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MICROBIAL PRODUCTION OF POLYHYDROXYALKANOATES (PHA) UTILIZING AGRO INDUSTRIAL WASTES AS CARBON SOURCE

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ABSTRACT

Polyhdroxyalkanoate (PHA) is biological polyester accumulated by wide range of bacteria. Bioplastics is a form of plastic made from renewable sources, which exhibit unique properties and can be produced from plants and crops wastes. Since there are many environmental concerns associated with the use of convenient plastics it is of immense importance to replace conventional plastics with bioplastics. And if the bioplastics are synthesized from agro-industrial wastes, a dual benefit of utilizing waste and simultaneously producing a valuable by product (PHA) can be obtained. This review presents the production of bioplastics and different uses of PHA and source of bio plastics. Recycling of wastes from agro-industries for PHA production is important economically and commercially. Thus, the review includes production of PHA from agro-industrial wastes which is cost-effective. Also, the utilization of cheap and renewable sources can be explored.

Keyword: Polyhydroxyalkanoates (PHA), Agro Industrial waste, Plant and crops, Bio plastics

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are family of biobased, biodegradable and biocompatible plastics. PHAs are of biological origin, which can be completely broken down to carbon dioxide and water by microorganisms found in soil, water and sewage. PHAs are water insoluble storage polymers. Many microbes are able to synthesize (PHA) polyhydroxyalkanoates as carbon and energy storage compounds.

Polyesters of hydroxyalkanoic acid are produced by a variety of bacterial species. Synthesized & stored intracellular as energy and carbon storage materials under limiting nutritional elements like N, S, P, O or Mg etc. More than 180 different PHAs are reported. There are mainly 2 groups of PHAs: short-chain length 3-5 carbon and medium-chain length 6-14 carbon, Copolyesters of PHAs containing monomers such as 3-hydroxybutyrate **[1]**. Beijerinck first observed PHAs as refractive bodies inside bacterial cells in 1888 **[2]**. Main factor that affects cost of PHA production is the substrate cost which can be reduced by utilizing waste products. In 1976, ICI of England explored if PHB could be satisfactorily produced by microbial fermentation. In 1993, Zeneca Bioproducts took over ICI's activities and in 1996 Monsanto bought the bioplastics production business from Zeneca. Some prominent ones are Metabolix, Proctor and Gamble, DuPont, General Motors, and Toyota etc. BIOPOL is a marketed bioplastic product.

Microbiology of PHAs synthesis:

Many natural microbes are PHAs producers including bacteria and archea. *Bacillus*, *Alcaligenes, Pseudomonas, azotobacter, Ralstonia Vibrio, Enterobacter, Cupriavidus, and Methylobacterium* etc. are some of the microorganisms involved in synthesis of PHAs.

Polyhydroxyalkanoates (PHA) are environmentally biodegradable thermoplastics or bioplastics when synthesized from agro-industrial wastes a dual benefit of utilizing waste and simultaneously producing renewable byproducts (PHA) can be obtained. Recycling of wastes from agro-industries for PHA production is important economically and commercially. This review presents the production of bioplastics and different uses of PHA, and source of bioplastics.

Information of Agroindustrial Waste:

Agroindustrial waste:-Agroindustrial wastes are generated during the industrial processing of agricultural or animal products. Those derived from agricultural activities including materials such as stems, straws, fruits and vegetable peels, legumes, crop wastes seed bagasses etc. **[3]**.

Agricultural wastes are divided into two categories: Agricultural residues and industrial residues.

Agricultural residues are further divided into another two categories - Field residues:- materials left in agricultural field after the crops has been harvested Example includes stems, leaves, seeds etc.. Process residues:- materials left in agricultural field after the crops is processed into a usable resources example including Bagasses molasses, husk, root, seeds etc.

Industrial-residues:-materials are left after processing of any food products. Examples are including potato peel, orange peel, cassava peel, ground nut oil cake, soya bean oil cake etc.

