Production of non-biodegradable biopolymers: current state and perspectives

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Biotechnology of New Materials –
Environment – Quality of Life

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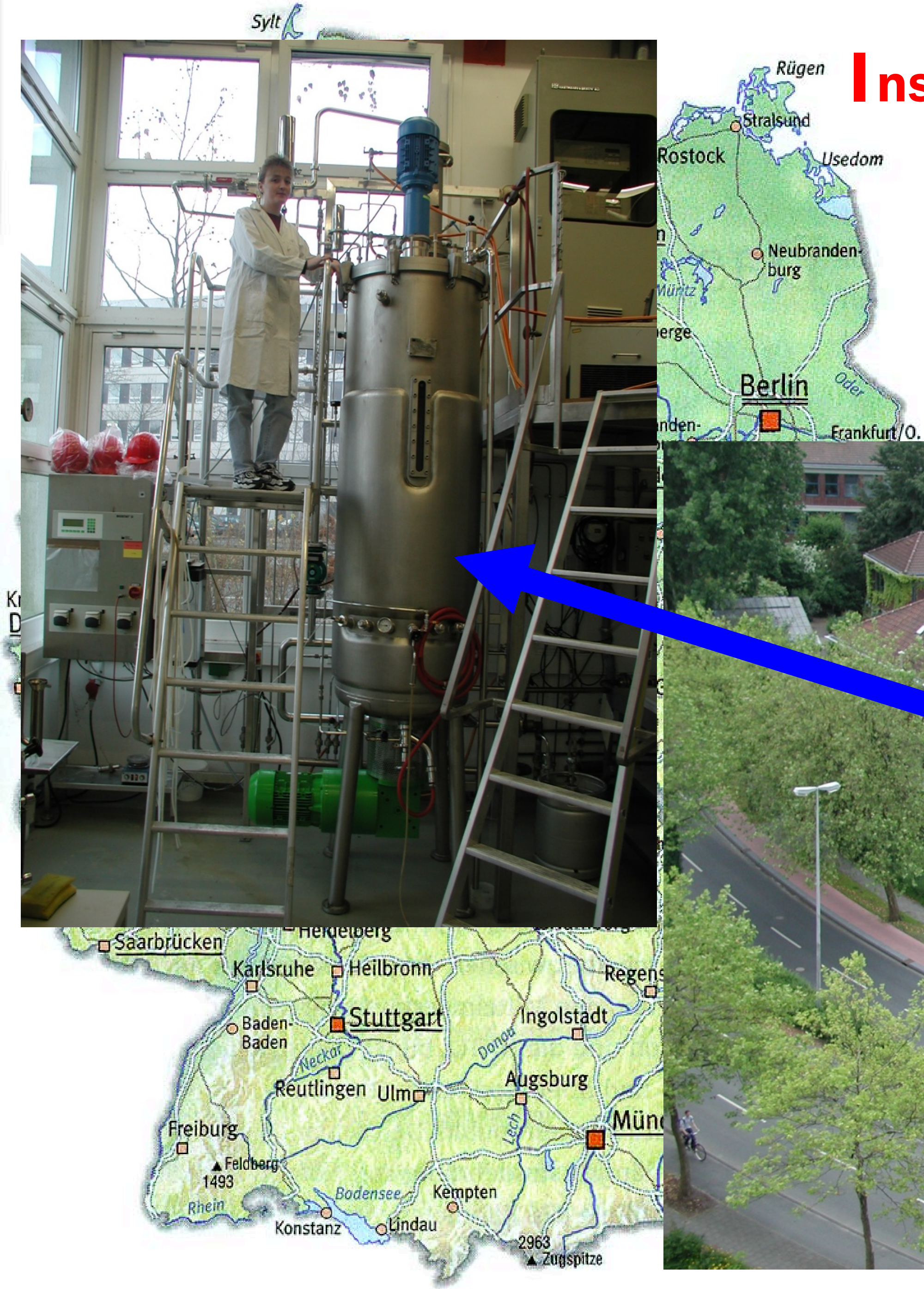
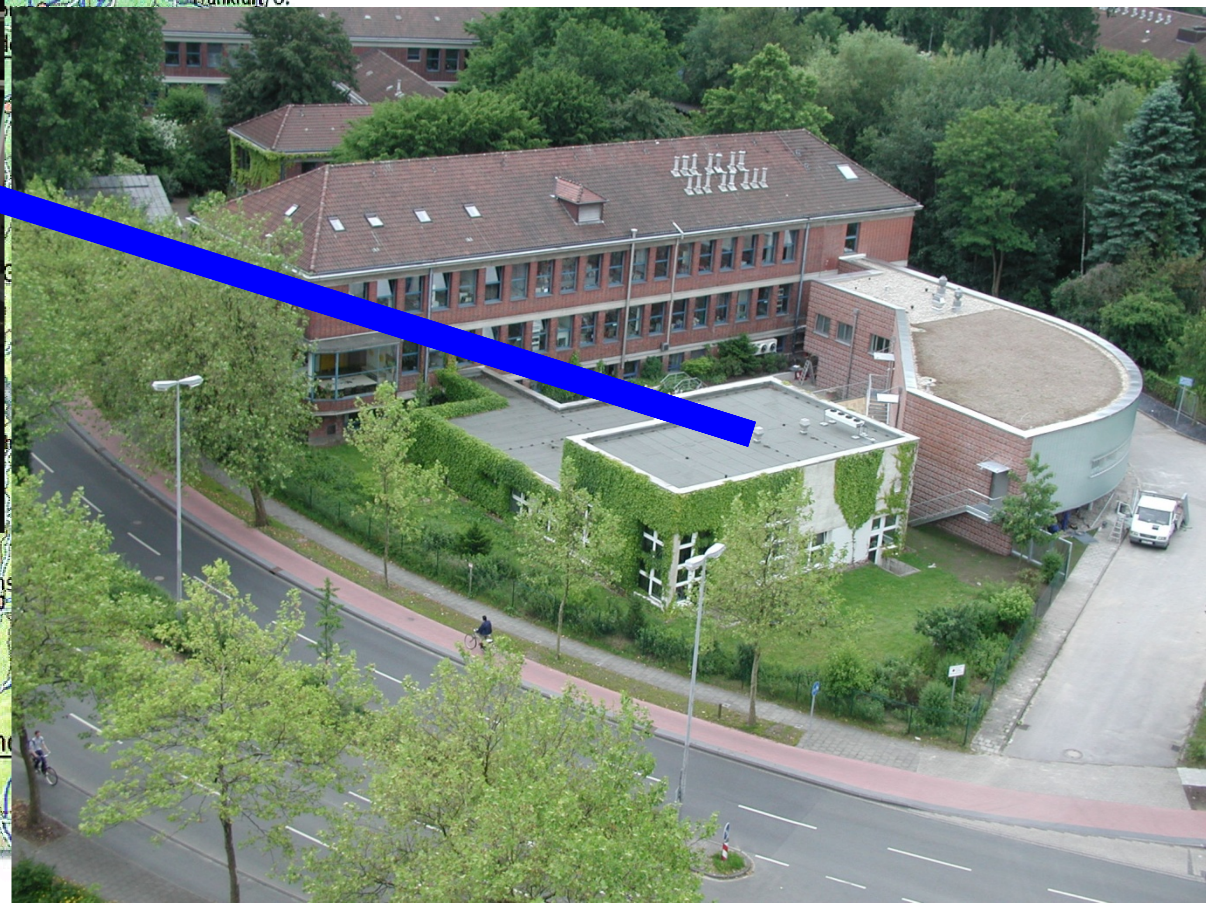
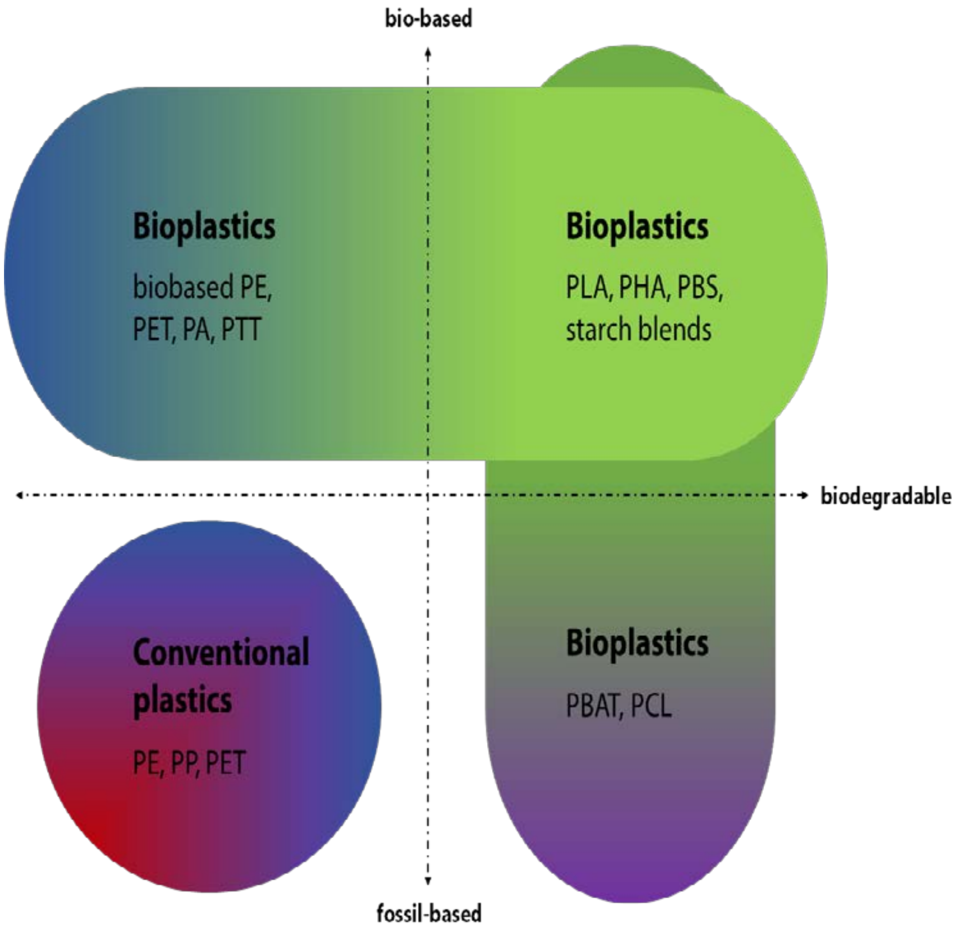
Institute of Molecular Microbiology and Biotechnology (IMMB)





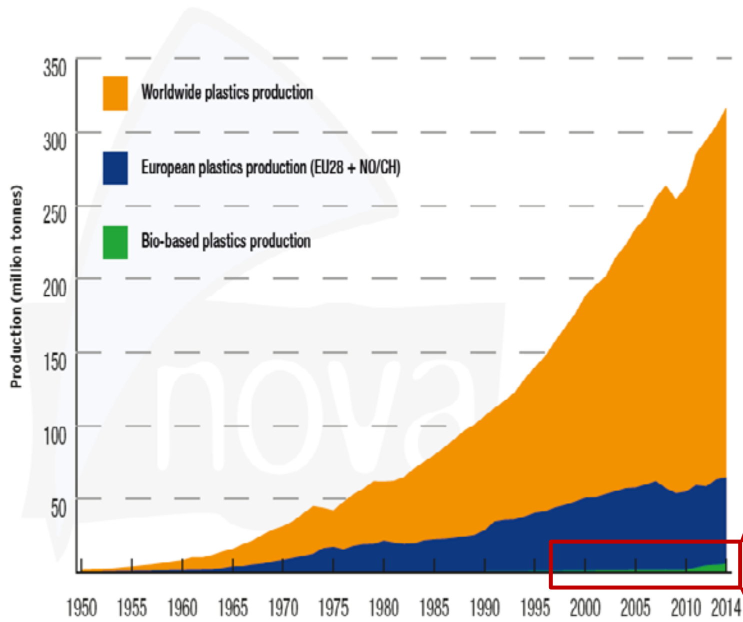
Plastics and biopolymers



Definition of bioplastic:

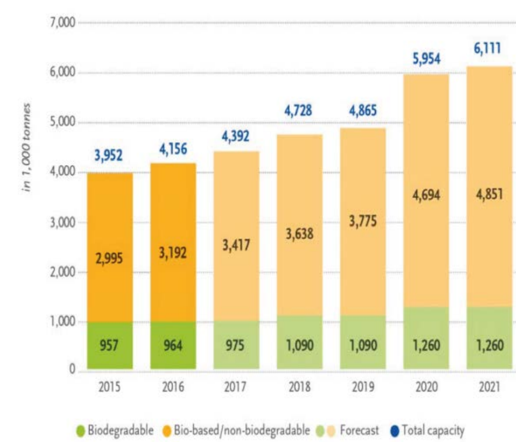
a) bio-based = derived from renewable resources

a) and/or biodegradable

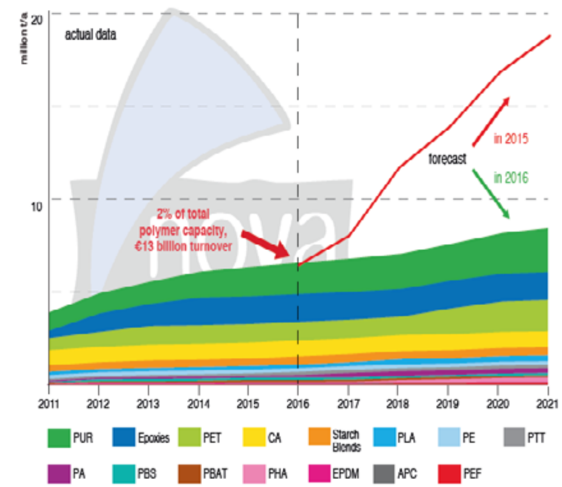


- **Global plastics production 2015:**
  - 330 million tons (1.3 % bio-based)
- **European Bioplastics:**
  - 4.2 Mio. t in 2016 → 6.1 Mio. t in 2021
  - dominated by non-biodegradable bioplastics

