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A Review on Strategies for Production of (Poly-3-Hydroxyalkanoates): The Green Materials for Sustainable Development - Current Status and Future Prospects

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ABSTRACT

One of the major paradigm shifts towards sustainable development is the substitution of nonrenewable resources with renewable resources. Petroleum derived plastics pose a great danger to the environment and also due to the finite supply of petroleum products, research efforts are now shifted towards finding alternatives to synthetic plastics, which are also environmentally friendly. Polyhydroxyalkanoates are one such class of biodegradable plastics, which have gained much attention in recent years. The present paper reviews various aspects of Polyhydroxyalkanoate production, including different strategies for production of PHAs in bacteria, transgenic plants and activated sludge., the various applications of PHAs, PHA production from inexpensive renewable resources, different fermentation strategies, methods for recovery of polyhydroxyalkanoates (PHAs) from biomass,economic considerations for cost effective PHA production, strategies to produce PHAs in activated sludge and transgenic plants. The current status and the future trends have been discussed.

KEY WORDS: Bioplastics, Polyhydroxybutyrate, renewable resources, biodegradation, polyhydroxyalkanoates.

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1. INTRODUCTION :

The replacement of petro derived plastics by biodegradable plastics is one of the major paradigm shifts towards sustainable development. It is now well established that petro derived plastics are non biodegradable and may remain in the environment for the next 250 – 450 years. Though plastic waste can be managed through source reduction, recycling, thermal destruction and land filling, the associated technologies for such management are not environmentally friendly.

Public awareness for the need to avoid endangerment of the natural systems that support life on earth i.e. the atmosphere, water soil and the living beings has also intensified. Thus the need of the hour is sustainable development in the manufacturing processes. There is a need to improve the efficiency of industrial processes thus minimizing wastage. Another approach for the development of sustainable technology is to design Eco-friendly products, which can be derived from the vast diversity of resources on the earth, and are also environmentally friendly. With this view the present paper reviews the research on Polyhydroxyalkanoates, the most promising substitute for synthetic plastics, which can be the future green materials as an alternative to non degradable plastics.

2. NEED FOR RESEARCH ON DEGRADABLE PLASTICS:

During the last two decades, the awareness for the clean environment has compelled scientists, engineers and technologists to search for alternatives to the non-degradable plastics. In the recent years it has been argued that degradable plastics could provide a solution for the management of plastics waste. Environmentally friendly plastic in terms of "Sustainable development" should possess the following properties:

- Appropriated thermo-mechanical attributes
- Recyclable
- Completely biodegradable
- Produced from renewable resources

Degradable plastics are either photodegradable or Biodegradable. Photodegradation occurs when plastics are exposed to ultraviolet light [1].

3. RECENT DEVELOPMENT IN BIODEGRADABLE PLASTICS:

The production of biodegradable plastics can be viewed within the wide context of the "greening of industry" with the use of renewable biomass as an alternative feedstock to fossil fuels in manufacturing eco-friendly products.

Most biodegradable plastics made from renewable resources are products of biotechnology, films suitable for certain packaging applications are made from microbial polysaccharides such as pullulan and xanthan [2], as well as from cellulose and chitosan [3] Polylactides, such as those marketed by Du Pont are produced from another fermentation product, lactic acid [4]. A number of plastic like materials consisting of chemically modified naturally polymers have been developed [5]. Materials made from exclusively modified starch which constitutes 70 – 90% of the total and remainder is plasticizers and additives which are also degradable. Manufacturers claim that this class of polymers performs similarly to biopolymers produced by microorganisms. They degrade in a matter of months and do not release toxic or metallic pollutants [5] .Table 1.0 gives the list of some of the companies producing biodegradable polymers.

