Poliesteri di origine microbica: produzione e applicazioni

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"I Poliesteri: produzioni e lavorazioni"
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OUTLINE

Biodegradable plastics

Biosynthesis of Polyhydroxyalkanoates (PHAs)

Production of PHAs by fermentation

Properties of PHAs

Applications of PHAs

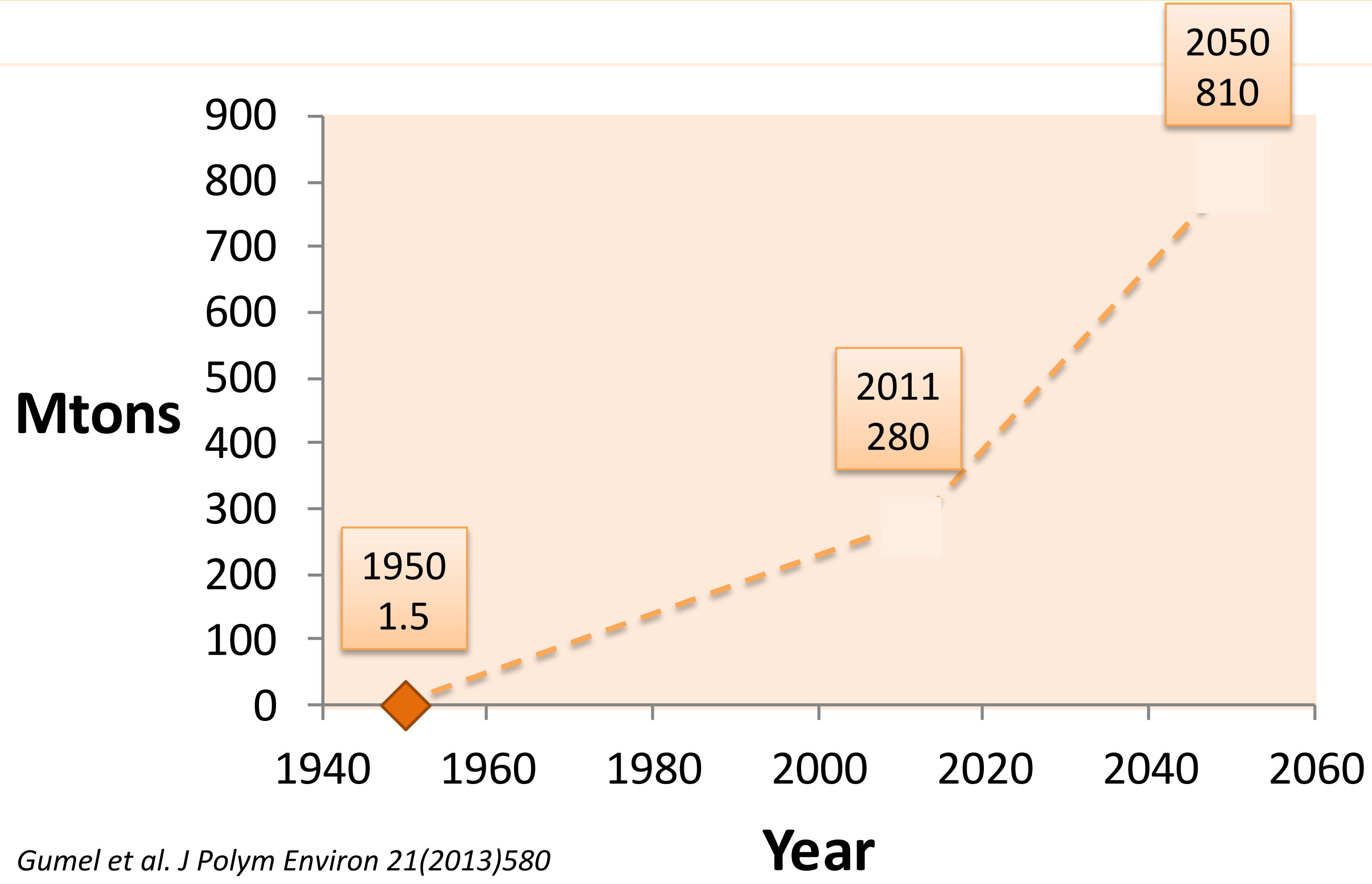
OIL-BASED PLASTICS

- Low production costs
- Chemical/mechanical inertness
- Excellent durability
- Versatile properties
- Wide range of applications:
    - Components of automobiles, home appliances, computers, packages, construction, sport, medicine, etc.

http://www.telegraph.co.uk/science/7450769/The-Biggest-Dump-in-the-World.html

- Single use application
- Non biodegradable
- If improperly discharged they accumulate in the ecosystem
- Burden on solid waste management
- High environmental impact

WORLD PRODUCTION OF PLASTICS



Gumel et al. J Polym Environ 21(2013)580

- Roughly 85% of plastics could technically be substituted with biobased plastics

BIODEGRADABLE PLASTICS

- Biosynthetic:
  - Sustainable production using renewable resources and biotechnological tools
- Biodegradable
- Biocompatible
- Recyclable
- Compostable (with organic waste)
- Zero toxic waste (H_2O, CO_2, ...)

POLYMERS FOR PLASTICS

Bio-based

Bio-based resources manufactured into non-biodegradable polymers:
- 1,3 propanediol (PDO) from corn sugar (Bio-PDO™)
- bioethanol-ba[...]
  polyethylene ([...]

