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Title: Polyhydroxyalkanoates (PHA): natural polymers produced by bacteria, an option for the replacement of plastics

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Introduction

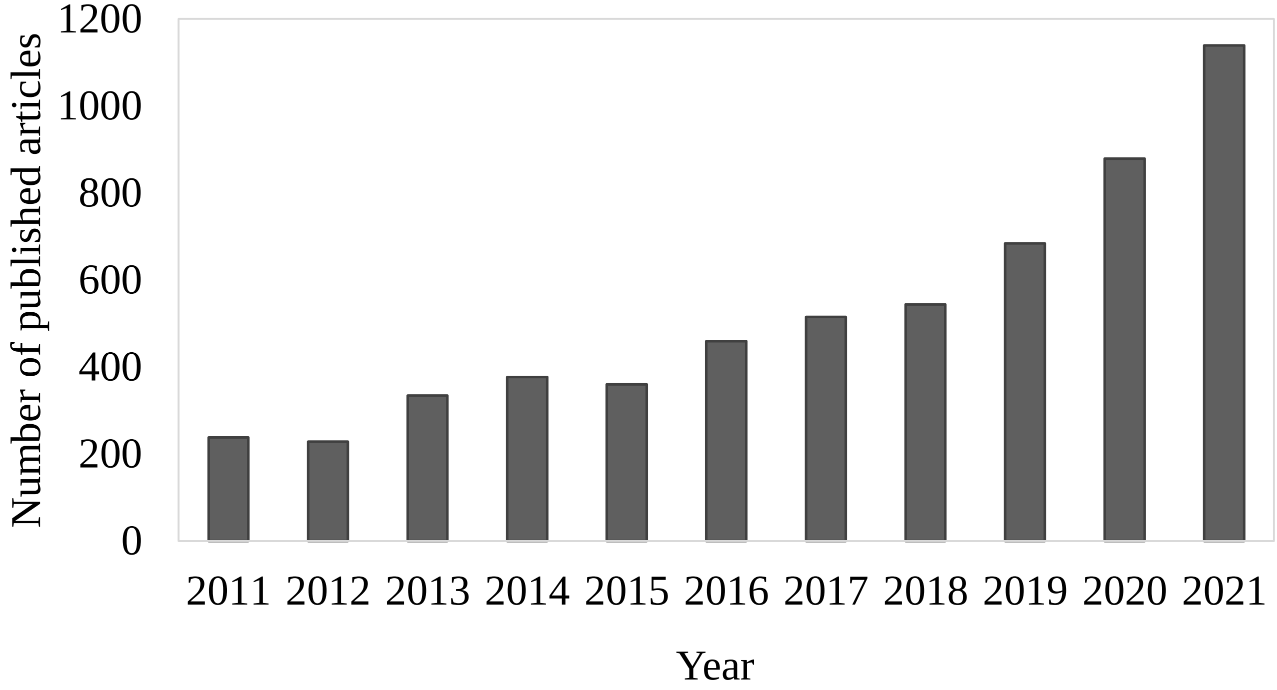
The production of plastics has increased significantly, reaching 350 million tons annually

Five countries originating the most significant amounts of plastic pollution are China, Indonesia, the Philippines, Vietnam and Sri Lanka

Polyhydroxyalkanoates

TianAn, a Chinese company - production of 10 thousand to 50 thousand tons per year.
Nodax, a U.S. company, - 91 thousand tons per year

Graph 1 Articles published in Science direct on Polyhydroxyalkanoates



Chemical structure of PHA

PHAs are linear polymers that form ester bonds between the carboxyl group of one monomer and the hydroxyl group of the next.

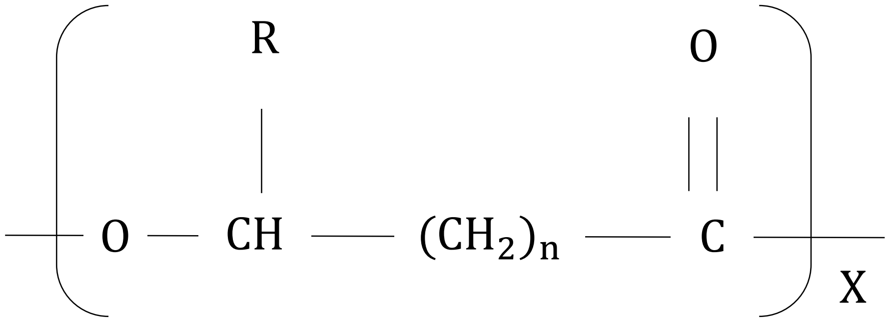


Figure 1. The general structure of PHAs

Table 2. PHA Classification

Chain size	Strains
Short (3-5 carbons)	*Rastonia eutropha* *Aeromonas vinosum* *Bacillus megaterium*
Medium (6-14 carbons)	*Pseudomonas aeruginosa*
Long (15 or more carbons)	