Table 1.0: Current players in PHA based resins (Polyhydroxyalkanoates)

S.No	Company	Country	S.No.	Company	Country	S.No.	Company	Country
1	Telles	USA	8	Biomer	Germany	15	ICI	UK
2	Tianjin GreenBioscience	China	9	PHB Industral	Brazil	16	BtF	Austria
3	Bio-on	Italy	10	Bioplastech	Ireland	17	BASF	Germany
4	Tianan Biologic	China	11	Mitsubishi Gas Chemical	Japan	18	Metabolix	USA
5	Biomatera	Canada	12	Chenzen Obioer	China	19	Monsanto	USA
6	Micromidas	USA	13	Kaneka	Japan	20	Tepha	USA
7	Meridian	USA	14	Chemie Linz	Austria	21	P&G	USA

The most interesting of other biologically produced replacements for synthetic plastics are the polyhydroxyalkanoates. PHAs have received much industrial attention because of their material properties similar to conventional plastics, their complete biodegradability and the ability to be produced from renewable resources. A number of review articles encompassing the general features of PHAs [6,7,8,9,10,11], the physiology, genetics of PHA producing microorganisms and molecular biology [12,13], the development of PHAs having novel monomer constituents [14,15,16], production processes and their economic evaluation [17,18,19,20,21], and biodegradability of PHAs have been published [22,23].

4.POLYHYDROXYALKANOATES(PHAs)- THE BIODEGRADABLE MICROBIAL THERMOPLASTICS:

Polyhydroxyalkanoates are polyesters of hydroxyalkanoates (HAs) synthesized by numerous bacteria as intracellular carbon and energy compounds and accumulated as granules in the cytoplasm of the cells. Many microorganisms accumulate large quantities of PHA intracellularly when their growth is limited by some element other than carbon. Such cultures are found in wide variety of niches. The list of PHA accumulating microorganisms is shown in Table 2.0.

Table 2.0 Poly (3-hydroxybutyrate)-Accumulating Microorganisms

Acinetobacter	*Gamphosphaeria*	*Photobacterium*
Actinomycetes	*Haemophilus*	*Pseudomonas*
Alcaligenes	*Halobacterium*	*Rhizobium*
Aphanothece	*Hyphomicrobium*	*Rbodobacter*
Aquaspirillium	*Lamprocystis*	*Rbodospirillum*
Azospirillium	*Lampropedia*	*Sphaerotilus*
Azotobacter	*Leptothrix*	*Spirillum*
Bacillus	*Methylobacterium*	*Spirulina*
Beggiatoa	*Methylocystis*	*Streptomyces*
Beijerinckia	*Methylosinus*	*Syntrophomonas*
Caulobacter	*Micrococcus*	*Thiobacillus*
Chlorofrexeus	*Microcoleus*	*Thiocapsa*
Chlorogloea	*Microcystis*	*Thiocystis*
Chromatium	*Moraxella*	*Thiodictyon*
Chromobacterium	*Mycoplana*	*Thiopedia*
Clostridium	*Nitrobacter*	*Thiosphaera*
Derxia	*Nitrococcus*	*Vibro*
Ectothiorhodospira	*Nocardia*	*Xanthobacter*
Escherichia	*Oceanospirillum*	*Zoogloea*
Ferrobacillus	*Paracoccus*	

Of all the PHAs, Poly-3-hydroxybutyrate (PHB) is the best known. It was first discovered and initially described in 1925, although it was not until Late 1950s that the metabolic pathways involved in the synthesis and degradation were elucidated. The first indication that there might be a role for PHB as a commercial plastic emerged in 1959 & 1960 when a US Patent for its use as a surgical suture was applied for.

PHB homopolymer shown similarities in its physical properties to polypropylene (PP). The main difference between the two is the biodegradability of PHB. Although other difference such as the density can have an impact on potential applications. PHB is much denser material than PP, so that whilst the latter floats, PHB will sink to the bottom of an aquatic ecosystem. This property allows PHB to be degraded in surface sediments by biogeochemical mechanisms. The comparison of properties of PHB with some of the common polymers is shown in Table 3.0.