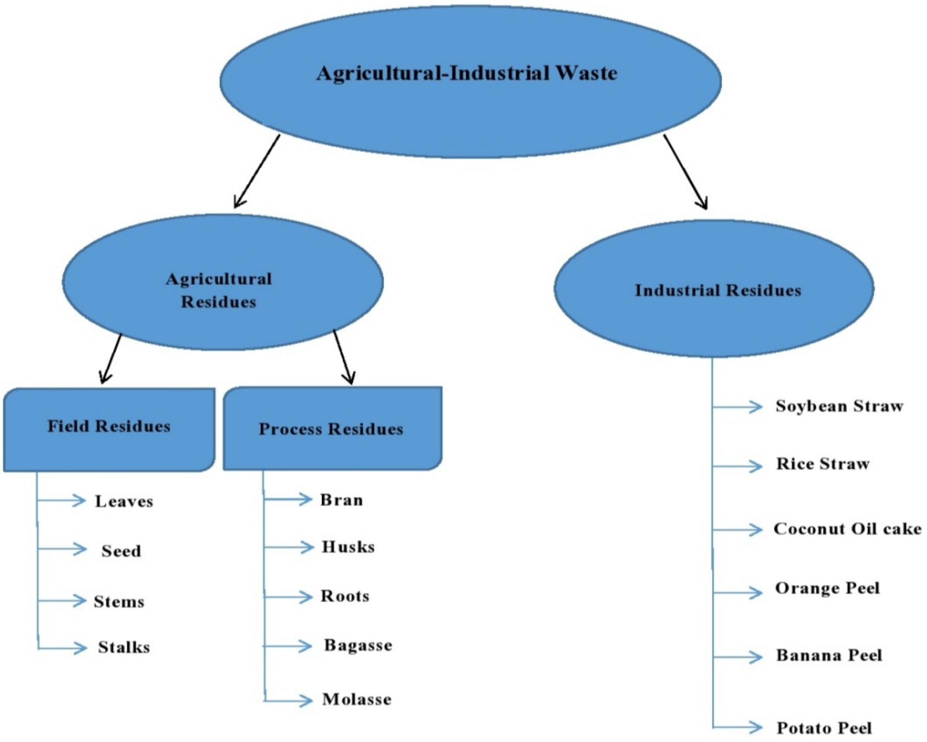

Figure 1: Agricultural wastes are divided into two categories: Agricultural residues and industrial residues

Alternatives

Biodegradable plastics:

Biodegradable plastic is similar to conventional plastics in all aspect with additional quality of being able to naturally decompose along with food. This will be of huge advantage because it includes Aaroindustrial wastes that are cheap, and their conversion solves the problem by converting wastes into useful products **[4]**.

Plastic that is made from biological sources such as sugar cane, potato starch, vegetable fats and oils, corn starch and pea starch. These are designed to biodegrade. Biodegradable bioplastics can break down in either aerobic or anaerobic environments, depending on how they are manufactured. (Bioplastics degrade in the natural environment. It is produced from biopolymers called PHA, which is completely degradable. Plastics made from petroleum are burned, they release toxic substances contained in petroleum into the atmosphere, leading to global warming.

Whereas the use of bioplastics offers advantages in economical and ecological sense. Use of renewable resources is promising alternatives for plastics industry.

Types of bioplastics: Starch-based, Cellulose-based, PHA, PHB, Lipid derived, etc.

Synthesis of PHA from Agroindustrial waste and cheap carbon sources:-PHA is polyester of various hydroxyalkanoates that is synthesized by many Gram-positive and Gram- negative bacteria from at least 75 different genera. These polymers are accumulated intracellular to level as high as 90% of the cell dry weight under the conditions of nutrients stress and act as a carbon and energy reserve. Non-storage PHA that are of low molecular weight, poly (3HB), have been detected in the cytoplasmic membrane and cytoplasm of *E. coli* **[5]**.

