Biopolymers and Their Applications

Prof. Dr. Özkan DANIŞ

Marmara Üniversitesi

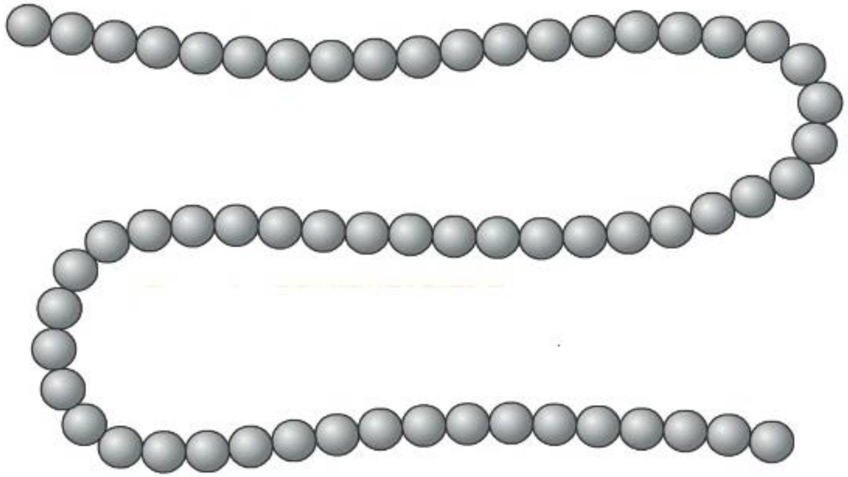
Fen Fakültesi

Kimya Bölümü

Biyokimya Anabilim Dalı

Polymer

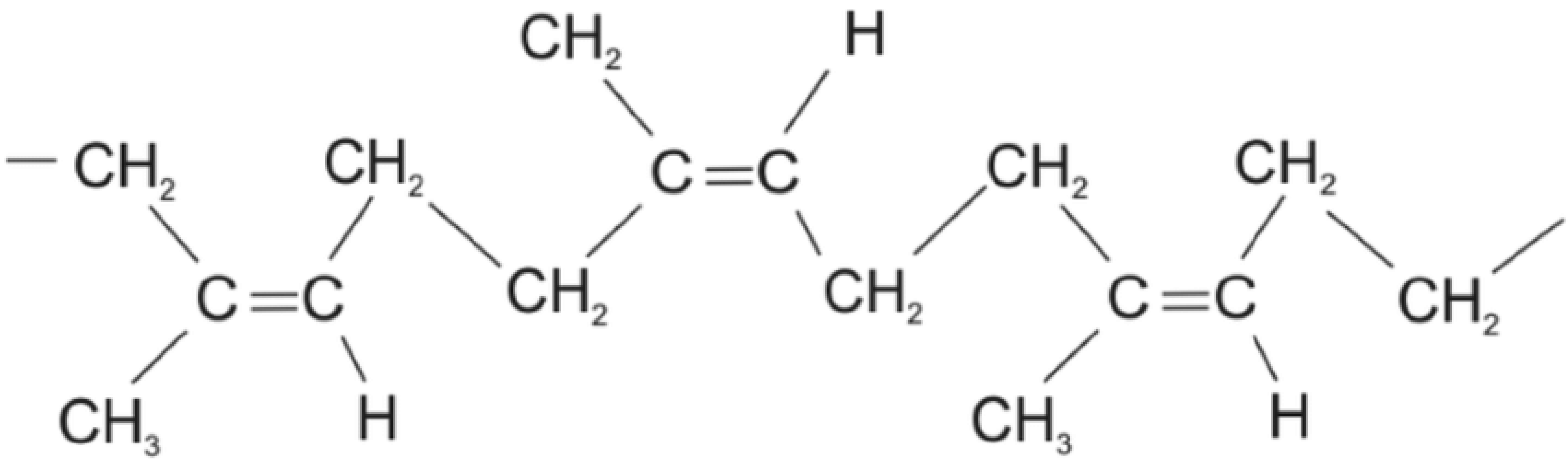
- **Polymers** are long-chain molecules formed by the addition of structural units called monomers one after the other.



- **Plastic** is the name given to polymers synthesized by humans.

HISTORY

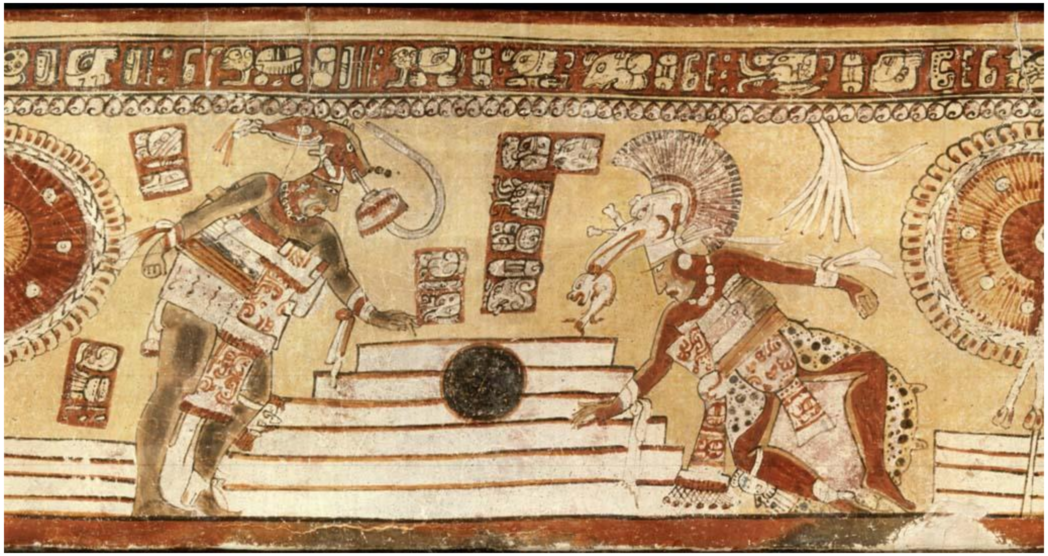
- Rubber, consists of polymers of the organic compound isoprene, with minor impurities of other organic compounds.



Natural rubber

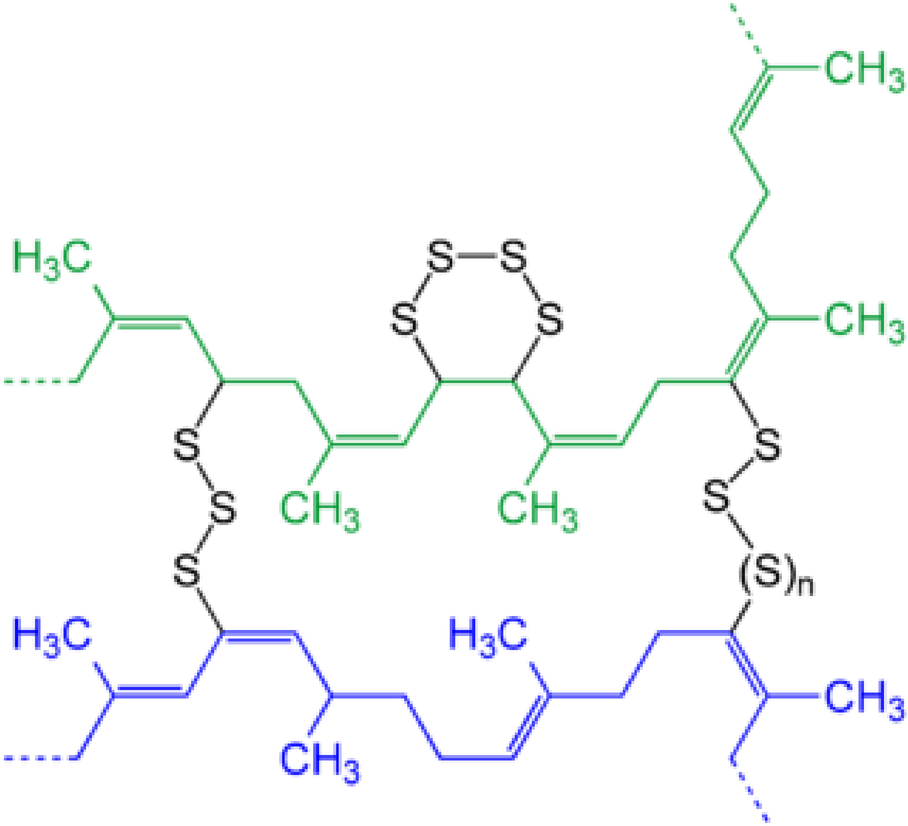
HISTORY

- The use of rubber is known in the Central American Mayan civilization in the 1500s.



HISTORY

- 1839 Charles Goodyear developed the vulcanization method.



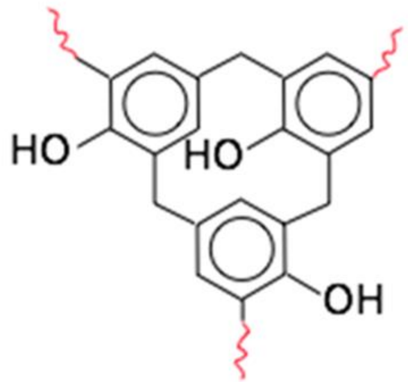
HISTORY

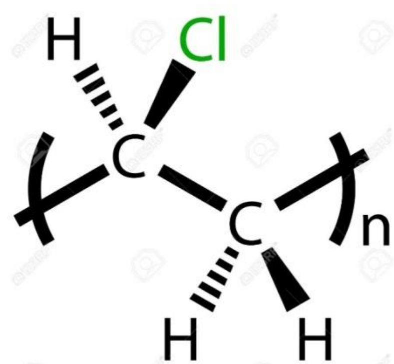
- Vulcanization of rubber creates di- and polysulfide bonds between chains, which limits the degrees of freedom and results in chains that tighten more quickly for a given strain, thereby increasing the elastic force constant and making the rubber harder and less extensible.

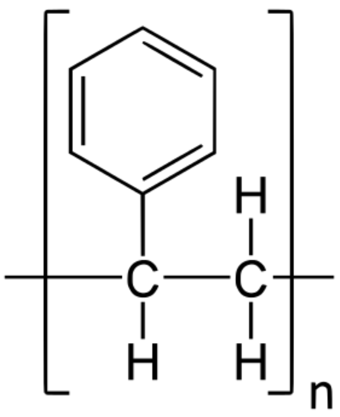
- It works by forming cross-links between sections of polymer chain which results in increased rigidity and durability, as well as other changes in the mechanical and electrical properties of the material.