R group	No. of carbons	PHA polymer
Metyl	C_4	Poly(3-Hydroxybutyrate)
Etyl	C_5	Poly(3- hidroxivalerate)
Propyl	C_6	Poly (3-hydroxyhexanoate)
Butyl	C_7	Poly (3-hydroxyheptanoate)
Pentyl	C_8	Poly(3-hydroxyoctanoate)
Hexyl	C_9	Poly(3-hydroxynonanoate)
Heptyl	C_{10}	Poly(3-hydroxydecanoate)
Octyl	C_{11}	Poly (3-hydroxyundecanoate)
Nonyl	C_{12}	Poly(3-hydroxydodecanoate)

Isolation sources and nutritional factors that affect its production of PHA

Soil samples

Dairy products

Used oils

Marine Sediments

Concentration and type of carbon source

Yüksekdag *et al.* (2007) results showed that the percentage of PHB increased when using sucrose (35.56%) compared to the control (glucose) (12.47%)

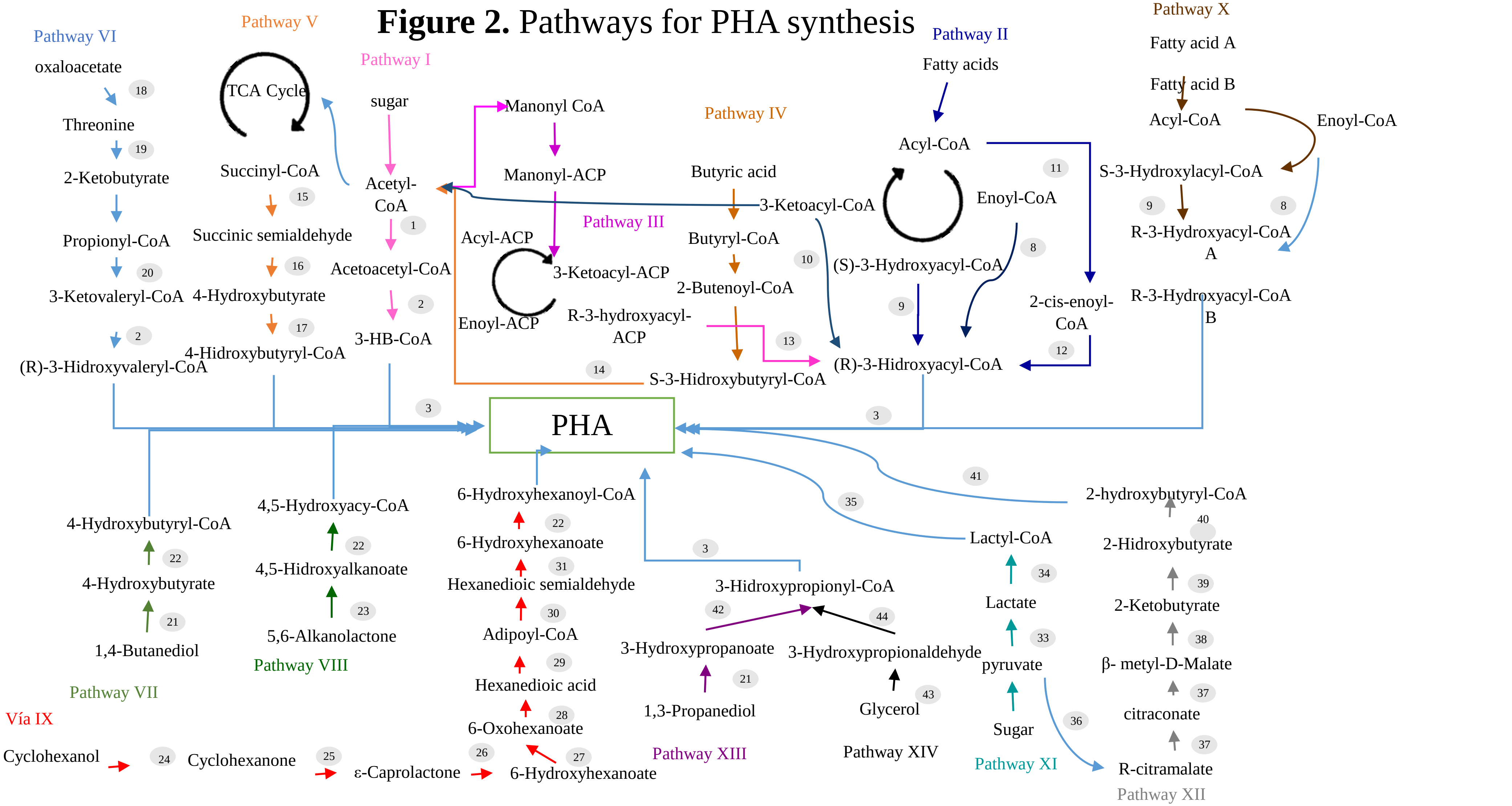
Nitrogen source concentration

Sharma *et al.* (2012), determined that the highest production occurred when using nitrogen at low concentrations

Metabolic pathways for the synthesis of PHA.

A total of 14 pathways have been reported for PHA synthesis

Figure 2. Pathways for PHA synthesis



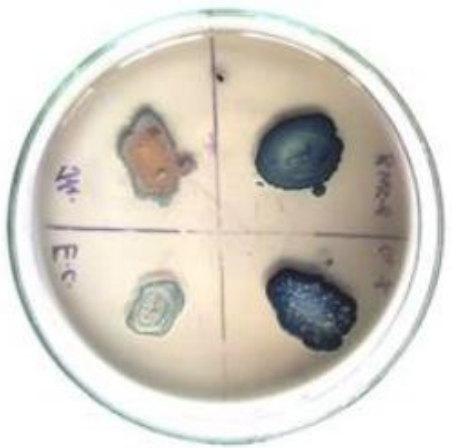
Enzymes for PHA synthesis: 1(β-Ketothiolase);2 (NADPH dependent acetoacetyl-CoA); 3(PHA synthase); 8 (R-Enoyl-CoA hydratase); 9 (Epimerase); 10(3-ketoacyl-CoA reductase);11(Acyl-CoA oxidase, putative); 12 (Enoyl-CoA hydratase, putative); 13 (3-Hydroxyacyl-ACP-CoA transferase); 14 (NADH-dependent acetoacetyl-CoA reductase);15 (Succinic semoaldehyde dehydrogenase); 16 (4-Hydroxybutyrate dehydrogenase); 