DSM 428, 531, and 545	Chicory Roots	Dried, milled, hydrolysis, detoxification, centrifugation, and filtration	Synthetic medium	1.00	0.020/ 0.010/ 0.030	Haas et al. (2015)
Bacterial sp./strain	Substrate	Pre-treatment	Additional supplement	Fermenter size (L)	PHA Yield (g/g substrate)	Reference
Pannonibacter phragmitetus ERC8	Crude glycerol	Dilution	Synthetic medium pH Control	3.00	0.160	Ray et al. (2016)
Halomonas hydrothermalis	Crude glycerol + oil cake hydrolysate	Hydrolysis	Synthetic medium, Seaweed derived crude levulinic acid	0.100	0.750	Bera et al. (2015)
H mediterranei	Cheese whey	Acid hydrolysis	Synthetic medium, HCl/ NaOH, Antifoam	2.00	0.750	Pais et al. (2016)
Not specified	Cheese whey	Anaerobic batch Fermentation, Centrifugation	Synthetic medium, NH_4Cl and/or KH_2PO_4	0.500	0.460	Valentino et al., 2015
Pseudomonas sp.	Olive mill wastewater	Water dilution, Anaerobic digestion (mesophilic), Centrifugation, Filtration, sterilization	Synthetic medium, phosphate buffer solution	2.40	0.180/ 0.680	Kourmentza et al. (2015)
H mediterranei	Olive mill wastewater	Dilution in nutrient limited medium	Synthetic medium	0.100	0.009	Alsafadi and Al-Mashaqbeh (2017)
Bacillus sp.	Pea-shells, potato peels, apple, pomace, onion peels (mixtures)	Hydrolysis, solid/liquid Separation, filtration	Glucose, Synthetic medium	0.200	0.040	Kumar et al. (2016c)
B. cereus EGU43	Pea-shell	Centrifugation, pH adjustment	Glucose, Synthetic medium	0.200	0.350	Patel et al. (2015)
E. coli and *R. eutropha* (Recombinants)	Rice bran	Hydrolysis, ultra-filtration, sterilized	Synthetic medium, NH_4OH Antifoam	1.00	0.026	Oh et al. (2015)
Not specified	Activated wastewater sludge	Solid/liquid separation, hydrolysis, water dilution	Water diluted nutrients	—	1.180	Pittmann and Steinmetz (2016)

2007). The yield of PHA is dependent on the type of carbon source utilized in bacterial growth media for the production of PHA. Fernandez et al. (2005) have reported the yield of PHA from *P. aeruginosa* 42A2 strain as 66.1%, 29.4%, 16.8%, and 54.6% of CDW by utilizing fatty acid components of waste, frying oils from the waste stream, glucose, and oleic acid in growth media, respectively. The culture conditions such as temperature, pH, duration, types of nutrients, and nutrient concentration are key parameters regulating different compositions as well as the higher yield of PHAs. For example, *P. aeruginosa* 47T2 showed varied yields of PHAs when it was grown at different temperatures, on various carbon sources, and limited nutrient concentrations (Haba et al., 2007). Production of scl PHAs and yields of 28.2% and 22.8% of CDW have been reported at 37 °C and 42 °C, respectively (Haba et al., 2007). To facilitate the allocation of carbon and energy source to the bacteria, lipases and rhamnolipids play crucial roles. Hydrolysis of lipids is performed by lipases, while rhamnolipids assist in the reduction of surface tension of the growth media and encourage oil emulsification which allows easier access of carbon source to bacterial cells (Abid et al., 2016). PHAs and rhamnolipids production have been

reported by *P. aeruginosa* (IFO3924) using oleic acid, glycerol along with palm oil. The major components of PHAs obtained were C_8 and C_{10} at both flask level and bioreactor level at 28 °C using 5–15 g/L palm oil as a substrate in basal salt medium (Marsudi et al., 2008). Increasing the concentration of palm oil from 5 to 7.5 g/L led to increase in the yield of PHAs; however, as the concentration went beyond (i.e., 10 and 15 g/L), it adversely affected the yield of PHAs (Marsudi et al., 2008). Recently, six bacterial strains were selected for production and estimation of PHAs using four different substrates such as diesel, waste frying oils, canola oil, and glucose. Among these strains, *P. aeruginosa* (KF270353) showed maximum accumulation (53.2%, wt/wt) of PHAs with waste frying oils, while glucose and canola oil as carbon source gave the yield of 37.8 and 34.4% (wt/wt), respectively (Tufail et al., 2017).

