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Bioplastics-For Sustainable Development: A Review

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Abstract

Bioplastics can be defined as plastics made of biomass such as corn, sugarcane etc. These substances have been increasingly spotlighted as means to saving fossil fuels, reducing CO_2 emission and plastic wastes. Biodegradability of Bioplastics has been widely publicized in society and the demand for packaging is rapidly increasing among retailers and the food industry at large scale. The plastic which is available in market is very dangerous as it is non-biodegradable. Therefore, it is the demand of the day that biodegradable plastics should be produced and used. The present review highlights all these points regarding the applications, production, types, challenges, sustainability, fermentation, process development and use of cheap substrates for bioplastics production.

Key words: Bioplastics, Fermentation, Polyhydroxyalkanoates, Biodegradability, Sustainable development.

Contents

1. Introduction

Bioplastics can be defined as plastics made of biomass such as corn and sugarcane. These substances have been increasingly highlighted as means for saving fossil fuels, reducing CO_2 emission and plastic wastes. Biodegradability of bioplastics has been widely publicized in society and the demand for packaging is rapidly increasing among retailers and the food industry at large scale.

Population growth has led to the accumulation of massive volume of non-degradable waste materials across our planet. The accumulation of plastic waste has become a major concern in terms of the environment (Saharan and Badoni, 2007). Conventional plastics not only take many decades during decomposition, but also produce toxins while degradation. Hence, there is need to produce plastics from materials that can be readily eliminated from our biosphere in an "eco-friendly" fashion (Gross and Kalra, 2002). Bioplastics are natural biopolymers synthesized and catabolized by various organisms (Saharan *et al*., 2007). These get accumulated as storage materials in microbial cells under stress conditions (Kadouri *et al*., 2005; Berlanga *et al*., 2006). However, the high production cost and the availability of low-cost petrochemical-derived plastics led to bioplastics being ignored for a long time. Currently, different types of biodegradable polymers are being studied for different applications including polyhydroxyalkanoates (PHAs), polylactide (PLA), poly(ε-caprolactone) (PCL), poly(*p*-dioxanone) (PPDO) and poly(butylene succinate) (PBS). The most extensively produced microbial bioplastics are polyhydroxyalkanoates (PHAs) and their derivatives (Witholt and Kessler, 2002). PHAs are one of the relatively newer families of biodegradable polymers that have great potential in the future due to their properties. Currently, intensive research has investigated the bacterial production of PHAs and a great effort is underway to improve this procedure (Braunegg *et al*., 2004; Khanna and Srivastava, 2005). However, the PHA production price is still far above the price of conventional plastics (Salehizadeh and Van Loosdrecht, 2004). In order to make the process economically viable, many goals have to be addressed simultaneously. Recombinant microbial strains are being developed to achieve both a high substrate conversion rate and close packing of PHAs granules in the host cell (Agus *et al*., 2006; Sujatha and Shenbagarathai, 2006). A more efficient fermentation process (Patwardhan and Srivastava, 2004), better recovery / purification (Jung *et al*., 2005) and the use of inexpensive substrates (Lemos *et al*., 2006) can also substantially reduce the production cost. Additionally, further research is required to enrich the physical properties of PHAs (Zinn and Hany, 2005). The current review will help the readers to make the basic understanding about the production and use of bioplastics in their day to day life. Keeping these points in mind, the present review has been written to meet the following objectives.

2. Objectives

- To explore possibility to produce a plastic which is biodegradable
- To reduce the production cost of Bioplastic
- To find out cheap substrate for the production of Bioplastic
- To test different kinds of bioplastics which are available in nature
- To understand about various types of Microorganisms which produce bioplastics
- To have the idea about the fermentation and process development for bioplastics production
- To make genetically engineered Microorganisms for the production of bioplastics

3. Polyhydroxyalkanoates

3.1 Structure

PHAs are polyesters of HAs (Hydroxyalkonates) with the general structural formula (Fig. 1). Beijerinck first observed lucent granules of PHA in bacterial cells in 1888 (Chowdhury, 1963). Lemoigne first described the composition of PHAs (Lemoigne, 1927). Non-storage PHAs that are of low molecular weight, poly (3HB), have been detected in the cytoplasmic membrane and cytoplasm of *Escherichia coli*. It is also a membrane constituent in yeasts, plants and animals.