Bio-based polymers biodegradable or compostable:
- polylactide (PLA) (Ingeo™)
- starch-basedmaterials (Mater-Bi™)
- [...] NatureFlex™)
- [...]s (Ecoflex®)

Bio-plastics

Non biodegradable

Biodegradable

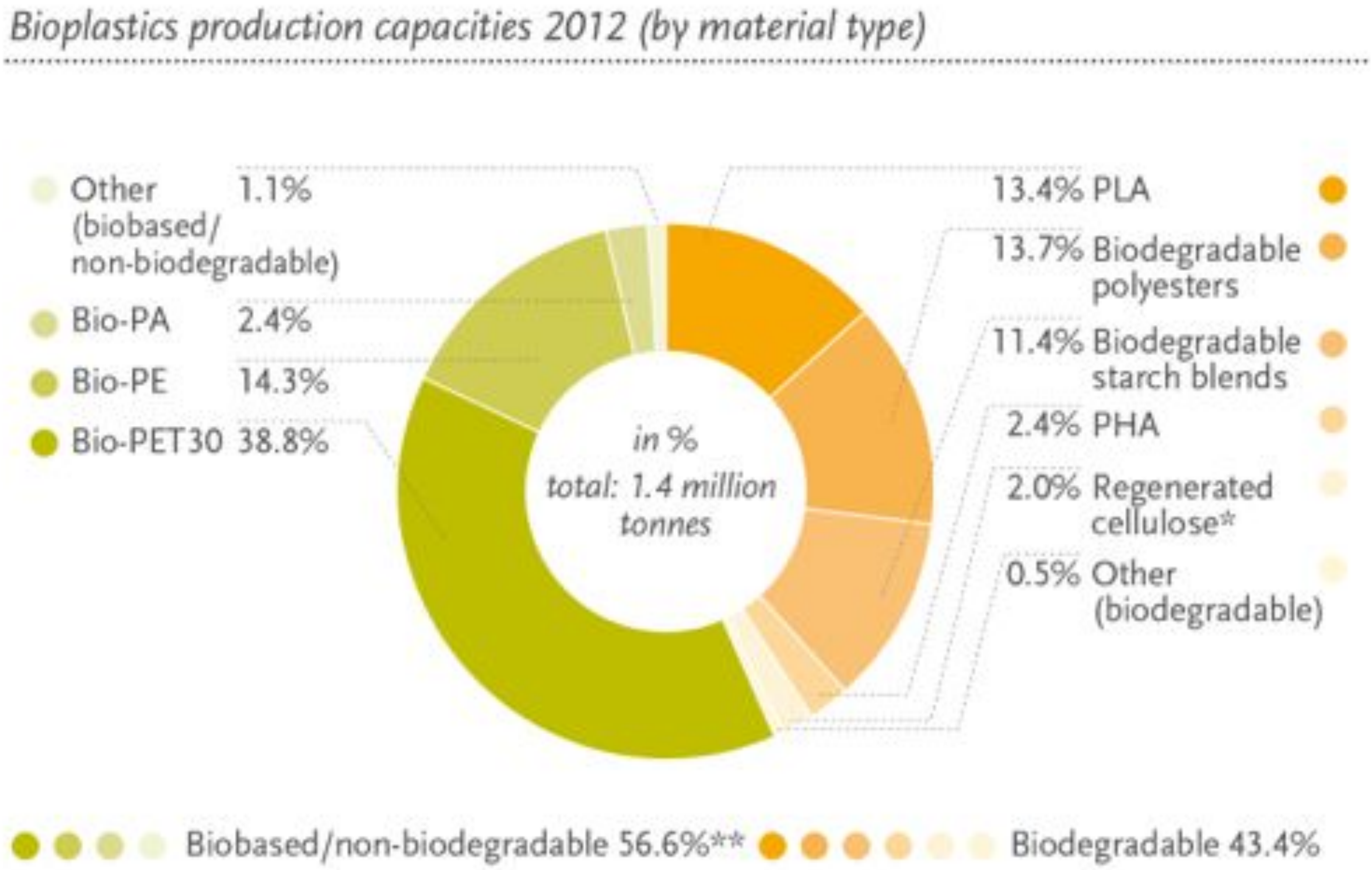
Conventional plastics:
- polyethylene terephthalate (PET)
- polyethylene (PE)
- polypropylene (PP)
- etc.

Biopolymers not bio-based but biodegradable or compostable:
- polybutylene terephthalate adipate (PBTA)
- polybutylene terephthalate succinate (PBTS)
- polybutylene terephthalate glutarate (PBTG)