6.3.3. Wheat, rice bran, and whey as carbon sources

Wheat is cultivated worldwide, and fulfills the food requirement of the large number of people. Bran is an important component of the wheat grain, and it consists of pericarp and aleurone layer. Carbohydrates and various minerals are major constituents of wheat bran that make it an excellent carbon source for the growth of microorganisms. Production of biomass and PHB by bacterial sp. *H. boliviensis* LC1 grown on wheat bran as substrate was 3.19 g/L and 1.08 g/L, respectively, which is 33.85% of CDW (Van-Thuoc et al., 2008). Along with wheat, rice is also produced worldwide. Using rice bran as carbon source supplemented with corn starch, bacterial sp. *Haloferax mediterranei* were able to produce 140 g/L biomass and the yield of PHAs was 55.6% of CDW (Huang et al., 2006).

Cheese and casein industry produces whey as a byproduct. Processed milk contains whey up to 90% of volume. About 50% of this side product is used as feedstock for the making of various useful foodstuffs, while the remaining half amount is castoff in the surrounding. Various studies have tried to utilize whey as a substrate for the growth of microorganism with subsequent accumulation of PHAs (Koller and Braunegg, 2018). *P. hydrogenovora* have been reported to produce PHAs and biomass yield of 1.27 g/L and 5.0 g/L, respectively, by utilizing whey as a carbon source (Koller et al., 2008). Thus, this effort provides an alternative route for the bioconversion of environmental wastes to a useful products such as PHAs (Favaro et al., 2019).

6.3.4. Wastewater and sludge for PHA production

Sewage treatment plants treat wastewater by removing gaseous and organic impurities. Wastewater is enriched with various organic and inorganic nutrients that can be used as a medium for microbial growth and production of PHAs along with lipids (Kumar and Thakur, 2018; Gupta et al., 2017). *Azotobacterv inelandii* strain has been known to produce PHAs (58% of CDW) by utilizing swine wastewater as growth media (Ryu et al., 2008). Simultaneous use of paper and pulp wastewater along with acetate leads to PHA yield of 43% of CDW (Yan et al., 2006). Successful biodegradation of dyes and production of PHAs have also been demonstrated by Tamboli et al. (2010) using textile wastewater. After secondary treatment of wastewater, the sludge obtained from clarifier contains mixed microbial consortia, which could be a possible source of PHA production (Kumar et al., 2018a).

6.3.5. Starch as a carbon source

Plants store food in the form of starch as storage materials. This storage material can be used as a substrate for the growth of microorganisms, as it is easily obtainable and cheaper (comparatively cost-effective) (Ciesielski et al., 2015). However, because of its complex structure, it is not hydrolyzed by many bacterial species due to lack of α-amylase activity in the strains. Therefore, the external supply of α-amylase is required to hydrolyze the starch that is further used by bacteria as a carbon source. The yield of biomass and PHAs of 1.14 g/L and 43% of CDW, respectively, was obtained from *H. mediterranei* by using enzymatic-extruded starch (Chen et al., 2006). Haas et al. (2008) obtained a yield of 179 g/L biomass and 55% CDW PHAs, respectively, by using potato starch as a substrate for growth of bacterial strain *R. eutropha* NCIM 5149 in feed-batch mode.

7. Extraction methods of PHAs from microbial biomass

The extraction of PHAs from cell biomass and further processing are tedious and costly processes as they are intracellular storage materials (Mohammadi et al., 2012). For extraction and downstream processing of PHAs, there are various methods reported, while every method has a certain advantage and disadvantage. Broadly applied methods for the extraction and recovery of PHAs from biomass are described in detail below.