PHA is typically produced as a polymer of about 104 monomers, which accumulate as inclusions of 0.2–0.5 μm in diameter. These inclusions or granules are synthesized and stored by both gram-positive as well as gram negative bacteria without any perilous effect to the hosts (Luengo *et al*., 2003). The molecular weight of these compounds varies from 2×10^2 to 3×10^3 kDa, depending on the micro-organism and the growth conditions (Byrom., 1994). PHA accumulates when the cells experience a nutrient imbalance such as excess carbon with limited nitrogen, phosphorus or oxygen (Steinbuchel and Fuchtenbusch, 1998). The bacteria store the excess nutrients intracellularly by forming insoluble biopolymers from soluble molecules. The biopolymers become mobilized when favourable conditions for normal growth

return. The structure, physio-chemical properties, monomer composition and the number and size of the granules vary depending on the organism (Ha and Cho, 2002).

3.2 Classification

PHAs can be subdivided into three broad classes according to the size of comprising monomers (Madison and Huisman, 1999):

i) scl-PHA: PHAs containing up to C5 monomers are classified as short chain length PHAs.

ii) mcl-PHA: PHAs with C6–C14 are classified as medium chain length PHAs.

iii) lcl-PHA: : PHAs with >C14 monomers are classified as long chain length PHAs respectively .

This difference is mainly due to the substrate specificity of the PHA synthases that can accept 3HAs of a certain range of carbon length (Anderson and Dawes, 1990). The PHA synthase of *Alcaligenes eutrophus* can polymerise 3HAs consisting of 3–5 carbon atoms whereas that present in *Pseudomonas oleovorans* can only accept 3HAs of 6–14 carbon atoms.

3.3 Properties **(**Byrom, 1987)

- PHAs can be degraded at a high rate (3-9 months) by many microorganisms into CO_2 and water using their own secreted PHA depolymerases (Jendrossek, 2001).
- Can be produced from renewable resources.
- Eco-friendly in nature.
- PHAs extracted from bacterial cells have properties similar to conventional plastics, such as polypropylene.
- Therefore, they are very good substitute of petrochemical thermoplastics (Poirier, 1999).

4. PHA production

4.1 Microorganisms Involved

PHAs are produced by many different bacterial cultures. *Cupriavidus necator* (formerly known as *Ralstonia eutropha* or *Alcaligenes eutrophus*) is the one that has been most extensively studied (Vaneechoutte *et al.*, 2004). A few important other strains that were recently studied include: *Bacillus* sp., *Alcaligenes* sp., *Pseudomonas* spp, *Aeromonas hydrophila*, *Rhodopseudomonas palustris*, *Escherichia coli*, *Burkholderia sacchari* and *Halomonas boliviensis* (Table 1).

4.2 Media used

The choice of media is important not only to supply optimal conditions for production but also to do so with high volumetric productivity so that a final product is economically competitive with the traditional plastics. As the major cost in production of PHAs is the medium (Saharan and Badoni, 2007), efforts are focused on finding cheap media. Significant cost reduction will be achieved if cheap media are found with the necessary requirements for PHAs production with high productivity (Ojumu *et al*., 2004). Cheap sources for fermentation include media containing molasses (Solaiman *et al.*, 2006), corn steep liquor (Nikel *et al*., 2006), whey (Koller *et al*., 2008), wheat and rice bran (Van-Thouc *et al*., 2008) starch and starchy wastewaters (Halami, 2008;), effluents from olive mill and palm oil mill (Bhubalan *et al*., 2007) activated sludge (Jiang *et al*., 2009) and swine waste (Cho *et al*., 1997). The choice of media, partly, depends on whether the microorganism is wild type or recombinant and whether it needs nutrient limiting conditions (Reddy *et al.*, 2003). Production of homopolymer or copolymer is another factor in the choice of media ingredients.

4.3 Fermentation process

Widespread and comprehensive studies have been carried out to develop efficient fermentations for the desired PHAs. Batch and particularly fed-batch (Kim *et al*., 1997) and continuous fermentations have been investigated (Durner *et al*., 2001; Zinn *et al*., 2003). Most PHAs fermentations are operated as two stage process (Tsuge, 2002). The main aim is to produce a high cell density culture in the first stage (growth) and then to increase PHAs concentration during the second stage which is usually a nutrient limited fermentation (Madison and Huisman, 1999). Fermentation conditions depend on the demands of the microbes. Usually temperature range of 30 to 37 ^{o}C along with low stirrer speeds, resulting in low dissolved oxygen tension, is adopted. pH is either left uncontrolled or is regulated linking to substrate (*e.g.* glucose) addition (Chung *et al.*, 1997). PHAs production in pure cultures is limited by an external nutrient whereas production in mixed cultures is induced by an intracellular limitation The use of open mixed cultures, such as activated sludge (Lemos *et al.* 2006) assist in decrease of PHAs cost, thus enhancing their market potential (Patnaik, 2005). It also increases the efficiency of fermentation (Tanaka *et al*., 1995). When cells are exposed to a

