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Review Paper

Microbial production of polyhydroxyalkanoates (PHA) from novel sources: A Review

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Abstract

The synthetic polymers find a lot of applications in the industry and are an important material in daily life. The unique properties of these make them irreplaceable in various applications. But the increasing environmental threat and depletion of fossil fuels required for their production there is an enormous requirement of suitable alternatives. Polyhydroxyalkanoates (PHAs) could make for more than appropriate replacement for the conventional plastics. PHAs being similar to conventional plastics with respect to properties are biodegradable in nature. PHA is produced by microorganisms when subjected to stressful conditions under the abundance of essential substrates. The microorganisms store the carbon source of nutrition into PHA as a source of energy in the form of water insoluble granules. However, bio based plastic production is more expensive than petrochemical plastics. Therefore, the uses of innovative new ways of PHA production including cheaper carbon sources, efficient process design, etc. are being sought after. In this review we will be looking at various C-sources and the respective microorganisms that can be used for PHA production and help in cost reduction. We would also look at the challenges that PHA pose and some ways in which we could overcome them.

Keywords: Polyhydroxyalkanoates, biopolyester, C-source, microorganisms, biodegradability, biocompatibility, mahua flower.

Introduction

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polymers that have a wide range of physical properties depending upon the monomeric composition in the polymer. PHA is a family of naturally occurring biopolyester synthesized by various microorganisms. PHA was first discovered by Lemogine in 1926[1]. Since then PHA has attracted much commercial and research interests due to its biodegradability, biocompatibility, chemical diversity and its manufacture from renewable carbon resources[2]. Biopolymers are in general referred to as polymers produced by microorganisms under controlled conditions. Some are already industrially produced at a large scale. However, many others are still to be optimised for commercial production. Biopolymers can be classified into four groups[3]:

(i) Amino acid based	E.g.: Silk, collagen,elastin.
(ii) Polysaccharides from bacteria	E.g.: Xanthan, dextran, cellulose
(iii) Polyphenol based	E.g.: Lignin, tannin
(iv) Polyesters	E.g.: Polylactic acid, shellac and PHAs

Bacteria synthesize PHAs as a carbon and energy source under the conditions of limiting nutrients in the presence of an excess C-source. Once the limiting nutrient environment is provided to the cells, these energy storage compounds are degraded and consumed [4].

R O
| ||
$-\!\!(O–CH–(CH_2)_n–C)\!\!-_{100\text{-}30000}$

n=1	R= hydrogen	poly(-3-hydroxypropionate)
methyl	poly(-3-hydroxybutyrate)	
ethyl	poly(-3-hydroxyvalarate)	
propyl	poly(-3-hydroxyhexanoate)	
pentyl	poly(-3-hydroxyoctanoate)	
nonyl	poly(-3-hydroxydecanoate)	
n=2	R= hydrogen	poly(-4-hydroxybutyrate)
n=3	R= hydrogen	poly(-5-hydroxyvalarate)

Figure 1: General structure of PHA [5]