7.1. Solvent extraction

Extraction of PHAs from biomass using solvent is the most generalized and comprehensive method because of its effortlessness and simplicity (Kumar et al., 2018a). Solvent extraction method involves various steps. Primarily, breaking the bacterial cells releases the stored PHA molecules. After that, the moleculaes are dissolved in an appropriate solvent and the dissolved PHA granules are precipitated using a non-solvent. Chlorinated solvents such as chloroform, propylene carbonate, dichloromethane, acetone, and ethylene carbonate are frequently applied to solubilize the PHA granules from ruptured bacterial biomass. The solubilized forms of PHA granules are generally precipitated by chilled methanol or ethanol. In general, extraction of PHAs by the solvent method is a preferred method when the PHA polymer is required in pure form, because there is no change in the property of the polymers, and it eliminates the bacterial toxins (endotoxins) attached with the PHA polymers (Jacquel et al., 2008). Besides this, non-chlorinated solvents such as 1, 2-propylene carbonate has lower toxicity compared to chlorinated solvents, and hence they are more preferable. Other solvents, which include 2-propanol, ethyl acetate, acetone, n-hexane, methylene chloride, and tetrahydrofurane (THF) were also used to extract PHAs from cells biomass. In the above list of solvents, methylene chloride recovered a yield of 86% of CDW at room temperature (Furrer et al., 2007). The recovered product by acetone along with ethyl acetate has less than 10% contaminant and precipitation of this product by chilled methanol leads to purity of 100%. The working temperature has an important role in the extraction of PHAs through the solvent method. At temperature close to 50 °C, n-hexane exhibited better efficiency, but when the temperature was around 40 °C, the efficiency reduced with simultaneous increase in purity of the recovered product (Furrer et al., 2007).

7.2. Floatation

To reduce the wastage of solvents, the floatation method is employed, which is a modified form of chemical extraction method. In the floatation method, the green solvents are applied for extraction of PHAs from cells biomass. Then, the removal of cell debris occurs by the self-floatation mechanism. In this process, the microbial cells are treated at 30 °C with chloroform for 72 h. Subsequently, the mixture is kept at room temperature for 12 h for separation of cells debris by self-floatation. This method has been reported to achieve recovery efficiency of up to 85% (wt/wt) of CDW along with 98% pure PHAs (Ibrahim and Steinbüchel, 2009). Reduction in waste generation and uses of green solvents are some other advantages of this method.

7.3. Supercritical fluid extraction

Application of supercritical fluids for extraction of PHAs from bacterial cell biomass is the latest, cost-effective, and less toxic technique. Use of carbon dioxide (CO_2) as a supercritical fluid is dominating the industrial PHA extraction from bacterial cell biomass, due to its adequate temperature and pressure. Lower viscosity, negligible surface tension, and higher diffusibility of supercritical fluid than the liquids and solvents used in extraction processes make this process faster. In this technique, the temperature along with pressure play crucial roles as excessive temperature, and higher pressure alter the cell membrane structure resulting in difficulty in the recovery of PHAs from cell biomass. According to Khosravi-Darani et al. (2003), the solubility of PHB molecules in supercritical CO_2 is highly dependent on both the temperature and pressure. The impurities such as bacterial endotoxins persist throughout with the PHA molecules after solvent extraction, and that product may cause allergic reaction when used in any biomedical applications (Koller et al., 2013a,b). Hejazi et al. (2003) have reported the recovery of PHAs up to 89% of CDW from the cell biomass of *R. eutropha* by applying the supercritical fluid technique. Hampson and Ashby (1999) have reported the recovery of mcl-PHAs as 42.4% of CDW by using supercritical CO_2 and chloroform from the cell biomass of *P. resinovorans* cultured on tallow and lard as carbon source. Ammonia and methanol have also been reported as supercritical fluid for recovery of PHAs besides CO_2 gas (Kunasundari and Sudesh, 2011). Compared to conventional extraction techniques, the supercritical solvent extraction is a more effective technique for recovery of PHAs for biomedical applications. The method delivers approximate 100% product purity, which is good for the application of PHAs in the biomedical field (Williams et al., 2005).

7.4. Aqueous two-phase extraction (ATPE)

ATPE is composed of two unique and heterogeneous phases in which one phase is water, and another phase is non-volatile liquid. ATPE is an advanced and environmentally friendly technique as compared to the solvent extraction processes (Leong et al., 2017). The watery phase of ATPE system facilitates the isolation, purification, and recovery of PHAs from the cells. In the ATPE system, the polyethylene glycol layer plays an important role, as the PHA is transferred to this layer, and the undesirable cells debris steadily settle down in the bottom part of the system. Therefore, ATPE is a non-solvent system that is able to extract purified PHA polymers from biomass (Leong et al., 2017). The ATPE is also a well-known process for the removal of unwanted contaminants from the desired end product (Kepka et al., 2003). Divyashree et al. (2009) have reported the recovery of PHAs up to 51% (wt/wt) from biomass of bacterial sp. *Bacillus flexus* by applying polyethylene glycol at a concentration of 12% (w/v) along with potassium phosphate at a concentration of 9.7% (w/v) in ATPE system.