Fossil-based

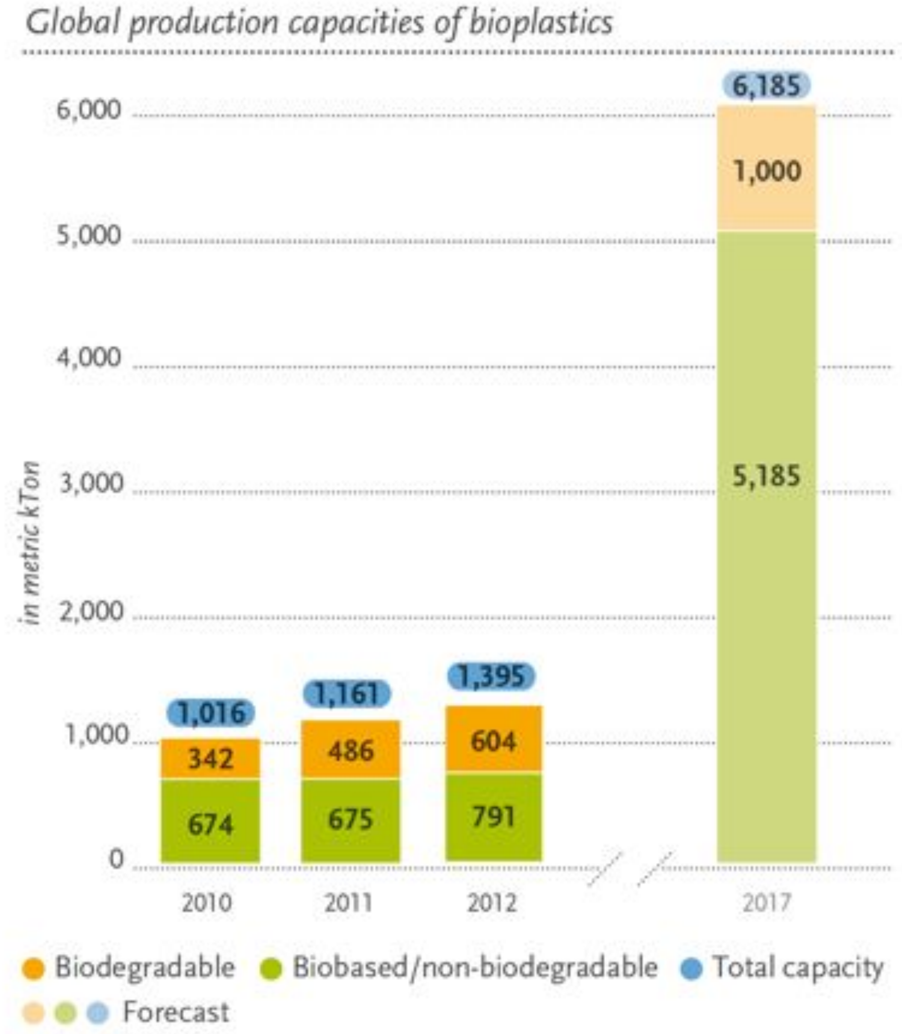
http://en.european-bioplastics.org/

BIOPLASTICS PRODUCTION



http://en.european-bioplastics.org/

BIOPLASTICS PRODUCTION



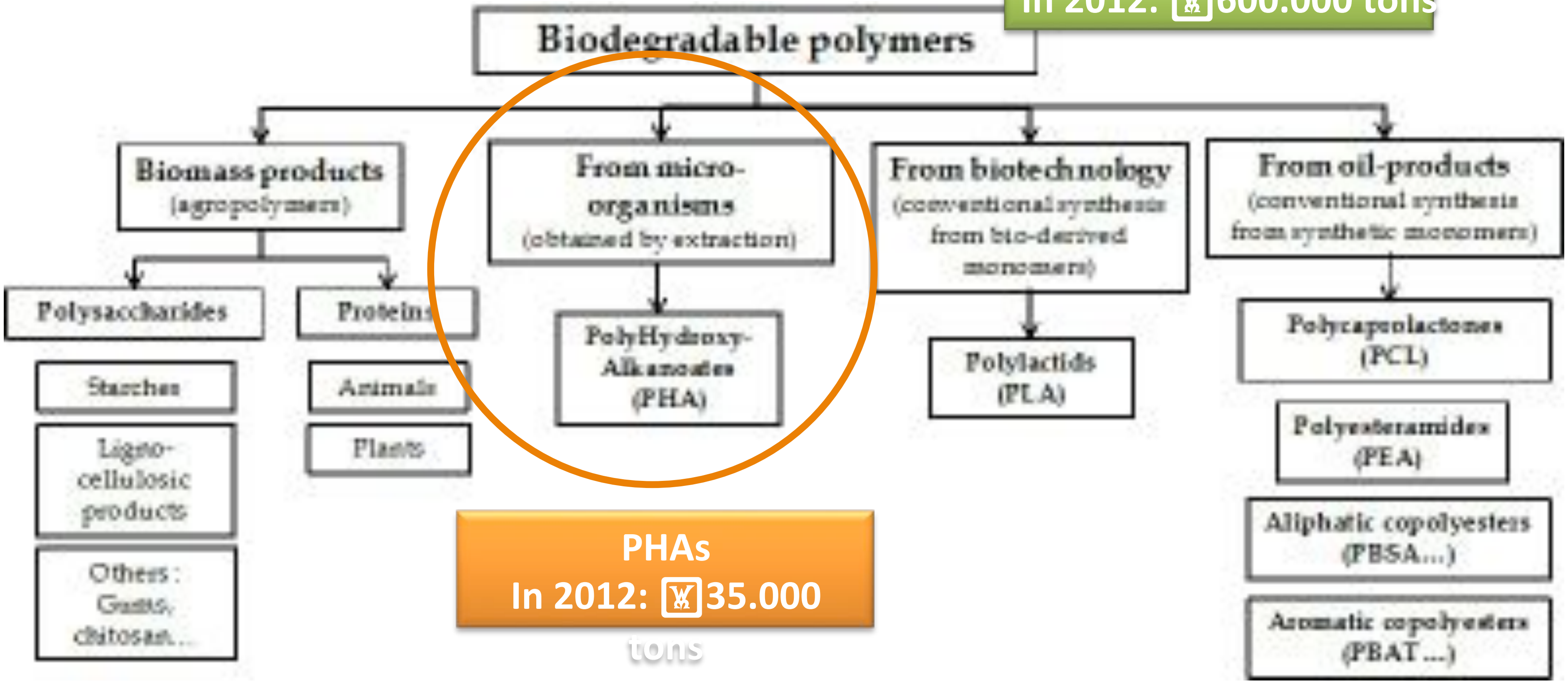
Growing demand for more sustainable solutions is reflected in growing production capacities of bioplastics:

- in 2012 production capacities amounted to approximately 1.4 million tonnes
- Market data of European Bioplastics forecasts production capacities will multiply by 2017 to more than 6 million tonnes

http://en.european-bioplastics.org/

BIODEGRADABLE POLYMERS

In 2012: ⌧600.000 tons



PHAs
In 2012: ⌧35.000 tons

Averous. J Macromol Sci. Polymer Reviews, C44(2004)231

POLYHYDROXYALKANOATES (PHAs)

- PHAs are a family of **biological polyesters**
- Synthesized by microorganisms (**bacteria**)
- Under conditions of **limiting nutrient** (N, P, S, Mg, O_2) and **excess of Carbon** source
- PHAs are storage compounds of carbon and energy

1920s: M. Lemoigne isolated a polymer (PHB) from the bacterium *Bacillus megaterium*

1959: W. R. Grace and Company produced PHB for commercial (US Patent No. 3225766)

1970: PHBV was commercialized by ICI/Zeneca BioProducts (Biopol™). In 1996 the technology sold to Monsanto and then to Metabolix, Inc. (recombinant *E. coli*)

1990s: Big players entered the PHB business: P&G, Kaneka Corp., Biomer Inc. (Germany), PHB Industrial S.A. (Brasil), Biomatera Inc. (Canada), Mitsubishi Gas Chemical, etc.