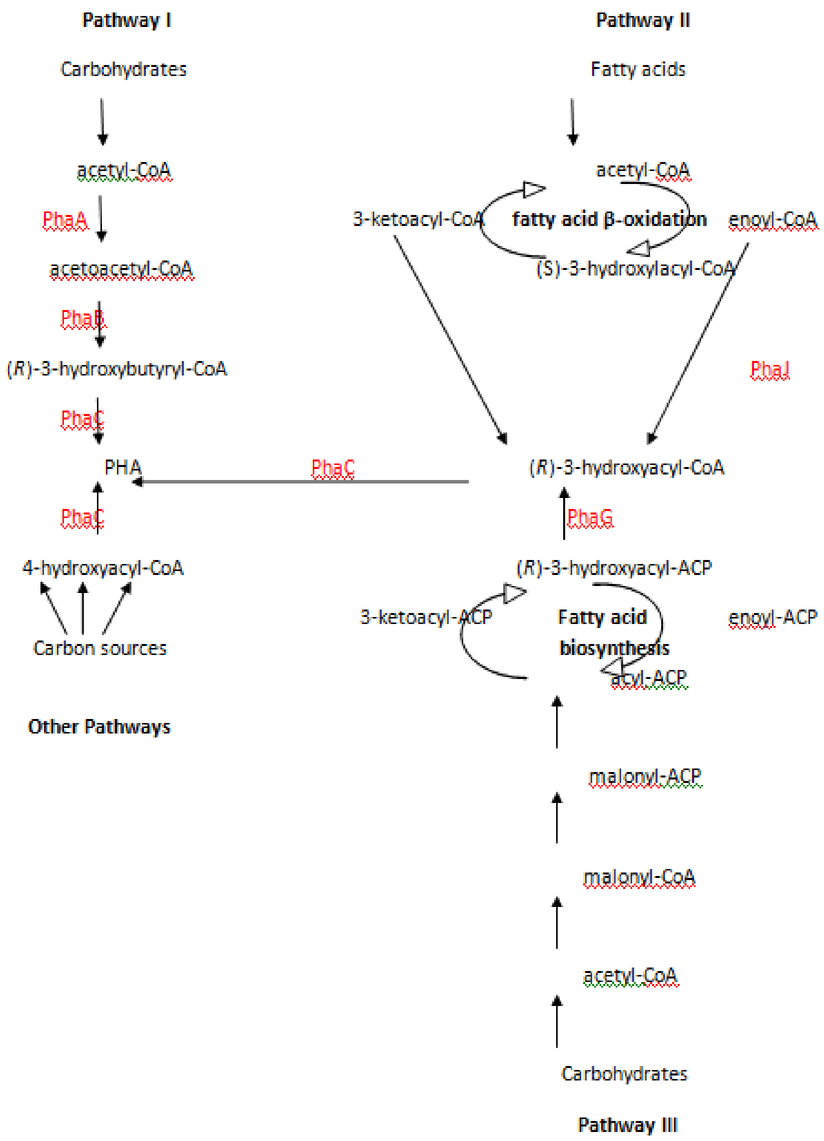

Figure 2: PHA biosynthesis in the context of microbial metabolism. The major enzymes involved in PHA biosynthesis are in red. Abbreviations: PhaA, 3-ketothiolase, PhaB, (R)-3-ketoacyl-CoA reductase (for PHB biosynthesis, this enzyme is acetoacetyl-CoA reductase), PhaC, PHA synthase or polymerase, PhaG, (R)-3-hydroxyacyl ACP: Co Atransacylase, PhaJ, (R)-specific enoyl-CoA hydratase [6].

A PHA molecule is typically made up of 600 to 35,000 *(R)* – hydroxy fatty acid monomer units. Each monomer unit harbors a side chain R group which is usually a saturated alkyl group but can also take the form of unsaturated alkyl groups, branched alkyl groups, and substituted alkyl groups although these forms are less common. Depending upon the total number of carbon atoms within a PHA monomer, PHA can be classified as either a short-chain length PHA (scl – PHA, 3 to 5 carbon atoms), medium-chain length PHA (mcl – PHA, 6 to 14 carbon atoms), or long-chain length PHA (lcl – PHA, 15 or more carbon atoms). They are also classified as homo-polymer or hetero-polymer depending on whether one kind or more than one kind of hydroxyalkanoate is found as the monomeric units. Molecular mass of these polymers range between $2×10^5$ Da and $3×10^6$ Da [4] depending on microorganism and the growth conditions [5].

A wide variety of C-sources like oils, whey, glucose, sugars, bagasse, lignin, etc. have been used for the production of PHA commercially and in research purpose. In this review we will look at some novel approaches of production of PHA.

Microorganisms producing PHA

A variety of microorganisms are identified as PHA producers based on the C-sources that they strive in. A list of microorganisms producing PHA is enlisted below in Table 1.