HISTORY

- 1905 Leo Bakeland - Bakelite - electrical insulation
- 1927 Large-scale use of polyvinyl chloride (PVC) resins – bottles, pipes
- 1938 Polystyrene and 1941 Styrofoam transition to widespread production



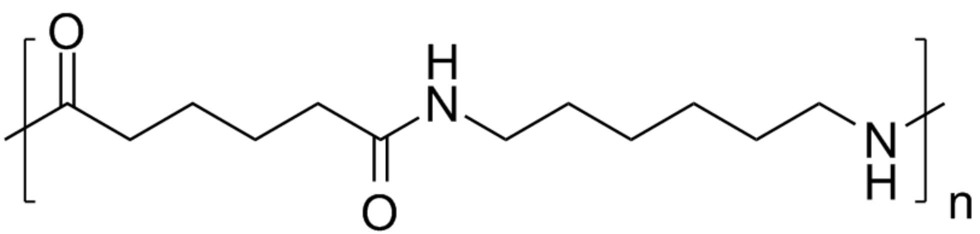


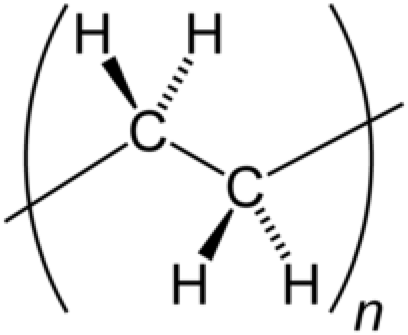


HISTORY

- 1938 Nylon (aliphatic or semi-aromatic polyamides)

- 1941 Polyethylene, the most commonly used plastic.





ENVIRONMENT

- Plastics have many superior properties compared to classical materials.

- However, most of them are produced from oil, which is an exhaustible resource.

- In addition, their long lifespan causes significant environmental problems.

ENVIRONMENT

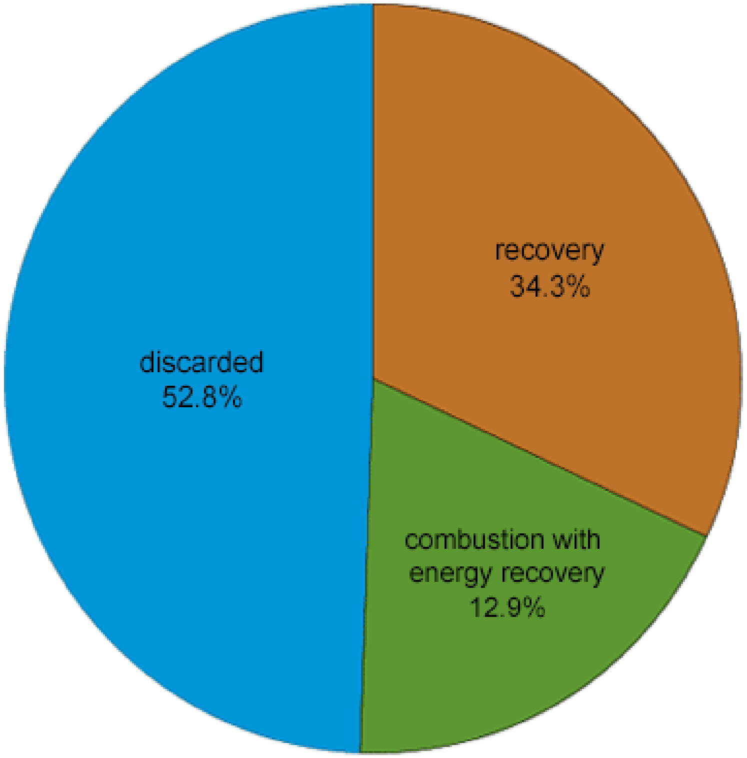
- A significant part of the plastic waste released during and after use is stored in landfills.

ENVIRONMENT

- Plastic waste thrown into the seas forms large piles in the oceans.

ENVIRONMENT

- Pyrolysis of waste plastics is both expensive and causes the release of harmful chemicals into the atmosphere.
- Recycling is a long process and not all plastics can be recycled.



ENVIRONMENT

- The best solution to this problem is

biodegradable plastics obtained

from renewable resources.

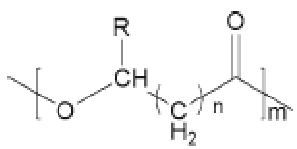
BIODEGRADABLE BIOPOLYMERS

- It is possible to examine it in three main classes:
- 1- Agro-polymer derived biomass products such as starch, cellulose and lignin.
- 2- Polymers obtained chemically from monomers of agricultural origin, such as polylactic acids.
- 3- Polymers obtained from microbial sources such as polyhydroxyalkanoates.

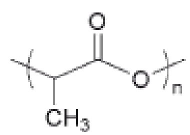
BIODEGRADABLE BIOPOLYMERS

Table 1 Comparison of various properties of bacterial plastics

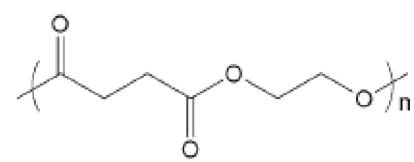
Bacterial plastics	Biosynthesized monomers	Polymerization approach	$M_w \times 10^4$	Polydispersity	T_m (°C)	T_g (°C)	$T_{d(5\%)}$ (°C)	Mechanical properties: Young's modulus (MPa)	Mechanical properties: Elongation at break (%)	Mechanical properties: Tensile strength (MPa)	References
PHA	Hydroxyalkanoates	Biological	10–1000	1.2–6.0	60–177	−50–4	227–256	Flexible	2–1000	17–104	See text
PLA	D,L-Lactic acids	Chemical	5–50	1.8–2.6	175	60	339	384–481	5.2–2.4	49.6–61.6	See text
PBS	Succinic acid	Chemical	3–20	2.0–6.3	115.8–146.5	−36.6 to −33	353	268.0	175.2	24.8	See text
PE	Bioethylene	Chemical	10–600	2.1–6.8	136.4	33.4.3	371	102	297.7	22–29	See text
PTT	1,3-Propanediol	Chemical	3.8	2	227.55	42.6	364	727.88	159.48	49.24	See text
PPP	*cis*-3,5-Cyclohexadiene-1,2-diols	Chemical	0.4–33	1.5–3.6	ND	173–232	380	ND	ND	ND	See text