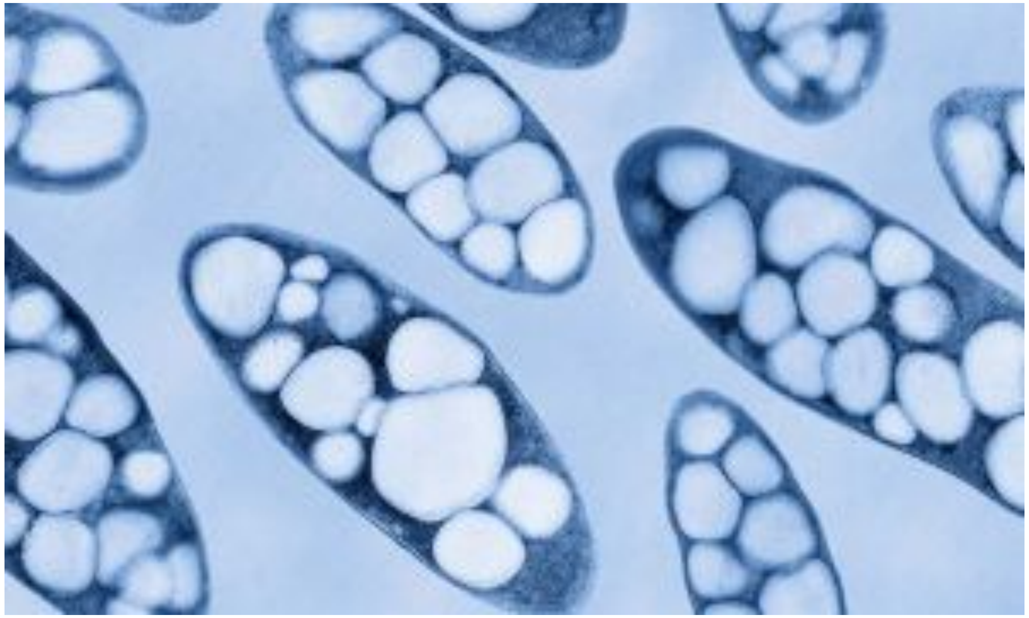
Today: More than 20 companies have been established globally for PHAs production

BIOSYNTHESIS OF POLYHYDROXYALKANOATES (PHAs)

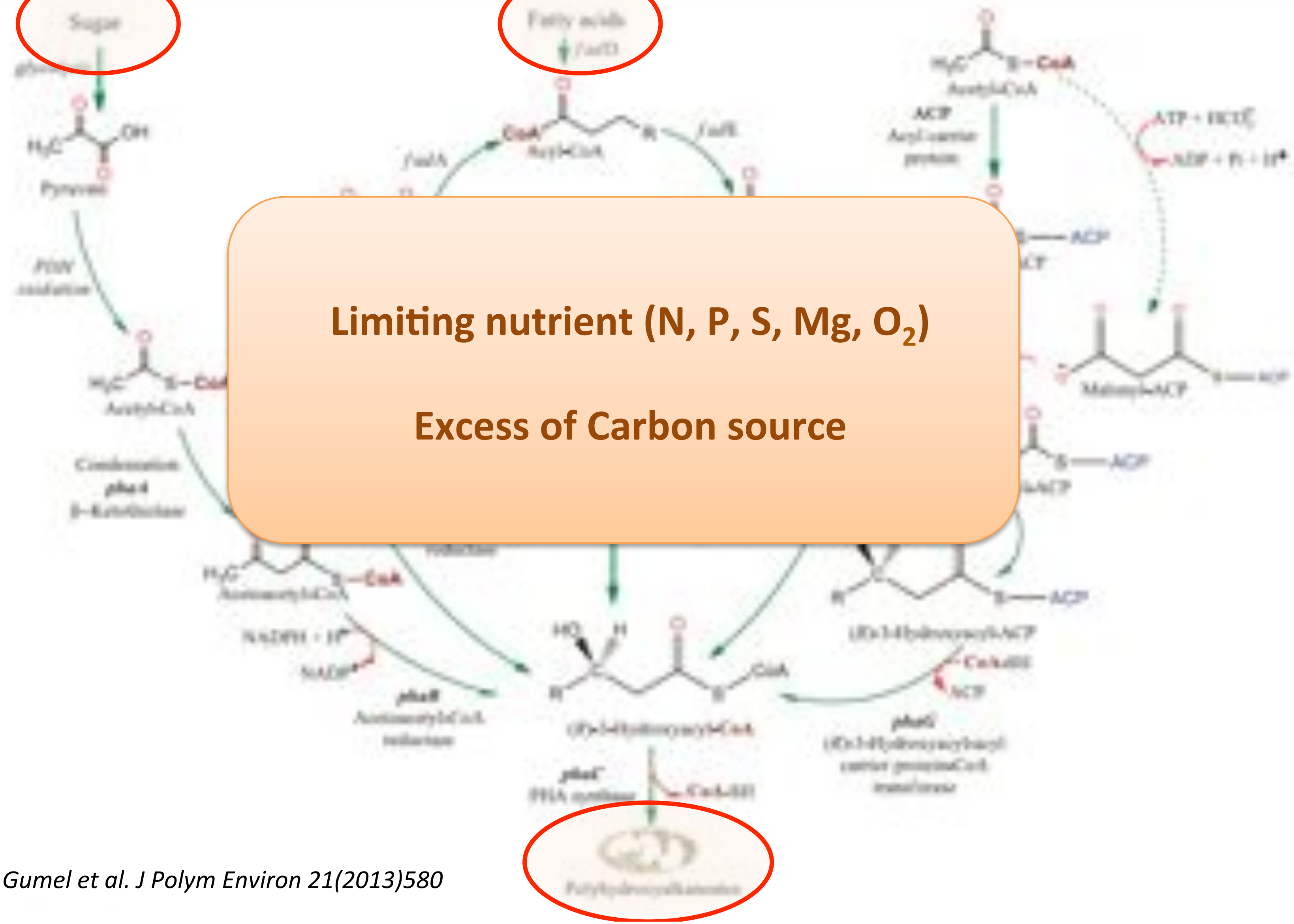
- >**300** PHA producers are known (bacteria)