Global production capacities of bioplastics



… and bio-based polymers



European Bioplastics & Nova Institute 2016

Persistant, non-biodegradable polymers/plastics

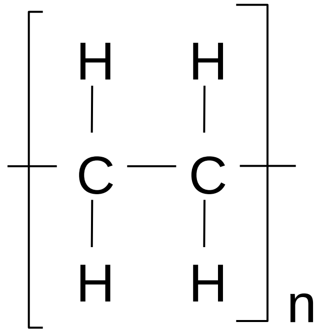
are mostly produced from fossil resources

(about 330 million tons/year)

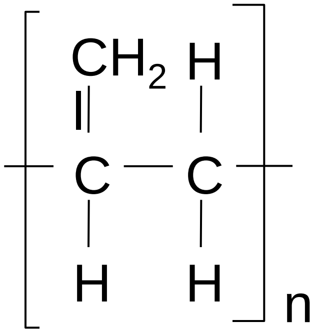
Plastic parts of an automobile

Persistant polymers

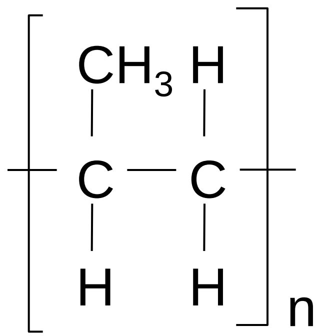
Polyethylene (PE)



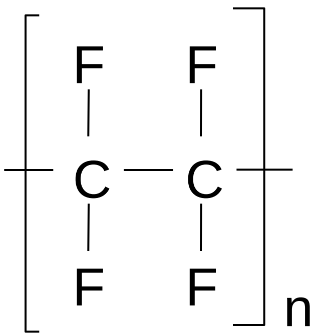
Polyvinylchloride (PVC)



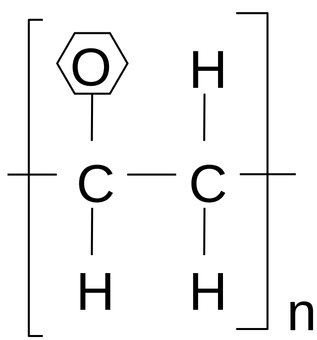
Polypropylene (PP)



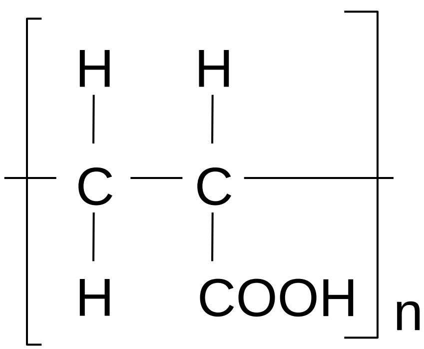
Polytetraflourethylene (PTFE = Teflon)



Polystyrol (PS)



Polyacrylic acid (PA)



Why is polyethylene not biodegradable ?

CH_4	Methane
$H_3C\text{-}CH_3$ to $H_3C\text{-}(CH_2)_{14}\text{-}CH_3$	Ethane to Hexadecane
$H_3C\text{-}(CH_2)_n\text{-}CH_3$	Polyethylene

Paradigm: biopolymers are like all other natural compounds biodegradable

However, synthetic polymers are not necessarily persistant,

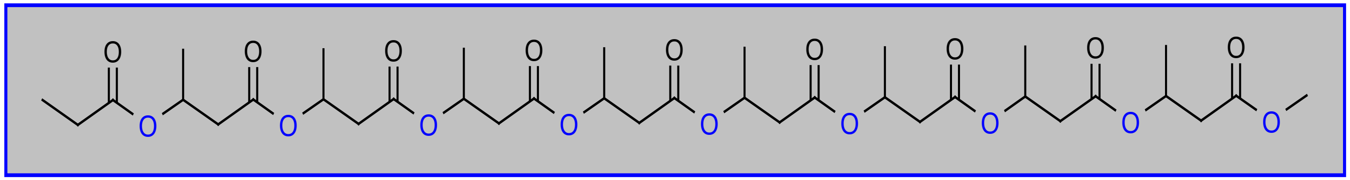
i.e. non-biodegradable

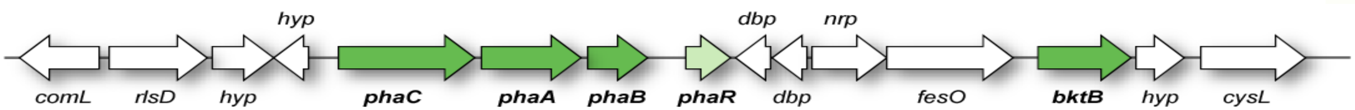
Biodegradable plastics from renewable resources?

Polyhydroxyalkanoates – PHA

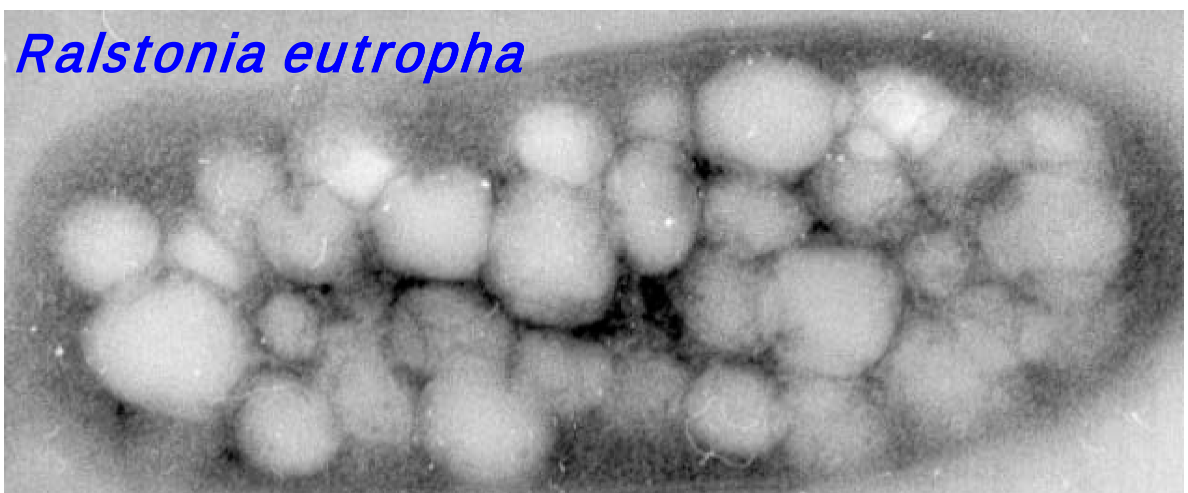
Bacterial storage compounds for carbon and energy

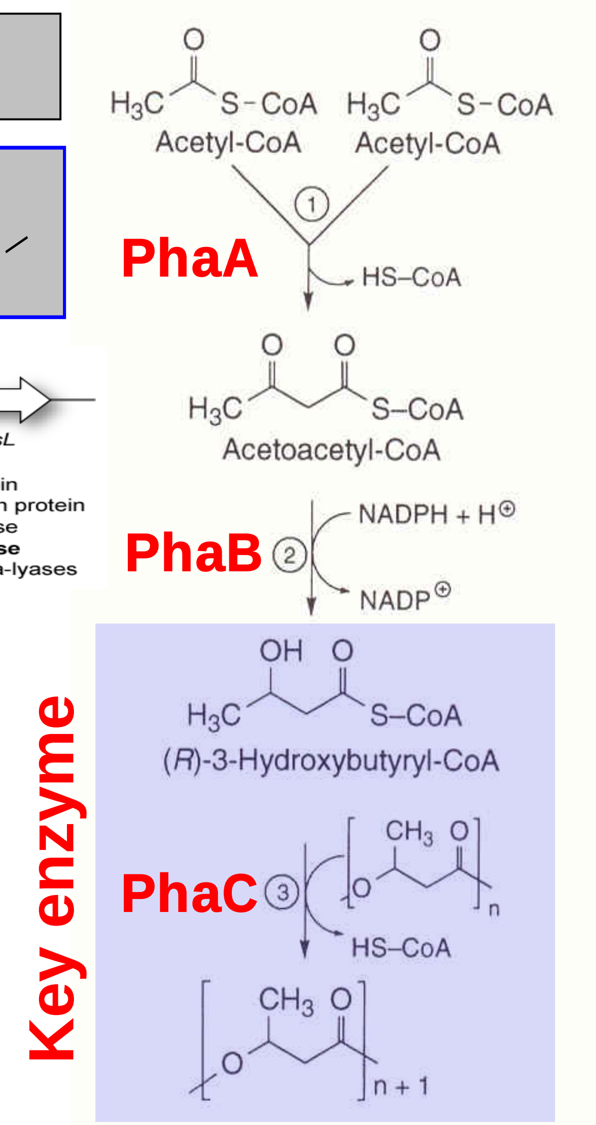
Best studied example: Poly(3-hydroxybutyrate)





comL	Lipoprotein, comL-family	***phaC***	**Poly(3HB)-Synthase PhaC**	*dbp*	DNA binding protein
rlsD	Pseudouridylate synthases, 23S specific	***phaA***	**Aceto-acetyl-CoA redutase**	*nrp*	Nitrogen regulation protein
hyp	hypothetical protein	***phaB***	**3-ketoacyl-thiolase**	*fesO*	FeS oxidoreductase
		phaR	**Regulator of Phasin expression**	***bktB***	**3-ketoacyl-thiolase**
				cysL	Cystathionine beta-lyases

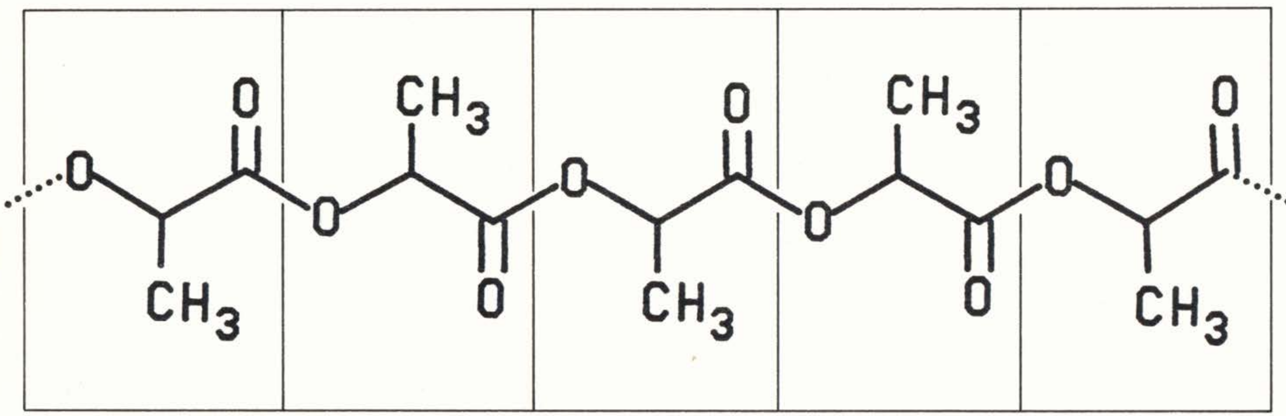




Cargill

NatureWorks LLC

Production of Polylactic acid



Glucose → Lactic acid bacteria → **Lactic acid**

Lactic acid → Chemical polymerization → **PLA**

(ca. 140.000 tons per year)

Must biopolymers be biodegradable ?

Biopolymers were in the past developed for applications where biodegradability is essential:

(■ compostable packaging material ■ Resorbable materials in medicine)

Persistant, non-biodegradable, corrosions-resistant Polymers are required in large amounts for different applications
(■ Construction ■ Automobiles)

The biotechnological production of non-biodegradable polymers will open new perspectives for the chemical industry and for the use of renewable resources

Persistant, non-biodegradable plastics from renewable resources?