PHA biosynthesis in natural isolates: The information on the P(3HB) metabolism, and biochemistry, and physiology since 1987 have been clone and characterized from a variety of microorganisms. Genetic studies have conferred insight into the regulation of PHA formation with respect to the growth conditions **[6]**.

Fermentation in production of Plastics: Fermentation is the use of microorganism to break down organic substance in the absence of oxygen. Today, fermentation can be carried out with genetically engineered microorganisms, specially designed for the conditions under which fermentation take place and for specific substance that is being broken down by the microorganisms. There are two ways by which fermentation can be used to create bio- polymers and bioplastics.

Bacterial polyester fermentation:- Bacteria are one group of microorganisms that can be used in fermentation, in fact, is the process by which bacteria can be used to create polyesters. *Ralstonia -eutropha b*acteria is commonly used for PHA production. The bacteria used the sugar of harvested plants, such as corn, to fuel their cellular processes. The byproduct of these cellular processes are the polymers which are then separated from the bacterial cells.

Lactic Acid fermentation: Lactic acid is fermented anaerobic process used to directly produce polymers by bacteria. In this fermentation process, the ultimate product of fermentation is lactic acid, rather than a polymer. Lactic acid is converted to poly lactic acid used for conventional bio plastics processes.

Production of PHA by genetically engineered plants: Crop plants are capable of producing large amount of a number of useful chemical at a low cost compared to that of bacteria or yeast. Commercialization of plant derived PHA will require the creation of transgenic crop plant, which in addition to high product yields have normal plants phenotype and transgenic that are stable over several generations. Production of PHA on an agronomic scale could allow synthesis of biodegradable plastics in the million ton scale compared to fermentation, which produce material in the thousand ton scale **[7]**.

Production of Polyhydroxyalkanoates (PHA) utilizing agro-industrial waste as carbon source

Table 1: Different strains used in production of PHAs

Bacterial Strain	Carbon source	Polymer produced
Alcaligenes latus	**Soya waste, milk waste, sesame oil**	**PHB**
Bacillus cereus	**Sugar beet, molasses**	**PHB,**
Bacillus spp.	**Alkanoates, soy molasses**	**PHB, PHBV**
Escherichia coli –mutants	**Molasses, palm oil waste**	**PHB**
Psuodomonas-areuginosa	**Molasses, oil waste**	**PHAs**

(Okamura *et al.*, 1996, [8] used soybean wastewater from a Japanese miso production process for the accumulation of an intracellular PHA blend by a recombinant Pseudomonas sp .61-3 The intracellular blend consisted of polyhydroxybutrate (PHB) homopolyester, (single type of polymer) and randomly distributed co-polyester (Two or more type of polymer) consisting of 3-hydroxybutyrate (3HB) and longer 3-hydroxyalkanoates with four to 12 carbon atoms **[9]**.

The bacterium *Alcaligens eutrophus* and *Alcaligens latus* are well known for their ability to Produce PHA. PHA accumulation by *Pseudomonas oleovorans,* which accumulates PHA only when alkenes are provided as carbon source for PHA production.

There have been many researches on the production of PHAs from cheap carbon sources by wild-type PHA producers or PHA producing microorganisms. Xylose is important component of hemicelluloses of hardwoods and crop residues, when xylose was used 2.6gL-1 biomass with 60% (w/w) PHB in shake flasks **[10]**. Hemicelluloses use for PHB production was comparable to that of sugarcane molasses and half that of bulk glucose **[11]**. Developed a recombinant *E.coli* strain harboring *the C. Necator* PHA biosynthesis genes which can produce p (3HB) from xylose. In a chemically defined medium added with Xylose, P (3HB) concentration and PHB content obtained were 1.7 g.L-1 and 35.8%, respectively, in flask culture. Addition of a small amount of complex nitrogen source such as soybean hydrolysate improved PHB production to a level of 74% of cell dry weight.

Since, recombinant *E. coli*, could be easily grown to high cell density by utilizing Xylose, recombinant *E. coli* would be useful for the production of PHB from Xylose or hemicelluloses hydrolyses **[12]**.