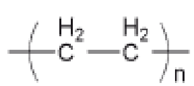
PHA



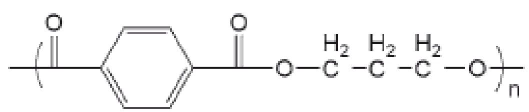
PLA



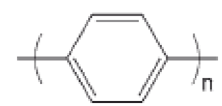
PBS



PE



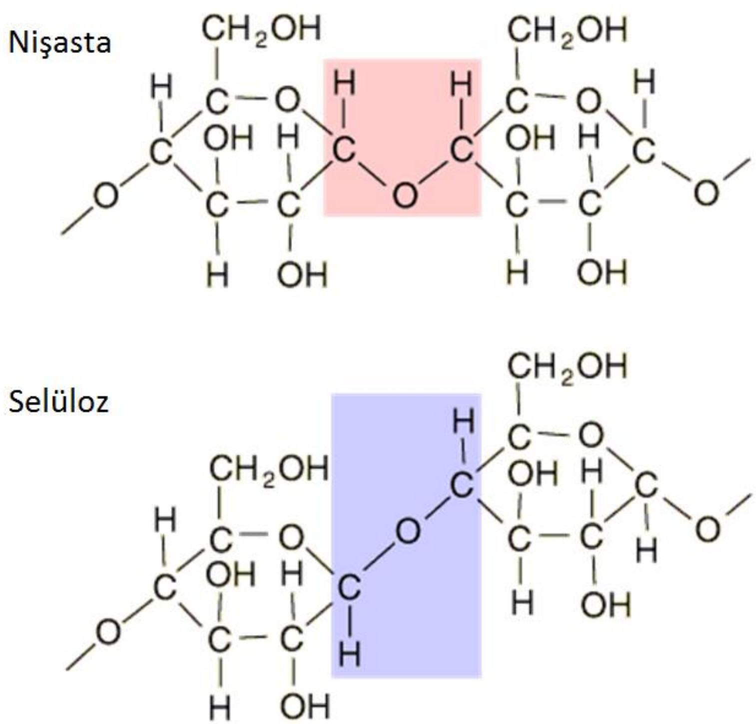
PTT

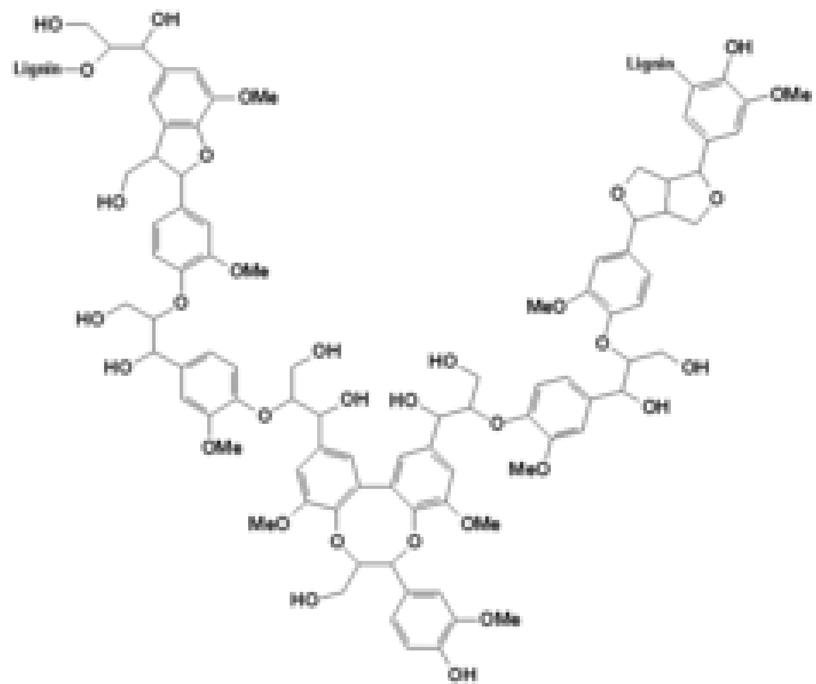


PPP

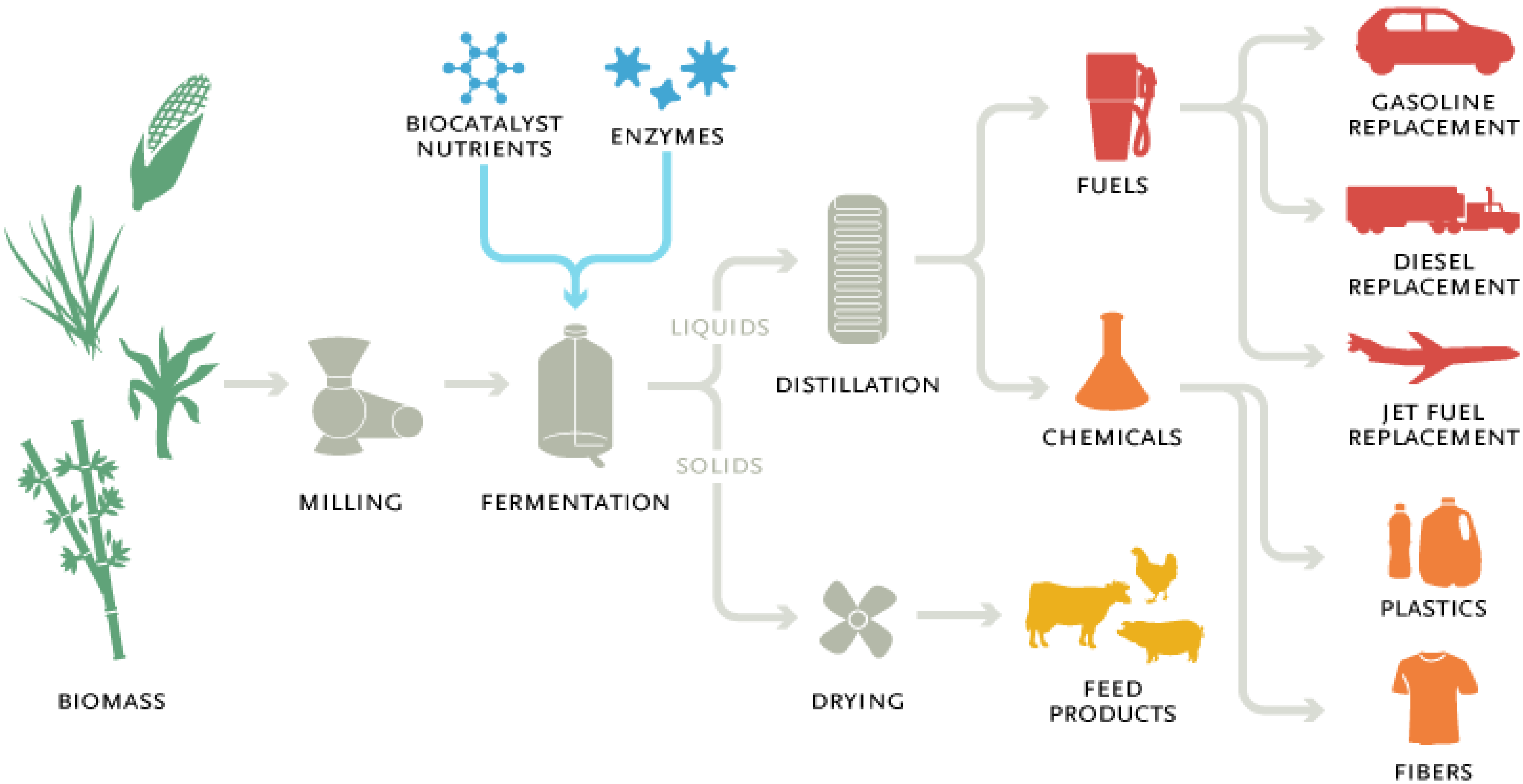
Agricultural Biomass Biopolymers

- Biomass products from agro-polymers such as starch, cellulose and lignin.



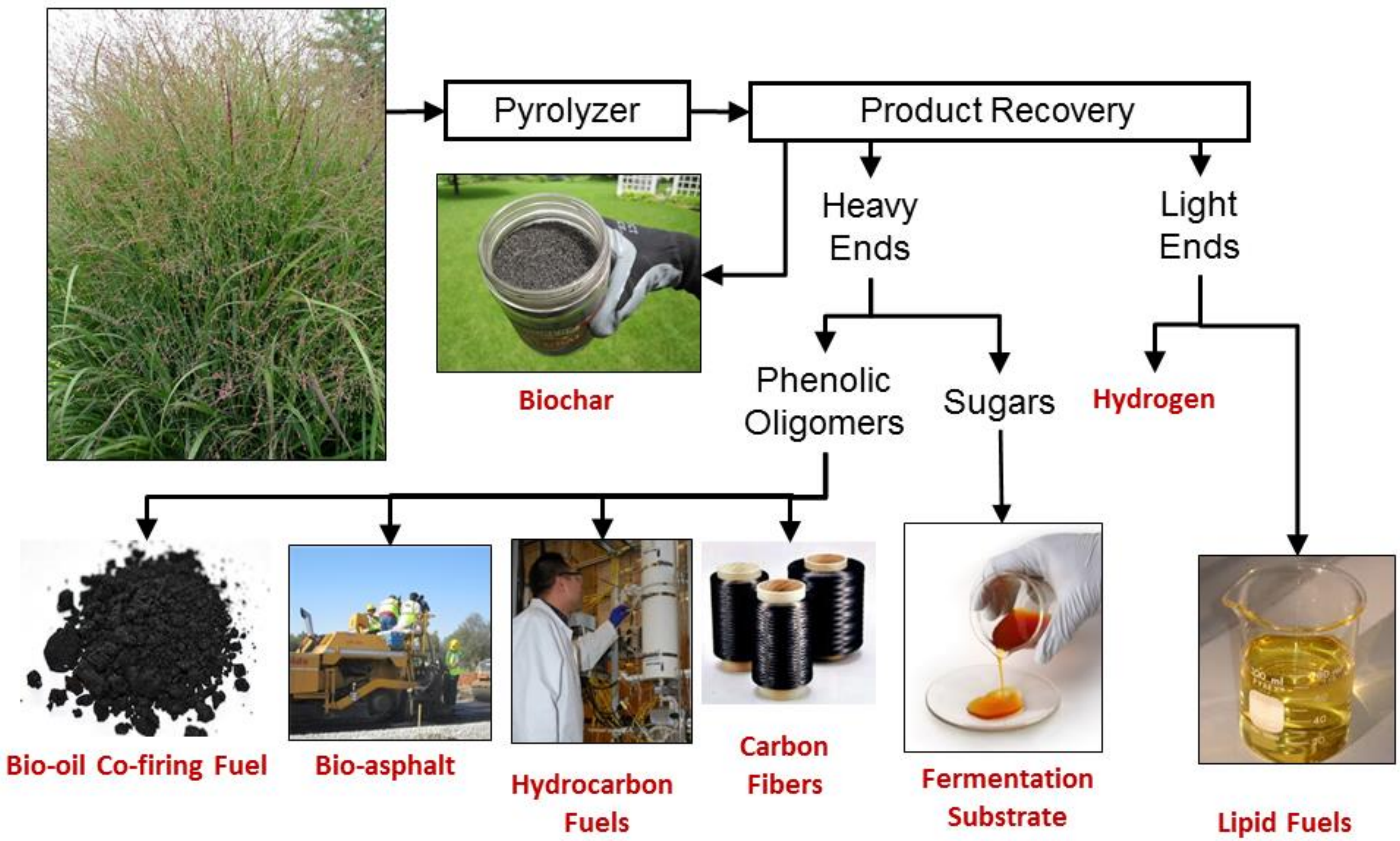


Agricultural Biomass Biopolymers



Agricultural Biomass Biopolymers

Biomass to Value-Added Products

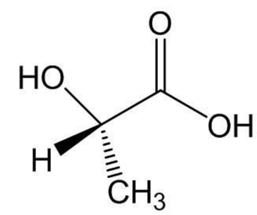


Biopolymers Obtained by Chemical Methods

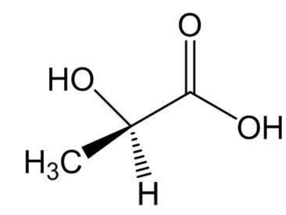
- Polylactic acid and polyethylene can be given as examples.

Polylactic Acid (PLA)

- Lactic acid is widely found in nature.



L-lactic acid



D-lactic acid

- Although it has been obtained synthetically in history, today more than 95% is obtained by microbial fermentation.

- PLA (polylactic acid) is both biodegradable and the most cost-effective.

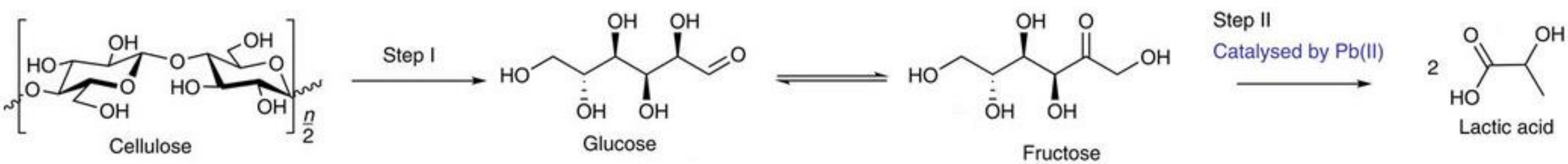
- Compared to traditional petroleum-based plastics, its cost is higher and its mechanical and physical properties are less desirable.

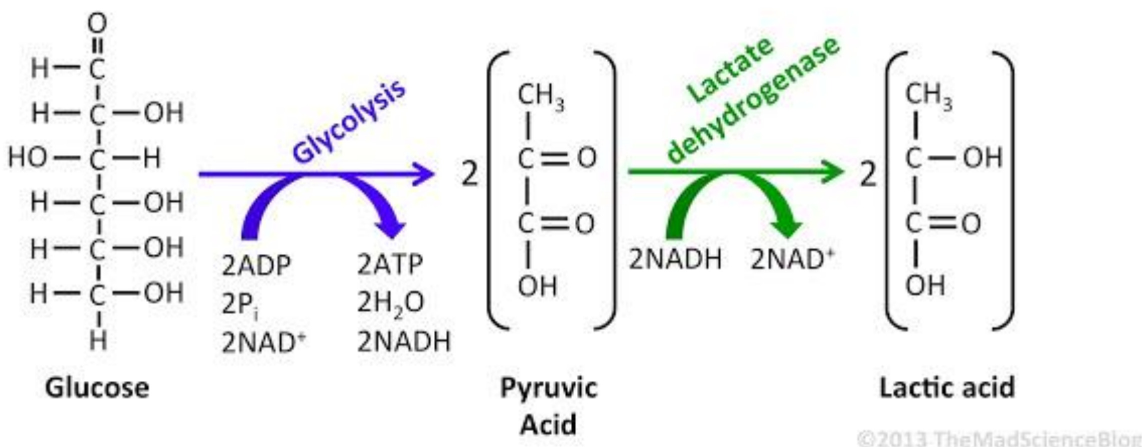
Polylactic Acid (PLA)

- Lactic acid and its derivatives are used in many different areas such as food, medicine, personal care, electronics, and polymers.

Polylactic Acid (PLA)

- Lactic acid can be obtained by chemical synthesis or microbial fermentation.