(biotechnological + chemical processes)

„Green“ polyethylene

- by Braskem S. A. (Brazil)
- Capacity: 200,000 tons/year
- Utilization of existing infrastructure and established knowledge for monomer synthesis
- well established polymer/material

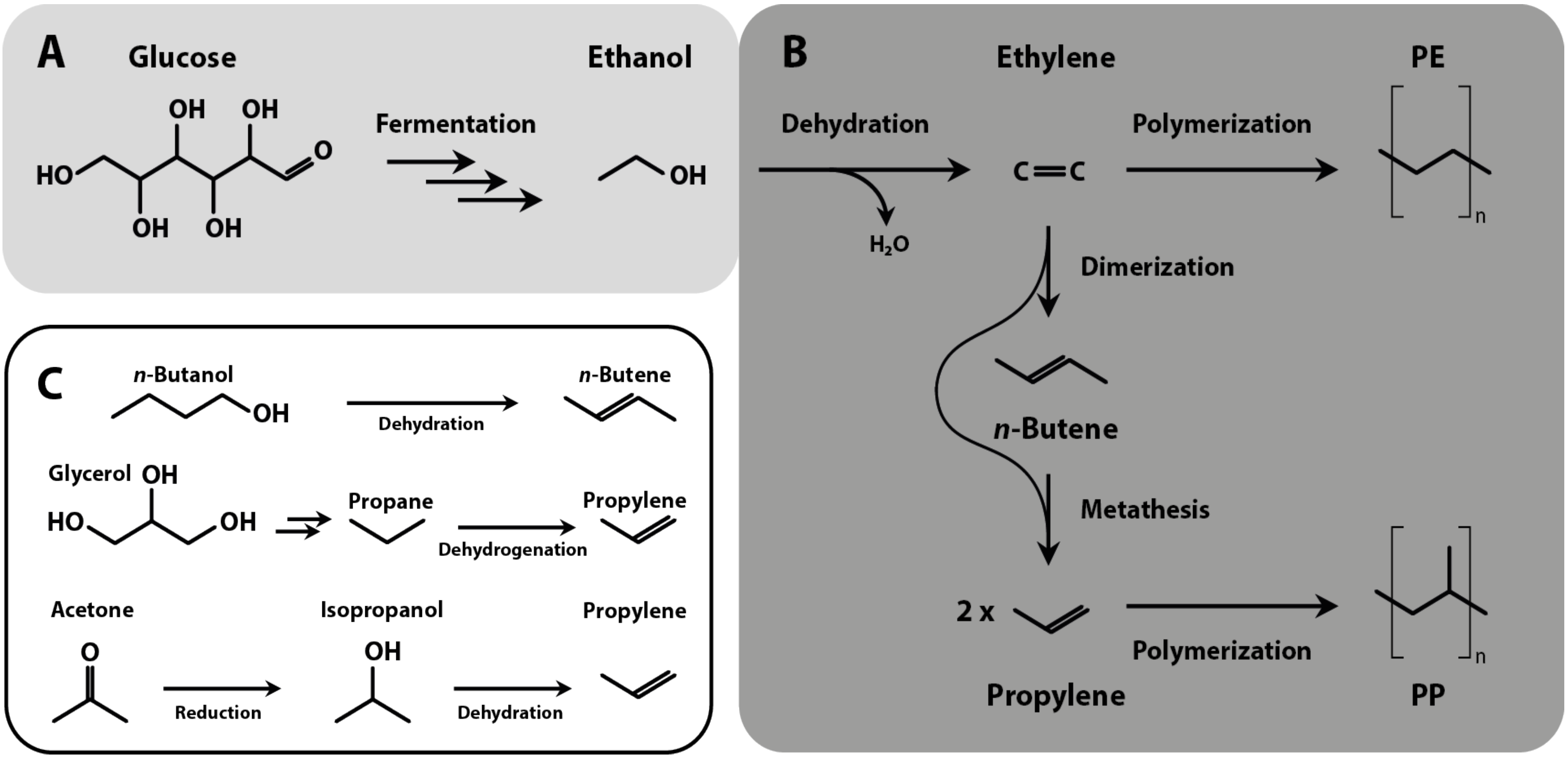
Strategy/Process:

(1) Fermentative production of ethanol

(2) Chemical conversion of ethanol to ethylene

(3) Chemical polymerization of ethylene

Production routes for biomass-derived polyethylene (A+B) and polypropylene (C+B)



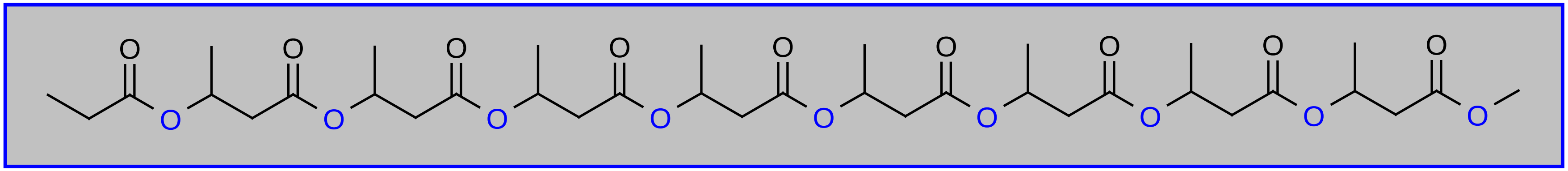
Persistant, non-biodegradable plastics from renewable resources?

(biotechnological processes)

Poly(3HB) ⇔ Poly(3MB)

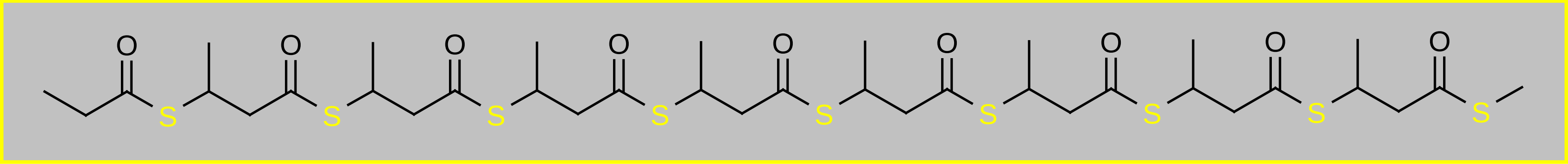
Polyoxoester: Poly(3HB)

Tg = 4 °C, Tm = 175 °C

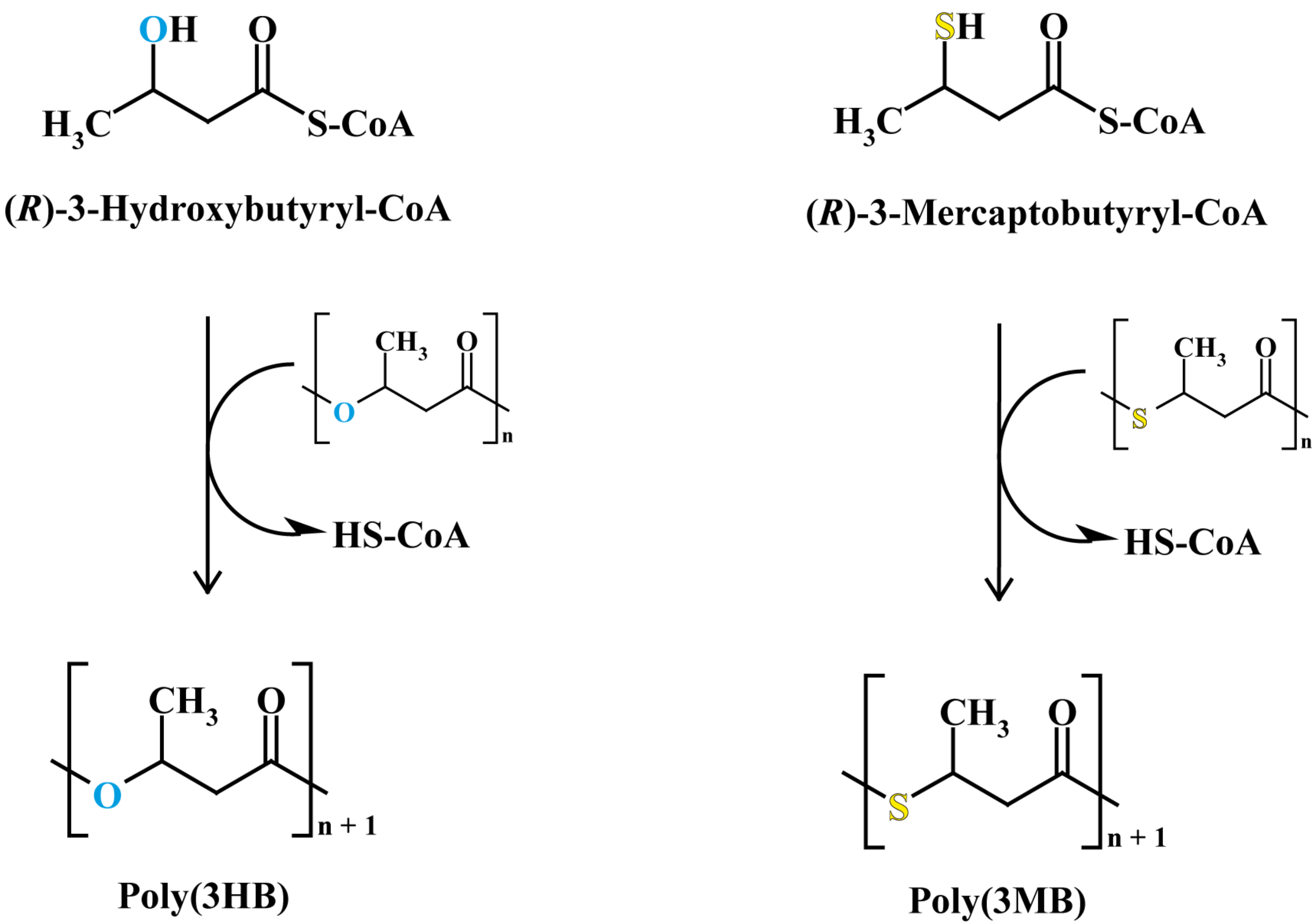


Polythioester: Poly(3MB)

Tg = 8 °C, Tm = 100 °C

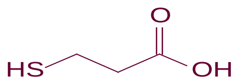


PHA synthases synthesize polyoxoesters as well as polythioesters

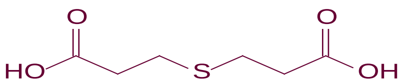


PTE copolymers produced by *Ralstonia eutropha* H16

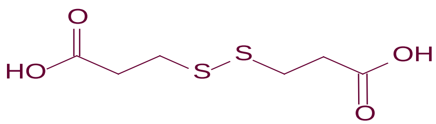
Precursor substrates | **Accumulated Polymer**



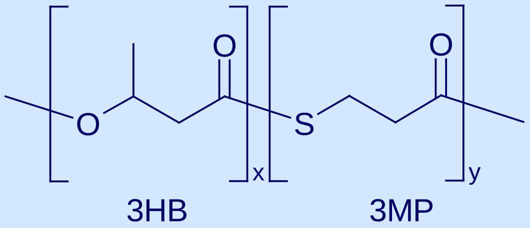
3-Mercaptopropionate (3MP)



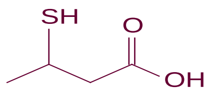
3,3‘-Thiodipropionate (TDP)



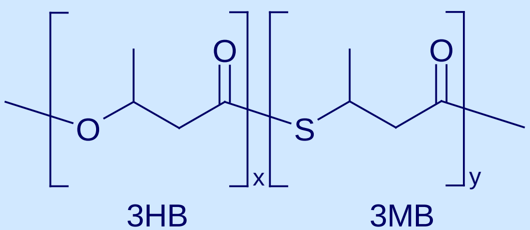
3,3‘-Dithiodipropionate (DTDP)



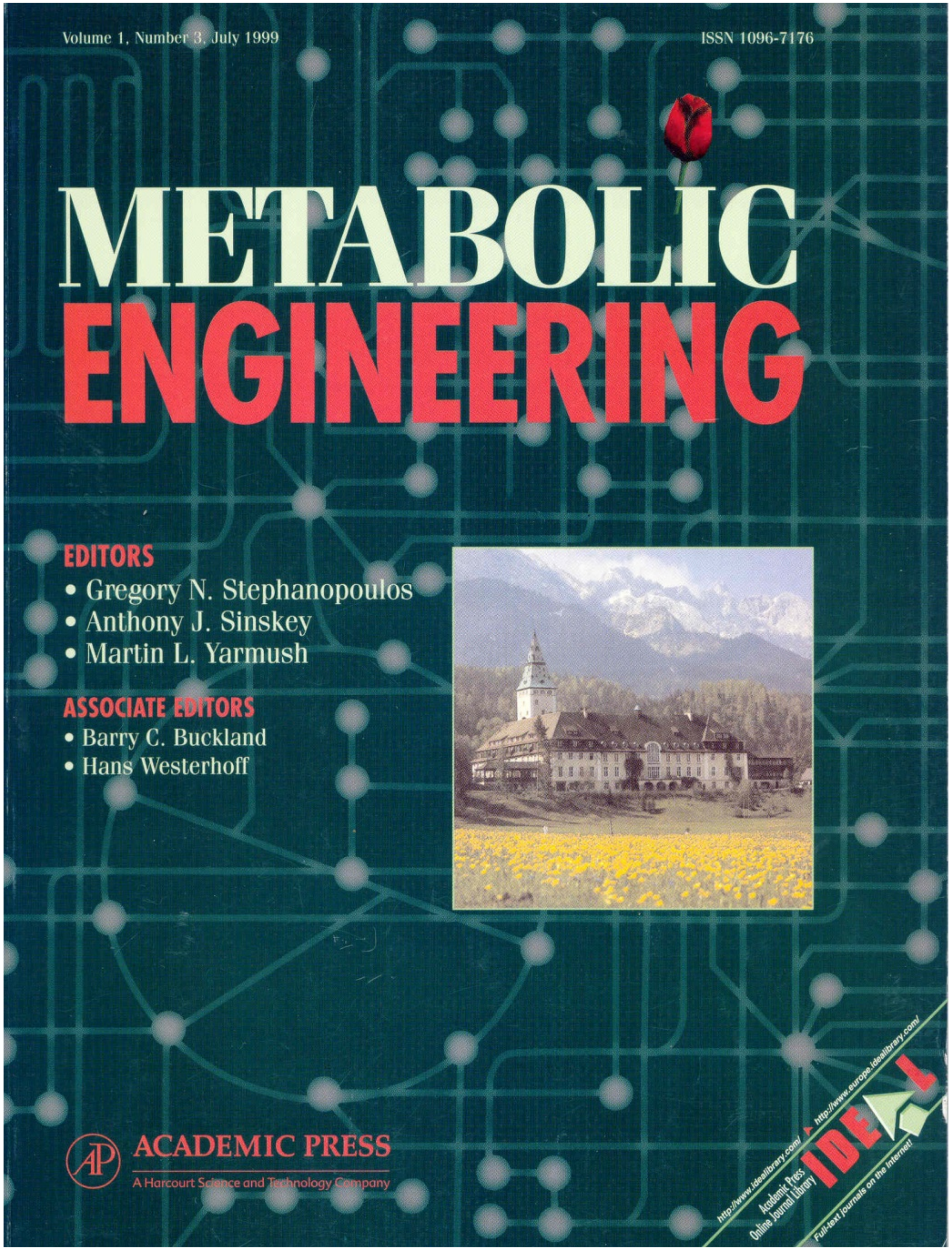
Poly(3HB-*co*-3MP)



3-Mercaptobutyrate (3MB)



Poly(3HB-*co*-3MB)



The metabolism of an organism is engineered :

- add new enzymes
- add new pathways

- inactivate genes
- eliminate existing pathways

- alter transport
- modify regulation
- ....