- PHA producers reside at ecological niches naturally or accidentally exposed to **high organic matter** or growth limiting conditions:
  - hydrocarbon **contaminated** sites
  - pulp and paper mill **wastes**
  - agricultural **wastes**
  - activated **sludges**
  - industrial **effluents**, etc.

- Few are exploited for PHA production:
  - *Cupriavidus necator*
  - *Alcaligenes latus*
  - *Azotobacter vinelandii*
  - *Pseudomonas oleovorans*
  - *Paracoccus denitrificans*
  - **Recombinant *E. Coli***



BIOSYNTHETIC PATHWAYS OF POLYHYDROXYALKANOATES (PHAs)



Limiting nutrient (N, P, S, Mg, O_2)

Excess of Carbon source

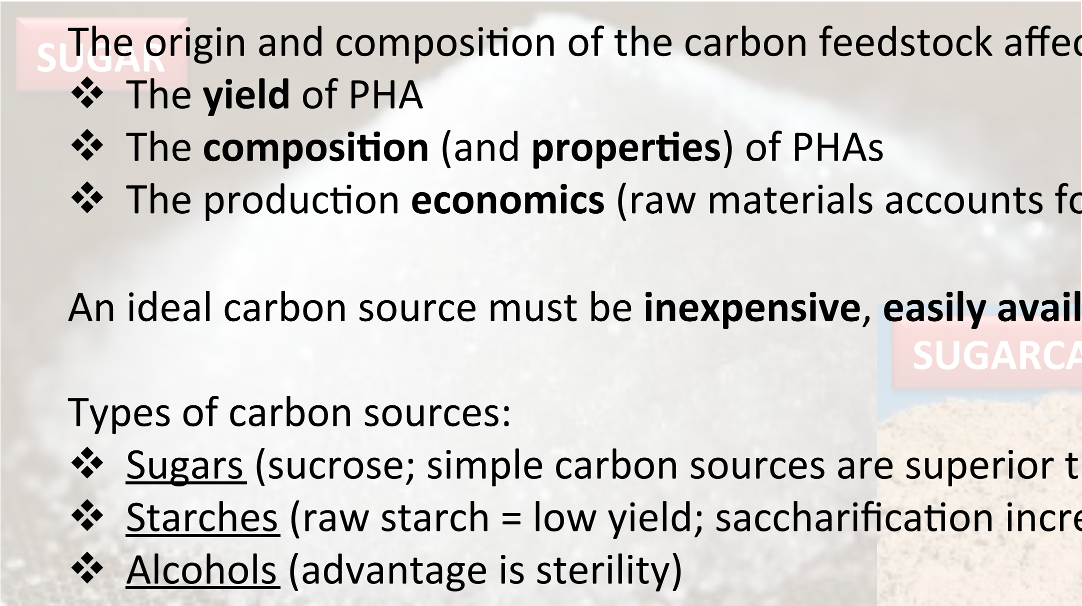
Gumel et al. J Polym Environ 21(2013)580

PRODUCTION OF PHAs BY MICROBIAL FERMENTATION

FERMENTATION PLANT

- Microbial fermentation is the conversion of organic materials into relatively simple substances by microorganisms
- <u>Selection of the microorganism</u>
  - Wild type or engineered *E.coli*
  - Single bacterial strain (need of sterility)
  - Mixed culture (enhance PHA productivity; no sterility needs; reduces overall costs)
- **<u>Selection of the carbon source</u>**
  - Optimum composition (C/N ratio)
  - Optimum feeding strategy
- <u>Selection of the operation method</u>
  - Mode of fermentation (batch, fed-batch, continuous; single or multi-stage)
  - Bioreactor type (air-lift reactor, continuous stirred tank reactor)
- <u>Optimization of fermentation conditions</u>
  - Control of microorganism metabolism
- **<u>Downstream processing</u>**
  - Extraction (cell membrane must be broken)
  - Purification of PHAs