PHA producers can also be classified as: (i) Hydrocarbon Degraders as PHA Producers (ii) Halophiles as PHA Producers (iii) Photosynthetic Bacteria as PHA Producers (iv) Plant Growth Promoting Rhizobia (PGPR) as PHA Producers (v) Antibiotic Producers as PHA Producers

Hydrocarbon Degraders as PHA Producers

Environmental stress, like presence of xenobiotic compounds, diverts physiological responses of residing organisms to produce more PHA [7]. Various bacterial strains capable of producing PHA while degrading oil have been isolated belonging *to the genera Pseudomonas, Acinetobacter, Sphingobacterium, Brochothrix, Caulobacter*, *Ralstonia, Burkholderia, and Yokenella* from oil contaminated sites [8]

Halophiles as PHA Producers

Archaea are considered as extremophiles since reported to reside at hot springs, marshlands, oceans, salt lake, and so forth and have been reported to produce PHA. These organisms require salts to sustain their growth. They grow optimally at 5% and at least tolerate 10% of salt NaCl (w/v) [9]. The first case of PHB accumulation by archaea had been reported in 1970 from Dead Sea named as *Halobacterium marismortui* analysed by freeze-fracture technique. Report shows that PHB and P(HB-co-HV) producers like *Halococcus* sp., *Halorubrum* sp., *Halobacterium noricense* DSM 9758, and haloakaliphiles (N*atronobacterium gregoryi* NCMB ,*Natronococcus occultus* DSM) require alkaline and salt conditions irrespective of complex medium and nutrient-rich or nutrient-limited conditions [10]. The halobacterium *Haloferax mediterranei* accumulates poly(β-hydroxybutyrate) (PHB) as intracellular granules. The conditions for PHB production in batch and continuous cultures have been studied and optimized. Phosphate limitation is essential for PHB accumulation in large quantities [11]. The production of PHAs by *Halococcus* sp. (*Halococcus morrhuae* DSM 1307^T, *Halococcus saccharolyticus* DSM 5350^T, *Halococcus salifodinae* DSM 8989^T, *Halococcus dombrowskii* DSM 14522^T, *Halococcus hamelinensis* JCM 12892^T, *Halococcus qingdaonensis* JCM 13587^T), *Halorubrum* sp. (*Hrr. coriense* DSM 10284^T, *Halorubrum chaoviator* DSM 19316^T, *Hrr. chaoviator* strains NaxosII and AUS-1), haloalkaliphiles (*Natronobacterium gregoryi* NCMB 2189^T,*Natronococcus occultus* DSM 3396^T) and *Halobacterium noricense* DSM 9758^T was reported recently. Most species synthesized PHAs when growing in synthetic as well as in complex medium [12].

Photosynthetic Bacteria as PHA Producers

Cyanobacteria are photosynthetic prokaryotes with short generation time, reported to produce PHA by oxygenic photosynthesis. Some Cyanobacteria were screened for the presence of PHA which was reported to be species specific, mostly producing PHB, stimulated by phosphorus deficient conditions and presence of excess amount of reducing equivalents [13]. *Synechococcus sp*. MA19 (accumulated up to 55% of CDW*), Nostocmuscorum and Spirulina platensis* produced PHB under phosphate limited conditions [13,14]. Use of Cyanobacteria ability to produce PHB with energy obtained from sunlight can result in reduction of cost and CO_2 a "greenhouse gas" as well [15].

Plant Growth Promoting Rhizobia (PGPR) as PHA Producers

Soil adjacent to roots of plants is termed rhizosphere which may harbour microbes enhancing the growth of roots and plants by secreting extracellularly metabolites [16]. Some microorganisms*, Burkholderia terricola, Lysobacter gummosus, Pseudomonas extremaustralis, Pseudomonas brassicacearum, and Pseudomonas orientalis* have been reported as PHA producers based on PCR technique having PhaC as targeted gene [17].

Antibiotic Producers as PHA Producers

Streptomyces are aerobic, gram positive filamentous bacteria known for their valuable metabolites production. Streptomyces are reported for PHA production intracellularly in granular form working as supplier of carbon units for antibiotic synthesis and sporulation [15]. *Streptomyces aureofaciens 84/25, S. griseus, S. olivaceous, S. fradiae, S. parvus, S. albus,* and so forth are reported to produce PHB with glucose as carbon source [18, 17].