PTE homopolymers produced by *E. coli* pBPP1

<u>Cultivation of *E. coli* pBPP1:</u>

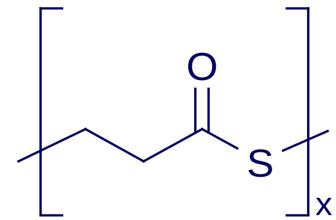
M9 mineral salt medium

+ glucose

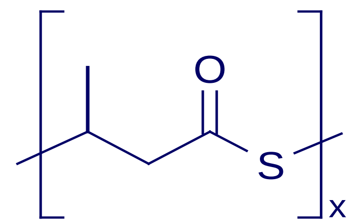
↓ 12 h

+ 3-mercaptoalkanoic acids

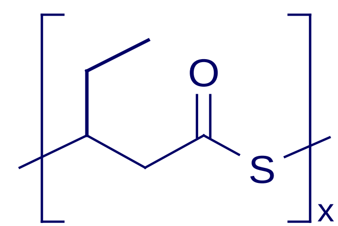
(3MP or 3MB or 3MV)



Poly(3-mercaptopropionate) (PMP)

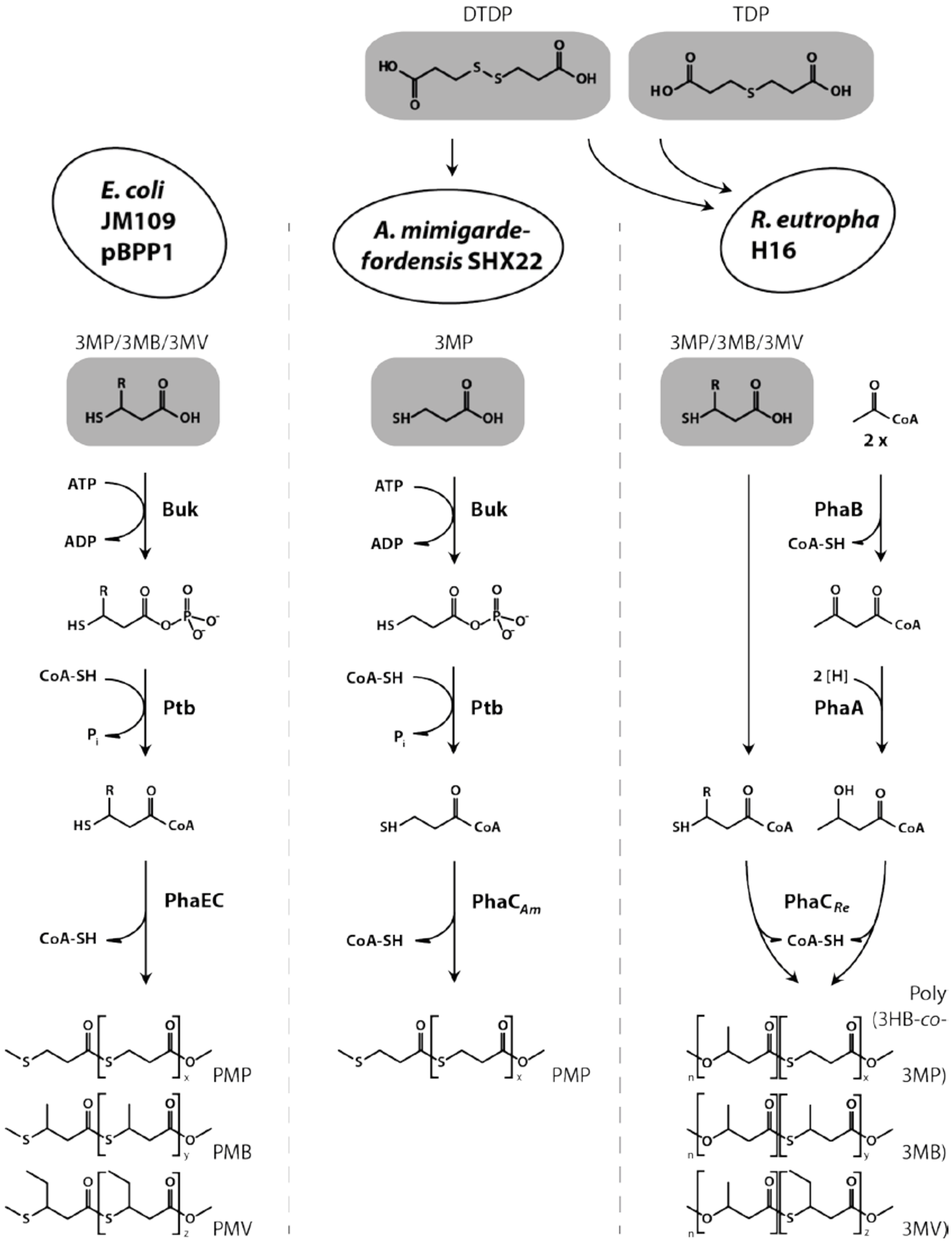


Poly(3-mercaptobutyrate) (PMB)

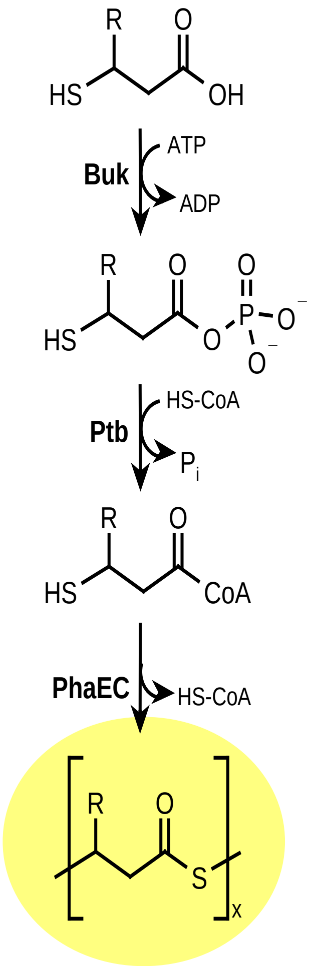


Poly(3-mercaptovalerate) (PMV)

Biosynthesis of polythioesters by microbial fermentation

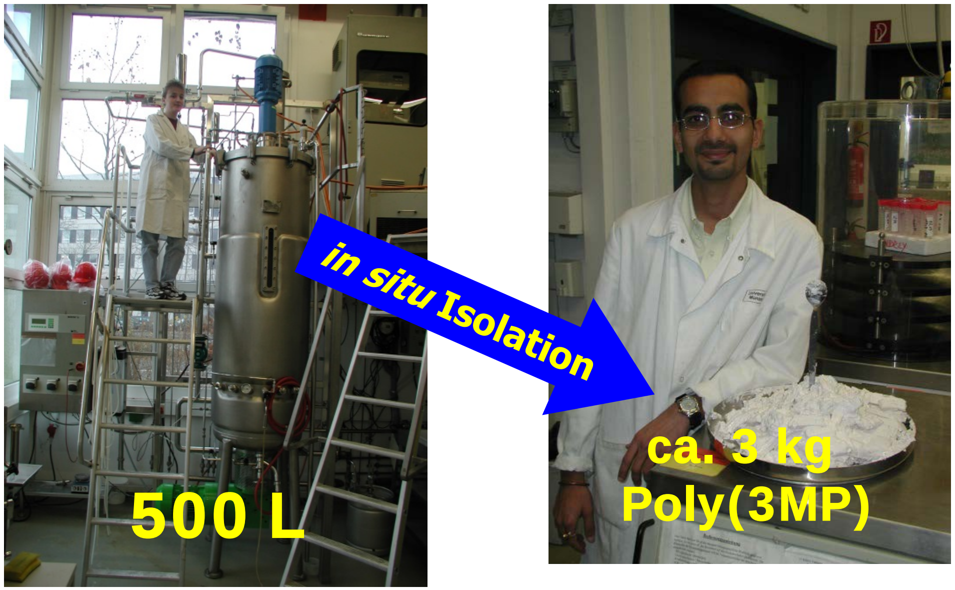


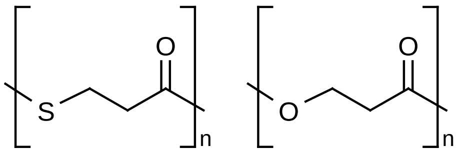
Polythioesters: Poly(3MP)



BPEC pathway

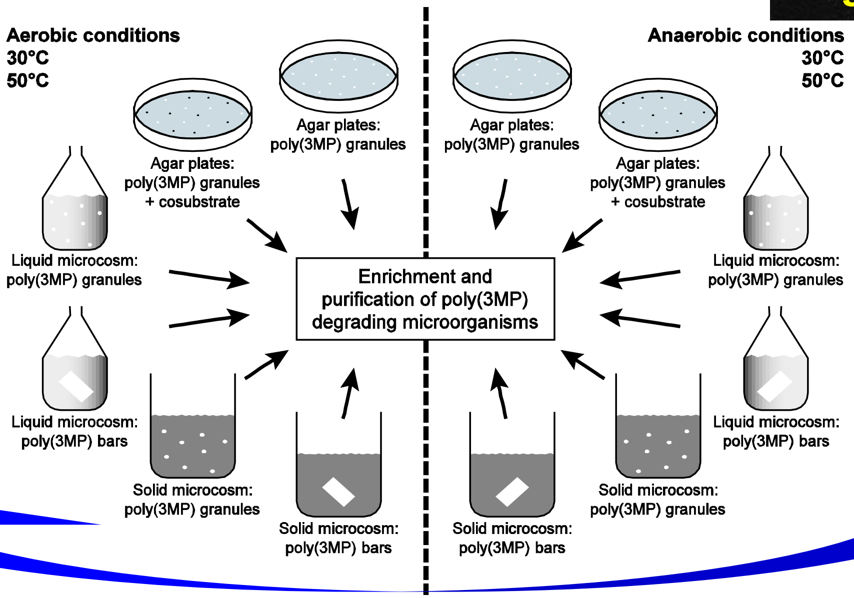
PTEs are persistant und not bio-degradable

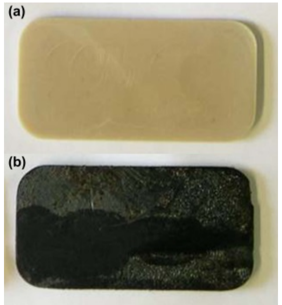




T_M = 170°C T_M = 121°C

T_Z = 277°C T_Z = 220°C





Poly(3-mercaptopropionate): A Nonbiodegradable Biopolymer?

Do Young Kim, Tina Lütke-Eversloh, Khaled Elbanna, Nehal Thakor, and Alexander Steinbüchel

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*Bio*MACROMOLECULES

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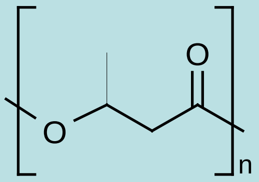
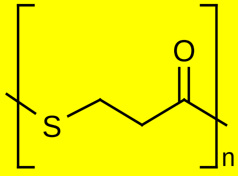
Modulation of biodegradability



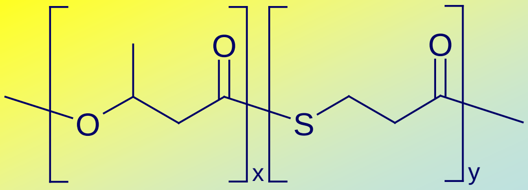
Poly(3HB)