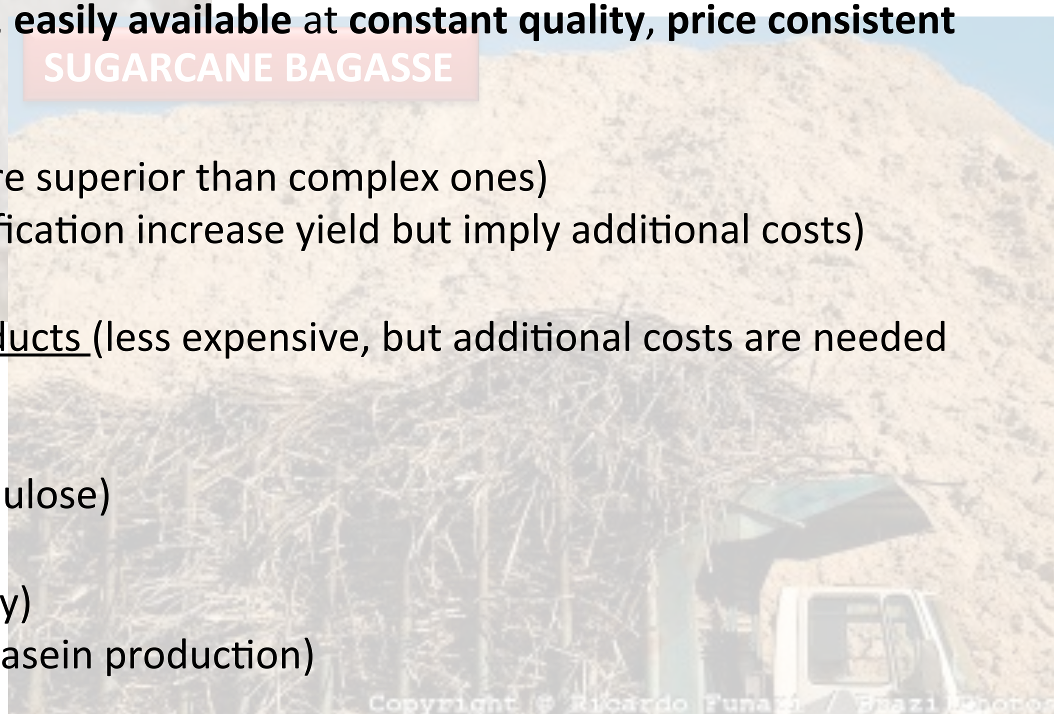
SELECTION OF THE CARBON SOURCE



The origin and composition of the carbon feedstock affect:

- The **yield** of PHA
- The **composition** (and **properties**) of PHAs
- The production **economics** (raw materials accounts for 30–40% of the total cost of PHAs)

An ideal carbon source must be **inexpensive**, **easily available** at **constant quality**, **price consistent**



Types of carbon sources:

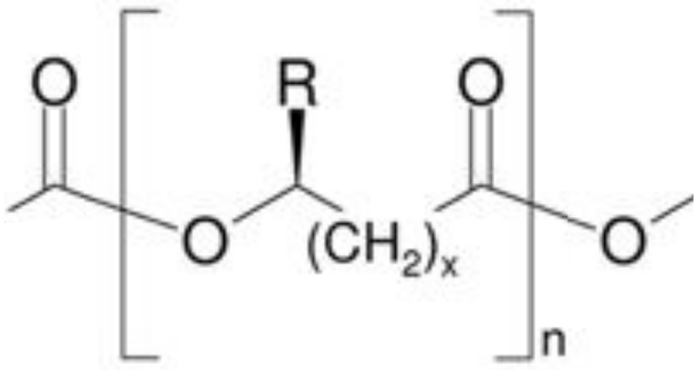
- Sugars (sucrose; simple carbon sources are superior than complex ones)
- Starches (raw starch = low yield; saccharification increase yield but imply additional costs)
- Alcohols (advantage is sterility)
- Agricultural wastes and industrial by-products (less expensive, but additional costs are needed for pre-treatment and purification):
  - Molasses (from sugar production)
  - Sugarcane bagasse (cellulose/hemicellulose)
  - Fatty acids and vegetable oils
  - Waste glycerol (from biodiesel industry)
  - Cheese whey (from milk, cheese and casein production)

DOWNSTREAM PROCESSING

Recovery method	Advantages	Recovery agent	Microorganism	Yield[a] (purity) (%)	References
Solvent extraction	High purity; endotoxin removal; limited polymer degradation	Chloroform, methanol	*Halomonas campisalis* MCM B-1027	36.82	[64]
		Chloroform, hexane	*Cupriavidus necator* A-04	78	[27]
		Chloroform, hexane	*Wautersia eutropha* ATCC 17699	90	[67]
		Dichloromethane, hexane	*Pseudomonas oleovorans*	38	[68]
		Chloroform	Mixed microbial culture	77	[43, 44]
Mechanical disruption	Less use of chemicals; reduced polymer degradation	Sonication, chloroform	*Alcaligenes latus* DSM1123	95	[70]
Chemical digestion	No polymer degradation; high purity; applicable to large volumes and high cell densities	NaClO, chloroform/ethanol	*Escherichia cloacae* SU-1	94	[71]
		SDS, LAS-99, ES702, AOS-04, Brij®58, NaOH	*Ralstonia eutropha*, *Escherichia coli*	99(90)	[72]
Enzymatic digestion (with or without mechanical treatment)	Good polymer recovery; high purity; reduced use of chemicals other than enzymes	Alcalase, SDS, EDTA	*Pseudomonas putida*	90(92.6)	[75]
		Benzonase, Alcalase, lysozyme, flavourzyme; microfluidizer	*P. putida* PGA1	(99.2)	[76]
Supercritical fluids	Low toxicity; low cost; high polymer purity	CO_2	Bacterial cells	90(99)	[79]

[a] Yield is given in terms of PHA content (% of cell dry weight)