medium with very little amounts of nutrient for a long time, the bacteria are altered physiologically (Daigger and Grady, 1982). Sudden increase of carbon substrate concentrations causes the cell to change their physiology again. As PHA-synthesis requires less adaptation than growth, the culture starts producing polymer. This kind of fermentation is referred to as 'feast and famine' (Lemos *et al*., 2006).

4.4 Recovery

Recovery of PHAs is another process that significantly enhances production cost. The development of a cheaper safe downstream process PHA recovery will have significant impact on industrial production of this versatile biopolymer. Various separation methods for the recovery of PHA have been described (Table 2). In all these methods, it is essential to concentrate cells in order to achieve high yield of PHA. Often centrifugation, cross flow filtration and flocculation are the methods of choice. Subsequently, either water based separation or solvent based extraction can be chosen as isolation methods.

Most methods to recover intracellular PHA involve use of organic solvents, such as acetone (Jiang *et al*., 2006), chloroform, methylene chloride or dichloroethane. The recovery yield of this process is better than the alternative cell disruption followed by aqueous extractions cell disruption decreases the biopolymer's molecular weight (Ojumu *et al*., 2004). For medical applications the solvent extraction is a good method as resulting PHAs have a high purity (Chen and Wu, 2005). However, the necessity of large quantities of solvent makes the procedure economically and environmentally unattractive (Braunegg *et al*., 1998). As an alternative to the unfavourable extraction with organic solvents, aqueous enzymatic procedures (Lakshman and Shamala, 2006), treatments with ammonia (Page and Cornish, 1993) or digestion with sodium hypochlorite and surfactants (Ryu *et al*., 2000) have been proposed. Supercritical fluid disruption (Hejazi *et al*., 2003; Khosravi-Darani *et al*., 2004), dissolved-air flotation (Van Hee *et al*., 2006) and selective dissolution of cell mass (Yu and Chen, 2006) for the recovery of PHAs are recently used methods. All of these methods are promising alternatives to solvent extraction but none of these possess all the necessary requirements for an efficient and economical large scale process. The major drawbacks are cost, safety and scalability.

5. PHA synthesis in Microorganisms

5.1 Genes and enzymes involved in PHA synthesis

Many species of bacteria, which are members of the family *Halobactericeae* of the *Archaea*, synthesize PHAs. The list of such microorganisms is increasing and currently contains more than 300 organisms (Berlanga *et al*., 2006). The chemical diversity of PHAs is large; of which the most well-known and widely produced form is PHB (Kim and Lenz, 2001). Synthesis of PHB is considered through the simplest biosynthetic pathway. The process involves three enzymes and their encoding genes (Fig. 2) (Reddy *et al*., 2003). All these genes are clustered and organised in one operon phbCAB. phaA gene encodes β-ketothiolase, the first enzyme for the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA catalyzed by the acetoacetyl-CoA reductase (Steinbuchel and Schlegel, 1991). The enzyme is encoded by the phaB gene and is NADPH-dependent. The last reaction is the polymerization of (R)-3-hydroxybutyryl-CoA monomers catalyzed by PHA synthase, which is encoded by the phaC gene (Rehm, 2003).

6. Various systems for the production of bioplastics

6.1 Individual Bacterial system for the production of bioplastics

PHAs producing bacteria can be divided into two groups according to the culture conditions required for PHA synthesis. The first group requires the limitation of an essential nutrient(s) for the production of PHAs. Bacteria in this group include *Cupriavidus necator, Rhodopseudomonas palustris* and *Methylobacterium organophilum.* The second group synthesize PHAs alongside growth in the cultivation medium. Bacteria in this group include *Alcaligenes latus* and recombinant *E. coli* containing the PHA biosynthetic genes.