Omar reported the ability of *Bacillus megaterium* to grow on various carbon sources such as date syrup, beet molasses, fructose, lactose, sucrose, glucose in mineral salts medium Best **[13, 14]**. Results in relation to growth and PHB production were obtained in the cheaper carbon sources like date syrup and beet molasses.

Beet molasses has proven to be an excellent feedstock for polyhydroxyalkanoates (PHA) production by *Azotobacter vinelandii* UWD. The substrate-cost for PHA production from beet molasses in fed-batch culture was one-third of that using glucose. Copolymers containing β-hydroxyvalerate were readily formed in beet molasses medium when valerate was used as a precursor. Production of P(3HB) by *Rhizobium meliloti* and by *Bacillus cereus* M5 strain was demonstrated using sugar beet molasses as a carbon source. P3HB **[15]** contents of 56 and 74 % of cell dry weight for *R. meliloti* and *B. cereus*, respectively, were reported. Solaiman screened several mcl-PHA-producing *Pseudomonas* species and identified *P. corrugata* as capable of growing and producing biopolymer when cultivated on soy molasses medium **[16]**. The biopolymer yields from *P. corrugata* cells grown in shake-flask cultures with a chemically defined medium supplemented with 5 % (w/v) soy molasses were 0.6 gL-1mcl PHA.

Yezza *et al.*, 2006, isolated a new bacterial strain, from groundwater contaminated with isolated genus Methylobacterium **[17]**. The bacterial isolate as strain GW2 was found capable of producing the homopolymer poly-3-hydroxybutyrate.

Methanol is a best substrate for the production of PHB reaching 40% w/w dry biomass. Optimal growth occurred at 0.5 % (v/v) methanol concentration and growth was strongly inhibited at concentration above 2 % (v/v). *Methylobacterium sp.* strain GW2 was also able to accumulate the co polyester poly-3-hydroxybutyrate-poly-3-hydroxyvalerate (PHB/HV) when valeric acid was supplied as an auxiliary carbon source to methanol **[18].**

Shunsaku demonstrated that two types of methylotrophic bacterial strains, *P. denitrificans* and *M. exotorquens,* synthesized the copolyester poly (3-hydroxybutyrate-co-3-hydroxyvalerate) when methanol and n-amyl alcohol were added together to N-limited medium **[19]**. The composition of the copolyester differed considerably between the 2 strains: the copolyester from *P. denitrificans* was comparatively rich in 3-hydroxyvalerate (3HV). Its maximum content was 91.5 mol%. In *M. extorquens*, the maximum 3HV content was confined to only 38.2 mol%.

Loo *et al.*, 2005, studied the suitability of palm kernel oil, crude palm oil and palm acid oil as substrates for scl-PHA synthesis by *C. necator*, PHB4 containing the PHA synthase gene of *Aeromonas cavia*. **[20]**. Copolymer P(3HB-co-3HHx) was synthesized at yields ranging from 1.5-3.7 g.L-1 and the content of 3HHx in the polymer was 5%. Alias and Tan isolated a Gram-negative bacterium FLP1, from palm oil mill effluent by using a culture enrichment technique and tentatively identified it as closely related to *Burkholderia cepacia*, when this organism was grown on crude palm oil and palm kernel oil it produces P(3HB) and supplementation of odd number fatty acids resulted in production of poly(3-hydroxybutyrate co-3-hydroxyvalerate). **Alias *et al.*, 2007 [21]**, employed maple sap, an abundant natural product rich in sucrose as carbon source to *Alcaligenes latus* for the production of PHB and obtained 77.6±1.5 % PHB in shake flask and PHB content of 77.0±2.6% in Batch fermentation **[18]**.