Table 3.0 : Chemical and Physical Properties of PHB v. PP

Property	PHB	PP
Melting point (°C)	171 – 182	171 – 186
Glass transition temperature (°C)	5 – 10	15
Crystallinity (%)		
Density (8/cm)	65 – 80	65 – 70
	1.23 – 1.25	0.90 –0.94
Molecular wt		
Molecular wt distribution	100000 – 800000	220000 – 700000
Flexural modulus (Gpa)	2.2 – 3.0	5.0 – 12.0
Tensile Strength (Mpa)	3.5 – 4.0	1.7
Extension to break (%)	40	39
UV resistance	6 – 8	400
Solvent resistance	Good	Poor
Good Oxygen permeability		Poor
(Cm3/m2XatmXd)		
Biodegradability	45	1700
	+	–

5. PHB METABOLISM:

PHB synthesis involves three enzymatic steps. The pathway of PHB synthesis starts with conversion of an appropriate carbon substrate (sugar, alcohol, organic acids or carbon dioxide) into acetyl CoA. Two molecules of acetyl-CoA are condensed by the action of 3-ketothiolase (acetyl-CoA acetyltransferase). The intermediate is reduced to D(-)-3 hydroxybutyryl CoA by NADPH_ dependent acetoacetyl CoA- reductase. PHB is then produced by the action of PHB synthase (polymerase) key regulatory enzyme in PHB metabolism is acetyl CoA- acelytransferase, which is inhibited by high concentrations of free coenzyme A. Under balanced growth conditions CoA-SH levels are high and the synthesis of PHB is inhibited. In nutrient limited but carbon excess condition, build up of NADH inhibits citrate synthase, which results in augmentation of acetyl CoA level to a point where inhibition by CoS-SH is overcome. The condensation reaction to acetoacetyl CoA proceeds and PHB is polymerized with the action of PHB synthase [24].

6. BIODEGRADATION OF PHAs:

PHAs can be completely mineralized to H_2O and CO_2 in aerobic systems [25], H_2O, CO2 and CH_4 are the final end products when PHA is degraded under anaerobic conditions [26]. PHA degradation in lakes has been described [27,28]. There seems to exist a wide distribution of PHA degrading abilities among fungi also [29,30].

Polymer degradation is controlled through the oxidation of monomeric 3-hydroxybutyrate, by the enzyme 3-hydroxybutyrate dehydrogenase. This enzyme is subject to product inhibition by acetoacetate and NADH. These main regulatory elements of the cycle are supplemented by a second level of control: intermediates of tricarboxylic acid cycle cause feedback inhibition of the enzymes. Thus synthesis and breakdown of PHB is linked to the metabolic status of the cell and the carbon flux through intermediary metabolism. The degradation of PHAs and the composition of microbial destructors under natural conditions were studied by Volova et.al. [30]. Biodegradation of PHAs have been reviewed by Reddy et al. [31], and the degradation by thermophilic *streptomyces* has been reported [32].

7. APPLICATION OF PHAs:

PHAs have been drawing considerable industrial interest as candidates for biodegradable and/or biocompatible plastics for a wide range of applications, some of which are listed in table 4.0.

Table: 4.0 Applications of polyhydroxyalkanoates

Sr.No.	Applications
1.	Packaging films (for food packages) bags, containers, paper coatings
2.	Biodegradable career for long term dosage of drugs, medicine, insecticides, herbicides or fertilizers
3.	Disposable items – such as razors, utensils, diapers, feminine hygiene products, cosmetic containers, shampoo bottles, disposable cups
4.	Starting materials for chiral compounds
5.	Medical applications- Surgical sutures, staples, swabs, wound dressings, bone replacements and plates, blood vessel replacements, stimulation of bone growth by piezoelectric properties

There are consumer focused applications which will benefit from both the product's biodegradability and the fact that, it is made from renewable resources. PHAs can be used in many fields and new potential applications are still emerging [33]. Applications ranging from denitrification in water and wastewater treatment [34], to tissue engineering materials [35], to the applications of PHA nano/microparticles in biotechnological and biomedical applications [36], and sustained drug release have been reported [37].