3HB Homopolymers
from *R. eutropha:*
fully biodegradable



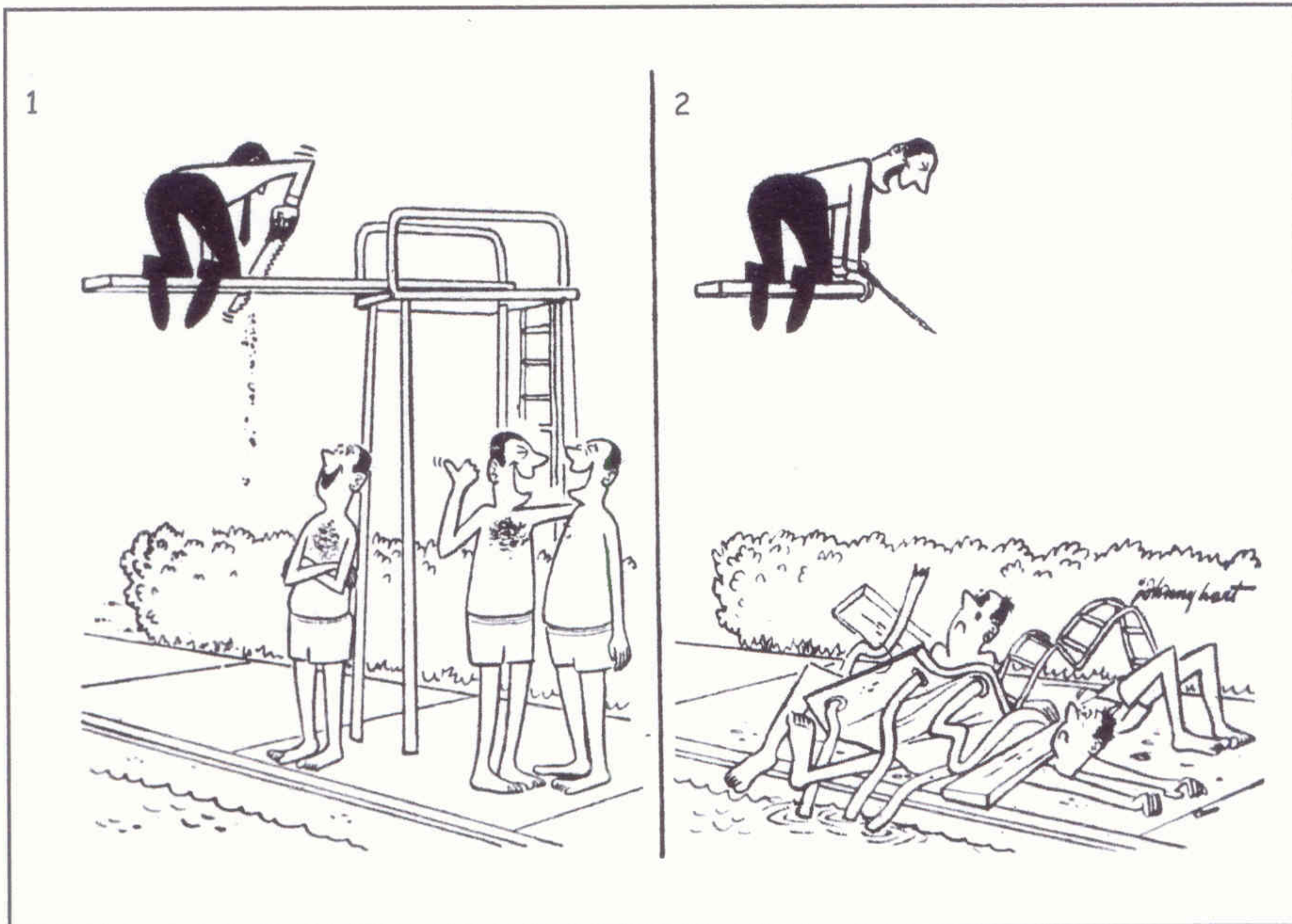
Poly(3MP)

3MP Homopolymers
from recombinant *E. coli:*
fully persistant



Poly(3HB-*co*-3MP)

3MP/3HB Copolymers
from *R. eutropha:*
partially biodegradable
with designed degradation rate



Never be prejudiced in experimental science!

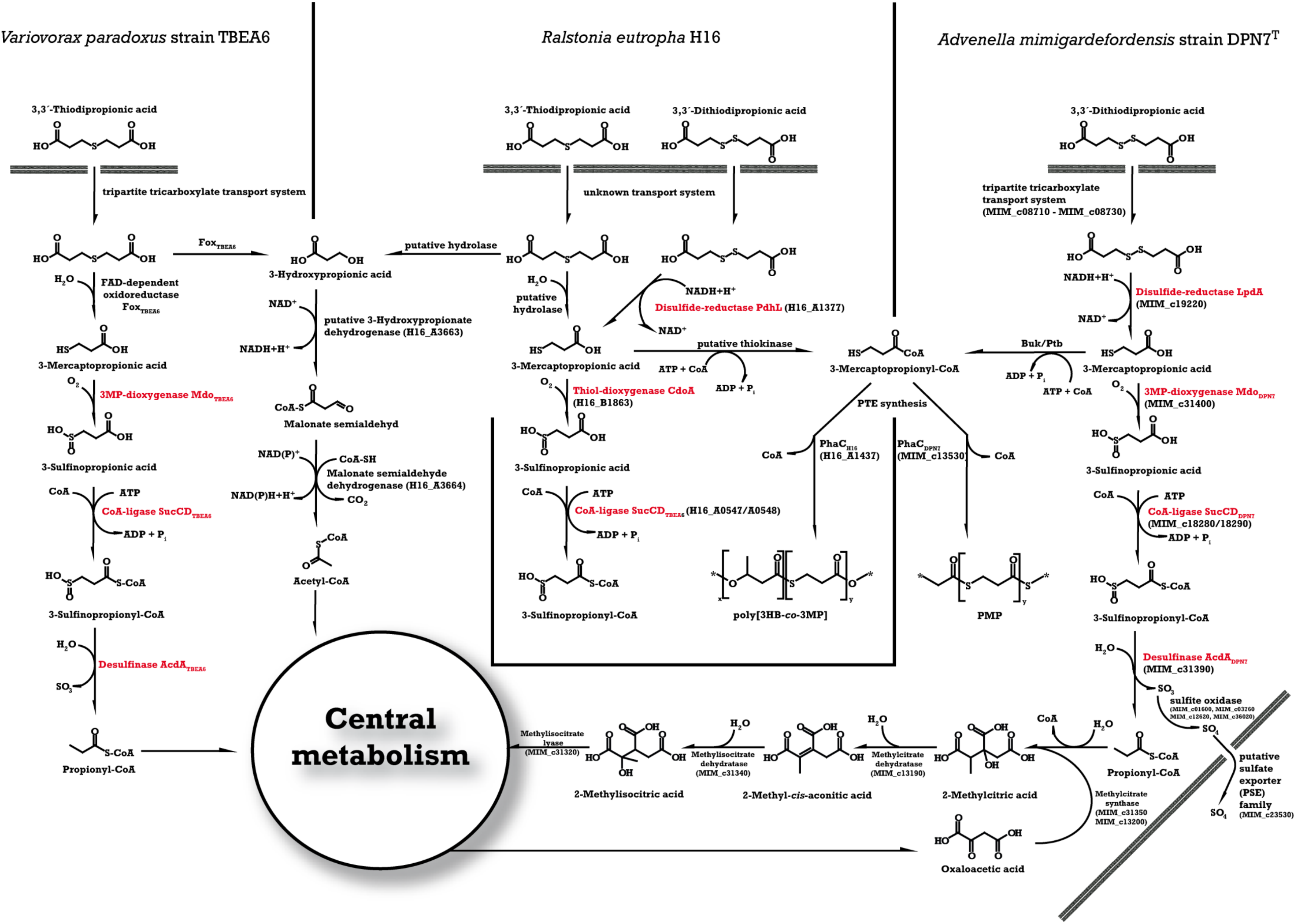
Perspectives for PTEs

- are only obtained from organic thiochemicals
- these chemicals are too expensive
- these chemicals are often toxic for bacteria

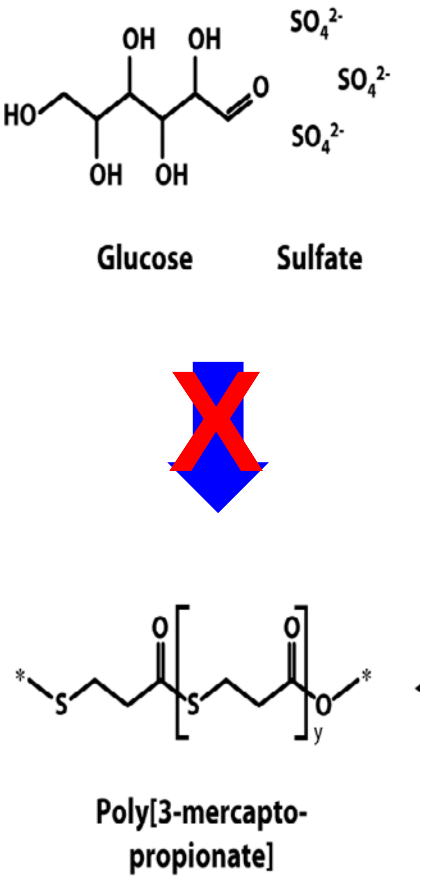
Engineering of the metabolism of suitable production organisms

⇩⇩⇩

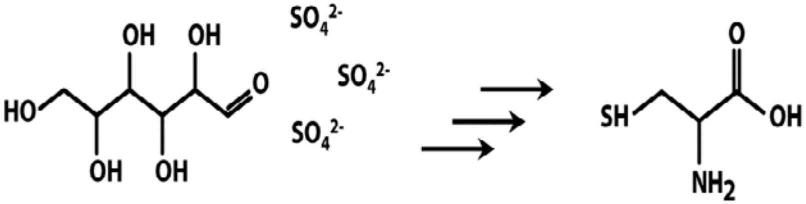
Production of PTEs from simple carbon sources and sulphate


Variovorax paradoxus strain TBEA6
Ralstonia eutropha H16
Advenella mimigardefordensis strain DPN7T
3,3´-Thiodipropionic acid
tripartite tricarboxylate transport system
Fox TBEA6
FAD-dependent oxidoreductase Fox TBEA6
3-Mercaptopropionic acid
3MP-dioxygenase Mdo TBEA6
3-Sulfinopropionic acid
CoA-ligase SucCD TBEA6
ADP + Pi
3-Sulfinopropionyl-CoA
Desulfinase AcdA TBEA6
Propionyl-CoA
3-Hydroxypropionic acid
putative 3-Hydroxypropionate dehydrogenase (H16_A3663)
Malonate semialdehyd
Malonate semialdehyde dehydrogenase (H16_A3664)
Acetyl-CoA
putative hydrolase
3,3´-Dithiodipropionic acid
unknown transport system
Disulfide-reductase PdhL (H16_A1377)
putative thiokinase
Thiol-dioxygenase CdoA (H16_B1863)
CoA-ligase SucCD TBEA6 (H16_A0547/A0548)
3-Mercaptopropionyl-CoA
PTE synthesis
PhaC H16 (H16_A1437)
PhaC DPN7 (MIM_c13530)
poly[3HB-co-3MP]
PMP
tripartite tricarboxylate transport system (MIM_c08710 - MIM_c08730)
Disulfide-reductase LpdA (MIM_c19220)
Buk/Ptb
3MP-dioxygenase Mdo DPN7 (MIM_c31400)
CoA-ligase SucCD DPN7 (MIM_c18280/18290)
Desulfinase AcdA DPN7 (MIM_c31390)
sulfite oxidase (MIM_c01600, MIM_c03760 MIM_c12620, MIM_c36020)
putative sulfate exporter (PSE) family (MIM_c23530)
Central metabolism
Methylisocitrate lyase (MIM_c31320)
2-Methylisocitric acid
Methylisocitrate dehydratase (MIM_c31340)
2-Methyl-cis-aconitic acid
Methylcitrate dehydratase (MIM_c13190)
2-Methylcitric acid
Methylcitrate synthase (MIM_c31350 MIM_c13200)
Oxaloacetic acid

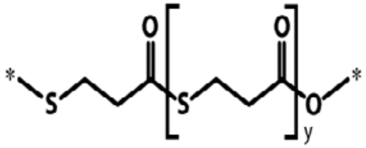
Synthetic pathways for PTE production from cheap and non-toxic substrates