Nehal *et al.*, 2005, investigated that Comamonas *testosteroni* accumulated PHAs up to 78.5–87.5% of the cellular dry material during cultivation on various vegetable oils **[22]**. The efficiency of the culture to convert oil to PHAs ranged from 53.1% to 58.3% for different vegetable oils. The composition of the PHAs formed was not substrate dependent as PHAs obtained from *C. testosteroni* during growth on variety of vegetable oils showed similar compositions; 3-hydroxyoctanoic acid and/or 3-hydroxydecanoic acid being always predominant.

Based on the carbon source and bacterial strain used in the fermentation process for PHB production, it is possible to produce related biopolymers having properties ranging from hard and breakable plastics rubbery plastics **[23]**.

Applications of PHA

PHA have applications in many fields including-

Packaging application: Packaging film (for food packages) paper, coatings, bags, containers etc.

Medical application: surgical pins, sutures, staples, swabs, dressing, bone replacement and blood vessel replacement. Disposable items such as cosmetic container shampoo bottles, cups, diapers etc.

There are many future applications for PHA produced by microorganism within the medical, food, dairy and pharmaceutical industries **[24]**.

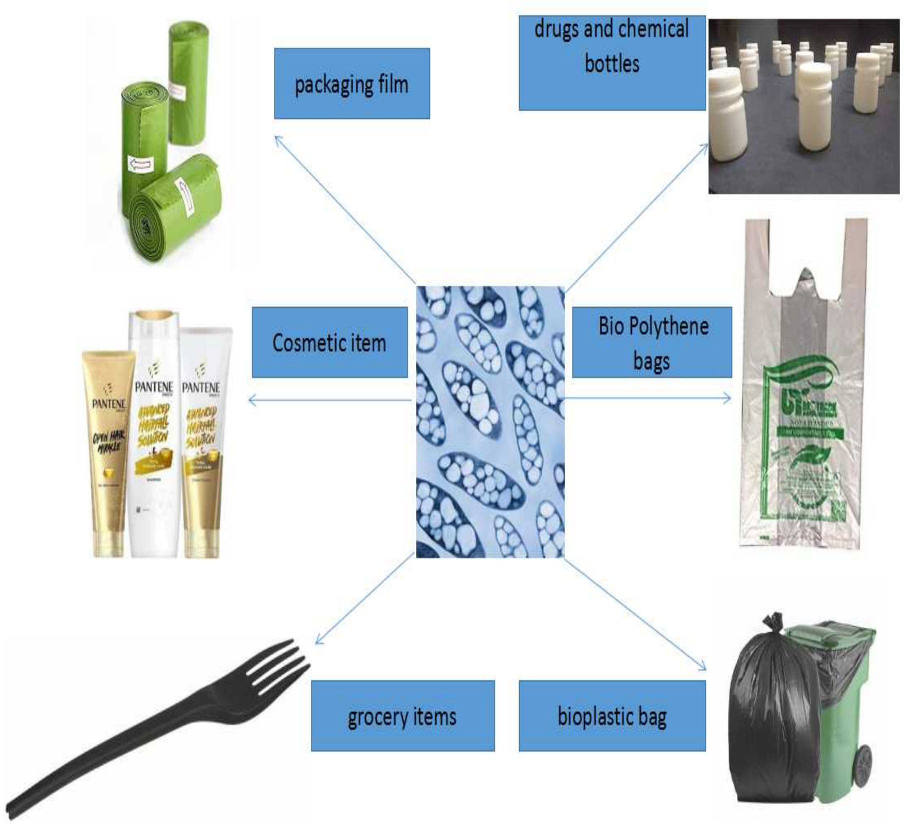

Figure 3: Applications of PHA in different fields

CONCLUSION

Conventional plastics which are not easily biodegradable and which are produced from petroleum is hazardous to the environment. Plastics produced through the biological means (plant & crop wastes, microbes, etc.) are biodegradable which is safe for the environment. Thus, the utilization of waste and carbon sources will provide green plastics through nonconventional means. The renewable nature and biodegradability of PHAs make them suitable materials instead of the use of synthetic plastics in many applications. Currently these plastics are in their first stage of development, further research on the use of inexpensive substrates can reduce the production cost.

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