Substrates that can be used for PHA production

PHA can be produced from various cheap substrates. They are as follows:
(i) Molasses : Sugarbeet, sugarcane and Soy molasses[18]
(ii) Whey and whey hydrolysates : Hydrolyzed soy and malt, hydrolyzed whey and whey[16]
(iii) Lignocellulosic raw materials : Wood, xylose, hemicellulosichydrolysates, wheat bran, etc.[19]
(iv) Fats, vegetable oils and waste cooking oils: olive oil, coconut oil,soybean oil, palm oil, etc.[20]
(v) Glycerol[21]
(vi) Wastewater : Alpechin, wastewater[22]

Some new novel C-sources include harvestable parts of plants, plant extracts, flower & fruit extracts, etc.

Use of Mahua flower as C-source: Utilization of mahua flowers, a natural substrate for bacterial fermentation aimed at PHA production, had additional advantage, as the sugars and organic acids present in the flowers were metabolized by *Bacillus* sp-256 to synthesize P(3HB-co-3HV) copolymer. Mahua flower is a natural substrate, which contains nearly 60% of sugar, and it additionally contains organic acids, which are essential for copolymer synthesis. In PHA production medium, nearly 50% of the cost is due to carbon sources such as sugar and organic acids. This can be economized by using industrial by products or natural substrates. Next to molasses, mahua flower can be considered as a cheaper source of carbon for the synthesis of PHA copolymers [29].

Study of best cheap carbon sources among Rice bran, Paddyhusk, pigeon pea waste, Sugarcane bagasse, and waste frying oil was done and the results suggested that Sugarcane bagasse was found to be the most suitable carbon substrate on selected bacterial species of *Bacillus subtilis* and *Pseudomonas aeruginosa* used in this study, as it enhanced the highest polymer yields [30].

Use of oils as C-Source: *P. oleovorans* produced mcl-PHAs from linoleic acid, corn and laurel seed oil acids. Mcl PHAs obtained by *P. oleovorans* contained the same functionalities as their substrates. Functional groups of substrates can be inserted into PHAs using *P. oleovorans* but not *A. eutrophus*. Rose oil, limonene and laurel leaf oil cannot be considered to be a substrate to produce PHAs. Laurel leaf oil and limonene also did not grow bacterium. This work reported the fermentation results of some new substrates for PHA production from *A. eutrophus* and *P. oleovorans* [31].