17(4-Hydroxybutyrate-CoA transferase); 18 (Aspartokinase I, Homoserine kinase, Threonine synthase); 19 (Threonine deaminase); 20 (BktB(PhaA)); 21 (Alcohol dehydrogenase, Aldehyde dehydrogenase); 22 (Hydroxyacyl-CoA synthase, putative); 23 (Lactonase, putative); 24 (Cyclohexanol dehydrogenase); 25(Cyclohexanone monooxygenase); 26 (Caprolactone hydrolase); 27 (6-Hydroxyhexanoate dehydrogenase); 28 (6-Oxohexanoate dehydrogenase); 29 (Semialdehyde dehydrogenase, putative); 30 (6-Hydroxyhexanoate dehydrogenase, putative); 31 (Hydroxyacyl-CoA synthase, putative); 32 (3-Ketoacyl-CoA thiolase, 3-hydroxyacyl-CoA dehydrogenase); 33 (Lactate dehydrogenase); 34(Propionate CoA-transferase); 35 (Type II PHA syhthase); 36(α- Isopropylmalate synthase); 37(3-Isopropylmalate dehydratase); 38 (3-Isopropylmalate dehydrogenase); 39 (2-Hydroxybutyrate dehydrogenase); 40 (Propionate CoA-transferase);41(Type II PHA syhthase); 42 (Propionyl-CoA synthase); 43 (Glycerol dehydratases); 44 (Propionaldehyde dehydrogenase). ***Source****:* Modified from Meng *et al.*,2014

Physicochemical properties of PHAs

Samples	Tm	σ (MPa)	ε (%)
PHB	177	43	5
P(3HB-co-20%mol3HV)	145	20	50
P(3HB-co-16%mol4HB)	150	26	444
P(3HB-co-15%mol 3HHx)	115	23	760
P(HB-co-10%mol HV)	150	25	20
P(HB-co-20%mol HV)	135	20	100
P(HB-co-10%mol HHx)	127	21	400
P(HB-co-17%mol HHx)	120	20	850
Polypropylene	170	34	400
Polystrene	110	50	-
Polyethylene	130	-	500
HDPE	135	29	620
LDPE	130	10	7300
PET	262	56	-