7.5. Chemical and enzymatic digestion

To extract the PHA from bacterial cells, these methods are applied as a substitute for the solvent extraction method. the cells are lyzed by either using solvents or biocatalysts (enzymes) for PHA granules to come out. Both chemical and enzymatic techniques break the microbial cells and facilitate the release of PHAs granules (Kumar et al., 2018a). Typically, sodium hypochlorite or surfactants are applied as a chemical to facilitate the extraction of PHA granules from the bacterial biomass. The surfactants such as sodium dodecyl sulfate, Triton X-100, palmitoylcarnitine, betaine, together with chemicals such as sodium hypochlorite has been applied to minimize the degradation of PHAs molecules (Koller, 2016). Use of sodium hypochlorite alone in the digestion process may affect the quality of the product by degrading the polymer up to 50%. The recovery efficiency of the process decreases when both sodium hypochlorite and surfactants have been applied separately; therefore, the combination of these two chemicals was investigated for improving the recovery efficiency of the process (Don et al., 2000). There are various groups of chemicals, including chloroform, propylene, dichloroethane (DCM), methylene chloride, and carbonate that are toxic but still are frequently employed for the extraction of PHA from cell biomass. As compared to other methods such as dispersion method (1.1 g/L) and chloroform extraction method (0.63 g/L), chemical and enzymatic method delivered a PHB yield of 5.6 g/L (Sayyed et al., 2009). Extraction of PHAs from cells biomass by enzymatic digestion is a complex process and has several steps, including heat treatment, hydrolysis, surfactant washing, and finally recovery of the product. Because of the specificity of the enzymes, the PHA recovery could be maximized (Jacquel et al., 2008).

8. Applications of PHAs

The inherent properties of PHAs such as biocompatibility and biodegradability make this polymer an important biological material having diverse applications in numerous fields. The polymers can be used as biofuels (hydroxylalkanotes methyl ester) through the transesterification with methanol in the presence of catalysts (Kargbo, 2010), packaging materials, cosmetic containers, sanitary products, carriers for long term release of herbicides or insecticides fabrication of pharmaceutical, and biomedical devices (Możejko-Ciesielska and Kiewisz, 2016; Chen and Tong, 2012). PHAs can be applied to produce ultra strong fibers for fisheries industry (Bugnicourt et al., 2014). Considering the inherent propertis of PHAs as discussed above and its production cost with existing technologies, PHAs can be promisingly applied in biomedical fields, which have been discussed below in detail.

8.1. PHAs as drug delivery carriers

Due to the biodegradable and biocompatible nature of PHA polymers, there is immense curiosity to use these polymers as drug delivery carriers (Ali and Jamil, 2016; Ray and Kalia, 2017). Micro and nanospheric compartment of PHA polymers in which the specific drugs are used, and after degradation of the polymeric compartment, drugs are automatically released from the compartment. The PHA polymers as drug delivery or vaccine carrier vehicle have been used in several animals such as cattle, mice, dog, and in humans to cure gingivitis (Valappil et al., 2006a,b). For the cure of chronic and implant osteomyelitis, drugs such as sulbactam-cefoperazone have been loaded to rods of PHBV (Yagmurlu et al., 1999). PHB polymers have also been used as a vehicle in transdermal tissue along with polyamidoamine dendrimer. To increase the transdermal permeability of tamsulosin drug, the dendrimer is added to PHB (Gurselt et al., 2002).

8.2. Bio-implanting material

Biodegradable, biocompatible nature, low inflammatory response, and tissue regeneration potency of PHAs polymers make this biological molecule as a potential material for application in the human body as bio-implant (Chen and Wu, 2005). The transmural problems of the gastrointestinal or gastric tract are very difficult to treat. The PHB polymer can be applied as bio-implant materials for treatment of gastrointestinal tract defects (Chen and Wu, 2005). In cats, the orthopedic implants of PHB grounded

polymer have been applied (Alves et al., 2011) whereas bio-composites of butyrate and hydroxyapatite (HA) have been demonstrated as a replacement for rabbit bone because of its biocompatible nature (Reis et al., 2010). The piezoelectric property of PHAs has been applied to repair the damaged nerves (Bugnicourt et al., 2014). In mice, the butyrate polymer has also been applied as a substitute for nerve tissue and exhibited encouraging outcomes (Hazari et al., 1999). These polymers are also applied in the dressing of wounds as well as preparation of scaffolds (Shishatskaya et al., 2016; Volova, 2004).