Cupriavidus necator has been widely studied because of its potential in producing significant amounts of P(3HB) from simple carbon substrates such as glucose, lactic acid and acetic acid (Ryu *et al*., 1997). Olive oil, corn oil and palm oil also have been used to produce approximately 80% dry cell weight (dcw) P(3HB) of dry cell mass from the organism (Futui *et al*., 1998). *Methylobacterium organophilum* uses only methanol as cheap carbon source to produce PHAs whereas *Rhodopseudomonas palustris,* a non-sulphur photosynthetic bacterium, has potential to produce P(3HB) and P(3HB-co-3HV) using different carbon and nitrogen sources. *Rhodospirilium* and *Rhodobacter* are two other non-sulphur photosynthetic bacterial genera that have been found to be particularly versatile in the production of the copolymer, P(3HB-co-3HV),

using various carbon sources such as malate, acetic acid and *n*-alkanoic acid(Carlozzi *et al*., 2001).

Alcaligenes latus is another organism that produces PHAs using carbon sources such as glucose, molasses and sucrose with good yields. Investigations with different nitrogen sources have proven that *A. latus* is able to grow and produce PHAs with ammonium chloride and ammonium sulphate as nitrogen sources. Ammonium nitrate and urea could not support PHA production in the organism (Grothe *et al*., 1999). It requires a temperature of 35 ^{o}C, which makes P(3HB) production from the organism economical by lowering the demand for cooling during fermentation.. Optimum biomass and PHA accumulation can be further enhanced by feeding the organism with enriched medium.

6.2 Mixed or Co-culture systems

Mixed or co-culture systems have also been shown to be effective for PHA production (Tanaka *et al*., 1995). *Cupriavidus necator* is unable to metabolize sugars, molasses, whey or starchy waste. Consequently, mixed cultures of lactic acid producing bacteria such as *Lactobacillus lactis* (Tanaka *et al*., 1995), *Propionibacterium* (Tohyama *et al*., 2002) and *L. delbrueckii* (Patnaik, 2005) and *C. necator* have been used in a single-stage fermentation system. The original sugar substrates are converted into lactic acid first, which is later taken up by *C. necator* to produce PHAs (Patnaik, 2005). In a two-stage system, xylose is first converted to lactate using *Lacticoccus lactis.* The lactate is further converted to P(3HB) by *C.necator*. *Lactobacillus delbrueckii* can also be used to convert glucose to lactate which is later converted to P(3HB) by *C. necator* (Tohyama *et al*., 2000).

Use of an anaerobic–aerobic sludge system could enhance PHA production (Satoh *et al*., 1992). It can be further enhanced by microaerophilic-aerobic conditions, by introducing limited amount of oxygen into the anaerobic zone of the anaerobic–aerobic system. Hence, it is concluded that the cost of PHA production can be reduced by using open or mixed cultures along with activated sludge system (Liu *et al*., 2008).

6.3 Genetically engineered bacteria

Recombinant organisms have been developed for enhancing PHA production. *E. coli* has been one of the most favoured host since growth-related PHA production is possible. Also, the ability to grow fast, to achieve high cell density from several inexpensive carbon sources (molasses and whey), and easy purification of the polymer from *E. coli* contribute to its popularity (Hahn *et al*., 1995). Owing to the massive acquaintance regarding *E. coli* genetics and its metabolic pathways, it plays a significant role in the commercial production of PHAs. Recombinant *E. coli* harbouring the *C. necator* PHA biosynthetic genes is able to accumulate P(3HB) with a yield of 80–90% dcw in fed-batch cultivation while a P(3HB) content of 76% dcw can be obtained in a pH-stat fed-batch culture (Kim *et al*., 1992). Liu and coworkers, obtained a P(3HB) concentration of 80% dcw when recombinant *E. coli* containing the *C. necator* PHA biosynthetic genes was grown on molasses (Liu *et al*., 1998). Genetically engineered (recombinant) *E. coli* with the *Aeromonas hydrophila* biosynthetic genes (*orf1*) can produce terpolymers of P(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3- hydroxyhexanoate) using decanoate and odd-chain fatty acids as carbon sources (Park *et al*., 2001).

7. Applications of PHA

PHAs are non-toxic, biocompatible, biodegradable thermoplastics produced from renewable resources. They have a high degree of polymerization, highly crystalline, optically active and isotactic (stereochemical regularity in repeating units), peizoelectric and insoluble in water. These features make them highly competitive with polypropylene.