8. COMMERCIAL PRODUCTON OF PHA IN BACTERIA:

Zeneca Bioproducts Billingham UK had developed PHB and PHB/V under the trade name Biopol since mid 1970s, and is the commercial producer of PHB and PHB-CO-HV. The production being around 1000 tons per annum. Biopol was being sold at US $ 16/kg. If we consider the price of conventional plastics, such as polyethylene and polypropylene, which is less than the US $ 1/kg. PHAs may be considered too expensive to be used as bulk plastic materials. However, it is unfair to compare the price of PHAs with polyethylene or polypropylene since the latter is not biodegradable. Therefore the comparisons should be made with other biodegradable polymers such as Polylactides, diol-diacid based aliphatic polyesters and starch based polymers which are currently sold at US $ 5-15/per kg.

Even though the prices of PHAs are still very high, there are several companies producing PHA to meet the demands of the market. PHB and PHBV are still the main members of PHAs that are produced on commercial scale. Presently there are some PHAs products in the markets such as Biopol, Mirel and Nodax made in USA, Biomer in Germany, Biocycle in Brazil, Degrapol in Italy, Tiannan PHBV and PHB in China. Most companies have started to increase their production capacities of PHAs to several thousand tons or 10 thousand tons per year e.g. Tianjin Green Biosciences Ltd., company has been building a new factory to produce PHAs at the capacity of 10 thousand tons per year [38]. There are now about 75,000/- metric tons of annual capacity globally to produce PHAs from five companies. Telles (USA), Green bioscience/DSM (China), Bio-on (Italy) Tianan Biologiy material co (China), Biomatera (Canada); Nine other companies are conducting developmental work on PHAs some with pilot plants micromidas (USA), Meridian (USA), Biomer (Germany). PHB Industrial Brazil, Bioplastic (Ireland). The salt and marine chemical Res Institute (India), Mitsubishi gas chemical (Japan), Shenzen Obioer China and Kaneta (Japan).

9.PHA PRODUCTION FROM LOW COST RENEWABLE RESOURCES:

For the commercialization of PHAs, much effort has been devoted to reduce the cost by employing efficient bacterial strains and more efficient fermentation/recovery process. Many bacteria such as *Alcaligenes eutrophus, Methylopophs, Pseudomonas oleovorans* synthesize PHAs under limitations of nutritional element such as N, P, Mg, K and /or O or S in presence of excess of carbon source. For the cultivation of these bacteria, two-step cultivation method is employed. Cells are first grown without nutrient limitation. Nutrient limitation is applied for PHA synthesis in the second stage.

In order to make PHAs production cost effective and economical, research on PHAs production from inexpensive raw materials a substrates have shown that even though PHA could be produced from low cost substrates but in most cases the cell density and PHA contents were at low level, making it more difficult for PHAs to be separated from the dilute culture broth.PHA production from inexpensive carbon sources such as agricultural wastes and surplus materials [39], plant oils [40,41,42,43], molasses [44,45,46], starch [47,48,49], whey [50,51], other inexpensive carbon sources [52,53,54,55] have been reported.

As compared to processes using single pure culture, the use of mixed cultures as a cheaper method to produce PHAs has gained much attention in recent years. Mixed cultures could become the most effective and potential means of producing PHAs in the future [56].

The use of mixed culture using industrial wastes was reported by Dias et al. [57]. PHA production using mixed culture and saponified sunflower oil and mixed culture with sugarcane molasses have been reported recently [58,59,60].

Review articles on production of polyhydroxyalkanoates have been published recently [61,62]. In most cases of mixed cultures PHAs were produced from the organic acids contained in the wastewater or transformed from other industrial wastes. Thus mixed culture strategy not only helps to solve the problem of environmental pollution but also simultaneous production of value added materials which is important for sustainable development and environmental protection. Two recently published reviews have included studies in production of PHAs from agro-industrial byproducts and low cost sustainable raw materials [63,64].

10. RESEARCH ON NEW PHA PRODUCING SPECIES:

The best PHA producing species should satisfy several demands such as, it should be fast growing, should be able to utilize inexpensive carbon sources, have a high conversion rate. Such strategies include isolation of efficient strains from natural environments and designing new recombinant strains.