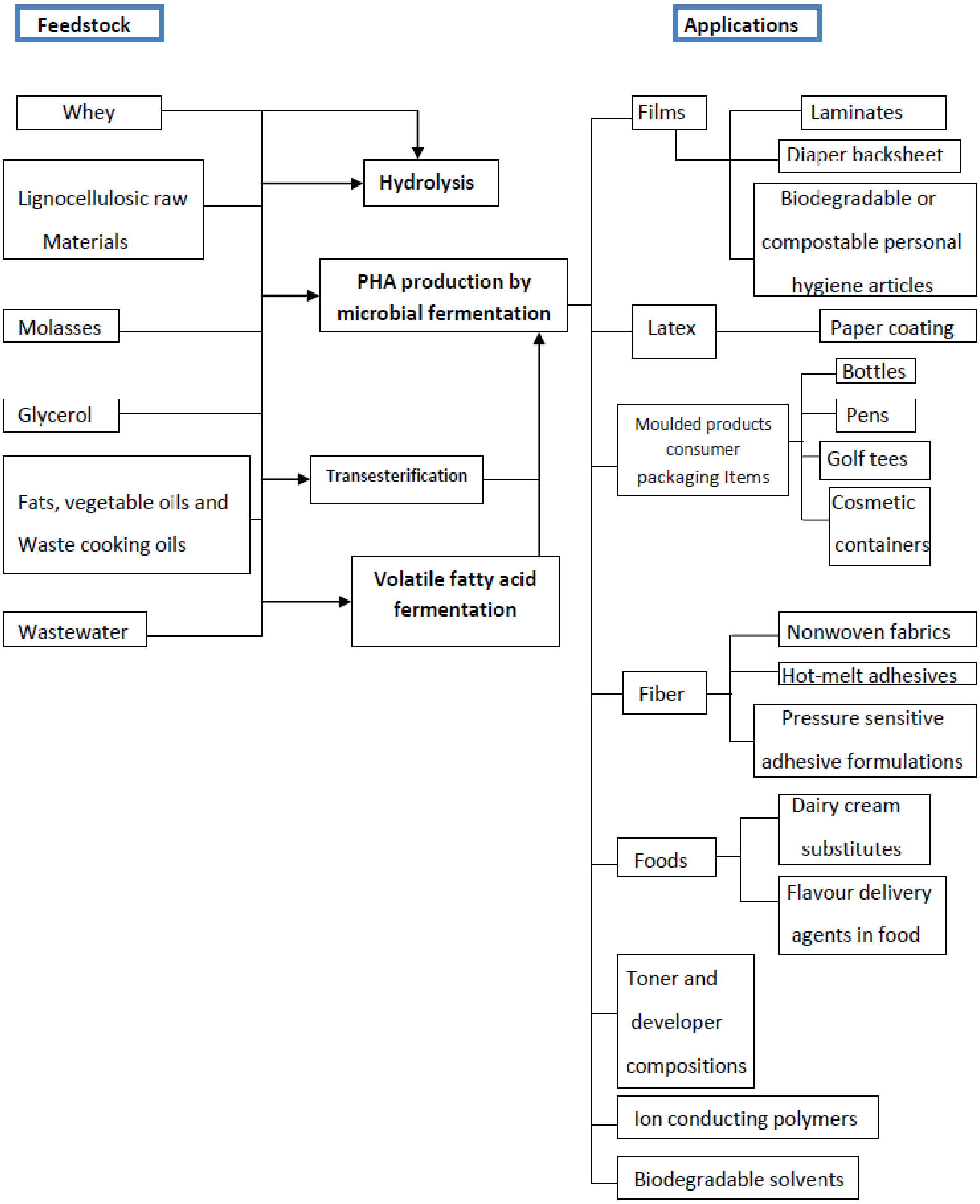

Figure 3: Feed stocks and applications of microbial PHA productions by different processing technologies

The transformation of aromatic pollutants into valuable aliphatic and biodegradable bioplastics was studied. Since benzoic acid was found to be the key compound for such bioremediation processes, its transformation, and metabolic pathways of digestion, by *Cupriavidus necator* were specifically analysed. It was found that the degradation of aromatic compounds follows the 2,3-dioxygenase pathway in this strain and that the batch transformations of benzoic acid with either fresh or adapted cells were limited to an initial concentration of 2.5 g/L of pollutant.[32] Sodium terephthalate (TA) produced from a PET pyrolysis product and waste glycerol (WG) from biodiesel manufacture were supplied to *Pseudomonas putida* GO16. PHA accumulated from TA or WG was predominantly composed of 3-hydroxydecanoic acid. PHA monomers 3- hydroxytetradecanoic acid and 3-hydroxytetradecenoic acid were not present in PHA accumulated from TA alone but were present when WG was supplied to the fermentation. When WG was either the sole carbon source or the predominant carbon source supplied to the fermentation the molecular mass of PHA accumulated was lower compared to PHA accumulated when TA was supplied as the sole substrate. [33]