These have a wide range of applications owing to their novel features. Initially, these were used in packaging films mainly in bags, containers and paper coatings. Similar applications as conventional commodity plastics include the disposable items, such as razors, utensils, diapers, feminine hygiene products, cosmetic containers, shampoo bottles and cups. These are also useful as stereo regular compounds that serve as chiral precursors for the chemical synthesis of optically active compounds (Senior and Dawes, 1973). Such compounds are particularly used as biodegradable carriers for long term dosage of drugs, medicines, hormones, insecticides and herbicides. They are also used as osteo synthetic materials in the stimulation of bone growth owing to their piezoelectric properties, in bone plates, surgical sutures and blood vessel replacements. However, the medical and pharmaceutical applications are limited due to the slow biodegradation and high hydraulic stability in sterile tissues (Wang and Bakken, 1998).

8. Biodegradation of PHAs

The property that differentiates PHA from petroleum based plastics is their biodegradability. PHAs are degraded upon exposure to soil, compost and marine sediment. Biodegradation is dependent on a number of factors such as microbial activity of

the environment, exposed surface area, moisture, temperature, pH and molecular weight of the bioplastics (Boopathy, 2000). The nature of the monomer units also has been found to affect degradation. Copolymers containing PHB monomer units have been found to be degraded more rapidly than either PHB or 3HB-co-3HV copolymers. Biodegradation of PHA under aerobic conditions results in CO_2 and water, whereas in anaerobic conditions the degradation products are CO_2 and methane. PHAs are compostable over a wide range of temperatures, even at a maximum of around 60 ^oC with moisture levels at 55%. About 85% of PHA can be degraded in seven weeks (Flechter, 1993).

9. Renewable nature and life cycle

PHAs production is biological and based on renewable resources (Braunegg *et al*., 2004). Fermentative production of PHAs uses agricultural feeds such as sugars and fatty acids as carbon and energy sources (Kadouri *et al*., 2005). The synthesis and biodegradation of PHAs are totally compatible to the carbon-cycle. PHAs receive general attention because they are based on renewable compounds instead of on fossil fuels (Gavrilescu and Chisti, 2005). The fermentation process to make PHAs is still to be optimized, while the production of petrochemical plastics is fully developed (Kim and Dale 2005).

10. Conclusions and future prospects

Mineral oil prices will get increase substantially in the next century, imposing the world to consider alternatives for petrochemical plastics. The renewable nature and biodegradability of PHAs make them appropriate resources to substitute synthetic plastics in many applications. Currently their production is expensive, but these plastics are only in their first stage of commercial development. Further research on recombinant microbial strains, mixed cultures, efficient fermentations, recovery / purification and the use of inexpensive substrates can substantially reduce the production cost. Therefore, the future of bioplastics depends on the efforts towards fulfilling price as well as performance requirement. Microbial synthesis of PHA seems to be an inexhaustible game; we can either make homopolymers with diversified monomers, or copolymers or block copolymers of various combinations. Because of their special characteristics and broad biotechnological applications, PHAs have an extremely promising future.

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Table 1: List of Bioplastics produced by various organisms along with their substrates.

Polymer(s)	Bacteria	Carbon Source(s)	Reference(s)
PHB	*Alcaligenes eutrophus*	Gluconate Propionate Octanoate	Liebergesell *et al*. (1994)
	Bacillus megaterium	Glucose	Mirtha *et al*. (1995)
	Klebsiella aerogenes recombinants	Molasses	Ackermann and Babel (1997)
	Methylobacterium rhodesianum	Fructose/Methanol	Borque *et al*. (1995)
	M. extorquens	Methanol	
	P. putida	Octanoate	Liebergesell *et al*. (1994)
	Sphaerotilus natans	Glucose	Takeda *et al*. (1995)
PHA	*Psuedomonas aeruginosa*	Euphorbia Castor Oil	Eggink *et al*. (1995)
	P. putida	Palm kernel oil Lauric acid Myristic acid Oleic acid	Tan *et al*. (1997)
P(3HV)	*P. denitrificans*	Methanol Pentanol	Yamane *et al*. (1996)
	P. oleovorans	Gluconoate Octanoate	Liebergesell *et al*. (1994)
5POHV	*P. putida*	11-Phenoxyun-decanoic acid	Song and Yoon (1996)

PHB—(polyhydroxybutyric acid), P(3HV)—polyhydroxvaleric acid, 5POHV—poly(3 hydroxy-5-phenylvalerate

