



REVIEW

Development of polyhydroxyalkanoates production from waste feedstocks and applications

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Polyhydroxyalkanoates (PHA) are naturally occurring biopolymers, obtained from microorganisms. Properties like biodegradability and biocompatibility make PHA a part of today's commercial polymer industry. However, the production cost of PHA has been a great barrier to extend its application to large scale production. Substrates and usage of pure cultures constitute the main reason for its high production cost. On the other hand, rapid industrialization i.e., industrial sectors such as sugar, pulp and paper, fruit and food processing, dairies, slaughterhouses, and poultries, has resulted in the generation of the huge quantity of wastes. Consequently, becoming large source of environmental pollution and health hazard. This review emphasizes on the usage of various waste feedstocks obtained from industrial and agricultural industries as an alternate substrate for PHA production. As these waste materials are rich in organic material and also microbes, they can be the good starting material for PHA production. Additionally, advantages and economic importance of mixed cultures and also PHA applications are discussed. Future prospects and challenges in PHA production from waste feedstocks are also highlighted.

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[**Key words:** Polyhydroxyalkanoates; Industrial waste; Agricultural waste; Mixed cultures; Medical applications]

Polyhydroxyalkanoates (PHA) are unique biodegradable polymers derived from the living organisms with the properties of commercial polymer/plastic. They are found to be deposited intracellularly as carbon and energy source in microorganisms (1). PHA was first identified in *Bacillus megaterium* by Lemoigne in 1926 (2). Many types of PHAs are available, out of those, polyhydroxybutyrate (PHB) and copolymer of hydroxyl butyrate and hydroxyvalerate (PHBV) are the most studied (3). Nowadays, synthetic polymers are replaced by biodegradable polymers due to their environmental friendly nature and also due to depletion of petroleum resources (4). PHA is considered as biopolymer since it is obtained from microorganisms, this in turn increased the demand for PHA. Thousands of tons of PHA are being produced every year by industries (5,6). Table 1 lists out some of the applications, companies producing PHA and starting material, microorganisms used in production process (5,7–9).

The biodegradability and biocompatibility of PHA and its copolymers, make them appropriate for medical and industrial applications (9). Besides, its toxic free nature, mechanical and thermoplastic properties of PHA can be an enduring alternative to conventional polymers. Industrially, PHA was synthesised by

fermentation, aerobic dynamic feeding (ADF) and batch reactors. The composition and quality of PHA depends on culture used, growth conditions of bacteria and amount of carbon source supplied (10). Numerous types of monomers are found to be the constituents of PHA, which made it difficult to know the exact properties of PHA (11). Meanwhile, the commercial production of PHA will use carbon rich sources like carbohydrates and fatty acids. In this production, pure microbial cultures are preferred for fermentation and further PHA accumulation (12). Whereby more than 50% of production cost was associated with substrates and also with cultures used (13). However, this high cost of production stands as a main constraint in PHA applications.

Recently, this problem is approached by considering waste feed stock for carbon source, mixed cultures for fermentation and aqueous two phase systems for purification and recovery (14,15) to make the PHA production more economical. Obtaining useful product from waste is anytime beneficial. Moreover, industrial and agricultural wastes are causing major problems by accumulating and disturbing the environment. Wastes obtained from palm oil industries, agro based industries, animal wastes, molasses, corn steep liquor whey, rice and wheat bran and many more wastes are considered as potent substrates for PHA formation (16). On the other hand, usage of mixed culture reduces the equipment cost and control measures to be taken, while increasing the utilisation of substrates to its maximum (17).

This paper presents overview of PHA production using different wastes feedstocks produced from different sources. In addition, the

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TABLE 1. List of current PHA industrial producers.