Newer and newer strains continue to be reported, but most of the studies are at just shake flask level, or batch culture, so that the cell densities and PHA contents are not so high. Much research efforts are needed to be focused in this area.

However, some bacteria such as *Alcligenes latus*, *Azobactor vinelandii* and recombinant *E.coli* do not require nutritional limitation for the synthesis of PHAs and can accumulate PHAs during growth. For these bacteria, nutrient feeding strategy is most important for the success of fermentation and should be optimized for each bacterium. The current strategies for production of optimization of PHA production have been reviewed by Robin Green [65].

11. RECOVERY OF PHAs FROM MICROBIAL BIOMASS:

Several methods have been developed for the recovery of PHAs, (Mostly PHB) from the cells. PHA containing biomass can be recovered by centrifugation or filtration and may be separated from the non PHA portion of the biomass by extraction using solvents such as chloroform, methylene chloride, propylene carbonate and dichloromethane [66].

However, the extracted polymer solution containing more than 5% (W/V) PHB becomes very viscous and removal of cell debris is difficult. The large amount of solvent required makes this method economically unattractive even after the recycle of solvents. Several other methods have been developed involving use of sodium hypochlorite for the differential digestion of non-PHA cellular materials [67], however during the digestion of non PHA cellular material (NPCM), severe degradation of PHB has been observed. Surfactant pretreatment and hypochlorite digestion under optimized conditions resulted in higher purity of PHB with less degradation [66]. An enzymatic digestion method developed by Zeneca consists of thermal treatment of biomass, enzymatic digestion and washing with an anionic surfactant to solubilize NPCM [68]. A process for PHB recovery using a dispersion of sodium hypochlorite and chloroform has been developed [69,70]. It was suggested that chloroform immediately dissolves the isolated PHB by hypochlorite, and thus protects polymer from degradation.

Process analysis and economic evaluation for PHB production processes using different microorganisms and various recovery processes by Lee et al.[71] have demonstrated that the cost of carbon source contributes significantly to the overall economics and various recovery processes have demonstrated that the cost of carbon source contributes significantly to the overall economics in large production scale. For the production of 2,850 tons of purified PHB, the process employing *A. eutrophus* with recovery method of surfactant- hypochlorite digestion resulted in lowest price of PHB, \$ 5.58/kg. As the scale of production increase to one million tons per year the price will drop to \$ 4.75/kg [71].The strategies for isolation and recovery have been reviewed recently by Kunasundari, Sudesh[72].

12. ECONOMIC CONSIDERATIONS:

Process analysis and economic considerations for PHB production process by Lee et.al.[71], have demonstrated that the cost of carbon source contributes significantly to the overall economics in large production scale. For the production of 2850 tons of purified PHB, the process employing *A. eutrophus* with recovery process of surfactant-hypochlorite digestion could result in lowest price of PHB$ 5.58/kg. As the scale of production increases to one million tons per year the price will drop to 4.75/kg.

There are continued efforts to reduce the production cost of PHB so as to make them comparable to the currently available thermoplastics. The production cost could be considerably lowered when inexpensive carbon substrates such as whey, cane molasses, agricultural wastes or hemicellulosic hydrolysate are used for fermentation, since the cost of carbon substrate accounts for 70 – 80% of total raw material cost.

The use of whey as a substrate in a two stage fermentation process and use of non solvent based extraction and recovery process for PHB was demonstrated. The pre-design cost estimate of the process revealed that the cost of production of PHB could be reduced to $3.04/kg. [73], Even though this price is still higher than petrochemical based plastic materials, complete biodegradability of PHB could justify higher price.

Since fermentation strategies for the production of PHB have been well developed, adopting these strategies using cheaper carbon substrates for large scale production of PHB could further bring down the cost of PHB. Studies are underway to optimize fed- batch fermentation using whey, lactic acid and propionic acid produced from whey, in turn, can bring down the cost of copolymer production as these acids are inexpensive.