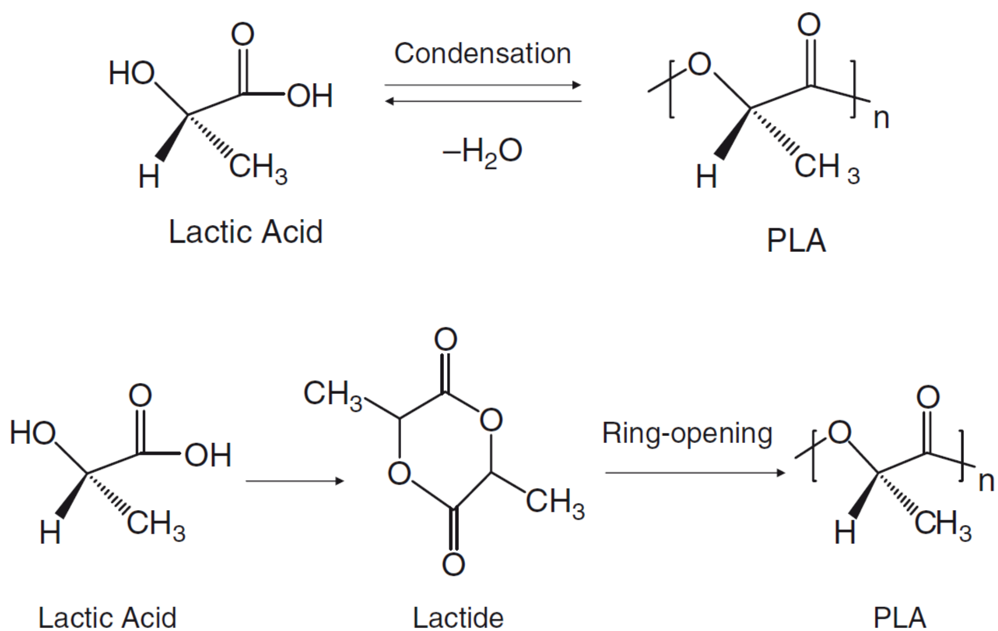




Polylactic Acid (PLA)

- Polylactic acid is the first biodegradable bioplastic produced commercially from annual, renewable resources.

- It is obtained by chemical polymerization of lactic acid.

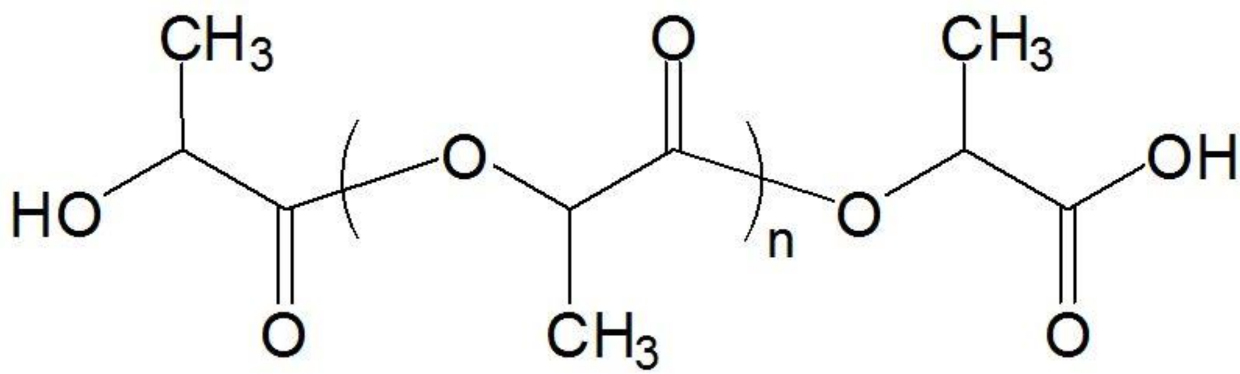


Polylactic Acid (PLA)

- PLA is a thermoplastic polyester that can be turned into fiber, knitted into rigid films, and injected into molds.

- It is primarily used in food packaging, textiles and injection molded parts.

- It must be blended with other polymers for flexible film applications such as shopping bags.



Polylactic Acid (PLA)

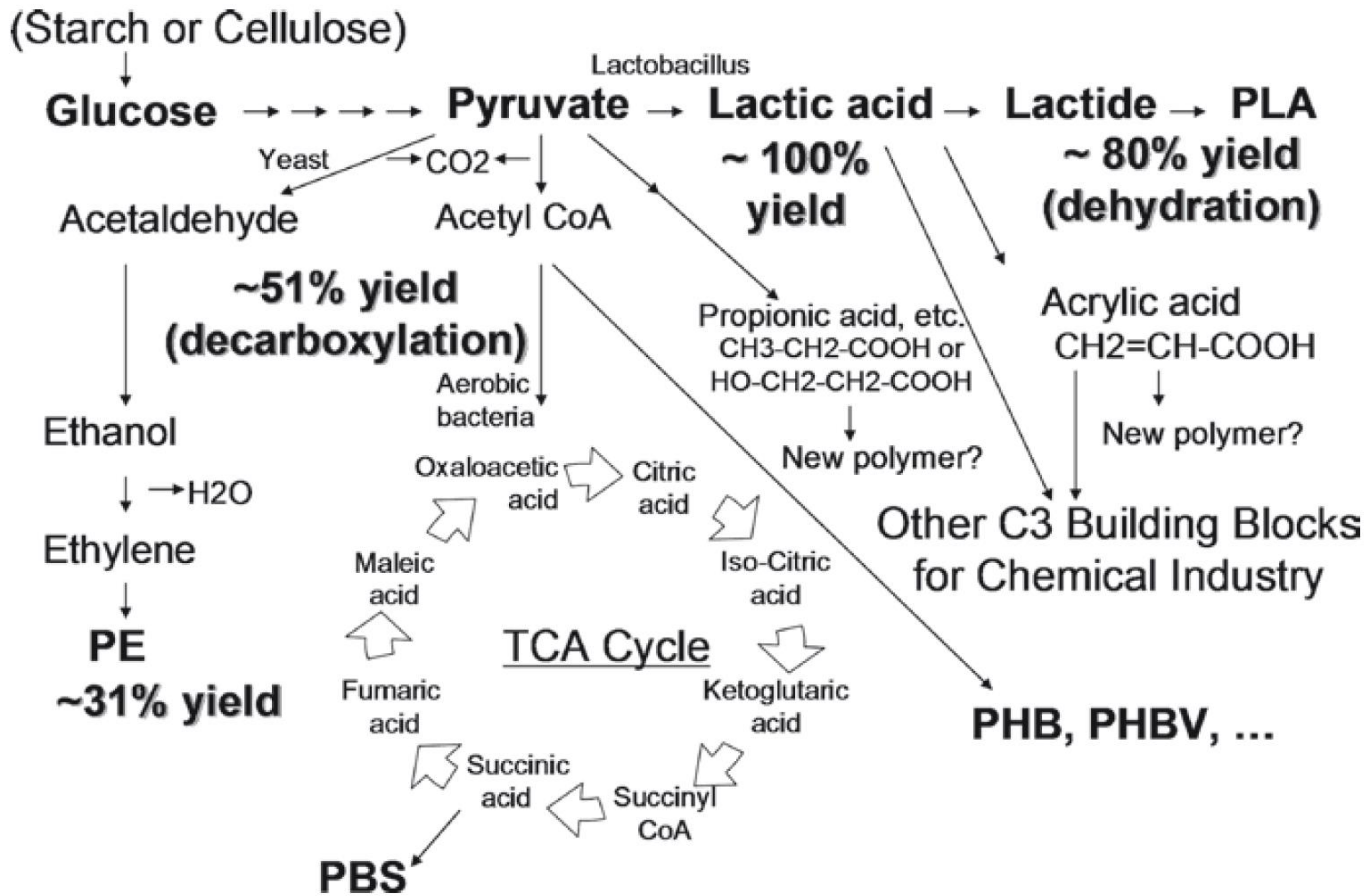
Polylactic Acid (PLA)

- As a rigid thermoplastic, PLA exhibits properties similar to polyethylene tetraphthalate and polystyrene.

- In addition, it has important unique features such as superior transparency, shiny appearance, inflexibility and fold retention ability.

- It also has high gas permeability compared to other similar polymers.

Polylactic Acid (PLA)

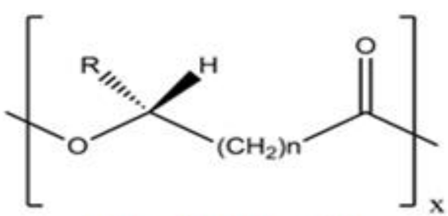


Bacterial Biopolymers, PHA

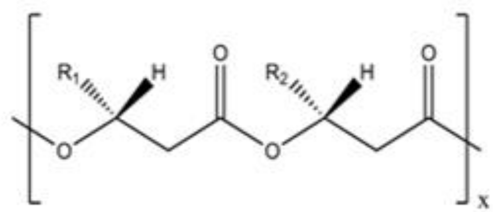
- Polyhydroxyalkonates are polyesters of hydroxy alkonates that are accumulated by many microbial species as a source of reducing power, carbon or energy in conditions where nutrient elements such as N, P, S, O or Mg are limited and an excess carbon source is available.

Polyhydroxyalconate, PHA

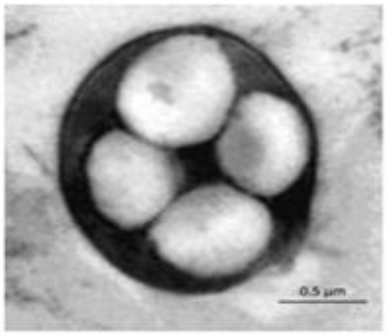
- These polyesters, whose physicochemical properties are similar to petrochemical plastics, are accumulated intracellularly in storage granules in microorganisms.



PHA homopolimer



PHA heteropolimer



scl-PHA:

n=1 R= -H poli(3-hidroksipropiyonat)
R= $-CH_3$ poli(3-hidroksibutirat)
R= $-CH_2CH_3$ poly(3-hidroksivalerat)

n=2 R= $-CH_3$ poly(4-hidroksibutirat)

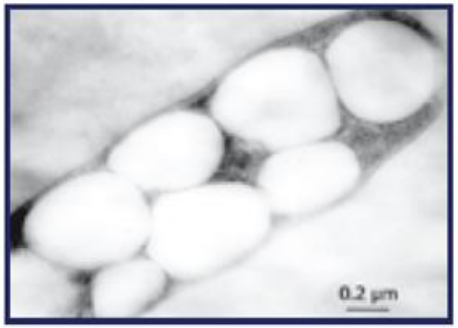
mcl-PHA

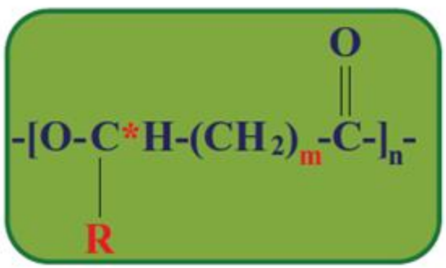
n=1 R= $-(CH_2)_2CH_3$ poli(hidroksihekzonat)
R= $-(CH_2)_4CH_3$ poli(3-hidroksioktanat)

R_1= $-CH_3$, R_2= $-CH_2CH_3$ poli(3-hidroksibutirat-co-3-hidroksivalerat)