8.3. Application of PHAs in tissue engineering

The PHA polymers such as PHB and PHBV are used as matrices in *in vitro* proliferation of human cells. The various human cells, such as endothelium cells, liver cells, and fibroblasts exhibit comparable adhesive property as PHAs when these polymers are applied as matrices (Sevastianov et al., 2003). Biopolymers were used in bone tissue engineering (BTE) and exhibited no chronic irritation or inflammation after twelve months of implantation. It also enhanced the bone regeneration close to bio-composite implant used for regeneration of osteoblastic cells (Porter et al., 2013). To improve the hardness of bio-composite material, HA is added to PHB polymer that has been used as a replacement in hard body tissues (Ni and Wang, 2002). The PHA polymers have also been used in cardiac tissue engineering. Several studies related to the application of PHA polymers, especially PHB, construct biodegradable scaffolds that can be used to replace defective valves in the human heart (Hong et al., 2009). The porosity of the material plays an imperative role in its application in the biomedical field. The PHBV microspheres prepared with poly (L-lactic-co-glycolic acid) (PLGA) scaffold exhibited greater porosity (>80%) (Huang et al., 2010). The load-bearing capacity and elasticity of PHBV-PLGA scaffold improved by increasing the amount of PHBV microspheres, and this scaffold was effectively applied in *in-vitro* BTE (Huang et al., 2010). The fabricated microspheres using PHBV polymers have been similarly applied in brain tissue engineering to support primary neurons (Chen and Tong, 2012).

9. Cost-effective production of PHAs: Opportunities and challenges

PHAs are the most potential substitute, which can replace synthetic petrochemical plastics due to its biocompatibility and biodegradability. PHAs are eco-friendly biomaterials and have several applications in the medical field. The production of PHAs is more costly than synthetic petrochemical plastics, which is one of the major stumbling blocks in expansion and growth of PHAs market (Koller et al., 2017). Microbial synthesis of PHAs produces mixed monomeric composition instead of a single type, but the big challenge is to segregate the pure monomers from complex mixtures. Research endeavors are being carried out on how to minimize the production cost of PHAs from synthesis to its downstream processes and recovery to make the polymers commercially viable.

Several methodologies and techniques have been investigated for the cost-effective production of PHAs, such as diverse energy and carbon sources, varying product yield, duration, the extraction process, and product purity (Koller and Braunegg, 2018). The carbon source for the growth of microbes along with the downstream process is crucial for cost-effective PHA production (Fig. 4). The purposeful application of the product is also a crucial factor that determines the importance and cost of the product (Cao et al., 2019). For example, a product produced from PHAs for a biomedical application would require high purity. If the product is fabricated as a disposable item for one-time use, the cost of down-stream processing would be the key factor. Kit et al. (2017) evaluated the cost of down-stream processing and reported that PHAs were produced at 5.77 US$/kg using ATPE process, which was considered as a green extraction process for the recovery of polymers from cells (Yau et al., 2015).

It is advantageous to utilize waste materials in the production of new products, which would better address the environmental concerns (Cao et al., 2018; Yu et al., 2019a). In this contest, biorefinery approach is gaining attention to convert waste into wealth without significant environmental cost (Mak et al., 2019; Xiong et al., 2019; Dutta et al., 2019; Yu et al., 2019). The utilization of environmental wastes as a substrate for the growth of microorganisms and the production of novel, useful biomaterials, such as biopolymers, makes the process economical (Kumar et al., 2020). Life cycle analysis of microbial PHA synthesis using whey waste as the substrate is commercially preferable (Koller et al., 2013a,b). Various waste materials as carbon source produce a diverse range of polymer yields and microbial biomass. The yield of PHAs from 8 to 89.10% of CDW (Table 2) have been described in the studies using different chemicals and environmental wastes as a carbon source. Recently, Eropian union-funded projects ANIMPOL and WHEYPOL utilized wastes such as crude glycerol, waste lipids, and whey from dairy as well as cheese making industry. The reported prices of PHA production were below 3 €/kg (WHEYPOL) and even less than 2 €/kg (ANIMPOL), respectively (Koller and Braunegg, 2018). The use of waste is profitable; however, due to the heterogeneous nature and presence of various impurities in the waste materials, most outcomes are not as expected for the forms of PHAs and biomass yield. Such variable output from these practices would need prior optimization of process parameters for PHA production at lab scale before going to large-scale production. For example, virgin oil as carbon source gives better yield of PHAs in comparison to waste frying oil in similar microbial culture settings, due to the occurrence of the undesirable contaminants in waste frying oil (Du et al., 2012). Even though the intention of utilizing leftover materials for the microbial production of biopolymers are concomitant with considerable assistances in lowering the environmental waste, until now, the final products obtained from these could not be applied in the medicinal field, in which non-toxic natureand a higher degree of purity are desirable.