13. PHAs FROM ACTIVATED SLUDGE:

PHAs are known to be temporarily stored by microorganisms in activated sludge especially in the anaerobic-aerobic process. When PHA is extracted from activated sludge, it is thermoplastic with the remarkable characteristics of biodegradability. Activated sludge as a possible source of biodegradable plastics has been reviewed by Satoh et. al. [74,75]. Various research papers on the augmentation of PHA content in activated sludge have been published [76,77,78].Much research has been focused on production of PHAs from activated sludge treating different wastewaters from industries like paper mill effluent [79],molasses spent-wash [80],food processing industries wastewater treatment plants[81],milk waste and dairy wastewater [82]. Studies on process optimization for optimum production of PHAs from activated sludge have been carried out [83,84,85]. The optimum yields of PHA obtained was 49.5% of the biomass. An efficient PHA production process from activated sludge has been reported [86]. A techno-economic evaluation of PHA production process from waste activated sludge was evaluated by Mudliar et.al. [87]. Research is now directed towards the biopolymer production from different wastewaters as an economic alternative for the cost effective production.

The attempt to produce PHA by activated sludge could provide a techno-economic solution to bioplastic production and commercialization; However further investigations are needed in these directions.

14. PRODUCTION OF PHA IN TRANSGENIC PLANTS:

With recent advances in plant molecular biology, allowing expression of foreign proteins in a variety of plants, genetic engineering of crop plants was aimed at both increasing the quantity and modifying the quality of the products produced in crop plants. In view of the flexibility of plants in expressing foreign genes, it was of interest to explore the feasibility of synthesizing PHAs in plants.

Transgenic plants harboring *A. eutrophus* PHA biosynthesis genes have been developed with the aim of ultimately reducing the price of PHA to close to that of starch. Up to 10mg/g PHB (which represents 10% of dry cell weight), could be produced in the model transgenic plant *Arabidopsis thaliana*. The concentration and yield of PHA will increase as our understanding of plant biochemistry and genetic engineering improves. It has been shown that PHA in potato, tobacco leaf, corn, sugarcane can be produced transgenically [88,89,90].Various researchers have reviewed the feasibility of producing PHAs in transgenic plants [91,92,93,94].The challenge of producing PHA involves expression of several genes along with optimization of PHA synthesis in the host. The idea is that if the carbon that is normally present in starch, sucrose or oils can be diverted to PHA, it may be possible to produce these PHAs at low cost and in large quantities in maize, potato, sugar beet, cereals or even oil containing seeds. It is assumed that after the necessary research and development,it will ultimately be possible to obtain comparable yield of PHAs,with around 2.5t PHA year^{-1} ha^{-1} might be anticipated. PHA could be produced by using fallow land and by investing presently unproductive land: production of bulk amounts of polyesters would require on the order of 4 X 105 ha as 4 X 10^3km^2 of farmland / 10^6 t of PHA [88]. The value of plant based PHAs in US$

would ultimately probably decrease to around $ 0.50 – 1.00/kg. Thus transgenic plants may become economically feasible alternative source of PHAs in the future.

15. CONCLUDING REMARKS:

Replacement of synthetic non degradable plastic with biodegradable PHA derived plastics may not significantly affect the consumption of fossil fuels but biodegradable plastics will have great contribution in minimizing problems related to environmental pollution.

At present, PHA is not cost competitive compared to fossil-derived products. Encouraging and intensifying research work on PHA is anticipated to enhance its

economic viability in the future. A lot of research is being done on production of PHAs to bring down the cost of production. Various reviews on production of PHAs have been published [61,95,96,97]. Efforts are being made to design or isolate new strains with high production capacities, optimize fermentation processes with increased yields, cost effective extraction and recovery processes [98,99,100].

Utilization of inexpensive carbon sources, and development of transgenic plants for economical production. While the processes are not yet economically competitive with that of petroleum bases plastics with further research however PHA products may soon become commercially viable. However in order to bring, biodegradable plastics to market, further research efforts are needed for techno economical feasibility of producing PHAs at competitive cost with synthetic plastics with all these efforts PHAs will become a major biodegradable plastic material in wide range of applications in near future.

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