R_1= $-CH_3$, R_2= $-(CH_2)_2CH_3$ poli(hidroksibutirat-co3-hekzonat

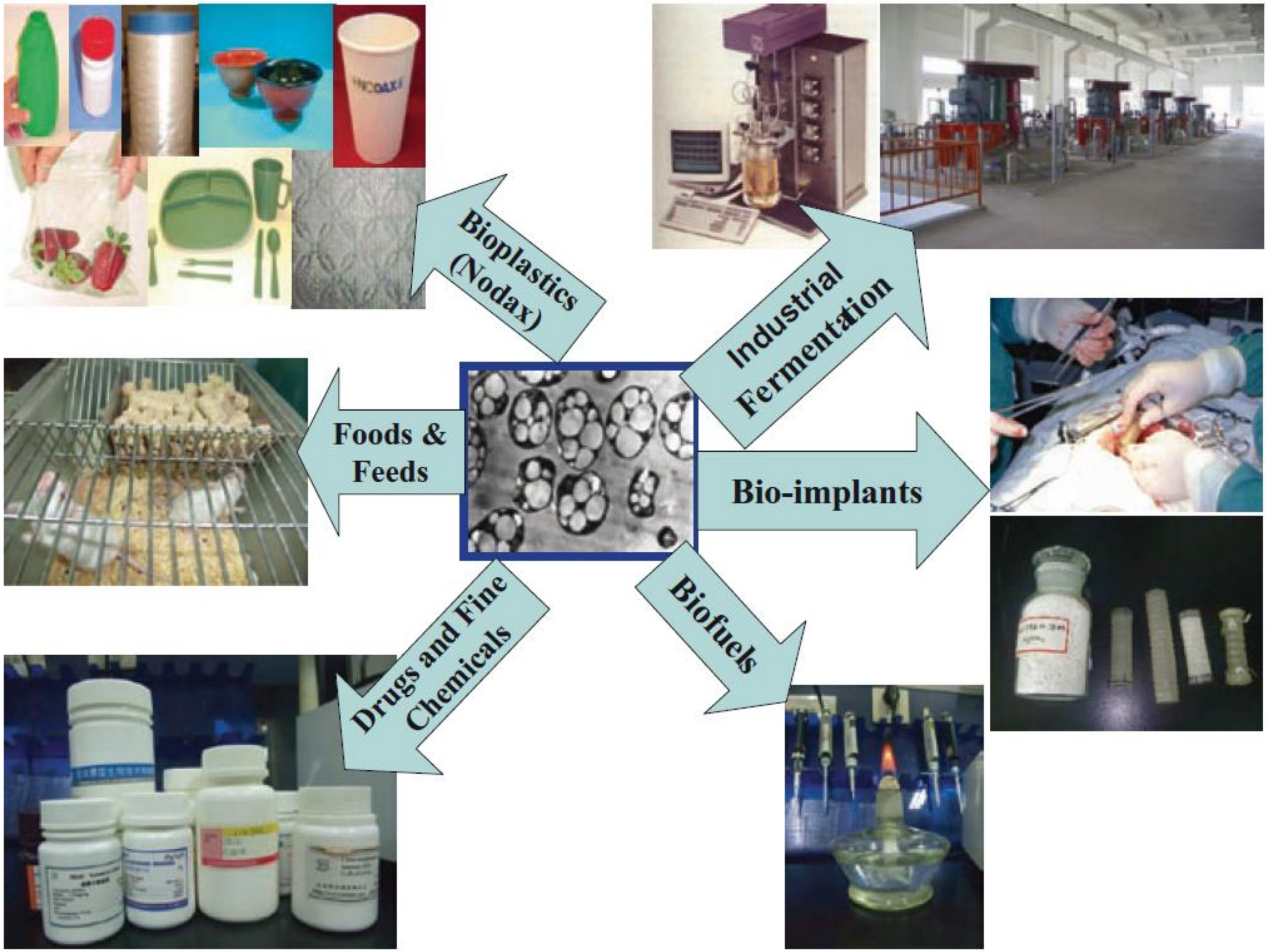
Polyhydroxyalconate, PHA





thermoplastic
biodegradable
biocompatible
piezoelectric
From fragile to elastic
Has functional groups
MA 20 kDa to 30,000 kDa
Chiral monomers
hydrophobic
gas tight
produced from renewable sources

Polyhydroxyalconate, PHA



Polyhydroxyalconate, PHA

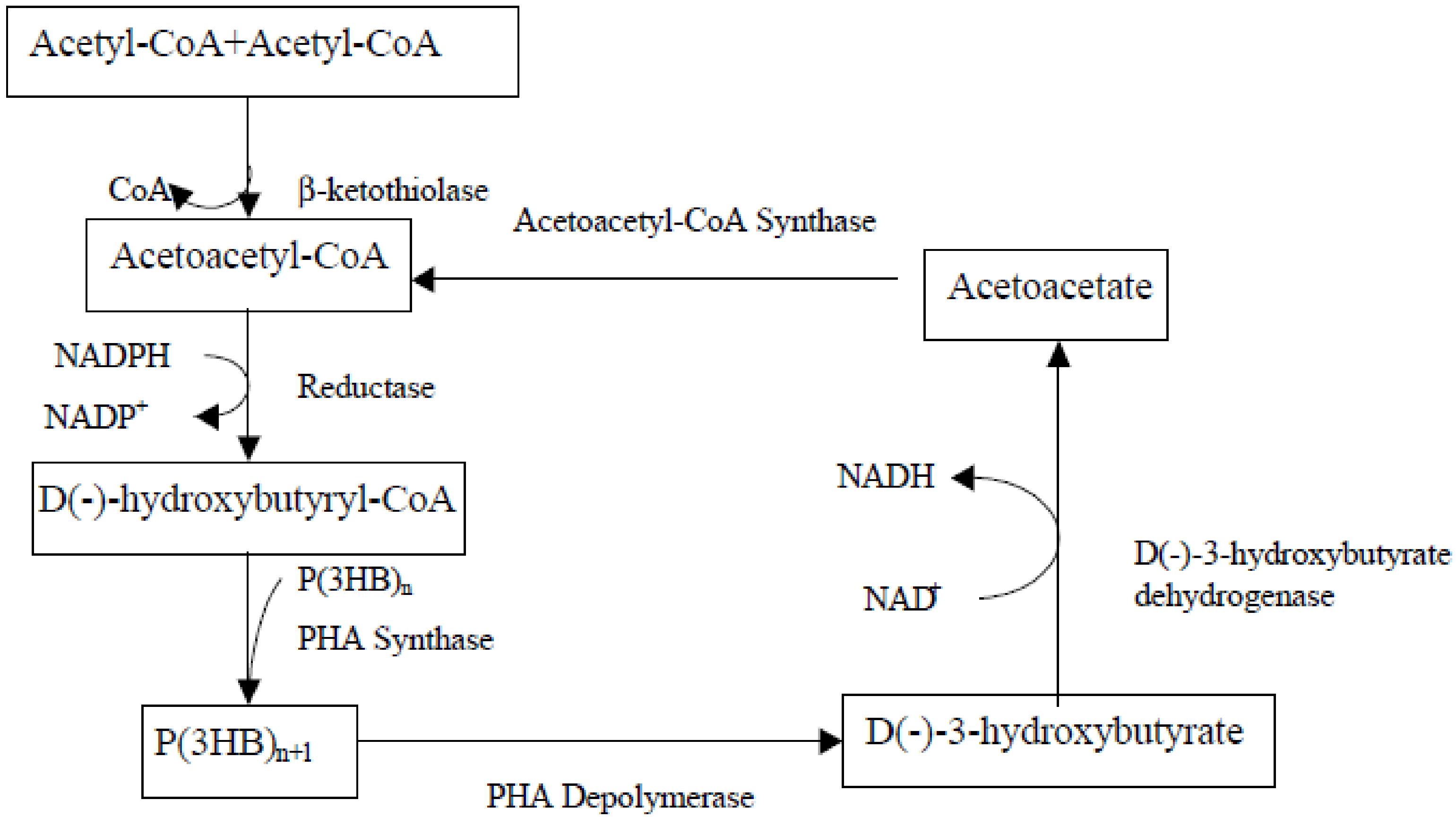
- The molecular structure and properties of synthesized PHAs vary depending on the bacterial and archaeal species used, the medium, and the substrate specificity of the enzymes in the biosynthetic pathways.

- Despite their many advantageous properties such as biocompatibility and biodegradability, they have not yet been able to replace petrochemical plastics due to their high costs.

Polyhydroxyalconate, PHA

- PHA is now available on an industrial scale from various microorganisms such as;
  - *Bacillus ssp.,*
  - *Alcaligenes ssp.,*
  - *Pseudomonas ssp.,*
  - *Azotobacter vinelandii,*
  - *Halomonas boliviensis* ve
  - recombinant *Escherichia coli*

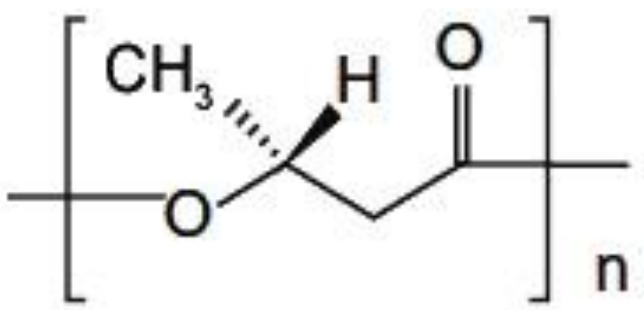
Polyhydroxyalconate, PHA

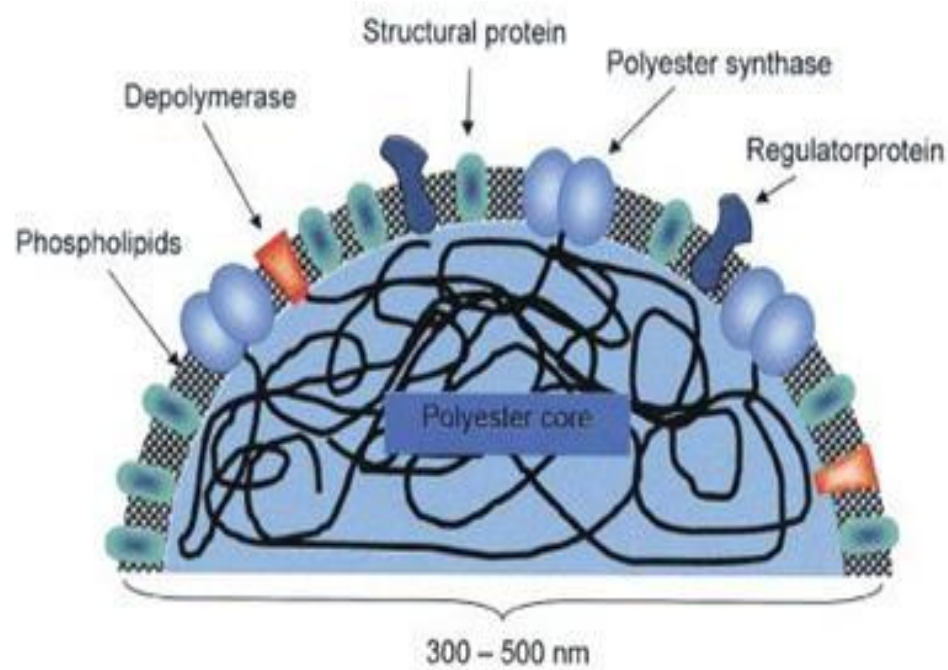


Polyhydroxybutyrate, PHB

- It is a water-insoluble polymer consisting of repeating units containing optically active D(-)-3-hydroxy butyric acid, with a methyl group in its side chain.