Synthetic pathways for PTE production from cheap and non-toxic substrates

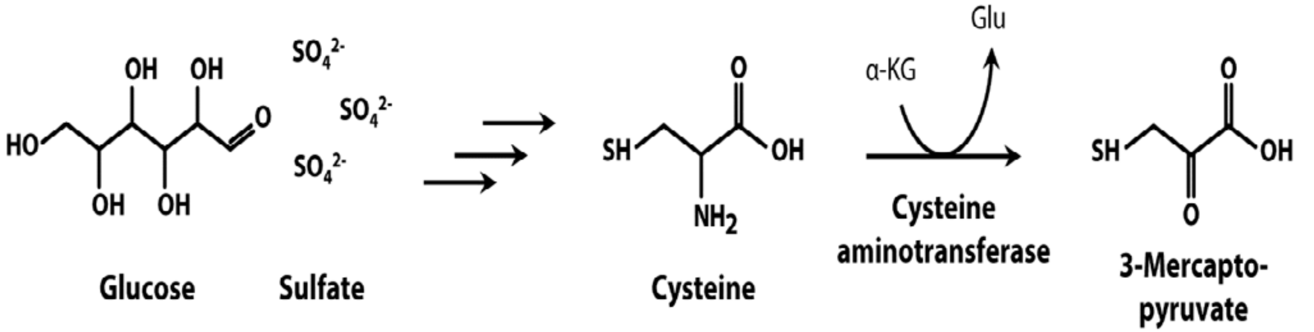


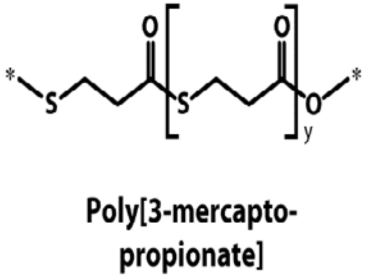
Glucose Sulfate Cysteine



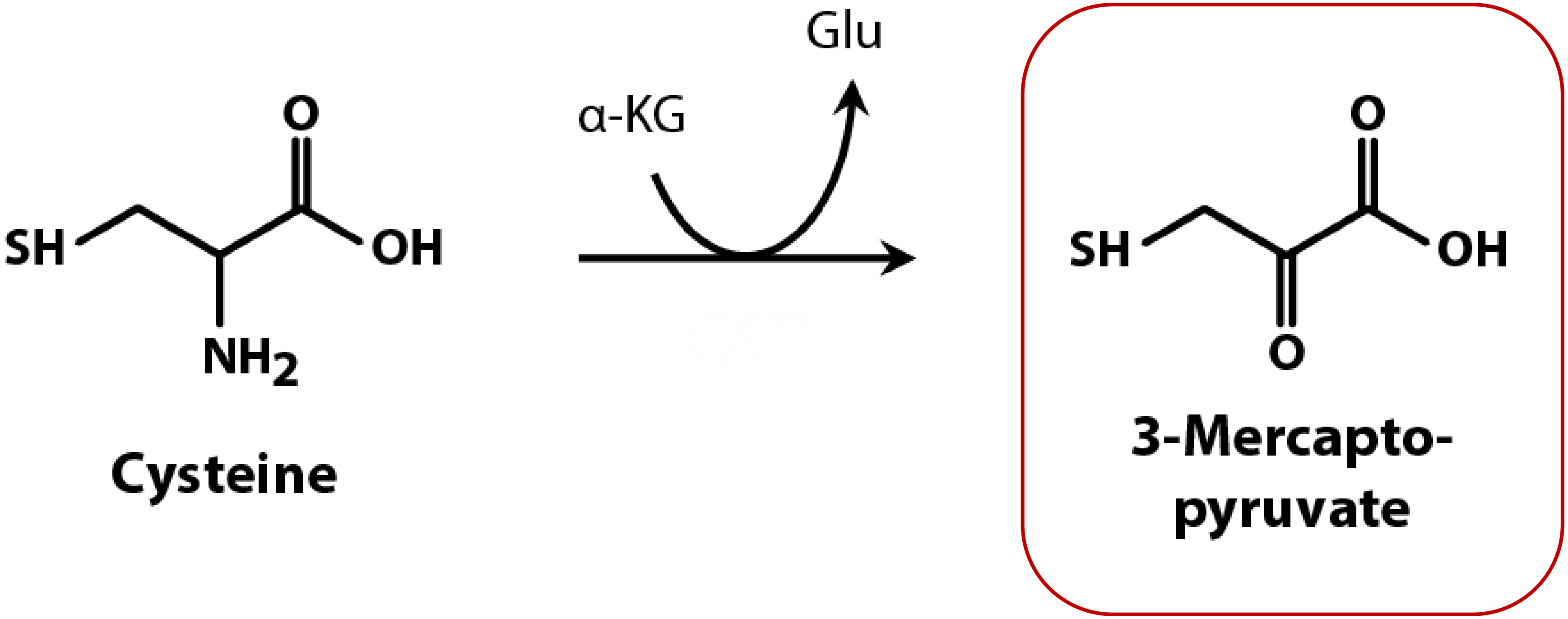
Poly[3-mercapto-propionate]

Synthetic pathways for PTE production from cheap and non-toxic substrates



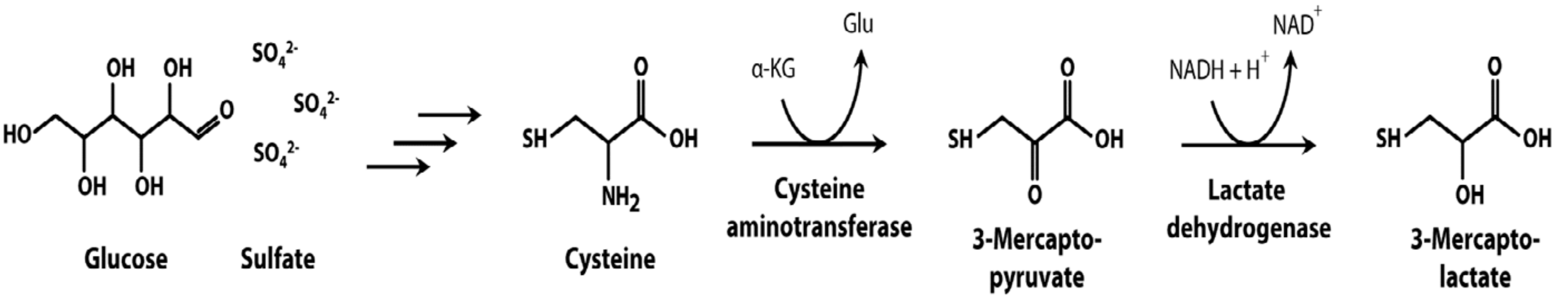


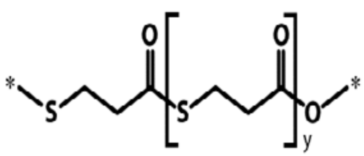
Conversion of cysteine into 3-mercaptopyruvate



(Aspartate) (Oxaloacetate)

Synthetic pathways for PTE production from cheap and non-toxic substrates



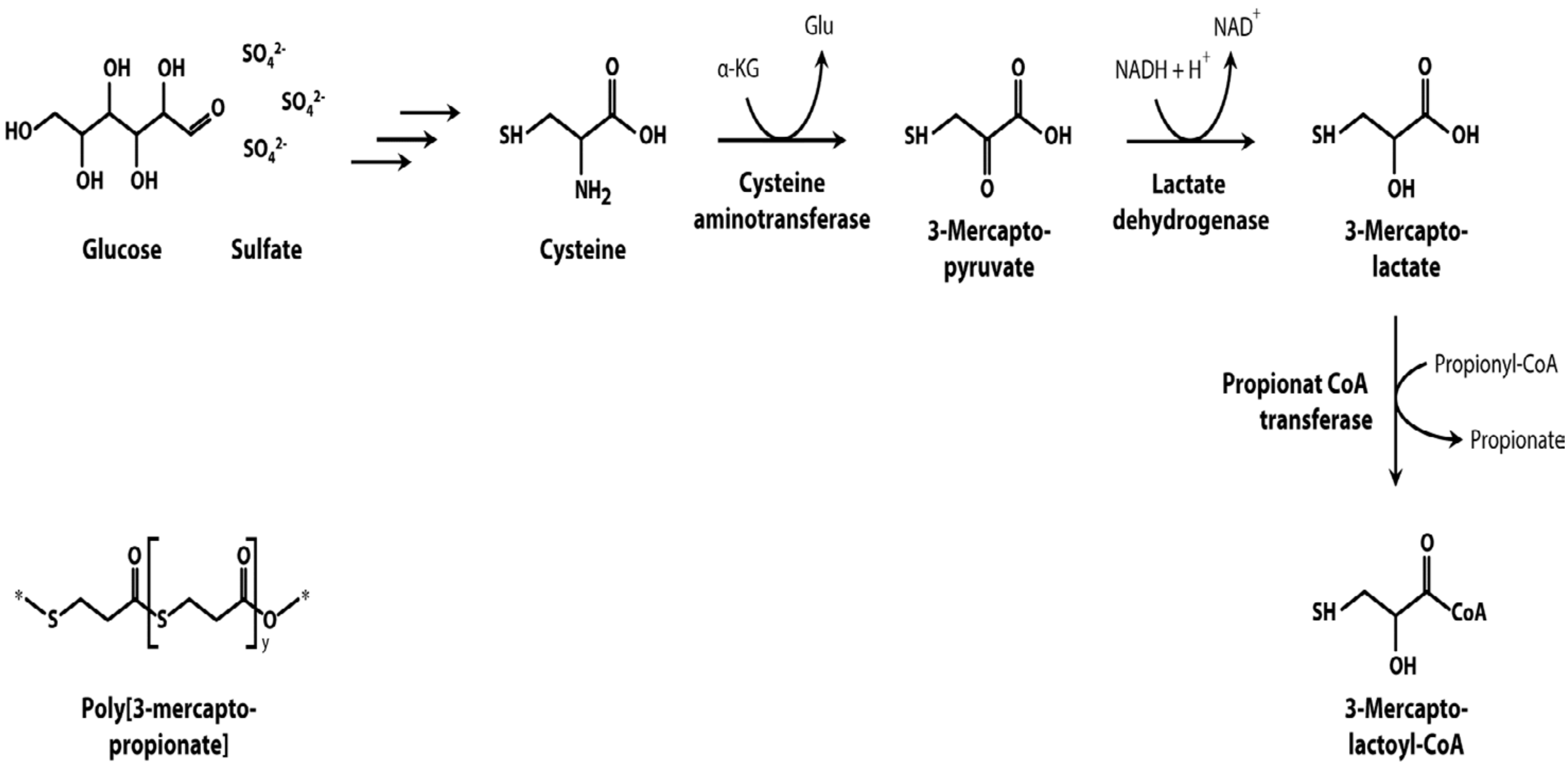


Poly[3-mercapto-propionate]

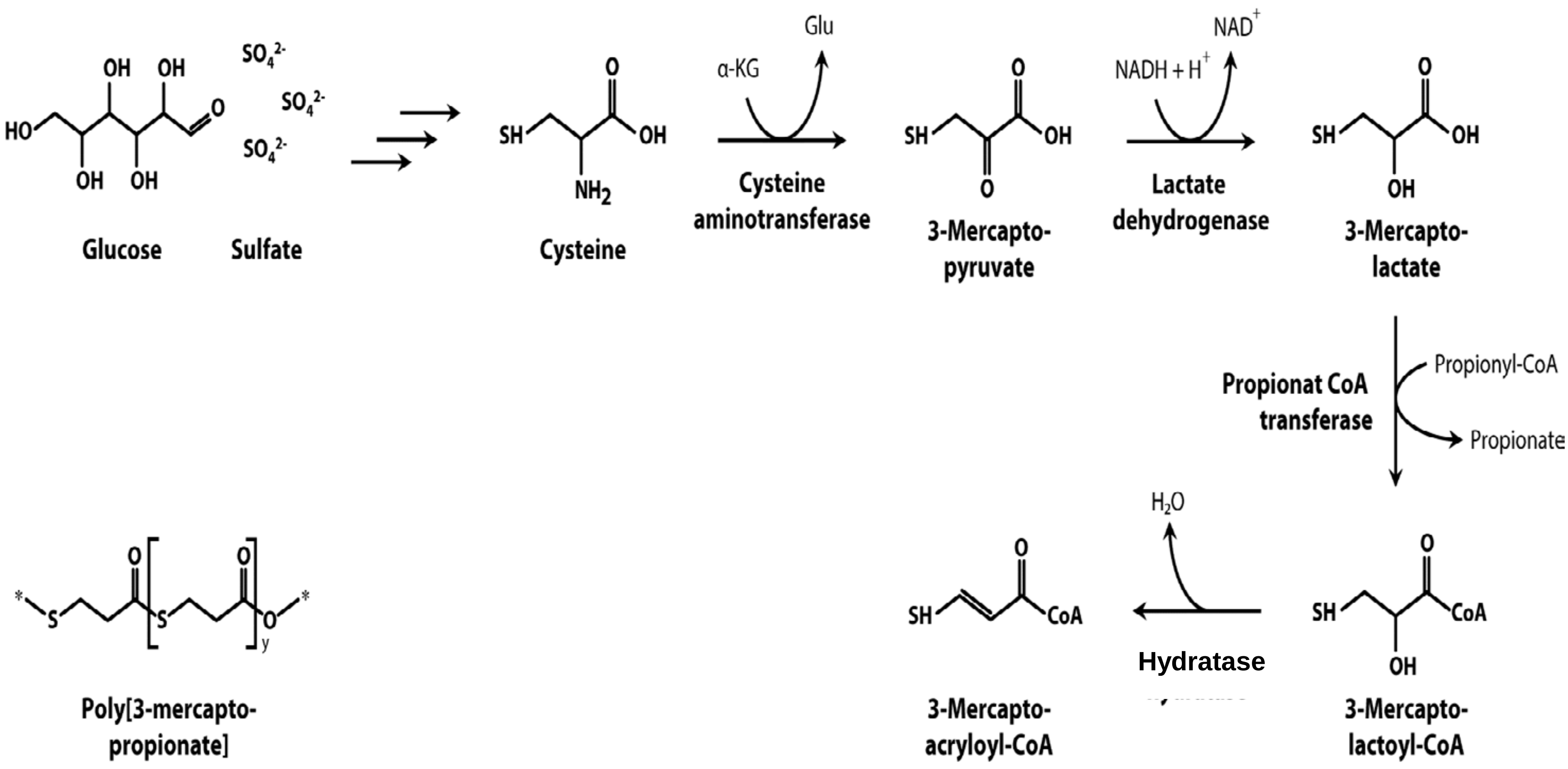
Studies on LDHs capable of reducing 3-Mercaptopyruvate

- ***In silico*** **analysis of LDH sequences**
- **Purification of selected bacterial LDHs which convert 3MPy to 3ML**
- ***In vitro*** **determination of specific activities with 3MPy**