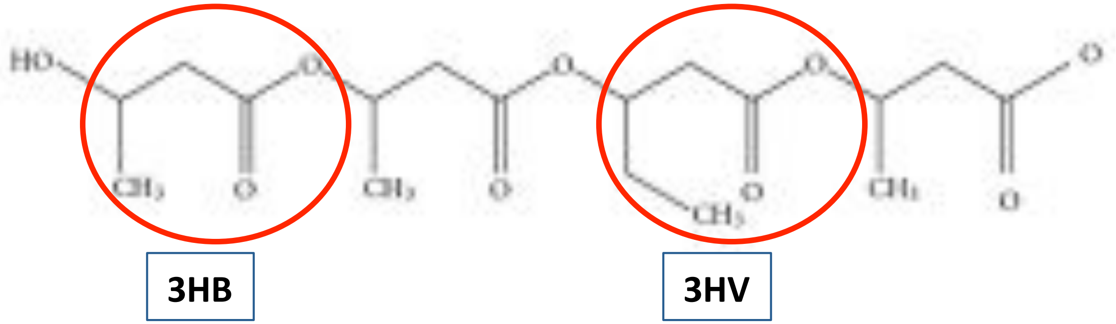
STRUCTURE OF POLYHYDROXYALKANOATES (PHAs)



R = **alkyl groups (C_1-C_{13})**
X = **1 – 4**
n = **100 – 30.000**
MW = **$2x10^5$ – $3x10^6$ D**

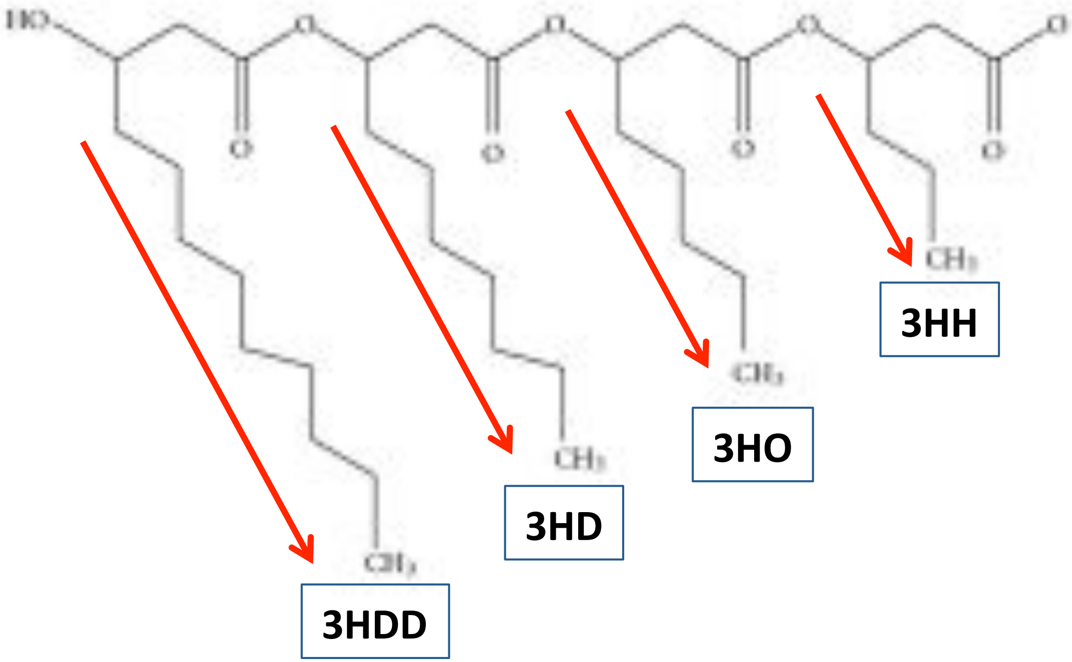
- >100 different monomer units
- >150 different monomer compositions
- ≠ Structures (homo-polymer, random copolymer, block copolymer)
- ≠ Properties (chemical, physical, thermal, mechanical, biological, …)
  - Distance between ester groups (C)
  - Structure of the side group (R) → flexibility, crystallinity, melting point, glass transition
  - Number of monomer units in the chain (n)
  - Monomer composition (homo, random, block-copolymers)
- ≠ Applications (industry, medicine, packaging, agriculture, etc.)

STRUCTURE OF POLYHYDROXYALKANOATES (PHAs)



3HB = 3-hydroxybutyrate (C_4)
3HV = 3-hydroxyvalerate (C_5)

<u>Short-Chain Length PHA (PHA_{SCL})</u>



3HH = 3-hydroxyhexanoate (C_6)
3HO = 3-hydroxyoctanoate (C_8)
3HD = 3-hydroxydecanoate (C_{10})
3HDD = 3-hydroxydodecanoate (C_{12})

<u>Medium-Chain Length PHA (PHA_{MCL})</u>

PROPERTIES OF POLYHYDROXYALKANOATES (PHAs)

- Composition, structure and properties of PHAs depend on:
  - Type of **bacteria** used
  - Type and amount of the **carbon source**
  - **Fermentation** conditions

- Main properties:
  - Semicrystalline
  - Brittle to elastomeric
  - Water **insoluble**
  - **Impermeable** to oxygen
  - Optically active and isotactic (stereochemical regularity in repeating units)
  - **Biodegradable**
  - **Biocompatible**
  - Piezoelectric

Short-Chain Length PHA (PHA_{SCL})
- Highly crystalline
- Brittle
- Stiff
- Piezoelectric

Medium-Chain Length PHA (PHA_{MCL})
- Thermoplastic elastomers
- Lower crystallinity
- Higher flexibility
- Softness
- More thermally stable

PROPERTIES OF POLYHYDROXYALKANOATES (PHAs)

Table 12.1 Comparison of the physical properties of P(3HB), a scl-PHA, P(3HO), a mcl-PHA and polypropylene [13, 23]

	scl-PHAs P(3HB)	mcl-PHAs P(3HO)	Polypropylene
Melting point (°C)	175	49	176
Glass-transition temp (°C)	15	−36	−10
Crystalline (%)	81	30	70
Young's modulus (GPa)	3.5	1.4	1.7
Tensile strength (MPa)	40	9	34.5
Elongation to break (%)	6	276	400

Panchal et al. Advanced Structured Materials (2013)

APPLICATIONS OF POLYHYDROXYALKANOATES (PHAs)

1990s: First consumer product: shampoos in **bottles** made of **Biopol®** (**Wella AG**)

Conventional commodity plastics: **disposable items**, such as razors, utensils, diapers, feminine **hygiene products**, bottles and cups, etc.