- PHB granules are 100-800 nm in diameter, contain 98% PHB and 2% protein, and are surrounded by a membrane 2-4 nm thick.





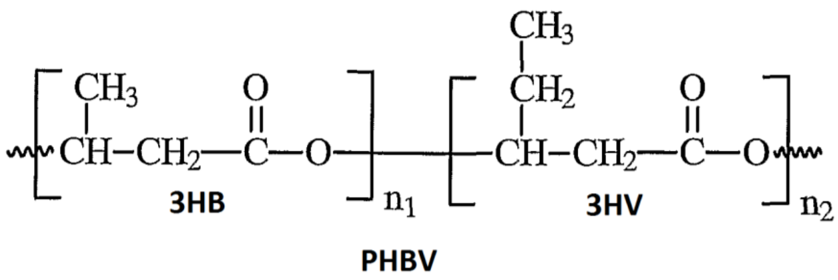
Polyhydroxybutyrate, PHB

- PHB granules do not separate from the lumen of the cell and cause the osmotic status of the cells to change.
- PHB molecules in the crystal structure have a right-handed helix structure.

Physical Properties	**PHB**
Tm [°C]	175
% Crystalinity [%]	80
MA [Dalton]	5 x 10^5
Tg [°C]	4
Density [g/cm3]	1.250
Tenzile strength [MPa]	40
Elongation at break [%]	6
UV resistance	Good
Solvent resistance	Weak
Source	Renewable
Cost[$/lb]	3.50

PHB-co-3-hydroxivalerate, PHBV

- Poly (3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV) copolymer is a biopolyester produced by a specific group of microorganisms under specific growth conditions.

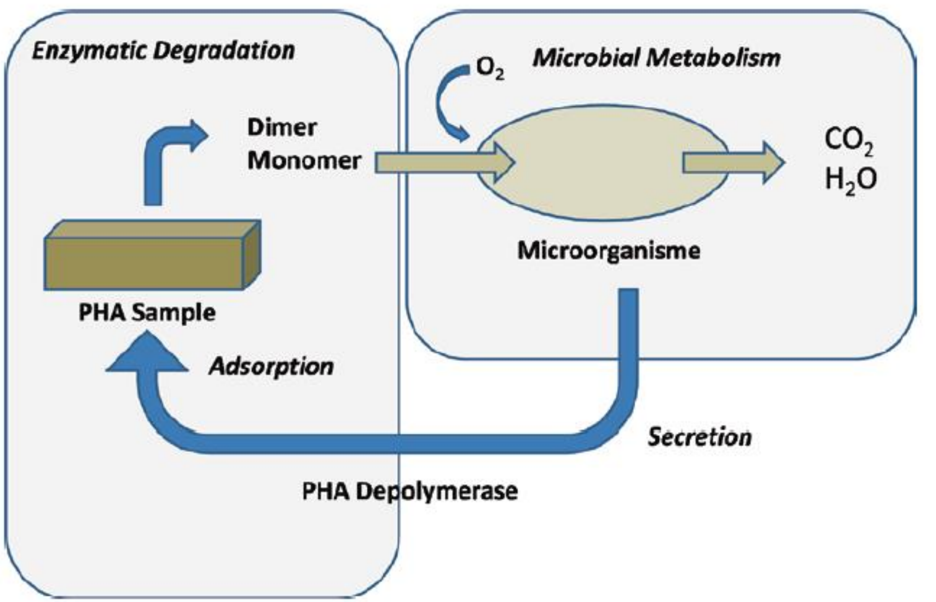


Mol % HV composition	Tm [°C]	Tg [°C]	Tensile strenght [MPa]	Elongation at break [%]	Elasticity [MPa]
0	175	9	45	4	3.8
11	157	2	38	5	3.7
20	114	- 5	26	27	1.9
28	102	- 8	21	700	1.5
34	97	- 9	18	970	1.2

Biodegradability

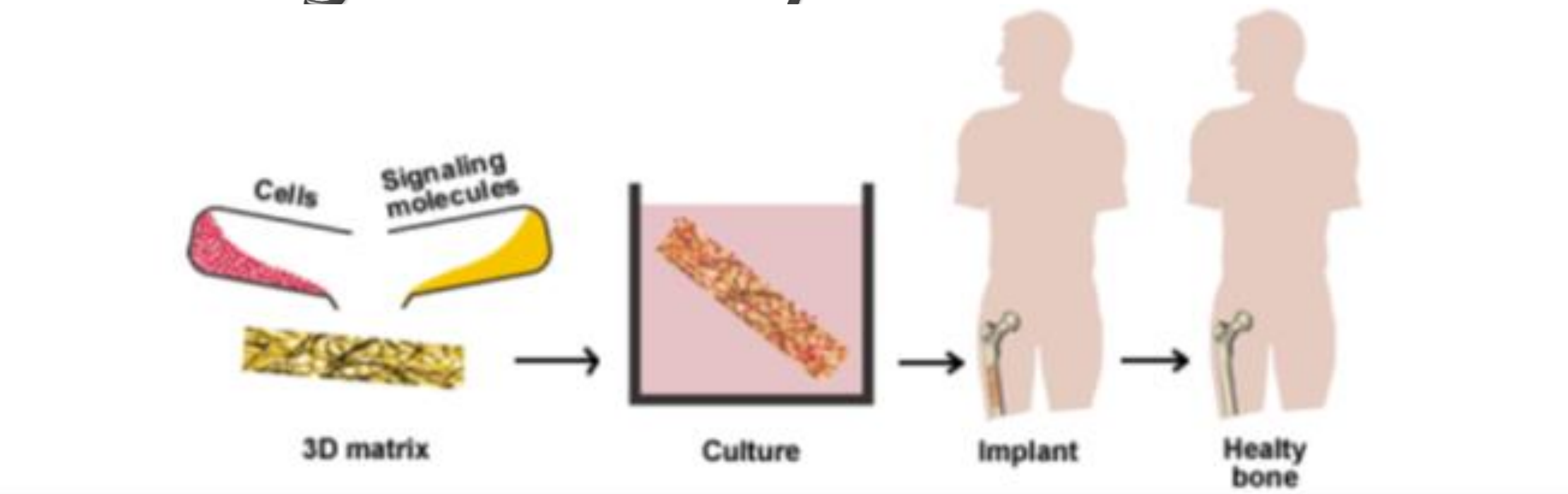
- Many microorganisms can degrade PHA in natural environments such as soil, sea and lake water.

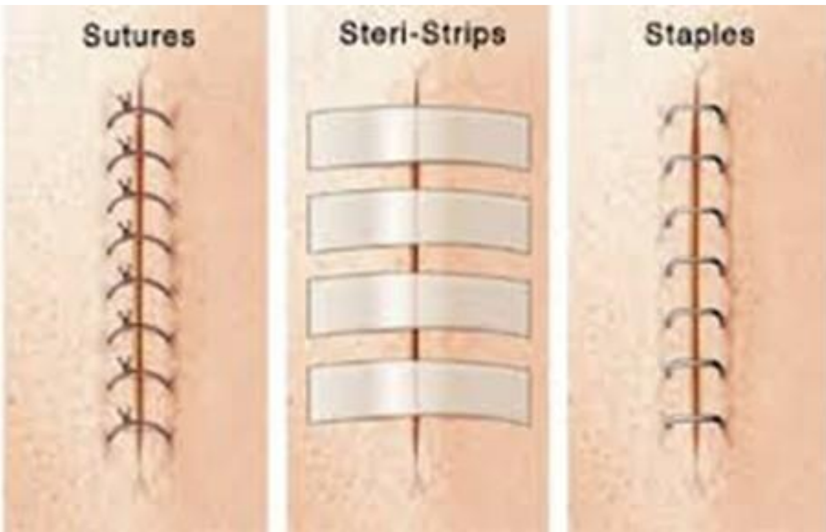
- Microorganisms colonize the surface of the polymer, secrete degradative enzymes and break down PHAs into their subunits.



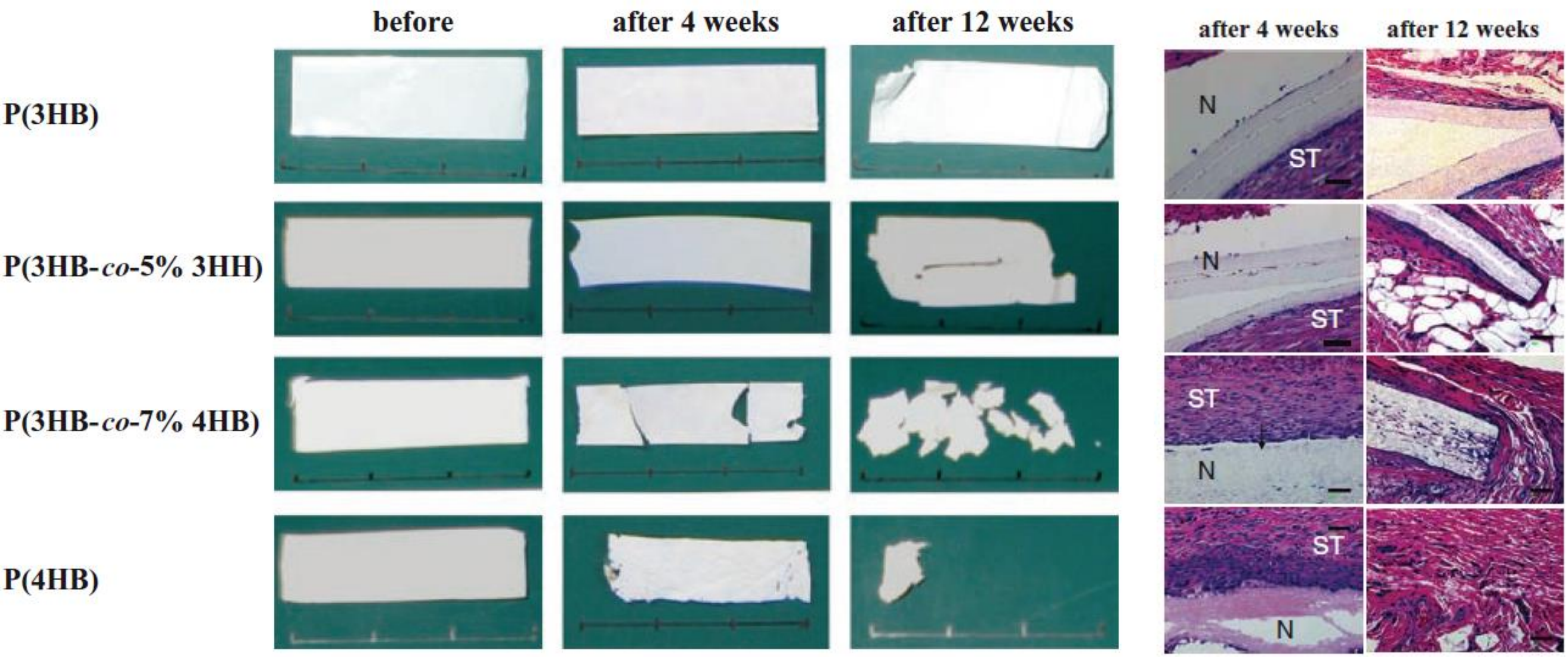
Biodegradability

Biodegradability





Biodegradability



Biodegradability

Table 8 Weight-loss (*WL*) biodegradabilities and biological oxygen demand (*BOD*) biodegradabilities of aliphatic polyester films in different natural waters for 28 days at 25°C (Kasuya et al. 1998)