Tm: melting temperature; σ: Tensile strength; ε: Elongation at break; HDPE: high-density polyethylene; LDPE:low-density polyethylene; PET: poly(ethylene terephthalate). *Source:* modified of Muhammady *et al.*, 2015

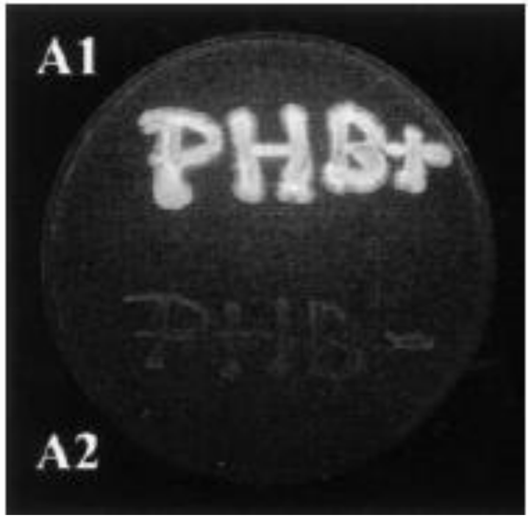
Method for the identification of PHA-producing bacteria



Transmission electron microscopy of the *Vibrio* MAT-28 bacterium isolated from the Ebro river, Catalonia.

- ❖ Sudan black dye B
- ❖ Ethanol
- ❖ Xylene

Mohammed *et al.* in 2019, isolated two bacteria (different *Bacillus* species) and tested positive using Sudan black B and Nile blue A.



- ❖ Nile blue A or Nile red
- ❖ Dimethyl sulfoxide
- ❖ Ultraviolet light

Ching *et al.* (2007) isolated bacteria from marine sediments and four colonies were positive in the Nile red staining test (the genus *Vibrio*)

Methods used for PHA Extraction

Cell disruption and removal of the protein sheet surrounding the PHA granules are necessary to extract PHA granules.

Solvent extraction

Samori *et al.* (2015) used this solvent to extract PHA from mixed cultures (*Amaricoccus sp.*, *Azoarcus sp.* and *Thauera*). They obtained a purity of 98 % and a polymer molecular weight of 1.2 MDa.

Chemical digestion

Ramsay *et al.* (1990) extracted PHB from *Alcaligenes eutrophus* using sodium hypochlorite and surfactants (SDS and Triton X-100). They obtained purity of 97 to 98 % with a molecular weight between 730000 Da and 790000 Da when using surfactants.

Physical methods

Hwang *et al.* (2006) obtained PHA and synthesized *Haloferax mediterranei* using ultrasonication with an amplitude of 20 kHz and a power of 525 W.

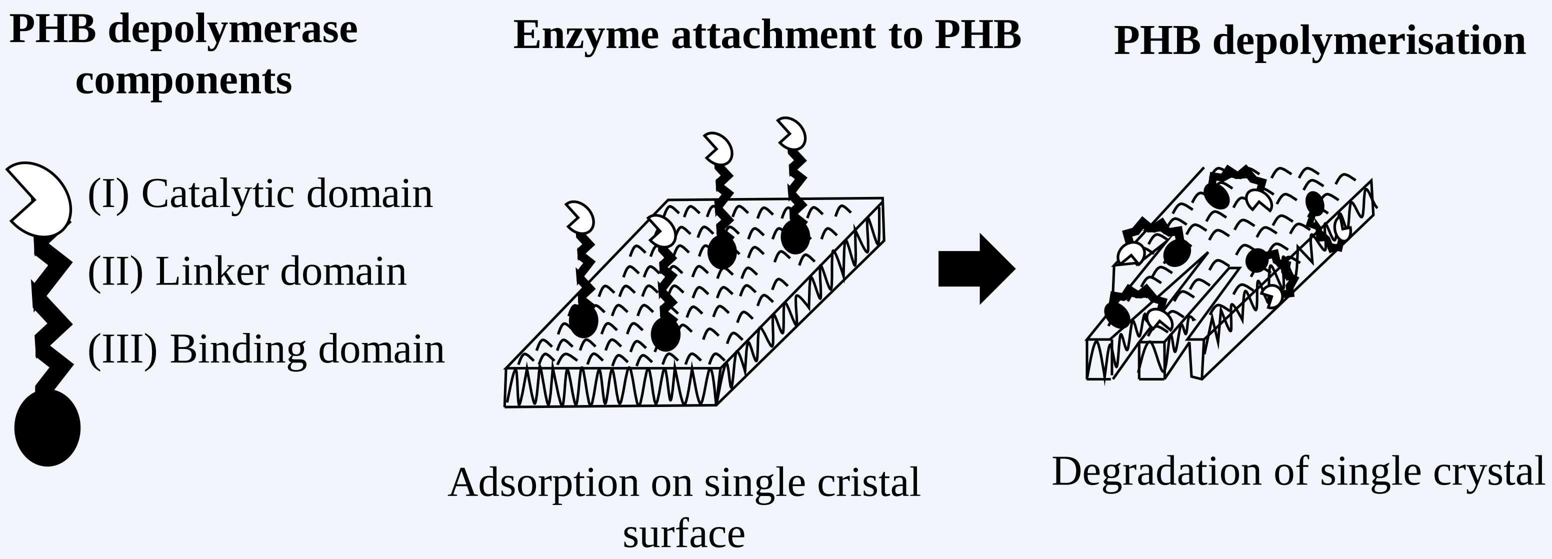
Methods for structural characterization of PHA