Table 1: PHAs fermentations using various cheap substrates

Carbon Sources	**Strains**	**PHAs (g/l)**	**Cell Density (g/l)**
Molasses			
Sugar beet molasses	*Azotobacter vinelandii* UWD	PHB, 19-22	N/A
	Azotobacter vinelandii UWD	PHB, 36	N/A
Sugar cane molasses	*Bacillus sp.* Jma5	PHB, 25-35% dw	30 (batch) 70(fed batch)
Molasses	Bacterial consortium	PHAs, 0.37-0.5 Cmol/Cmol VFA	2-3
	Bacterial consortium	PHAs, 0.47-0.66 Cmol/Cmol VFA	3.6-5.1
Sugar cane molasses	*Bacillus megaterium ATCC 6748*	PHB, 2.2(43% CDW)	5.0
	Bacillus megaterium BA-019	PHB, 30.5 (42% CDW)	72.6
Soy molasses	*Pseudomonas corrugata*	mcl-PHA 5-17%	1.5-3.6
	Bacillus sp. CL1	PHAs, 90%	3.42
Whey and whey hydrolysates			
Hydrolyzed soy and malt	*Bacillus sp. HF-1, HF-2*	PHAs, 18.42	32
Hydrolyzed whey	*Ralstonia eutropha* DSM545	PHBV, 2.25	4.5
	Haloferax mediterranei [23]	PHBV, 50% CDW PHV, 8% CDW	9.1
	Hydrogenophaga Pseudoflava [23]	PHBV, 40% CDW PHV, 5% CDW	12.5
	Pseudomonas hydrogenovora	PHBV, 1.44	1.84
Whey	Recombinant *E. coli*	PHB, 9.0	58.2 (fed batch)
	Recombinant *E. coli*	PHB, 5.2	6.4
	Thermus thermophiles HBS	PHA, 0.51	2.09
	Hydrogenophaga pseudoflava	PHA, N/A	N/A
	Methylobacterium sp. ZP24	PHA, 2.6-5.9	5.1-9.9
	Methylobacterium sp. ZP24	PHB, 6.12	N/A
	E. coli GCSC 6576	PHB, 109	87
	E. coli GCSC 4401	PHB, 96.2	119.5
Lignocellulosic raw materials			
Hemicellosic fraction of poplar wood	*Pseudomonas pseudoava*	PHB, 6.57	1.50
Xylose, xylose with propionic acid	*Burkholderi cepacia* ATCC 17759	PHB, PHBV, 1.6-3.7	2.6
Xylose with levulinic acid	*Burkholderi cepacia* ATCC 17759	PHBV, 1.3-4.2	Max 9.5
Hemicellulosic hydrolysates	*Burkholderi cepacia* ATCC 17759	PHB, 2.0	Max 5.1
Xylose from sugar cane bagasse	*Burkholderi cepacia* IPT 048 and *B. sacchari* IPT 101	PHB, 34.8	60
Xylose and glucose	*E. coli* PTS mutant	PHA, 0.476	2.3
Wheat bran hydrolysate	*Halomonas boliviensis* LC1	PHB, 4	9
Cellulose, in tequila bagasse	*Saccharophagus degradans* ATCC 43961	PHA, 1.5	2.55
Formic acid, acetic acid, furfural and acid soluble lignin	*Ralstonia eutropha*	PHB, 6.1-6.8	10.7-11.1

Carbon Sources	Strains	PHAs (g/l)	Cell Density (g/l)
Fats, vegetable oils and waste cooking oils			
Unsaponified olive oil	*Aeromonas caviae*	mcl-PHA, max 96 wt%	N/A
Lard, butter oil, olive oil, coconut oil, soyabean oil	*Pseudomonas aeruginosa and Pseudomonas resinovorans*	mcl-PHA, 2.1	1.6-2.8
Olive oil, corn oil and palm oil	*C. necator*	PHB, 3.4	4.3
Olive oil, corn oil and palm oil	*P. putida, C. necator*	PHA, 1.6	3.0
Castor seed oil, coconut oil, mustard oil, cottonseed oil, groundnut oil, olive oil and sesame oil	*Comamonas testosteroni*	The polymer contained HA units with 6 to 14 carbon atoms, 87.5% CDW	N/A
Lard and coconut oil	*Pseudomonas putida*	PHA, 0.9-1.6	2-4
Coconut oil and tallow	*Pseudomonas saccharophilia*	mcl-PHA, 0.8	N/A
Palm kernel oil, palm olein, crude palm oil and palm acid oil	*C. necator*	PHA, 3.3	8.3
Palm kernel oil	*C. necator*	P(3HB-co-3HV-co-3HHx), 6.79	7.9
	C. necator	PHA, 2.5	7.5
Soybean oil	*Pseudomonas stutzeri*	PHA, 1.4	2.7
	C. necator H16	PHA, 102	Max 138
	C. necator mutants	PHA, 1	Max 2.2
Jatropha	Marine bacteria, SM-P-3M	PHA. 0.306	0.404
Linseed oil	*Pseudomonas aeruginosa*	PHA, 1.8, 50.2% CDW	3.6
Brassica carinata oil	*Pseudomonas aeruginosa*	PHA, 5%	1.0
Waste cooking oil	*Pseudomonas aeruginosa*	PHA, 2.3	4.5
	Pseudomonas aeruginosa	PHA, 5.4	19.0
	Pseudomonas aeruginosa	PHA, 3.43	Max 6.8
Spent palm oil	*C. necator*	P(3HB-co-4HB), 4.4	5.5
Waste vegetable oil	*Pseudomonas sp.* strain DR2	PHA, 23.5% CDW	N/A
Animal-derived waste lipids			
Tallow-based biodiesel [24]	*Pseudomonas citronellolis*	mcl-PHA, 0.0036-0.004	26.6 wt%
Tallow-based biodiesel [24]	*Pseudomonas chlororaphis*	mcl-PHA, 0.137	22.1-29.4 wt%
FAME (glycerol and fatty acid methyl esters) combined with glucose and valeric acid [25]	*Cupriavidus necator* DSM 545	PHA, 10-12	N/A
Raw glycerol from biodiesel	*Haloferax mediterranei* [26]	PHA, 0.12 g/Lh	-
	Zobellella denitrificans MW1	0.25 ± 0.04 g PHB/g glycerol	-
	Burkholderia cepacia ATCC 17759 [27]	PHB, 7.4	23.6 g/l dry biomass
	highly osmophilic organism [28]	PHA, 16.2	-