Company name and country	Current status	Microbial strains used for PHA production	Production capacity	Product type	Trade mark of PHA product	References
Biomer, Germany	Partnered with Newlight Technologies since 2013	n.r.	1000 tonnes/year	P3HB	Biomer	5,7
Metabolix, USA (now Yield10 Bioscience)	Sell biopolymer business to Korea CJ Cheiljedang Corp	n.r.	n.r.	scl-PHA to mcl-PHA	n.r.	
Polyferm Canada, Canada	Founded by professors from Queen's University and partnered with PARTEQ Innovations since 2013	<i>Aeromonas hydrophila</i> and <i>Pseudomonas putida</i>	n.r.	mcl-PHA	VersaMer	8,9
Tepha Inc., USA	As a pioneer developer of implantable medical devices based on a resorbable polymer	<i>Escherichia coli</i>	n.r.	P4HB, P3HB-co-4HB	TephaFLEX	7
Tianan Biologic, PR China	Established since 2000	<i>Cupriavidus necator</i>	2000 tonnes/year	PHBV	ENMAT	5,9
Tianjin GreenBio Materials, PR China	Partnered with The DSM Venture Capital since 2009	n.r.	10000 tonnes/year	P3HB-co-4HB	Sogreen	5,7
Bio-On, Italy	The world largest fermenters for PHA bioplastic was constructed in November, 2017	n.r.	> 10000 tonnes/year	n.r.	Minerv	
Kaneka Corporation, Japan	The world's first completely bio-based polymer with soft and heat-resistant properties	n.r.	1000 tonnes/year since 2010	PHBH	KANEKA BIODEGRADABLE POLYMER PHBH; AONILEX Nodax	
Danimer Scientific, USA	Partnership with PepsiCo on biodegradable resin from PHA materials since 2017	n.r.	n.r.	PHB		

n.r., not reported.

mechanisms, metabolism and applications of PHA will be briefly highlighted.

STRATEGIES OF PHA FORMATION FROM WASTE FEEDSTOCK

In spite of numerous applications, the cost of PHA being comparatively higher than the other bio-based polymers is the prime constrain to maximise its usage in market. This is due to costly raw materials and synthesis procedures particularly processing and purification (18). For this reason, recent trends are developed choosing waste feedstocks as alternates instead of costly substrates. Furthermore PHA belonging to the bio based class it is synthesised by microorganisms almost from 300 different species (19). Until to date still research is going on to discover the capability of more microorganisms to synthesize PHA (20,21). That being said, there are many factors to consider when synthesising PHA via microorganisms, temperature, pH and mainly the type of cultures (22,23). Cultures are selected favouring the high storage capacity of PHA (17). In addition, multiple factors like cell density, bacterial growth, expenditure on substrates and extraction and purification methods of PHA play major role in achieving a reasonable economic microbial formation of PHA. Nevertheless, managing the quality or structure of PHA is hard due to involvement of various pathways which in turn are determined by culture types.

PHA production using pure cultures Initially, PHA synthesis started using pure cultures (single species). In 1959, Grace and company patented for PHA synthesis using pure cultures commercially. In 1970, it was commercialised as Biopol by Imperial Industries, later by Monsanto and Metabolix (24). Few commercially available PHAs namely Biopol, Biomer, Nodax and Biocycle are produced using pure cultures (17,25). This in turn paved a way to the PHA synthesis using pure cultures either with wild strains or recombinant strains (26). This production process generally involves two stages, first stage is providing the microorganisms with feed and second stage allowing the PHA to accumulate by controlling the feed (27). One of the advantages of using pure cultures is that more polymer content is obtained (more than 90%). Moreover, environmental impact of PHA synthesis by pure cultures was assessed by life cycle assessment (LCA) (28). This study investigated based on CO₂ emissions and internal rate of return (IRR) to evaluate financial viability along with environmental impact. Using mixed cultures was found to be more captivating financially than pure cultures (29). However, aseptic conditions are necessary to maintain these pure cultures, thus increasing the maintenance cost.

PHA production using mixed cultures Mixed cultures have been identified as effective systems in many fermentations process. Recently they gained lot of attention in PHA production. In mixed cultures, different group of microorganisms are considered to synthesize PHA. Wallen and Rohwedder (30) were the first to report on the formation of PHA by mixed cultures, which was initially used in the waste water treatment to remove for phosphorus. Mixed cultures are considered as the potential producers of PHA in substantial amounts due to reduced equipment for sterility and control requirements. Major advantage to consider the mixed cultures over pure cultures, is their ability to utilize a huge range of substrates including industrial and agricultural wastes (17). This factor is highly appreciable when wide variety/mixture of carbon sources are used as substrates. In mixed cultures; controlling the substrate concentration, maintaining the oxygen flow rate and control of the N and P values will increase the PHA storage in the cell (31). It's also considered as feasible process for PHA production as it can be continuous process and also due to its adaptive capacity to different substrates.

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