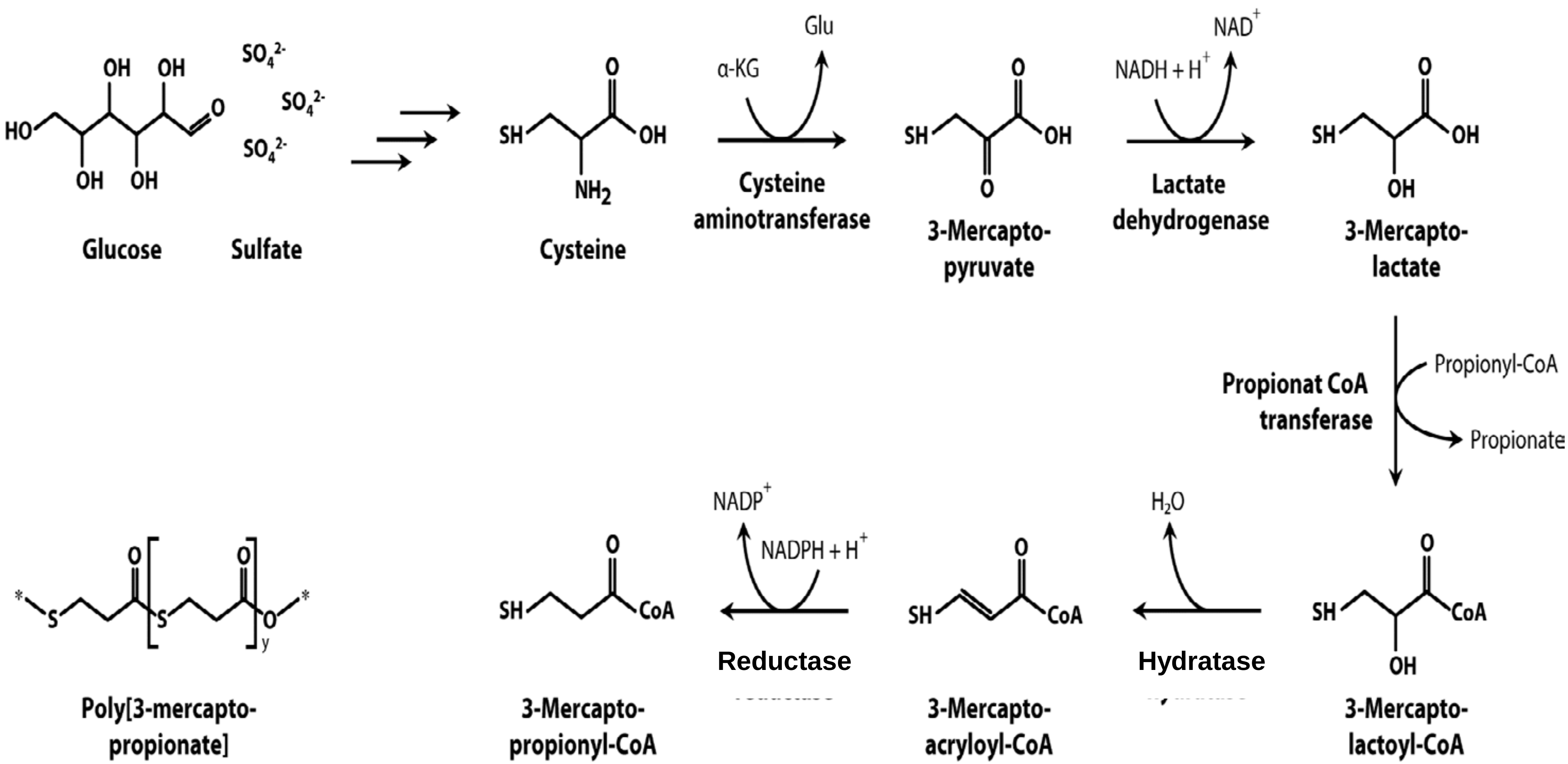
Synthetic pathways for PTE production from cheap and non-toxic substrates



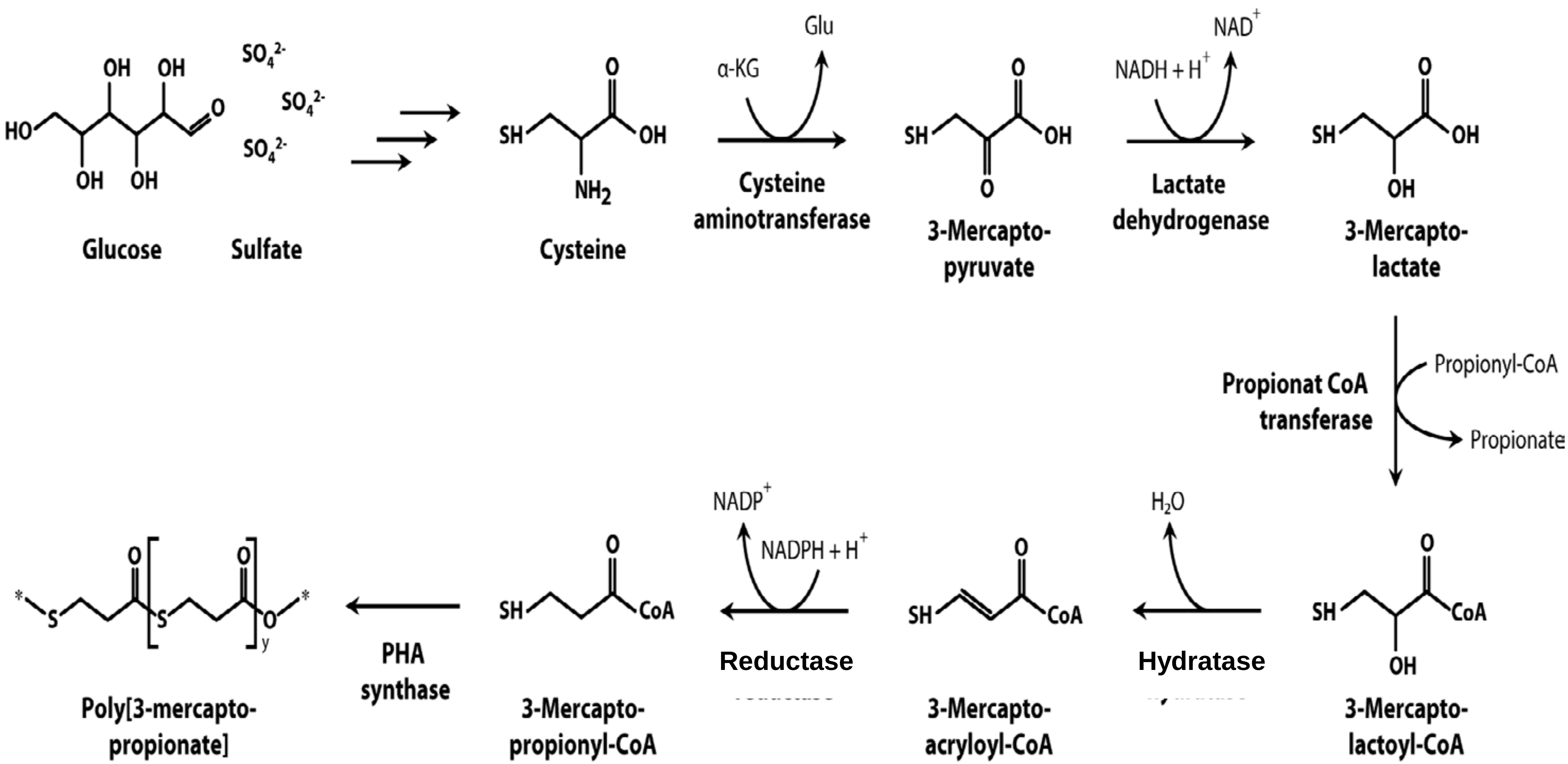
Synthetic pathways for PTE production from cheap and non-toxic substrates



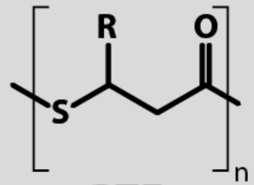
Synthetic pathways for PTE production from cheap and non-toxic substrates



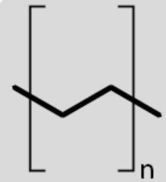
Synthetic pathways for PTE production from cheap and non-toxic substrates



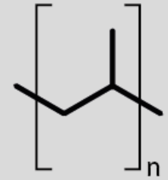
Chemical structures of persistent bioplastics



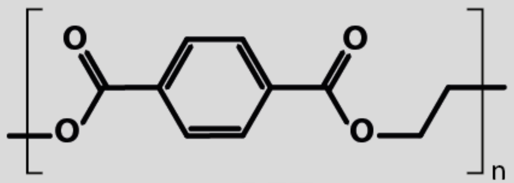
PTE



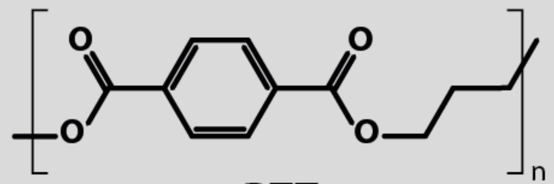
PE



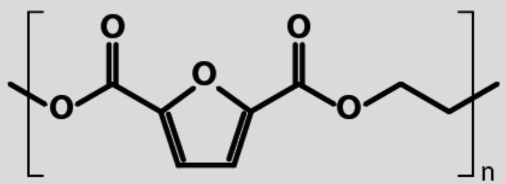
PP



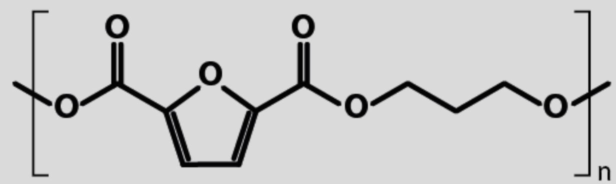
PET



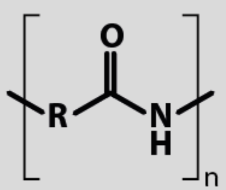
PTT



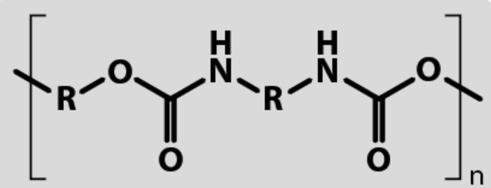
PEF



PTF



PA



PUR

Summary and Conclusions

- Persistant plastics are so far almost exclusively produced from fossil resources.

- However, non-biodegradable plastics can be also produced by fermentation (PTE) or by a combination of fermentation and chemical synthesis from renewable resources („Green“ PE).

- Polythioesters (PTE) are so far only obtained by the cultivation of microorganisms in presence of organic sulfur compounds (OSC).

- Catabolism of OSC is often achieved by unspecific enzymes constituting „patch work pathways“.

- The metabolism of OSC can be engineered (i) to improve production of PTE, (ii) to modulate the composition, (iii) to produce novel PTE, and (iv) to produce PTE from cheap substrates.