Wastewater			
Alpechin (wastewater from olive oil mill)	*Azotobacter chroococcum* H23	PHA, 70% CDW	N/A
Wastewater	*Enterobacter aerogenes* 12Bi	PHB, 5.2	6.2

Use of Styrene and phenylacetic acid as C-source: *Pseudomonas putida* CA-3 is capable of converting the aromatic hydrocarbon styrene, its metabolite phenylacetic acid, and glucose into polyhydroxyalkanoate (PHA) when a limiting concentration of nitrogen (as sodium ammonium phosphate) is supplied to the growth medium. PHA accumulation occurs to a low level when the nitrogen concentration drops below 26.8 mg/liter and increases rapidly once the nitrogen is no longer detectable in the growth medium [34].

Applications

The extensive range of physical properties of the PHAs and the extended performance obtainable by chemical modification [35] or blending [36, 37] provide a broad range of potential end-use applications. Earlier applications of PHA were mostly in packaging but now its importance in medical industry has become significant.

Industrial

❖ **Packaging** [38] :
- Cosmetic containers and films
- Shampoo bottles
- Pens and golf tees
- Personal hygiene articles such as diapers, diaper backsheet

❖ **Covers** [38, 39, 40]:
- Cardboards and papers
- Milk cartons and films, moisture barriers in nappies and sanitary towels
- Pens, combs, bullets
- Bulk chemical production using depolymerised PHA.

❖ **Food industry :**
- Dairy cream substitutes
- Flavour delivery agents in foods [18, 36]

❖ **Others :**
- Non-woven fabrics [41, 36]
- Source for the synthesis of chiral compounds Research is being conducted where 3HB and its derivatives are used to enhance learning and memory. [42]
- Mcl-PHA of poor quality as novel Biofuel [43, 44, 45]
- Raw materials for the production of paints 3HB derivatives (Monomers of PHA) have an inhibitory effect on cell apoptosis. [46]
- Bio-based Green solvents can be produced from PHA via pyrolysis process.

Medical and Pharmacological

The properties of biocompatibility and biodegradability play a major role in its application in medical industry [47, 48]

In pure form or as composites, PHAs are used in different medical applications as:

- Sutures, skin substitutes, dressing, dusting powders in wound management,
- Heart valves, vascular grafts in vascular system devices,
- Scaffolds for cartilage engineering, screws and bone graft substitutes in orthopaedy and regeneration of arterial tissues,

- Biomedical materials for drug delivery due to their unique physiochemical and mechanical properties[48]
- Urological and cardiovascular stents [47, 39]
- Plain membranes for guided tissue regeneration,
- Multifilament meshes or porous structures for tissue engineering [38], prodrugs and their efficacy in nerve and soft tissue repair,
- Dental and maxillofacial treatment (guiding tissue and bone regeneration),
- Tablets, implants, micro-carriers in drug delivery [49].