Nonwovens, binders, flexible **packaging**, thermoformed articles, synthetic paper and medical devices (**biocompatibility**)

Pressure **sensors** for **keyboards**, stretch and acceleration measuring instruments, shock wave sensors, gas lighters; **acoustics**: microphone, ultrasonic detectors, sound pressure measuring instruments; **oscillators**: headphones, loudspeakers, for ultrasonic therapy and atomization of liquid (**piezoelectric nature**)

Food **packaging**, plastic beverage **bottles**, paper and films for coated paper milk cartons, cover stock and the plastic film moisture barriers in **nappies** and **sanitary** towels, specialty paramedical film applications in hospitals (**gas barrier property**)

Today: The main markets are short term application for **packaging** and **agriculture**
Expansion to **textile**, **automotive**, and other durable applications

MEDICAL APPLICATIONS OF POLYHYDROXYALKANOATES (PHAs)

- PHAs are attractive materials for biomedical applications because:
  - Are of **natural** origin
  - Have enhanced **biocompatibility**, **biodegradability**, lack of cytotoxicity, ability to support cell growth and cell adhesion

- The high cost of PHA production has led to most of the current studies to be focused on applications that conventional plastics cannot perform and where their properties are more important than the cost, such as medicine and pharmacology:
  - Cardiovascular (pericardial patches to prevent postsurgical adhesions between the heart and the sternum, artery augmentation, atrial septal defect repair, vascular grafts and heart valves, biodegradable stents, etc.)
  - Wound healing sutures
  - Tissue engineering of peripheral nerves
  - Drug delivery (degradation mechanism by surface erosion)
  - Dental materials
  - Bone regeneration
  - Etc.

INDUSTRIAL PRODUCTION OF POLYHYDROXYALKANOATES (PHAs)

- Only a few PHAs have proceeded to the production stage in large quantities:
  - Polyhydroxybutyrate (**PHB**)
  - Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (**PHBV**)
  - Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (**PHBH**),

TABLE 1. The current and potential large volume manufacturers of polyhydroxyalkanoates [33].

Polymer	Trade names	Manufacturers	Capacity (tons)	Price (kg^{-1}) (in 2010)
PHB	Biogreen®	Mitsubishi Gas Chemical Company Inc. (Japan)	10,000	€2.5–3.0
PHB	Mirel™	Telles (US)	50,000	€1.50[a]
PHB	Biocycle®	PHB Industrial Company (Brazil)	50	n/a
PHBV and PHB	Biomer®	Biomer Inc. (Germany)	50	€3.0–5.0
PHBV, PHBV + Ecoflex blend	Enmat®	Tianan Biologic, Ningbo (China)	10,000	€3.26
PHBH	Nodax™	P&G (US)	20,000–50,000	€2.50
PHBH	Nodax™	Lianyi Biotech (China)	2000	€3.70
PHBH	Kaneka PHBH	Kaneka Corporation (Japan)	1000	n/a[b]
P(3HB-co-4HB)	Green Bio	Tianjin Gree Bio-Science Co./DSM	10,000	n/a
Polyhydroxyalkanoate from P&G	Meredian	Meredian (US)	272,000 (2013)	n/a

[a] Bacteria to produce bioplastics, BIOPRO Baden-Württemberg GmbH, September 24, 2009 available at http://www.bio-pro.de/magazin/thema/04308/index.html?lang=en&artikelid=/artikel/04310/index.html, accessed on March 2010.

[b] Full-scale development of the world's first completely bio-based polymer with soft and heat resistant properties, Kaneka Corporation, March 10, 2009 available at http://www.kaneka.co.jp/kaneka-e/news/pdf/090310.pdf, accessed on March 2010. n/a means price is not able to be found.

Chanprateep. J Biosci Bioeng 110(2010)621

PRODUCERS OF POLYHYDROXYALKANOATES (PHAs)

Metabolix

http://www.metabolix.com/

BIOCYCLE

http://www.biocycle.com.br/site.htm

Meredian

http://meredianinc.com/

BIOMER
biodegradable polymers

http://www.biomer.de/IndexE.html

Kaneka
KANEKA CORPORATION

http://www.kaneka.co.jp/kaneka-e/

MITSUBISHI GAS CHEMICAL

http://www.mgc.co.jp/eng/

P&G Chemicals
Delivering solutions, creating value

http://www.pgchemicals.com/case-studies/plastic-made-from-nature-nodax/

国韵 生物
GREEN BIO

http://www.tjgreenbio.com/en/

TianAn Biopolymer
Nature's Eco-Friendly Solution
ENMAT™

http://www.tianan-enmat.com/

ECONOMICS OF POLYHYDROXYALKANOATE PRODUCTION

Challenge → Reduce the unit cost of PHAs

- Cost is still higher than that of starch polymers and other bio-based polyesters due to:
  - High **raw material** costs
  - Small production **volumes**
  - High **processing** costs, particularly for purification

- Approaches:
  - Selection of the best producing microorganism
  - Use of less expensive carbon sources
  - Genetic engineering
    - Increase productivity
    - Made possible extracellular secretion of the polymer
    - Use yeast cells (larger cell size, easier to break)
  - Use genetically modified plants
  - Use cell-free enzymatic production
  - Optimization of the fermentation process (metabolic engineering)
  - Reduce downstream processing costs
  - Increase the scale of production facilities (from 1000 to 100.000 tons)

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