Table 2: List of Methods of recovery of Bioplastics

S.No	Method(s)	Advantage(s)	Disadvantage(s)	Reference(s)
1	Simple digestion	Efficient and Economical	Low Purity	Page and Comish (1993)
2	Solvent Extraction	High Purity	Require large quantity of solvent High production cost Use toxic and volatile solvents	Ramsay *et al* .(1994), Choi and Lee (1997), Saharan *et al.* (2011)
3	Hypochlorite Digestion	High Purity	Severe degradation of non cellular PHAs	Berger *et al.* (1989)
4	Enzymatic Digestion	High Purity	Complex and Expensive	Holmes and Lim (1990)
5	Dispersion Process	Low polymer degradation	Large amount of solvent is required	Hahn *et al.*(1993)

Figures

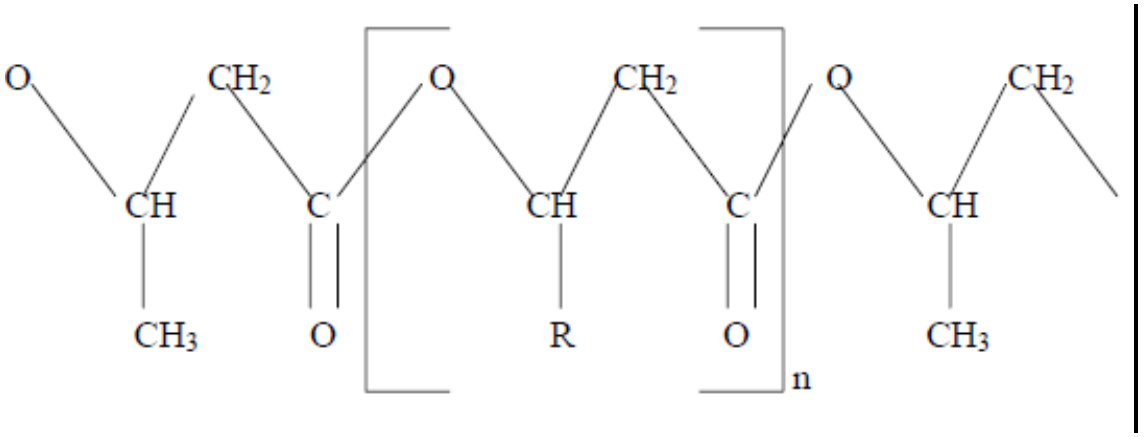

Fig.1: Structure of PHA (Courtesy: Lee, 1995; Brandl *et al*., 1990)

[*n varies from 600 to 35000; R= hydrogen Poly (3-hydroxypropionate); R=methyl Poly (3-Hydroxybutyrate); R=ethyl Poly (3-hydroxyvalerate); R=propyl Poly (3-hydroxyhexanoate); R=pentyl Poly (3-hydroxyoctanoate); R=nonyl Poly (3-hydroxydodecanoate)]

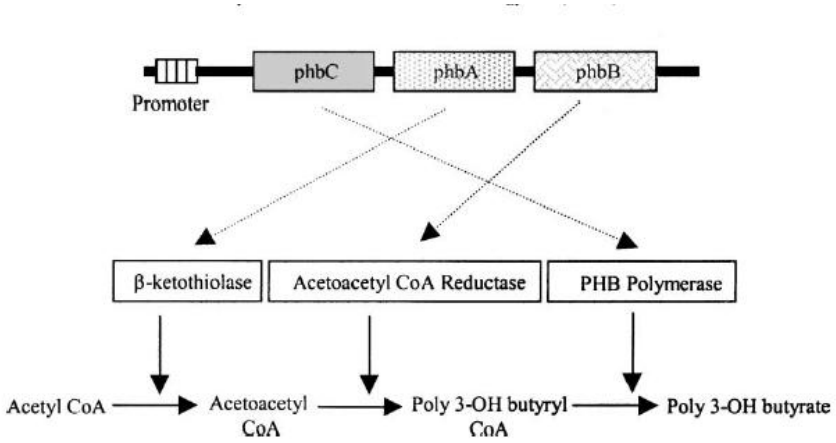

Fig. 2: Genes and enzymes involved in PHA synthesis (Courtesy: Madison and Huisman, 1999).

Biosynthetic pathway of poly(3-hydroxybutyrate). P(3HB) is synthesized by the successive action of b-ketoacyl-CoA thiolase (phbA), acetoacetyl-CoA reductase (phbB) and PHB polymerase (phbC) in a three-step pathway. The genes of the phbCAB operon encode the three enzymes.