Furthermore, PHAs synthesized from waste material could have multiple tracer components, such as genetic contagions, virus, and plasmids, as impurities, which impede their biomedical application. Various impurities associated with PHAs, such as extraction solvents including hypochlorite and antifoaming agents, along with inherent components including DNA, proteins, endotoxins, and lipids, have been noted (Koller et al., 2013a,b). The removal of undesirable components needs some sort of diverse post recovery procedures that impose an extra production cost and make production not economically viable (Volova et al., 2003).

Genetically engineered bacteria can accumulate more PHAs in the cellular biomass and also synthesize single selective monomers instead of a mixture of copolymers. The down-stream processing cost concomitant with PHA extraction and recovery should also be lowered down by utilizing more economical, advanced techniques. The economy of the processes is principally governed by the final application of the end products (Chen et al., 2017). The biopolymers are gaining consideration in the biomedical associated area, and for that, the purest form of a product is desirable, which is obtainable by applying economically-advanced down-stream processing. Application of advanced biological tools, such as genetic modification and synthetic biology also increases the production of non-toxic PHAs from the Gram-negative bacterial strains. These non-toxic PHAs could effortlessly be applied in biomedical fields. However, the toxin-free PHAs extracted from Gram-negative bacteria require post-cleaning actions. The post-cleaning process