Sample	Freshwater (river)		Freshwater (lake)		Seawater (bay)		Seawater (bay)	
	WL biodeg.[a] (%)	BOD biodeg.[b] (%)	WL biodeg.[a] (%)	BOD biodeg.[b] (%)	WL biodeg.[a] (%)	BOD biodeg.[b] (%)	WL biodeg.[a] (%)	BOD biodeg.[b] (%)
Poly(3HB)	100 ± 0	75 ± 16	93 ± 7	52 ± 7	41 ± 16	27 ± 10	23 ± 13	14 ± 10
Poly(3HB-*co*-14%3HV)	100 ± 0	76 ± 2	100 ± 0	71 ± 1	100 ± 0	84 ± 2	100 ± 0	78 ± 5
Poly(3HB-*co*-10%4HB)	100 ± 0	90 ± 1	74 ± 26	55 ± 17	70 ± 30	51 ± 27	59 ± 15	43 ± 14
Poly(ε-caprolactone)	100 ± 0	75 ± 8	100 ± 0	77 ± 1	100 ± 0	79 ± 2	67 ± 21	56 ± 9
Poly(ethylene succinate)	100 ± 0	83 ± 2	100 ± 0	77 ± 2	2 ± 1	1 ± 1	5 ± 2	3 ± 2
Poly(ethylene adipate)	100 ± 0	70 ± 3	95 ± 5	68 ± 8	100 ± 0	65 ± 13	57 ± 14	46 ± 13
Poly(butylene succinate)	2 ± 1	3 ± 1	22 ± 14	12 ± 8	2 ± 2	1 ± 1	2 ± 3	2 ± 0
Poly(butylene adipate)	24 ± 7	20 ± 4	80 ± 13	50 ± 10	34 ± 2	20 ± 2	11 ± 10	10 ± 5

3HB 3-hydroxybutyrate, *3HV* 3-hydroxyvalerate, *4HB* 4-hydroxybutyrate

[a] Weight-loss biodegradability

[b] BOD biodegradability

Biodegradability

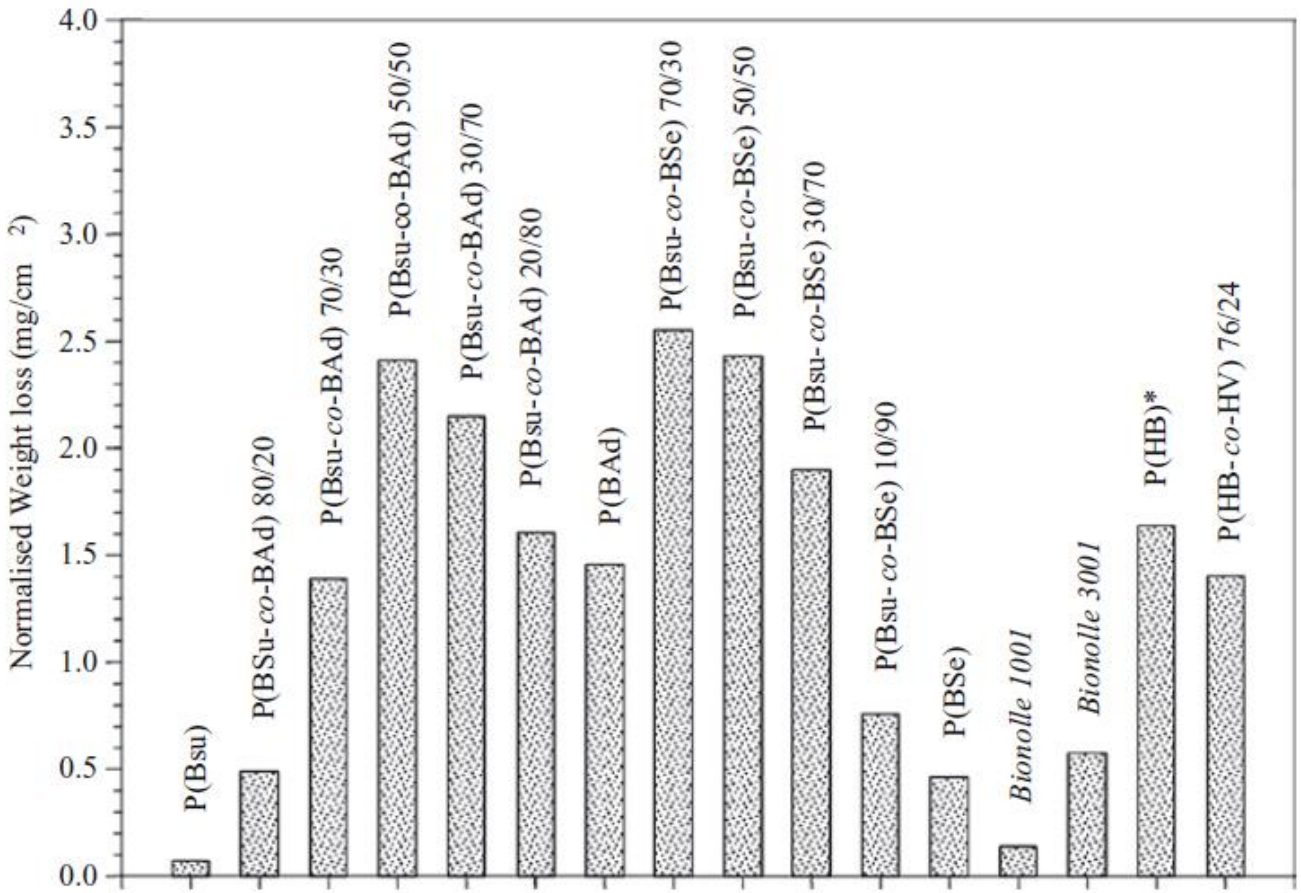
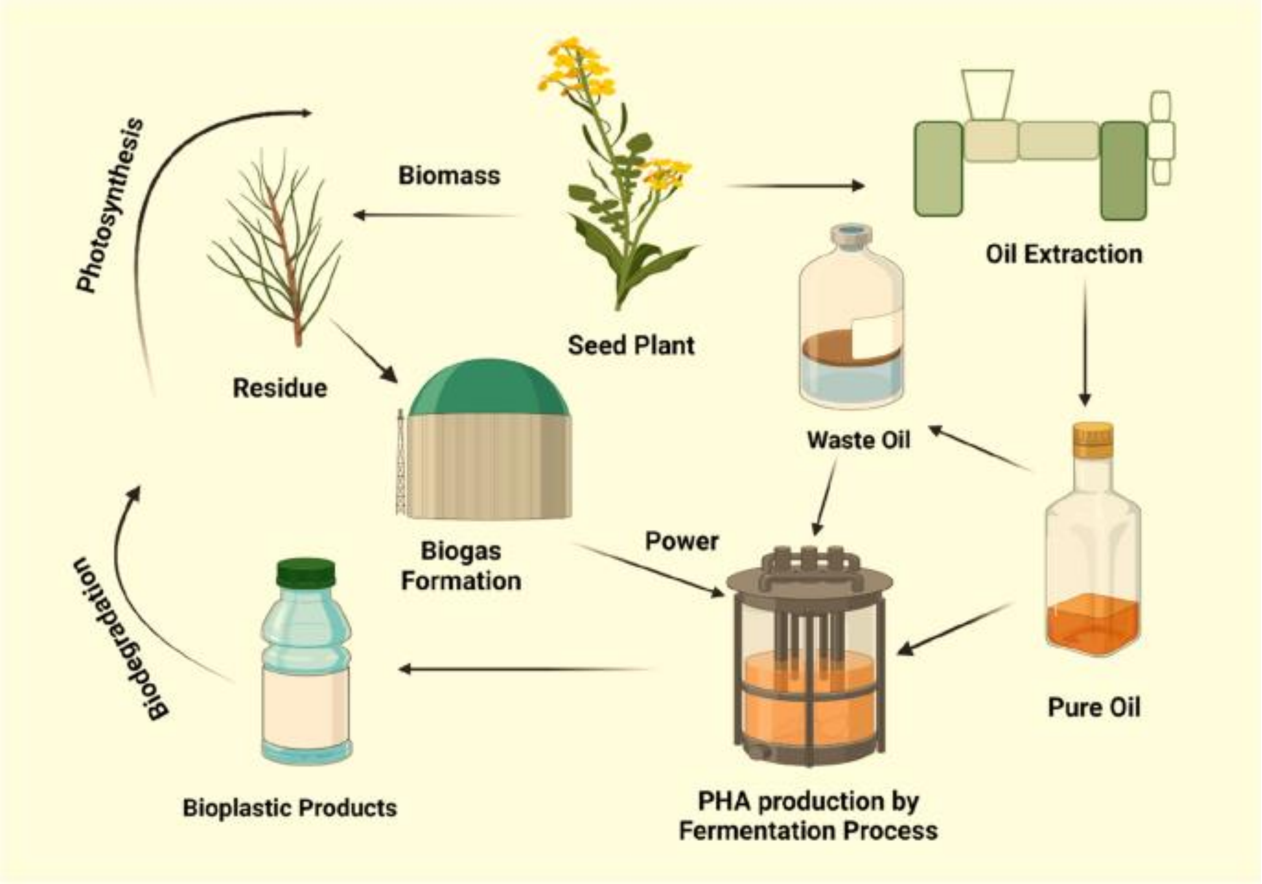


Fig. 23 Normalized weight losses for PBSA, poly(butylene succinate-*co*-butylene sebacate BSe), Bionolle, polyhydroxybutyrate and poly(hydroxybutyrate-*co*-hydroxyvalerate) 76/24 film samples in soil burial tests for 15 days (Rizzarelli et al. 2004)

Production from Renewable Resources

- PHAs can be produced by fermentation from renewable sources instead of fossil fuels, using agricultural raw materials such as sugars and fatty acids as carbon and energy sources.