Agricultural

PHAs have potential agricultural applications including the following [50, 40, 47]:

- Encapsulation of seeds
- Encapsulation of fertilizers
- Biodegradable plastic films for crop protection
- Biodegradable containers for hot house facilities
- Biodegradable carriers for long term dosage of insecticides and herbicides

Challenges and prospects of PHA

Table 2: Challenges and Prospects [51]

PHAs challenges	PHAs prospects
High cost of production and extraction	Reduction in cost related to the use of substrate for bacteria growth coming from by products or waste materials. Increase PHAs production by the use of mixed culture or modified bacteria or microalgae. Optimization of PHA extraction processes
Quality of PHA	Optimization of the quality and uniformity of PHAs produced in mixed culture.
Mechanical properties	Better understanding of PHAs kinetics of crystallization and proper choice of additives (nucleating agents, plasticizers) to achieve stability in mechanical properties, and improvement in elongation at break.
Production of blends and composites	Optimization in the use of PHAs in blends with other biodegradable polymers achieving a reduction in cost of the final product while still maintaining the outstanding properties of PHAs in terms of barrier properties. Modulus, high biodegradability in different environments.
Blends with natural additives	PHAs in processing are very sensitive to water presence, but proper drying of natural additives and proper choice of compatibilizers is promising for the preparation of blends of PHAs and natural polymers (starch, proteins, etc.) which can achieve the production of plastic items with high biodegradability, for example also in marine environment.

Conclusion

This review paper is a summary of various C-sources and the respective microorganisms required to produce PHA. PHA can be produced from various sources like lignocellulosic waste, dairy industry waste, waste water plants, biodiesel production wastes, molasses and various cooking and waste oils. This review also includes certain novel sources like mahua flower, pigeon pea waste, rice husk, essential oils like rose oil etc. for the production of PHA. The percentage production of PHA in mahua flowers was found to be about 51% of its biomass in *Bacillus sp-256.* Hence, they prove to be a cheap and efficient C-source for PHA production. In case of rose oil, laurel leaf oil and limoneneit was found that they do not produce PHA in *P. oleovorans* species.

The fixation and direct conversion of CO_2 into the useful PHA biopolymer by genetically engineered cyanobacteria are two processes of a promising technology for the coming low carbon economy. Although considerable researches are still required to realize the practical on-site applications of the present system to the industrial emission sites, such as thermal power plants, this technology can be a promising option for the biological conversion of CO_2 into useful industrial materials. [52]

PHA was developed as an alternative to synthetic plastics but it also finds wide applications in the field of medicinal, agricultural and food industries. PHA is used as covers, moulds, implants, surgical aids, fertilizers, crop protection agents, flavouring agents etc. They are majorly used as packaging materials. Unlike plastics, PHA does not release toxic products on degradation. PHA is non-polluting and has huge potential in various field applications.

Batch and fed-batch discontinuous fermentation modes are currently most commonly used techniques for the microbial PHA production. In contrast, the continuous production mode is a well-known tool for achieving high productivities, lower production costs and a constant product quality in biotechnological processes. Therefore, an increasing research is focusing on investigating and assessing the potential of continuous PHA-production processes. Recently, based on the kinetic considerations regarding biomass growth and PHA accumulation, the continuous production of PHB in a five-stage bioreactor cascade (5-CSTR) was investigated. This 5-CSTR acts as a device-related engineering substitute for a tubular bioreactor that is theoretically the best match for the process-engineering requirements for an efficient PHA production from a kinetic point of view [53,54].

The major challenge of PHA is its moulding and formulation into useful products since its mechanical properties are poor as compared to plastics. Knowledge about moulding and formulation of PHA into tough and strong articles is limited. Hence PHA as a replacement to plastic is still limited. Another problem faced is the high production cost of PHA which can be reduced by searching for cheaper C-sources or by elimination of unwanted steps for the production of PHA.

In addition to introducing the substrates, costs have to be reduced by optimizing the downstream processing required for the PHA recovery after a cell harvest. As intracellular products, PHAs have to be separated from the surrounding non-PHA cell mass (NPCM, also known as the residual biomass) that mainly consists of proteins, lipids, nucleic acids and polysaccharides [55]. PHA has a very high potential due to their properties of biocompatibility, chemical diversity, manufacture from renewable carbon resources, non-toxicity and release of non-polluting products on degradation. With the progress in research and development of new technologies to favour optimized production of PHA and improvements in its mechanical properties we could soon replace plastics with PHA and make a greener world.

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