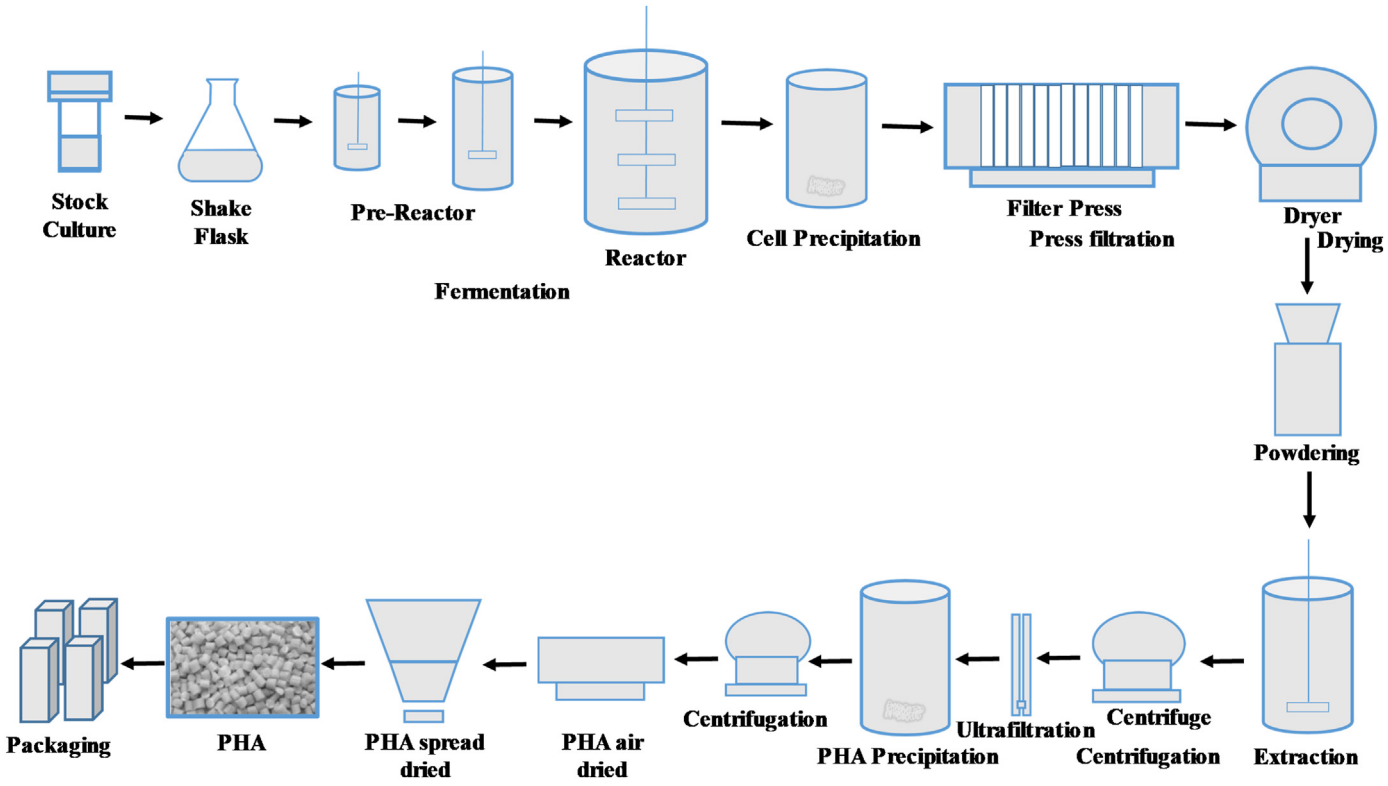

Fig. 4. Schematic representation of overall bacterial PHAs production process.

further makes the PHA production costlier. Research endeavors should focus more to obtain high-yield, toxin-free, and high-purity PHAs from bacterial strains belonging to Gram-negative bacterial strains, which are regularly applied in commercial-level production of PHAs these days. The selection and prospects of applying genetically manipulated Gram-positive bacteria should be scrutinized for the improved accumulation of PHA polymers. Gram-positive bacterial strains can be applied from laboratory scale to commercial level process for the production of cost-effective medical-grade PHAs.

Furthermore, the reduction in the production cost of PHAs can provide a new platform for its applications in biomedical fields and change the mindset and standard of the community. Application of PHAs can also change the traditional pharmacological technologies, and immobilization of proteins on PHA polymers may expand its advanced applications. This novel biocompatible and biodegradable material is a candidate of the future, which can partially substitute the traditional uses of synthetic petroleum-based plastics.

10. Conclusions and prospects

To substitute the use of synthetic plastics and food-based bioplastics, bacterial PHAs have gained enormous interests in scientific research and commercial uses. Although their production costs currently are high due to under-established production process, which is the main stumbling block in the commerical applications, researchers are trying to modify the bacterial pathways by applying genetic engineering tools to improve the accumulation of PHAs. Research endeavors also attempt to improve the economy of PHA production by utilizing waste materials as substrates for growth of wild and mutant bacterial strains. Potential utilization of agricultural feedstocks, industrial by-products, waste oils, wastewater, and sewage sludge is gaining attention for the production of PHAs while simultaneously solving the problem of waste disposal. To evaluate the environmental sustainability, LCA studies support that the production of PHAs is less energy-intensive as compared to synthetic plastics, while in terms of other environmental indicators the production of PHAs may require further improvement by process optimization and technological development.

Cost-efficient extraction method is also considered a crucial factor, which determines the economy of the green products. The ATPE process is considered as environmentally friendly and cost-effective extraction method of PHAs, which can be a viable technique for commercial-scale application. Bacterial PHAs present advantages of excellent biodegradability and bio-compatibility, which make them suitable for several uses such as fabrication of pharmaceutical, biomedical devices, applications in tissue engineering, and bio-implants. Nevertheless, there are various shortfalls. The production of bacterial PHAs is currently uneconomical. Recycling of PHAs poses an additional problem at the end of its service life, as there is no available cost-effective recycling process. Unsuccessful attainment of necessary physical properties also limits broad applications of bacterial PHAs limited in comparison to synthetic plastics. The path of the future research should include advanced application and economical production of PHAs, along with the enhancement of its overall environmental sustainability.

Declaration of competing interest

The authors declared no conflicts of interest in publishing this work.

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Appendix A. Supplementary data

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