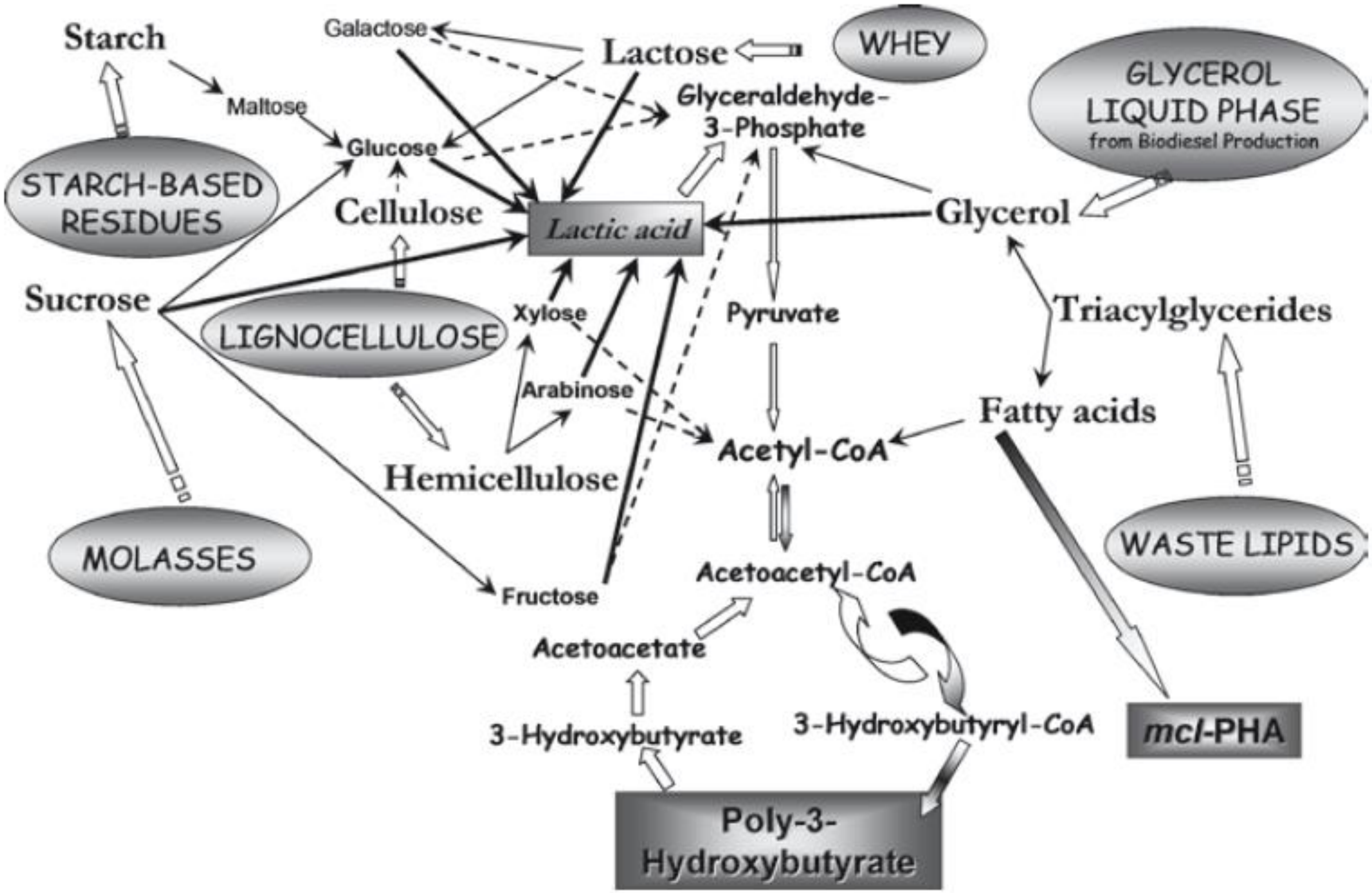


Production from Renewable Resources

- In PHA production;
  - Glycerol, a by-product in biodiesel and soap production,
  - sugar industry and waste (molasses and bagasse),
  - lignocellulosic materials and their by-products,
  - city sewage waste
  - solid and wastewater treatment plant wastes

can be utilized.

Production from Renewable Resources



Publications

Extremophiles (2015) 19:515–524
DOI 10.1007/s00792-015-0735-4

ORIGINAL PAPER

Preparation of poly(3-hydroxybutyrate-co-hydroxyvalerate) films from halophilic archaea and their potential use in drug delivery

Ozkan Danis · Ayse Ogan · Pınar Tatlican · Azade Attar · Emrah Cakmakci · Bulent Mertoglu · Meral Birbir

Test Isolates

2KYS1, 3TL4, 5TL6 (Birbir et al. 2007), 1TK1 (Birbir et al. 2004) and a novel isolate 1KYS1 were obtained from salt lake (TL), Tuzkoy Salt Mine (TK) and Kayacik saltern (KYS), respectively. All test isolates were maintained at 4 °C on Brown agar medium containing 250 g NaCl, 2.0 g $MgSO_4 \cdot 7H_2O$, 2.0 g KCl, 3.0 g $C_6H_5Na_3O_7 \cdot 5H_2O$, 5 g yeast extract and 2 g agar per liter (Brown 1963; Vreeland 1993).

Publications

Extremophiles (2015) 19:515–524
DOI 10.1007/s00792-015-0735-4

ORIGINAL PAPER

Preparation of poly(3-hydroxybutyrate-co-hydroxyvalerate) films from halophilic archaea and their potential use in drug delivery

Ozkan Danis · Ayse Ogan · Pınar Tatlican · Azade Attar · Emrah Cakmakci · Bulent Mertoglu · Meral Birbir

Test Isolates

2KYS1, 3TL4, 5TL6 (Birbir et al. 2007), 1TK1 (Birbir et al. 2004) and a novel isolate 1KYS1 were obtained from salt lake (TL), Tuzkoy Salt Mine (TK) and Kayacik saltern (KYS), respectively. All test isolates were maintained at 4 °C on Brown agar medium containing 250 g NaCl, 2.0 g $MgSO_4 \cdot 7H_2O$, 2.0 g KCl, 3.0 g $C_6H_5Na_3O_7 \cdot 5H_2O$, 5 g yeast extract and 2 g agar per liter (Brown 1963; Vreeland 1993).

Extremely halophilic archaeal isolates were separately inoculated into 1 L liquid Brown medium. Then, these cultures were incubated in a shaking incubator (120 rpm) at 39 °C for 10 days. After the incubation period, 15 mL of the culture broth was transferred into a 100 mL of basal minimal medium containing 0.375 g/L KH_2PO_4, 0.2 g/L NH_4Cl, 0.00838 g/L $FeCl_3 \cdot 6H_2O$, 250 g/L NaCl and 20 g/L only one carbon source (corn starch, sucrose, whey, apple, melon or tomato wastes) with pH 7.5 (Lillo and Rodriguez-Valera 1990). Later, these culture media were incubated at 39 °C in a shaking incubator (120 rpm). Maximum turbidity was determined by measuring the optical density of the samples at 550 nm at the end of 10 days of incubation time.

Publications

Table 1 Cell dry weights, maximum PHA concentrations and PHA yields of the isolates

Carbon source	1KYS1			2KYS1			3TL4			5TL6			1TK1		
	Cell dry weight (g/L)	Max. PHA conc. (g/L)	PHA yield (%)	Cell dry weight (g/L)	Max. PHA conc. (g/L)	PHA yield (%)	Cell dry weight (g/L)	Max. PHA conc. (g/L)	PHA yield (%)	Cell dry weight (g/L)	Max. PHA conc. (g/L)	PHA yield (%)	Cell dry weight (g/L)	Max. PHA conc. (g/L)	PHA yield (%)
2 % CS[a]	0.174	0.075	53.14	0.196	0.104	43.10	0.326	0.134	41.10	0.435	0.180	41.38	0.217	0.09	41.47
2 % S[b]	2.219	0.055	2.48	2.5665	0.048	1.87	2.5491	0.105	4.11	3.436	0.112	3.26	3.59	0.071	1.97
2 % W[c]	0.457	0.091	19.92	0.3915	0.077	19.66	0.435	0.104	23.90	0.4785	0.179	37.40	0.412	0.197	47.69
2 % MW[d]	0.371	0.039	10.50	0.555	0.146	26.30	0634	0.134	21.19	0.4576	0.079	17.26	0.593	0.068	11.46
2 % AW[e]	2.550	0.077	3.02	4.54	0.481	10.58	1.918	0.206	10.74	3.7627	0.296	7.85	3.266	0.494	15.25
2 % TW[f]	3.858	0.464	12.03	2.523	0.231	9.39	3.758	0.297	7.91	2.436	0.348	14.28	2.797	0.872	31.17

[a] 2 % Corn Starch Nutrient Broth

[b] 2 % Sucrose Nutrient Broth

[c] 2 % Whey Nutrient Broth

[d] 2 % Melon Nutrient Broth

[e] 2 % Apple Nutrient Broth

[f] 2 % Tomato Nutrient Broth

Publications

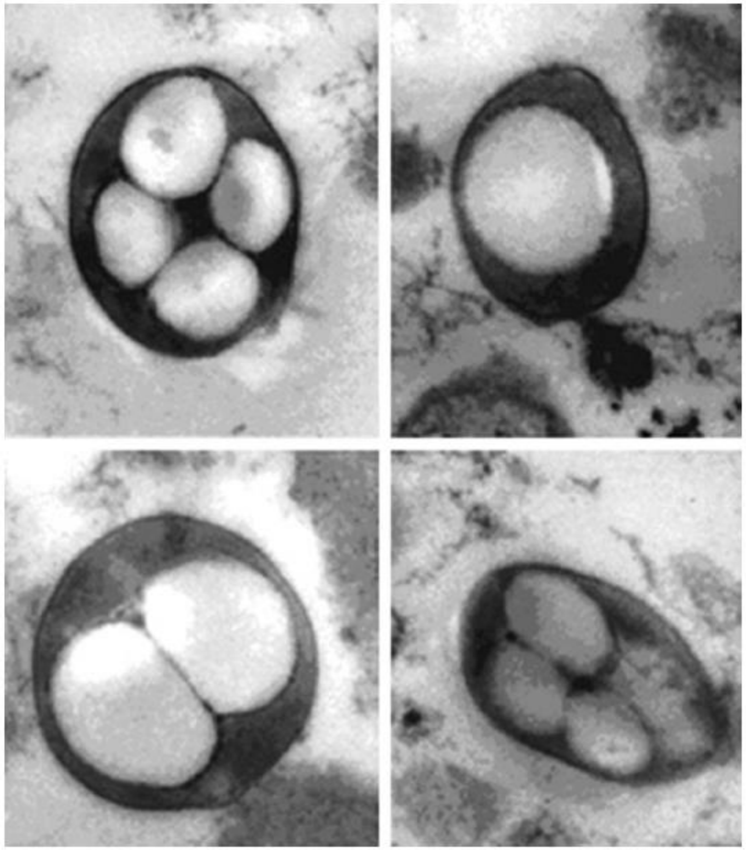


Fig. 3 Transmission electron microscope pictures of PHA granules in 1KYS1 surrounded by a homogeneous membrane. Magnification ×40,000 grown in basal minimal medium; supplemented with 2 % (w/v) corn starch. Cells were harvested on reaching maximum PHA accumulation

Publications

Fig. 1 Structure, [1]HNMR spectrum (**a**) and Fourier transform infrared spectra (**b**) of poly(3-hydroxybutyrate-co-hydroxyvalerate) polymer produced by 1KYS1, when grown on 2 % corn starch