The method used for the structural characterization of PHA is Fourier transform infrared spectroscopy (FTIR). This method can be combined with gas chromatography coupled to mass (GCMS) which helps to quantify and determine the proportion in which each PHA is present in the structure.

FTIR analysis		GC analysis:
Wave-number (cm^{-1})	Possible PHA components	**PHA monomer composition (%)**
1728 **1262**	PHB	100HB
1739 **1261** **2925**	HB and mclHA	92HB, 8HD
1739 **1260** **2924**	HB and mclHA	98HB, 2HO
1744 **1165** **2926**	mclHA	0.3HB, 58HO, 41HD
1744 **1162** **2926**	mclHA	22HO,78 HD

FTIR analysis		GC analysis:
Wave-number (cm^{-1})	Possible PHA components	**PHA monomer composition (%)**
1744 **1665** **2928**	**mclHA**	**0.6HB,19HO, 80HD**
1744 **1165** **2928**	mclHA	0.4HB,20HO, 80HD
1739 **1257** **2926**	HB and mclHA	23HB, 39HO, 38HD
1739 **1258** **2926**	HB and mclHA	14HB, 50HO, 36HD
1739 **1257** **2926**	HB and mclHA	22HB, 40HO, 38HD

Biodegradability of PHAs



(I) a binding domain responsible for surface adsorption and breakdown of the polymer structure
(II) a linker domain that joins the binding domain to the catalytic domain
(III) a catalytic domain that cleaves the PHA and any available dimer/trimer in two parts

Bacterial genera:
Bacillus
Clostridium
Comamonas
Enterobacter
Klebsiella
Pseudomonas
Staphylococcus
Streptomyces

Fungi:
Acremonium
Aspergillus
Candida
Paecilomyces
Emericelopsia

Applications of the PHA

Conrrent		*Futures*
Garbage, gift and transport bags	**Packaging**	Bottles
Disposable gloves, disposable aprons	**Food services**	Food containers and utensils
flowerpot	**Agriculture**	Slow release of fertilizers into the soil
Absorbable sutures and meshes	**Medical**	Microencapsulation, slow release drug for formulations, bone plates, gowns
Plastic additives	**Chemicals**	Adhesives, paints, coatings, fine chemicals
	Automotive	Automotive parts

Conclusions

- ❖ The accumulation of plastics in the soil and the oceans is becoming more evident and alarming.
- ❖ This problem is caused due to poor waste management by governments and the industry's increased plastic production.
- ❖ Recyclable materials have been proposed to reduce the accumulation of plastics. However, people's lack of awareness causes the recycling process to be inefficient. It is easier to throw garbage on the streets where we walk than find a recycling point.
- ❖ PHAs have the advantage of the show a wide variety of structures that can be used individually or in combination to improve their characteristics.
- ❖ PHA is that it can be degraded by enzymes of the PHA-producing bacteria or bacteria living naturally in soils and seas. They can be degraded in less than two months, depending on environmental conditions.
- ❖ The varied applications of PHA have led to their industrial production in different countries such as China, the United States and Canada.

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