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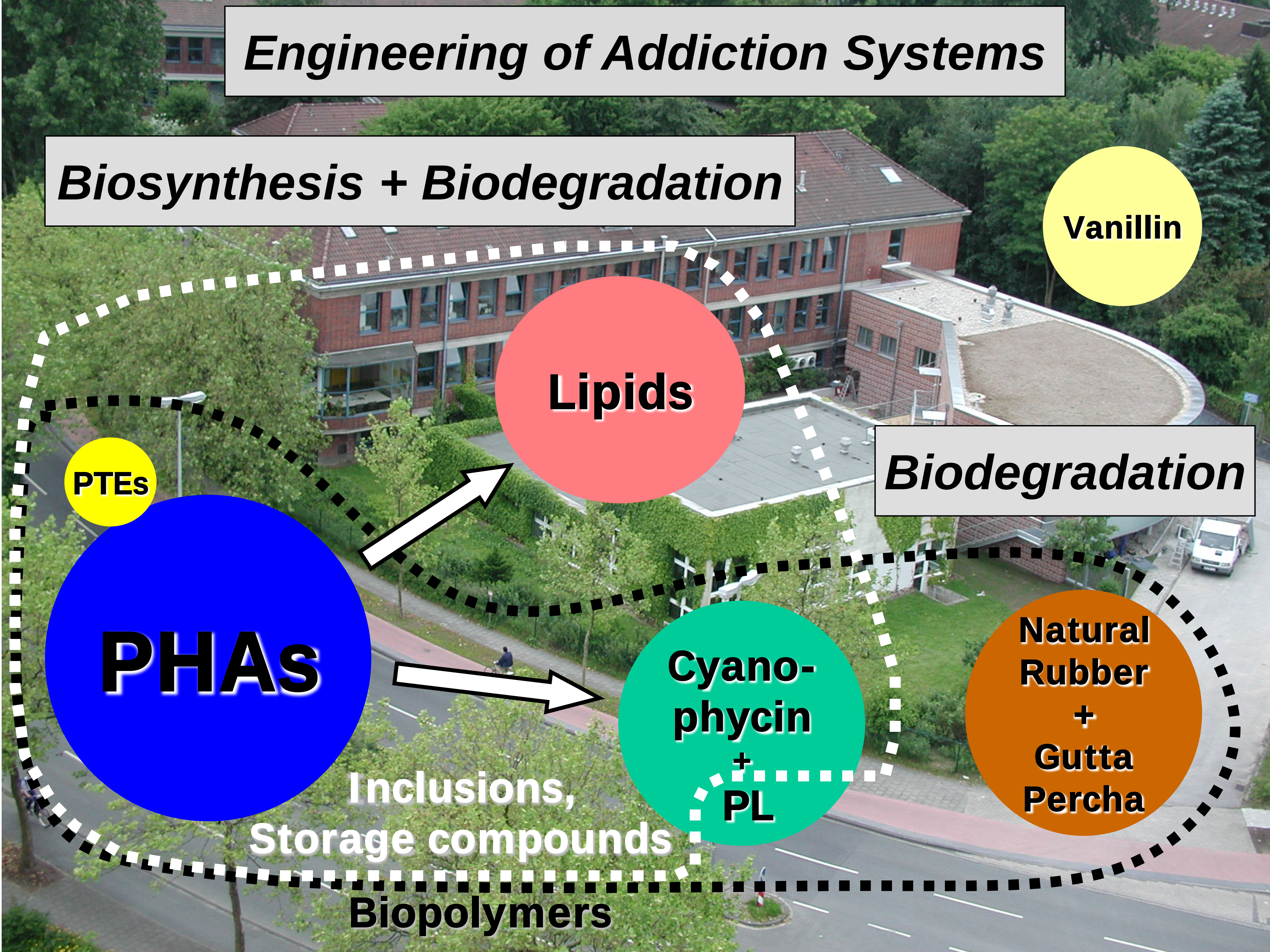
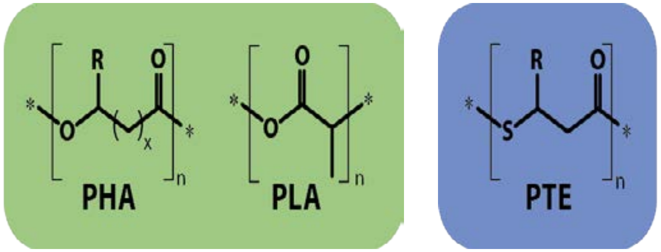
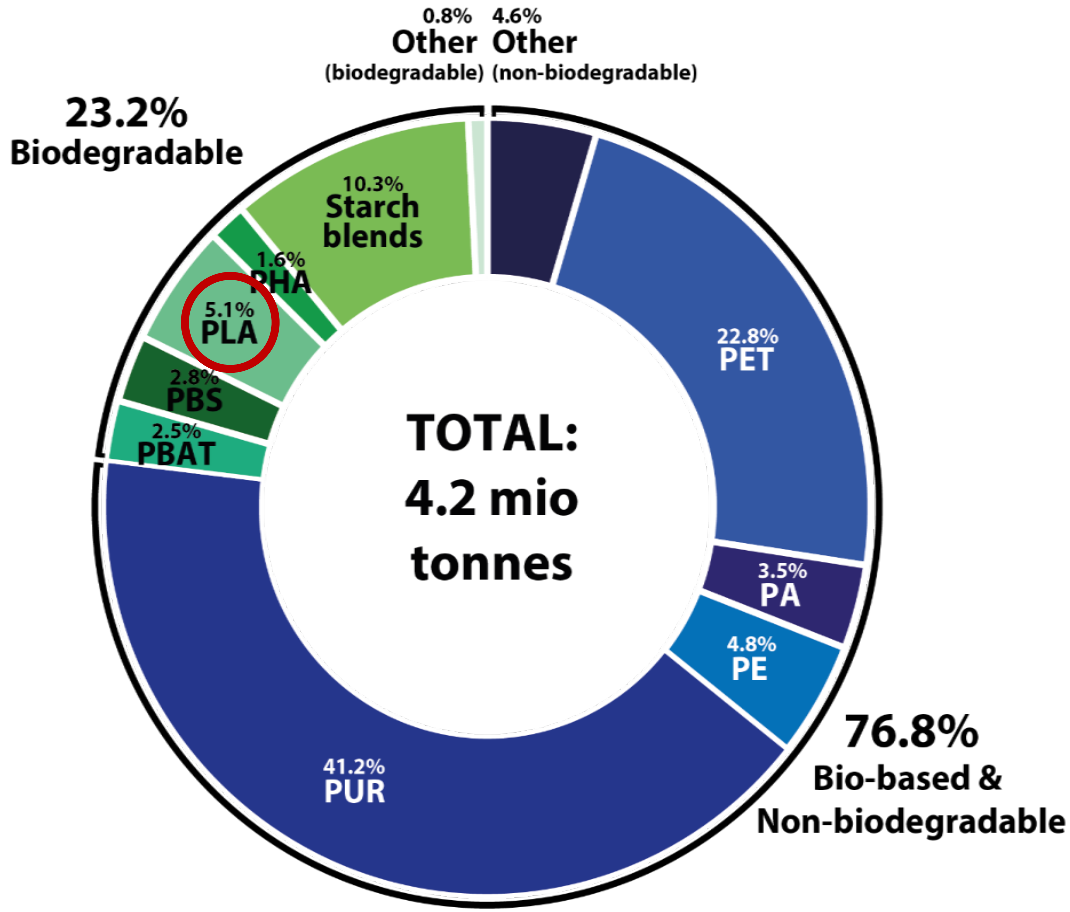

Engineering of Addiction Systems
Biosynthesis + Biodegradation
Vanillin
Lipids
Biodegradation
PTEs
PHAs
Cyano-
phycin
+
PL
Natural
Rubber
+
Gutta
Percha
Inclusions,
Storage compounds
Biopolymers

Bioplastics and polythioesters

- **> 75% persistent** plastic materials
- conventional plastics produced from renewable resources: PUR & PET
- most promising bioplastics among **biodegradable** polymers: **PLA** & **PHA**
- biosynthesis of polythioesters **(**PTE**s)** in 2001: persistent structural analog of PHA





PA, polyamide
PBAT, poly(butylene adipate-*co*-terephthalate)
PBS, polybutylene succinate
PE, polyethylene
PET, polyethylene terephthalate
PHA, polyhydroxyalkanoate
PLA, polylactate
PUR, polyurethane

Why are PTEs persistant ?

Material properties (only contributing)**:**

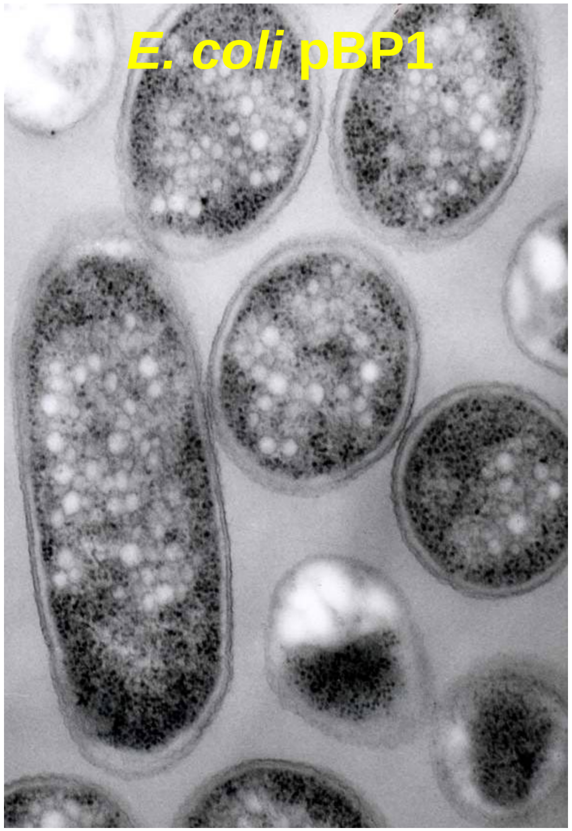
- solid material
- insoluble in water
- extremely hydrophobic

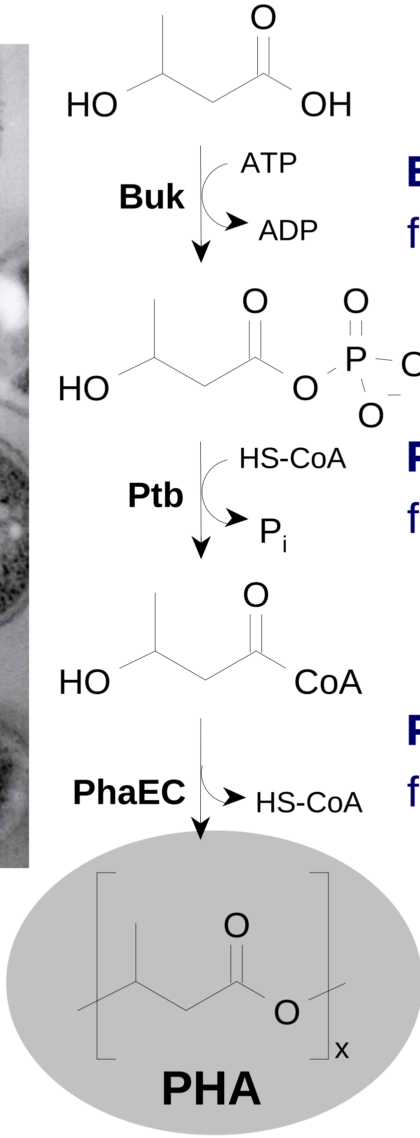
Biological reasons:

- only obtained from precursor carbon sources which do not (or only rarely) occur in natural habitats
- Existence of an unusual linkage type
- synthesized only in the lab by engineered microorganism possessing a synthetic/non-natural pathway
- PHA depolymerases are more specific than PHA synthases

The BPEC biosynthesis pathway:

a “non-natural“ biosynthesis pathway for production of (novel) biopolymers

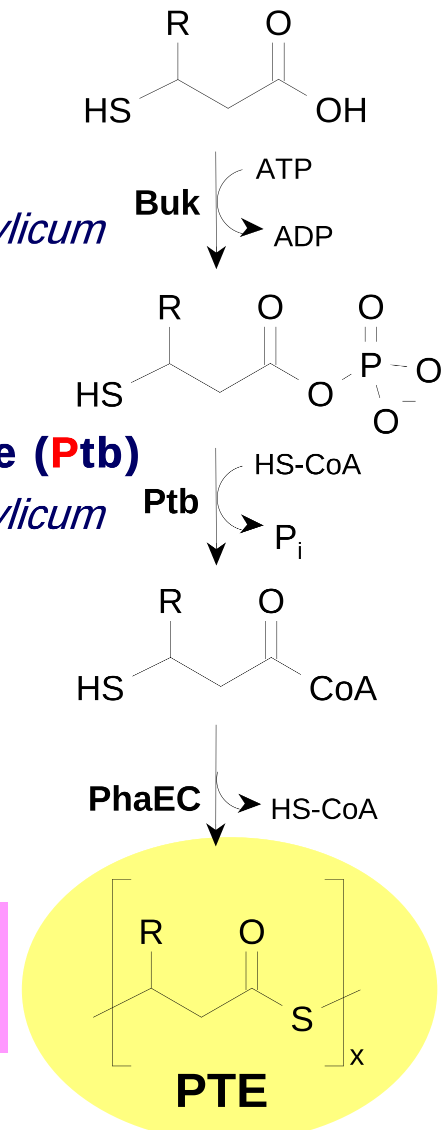




Butyrate kinase (Buk)
from *Clostridium acetobutylicum*

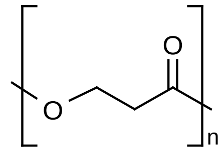
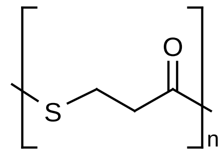
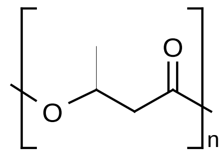
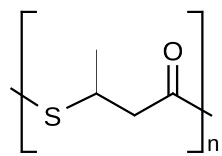
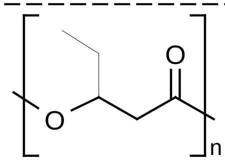
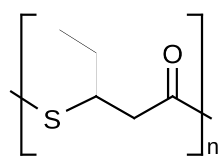
Phosphotransbutyrylase (Ptb)
from *Clostridium acetobutylicum*

PHA synthase (PhaEC)
from *Thiococcus pfennigii*



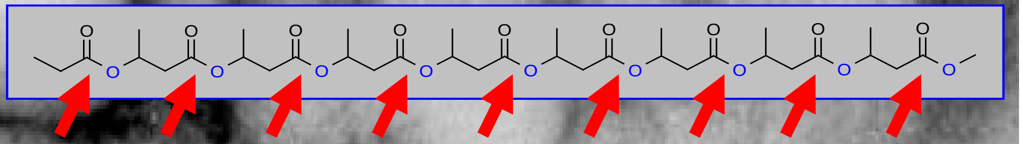
Homopolymers

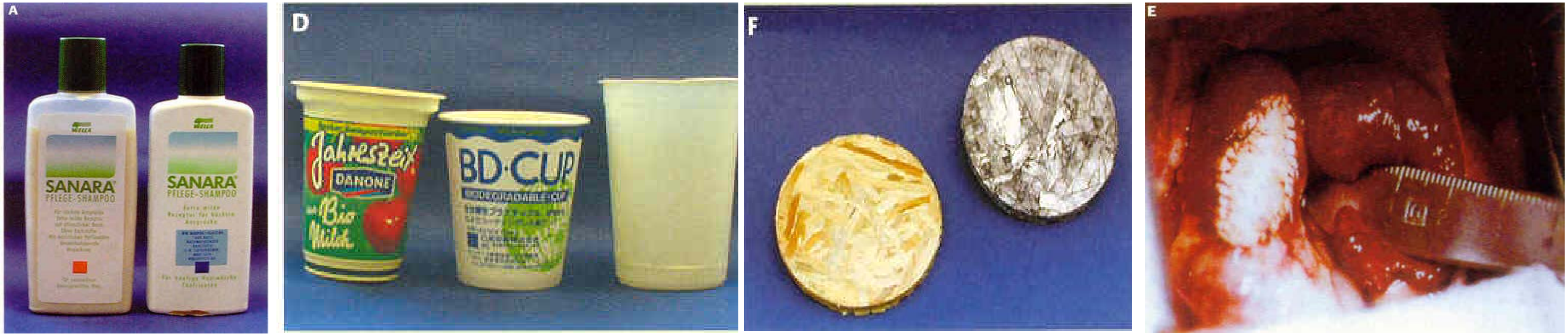
Thermal analysis of PTEs

Polymer	Formula	T_g [°C]	T_m [°C]
PHP Poly(3-hydroxypropionate)		-10	121
PMP Poly(3-mercaptopropionate)		-	170
PHB Poly(3-hydroxybutyrate)		4	175
PMB Poly(3-mercaptobutyrate)		8	100
PHV Poly(3-hydroxyvalerate)		-10	115
PMV Poly(3-mercaptovalerate)		-1	84

Thermoplastics (Polyesters) from Bacteria

- thermoplastic
- insoluble in water
- biodegradable
- non toxic
- from renewable resources <u>or</u> from precursors

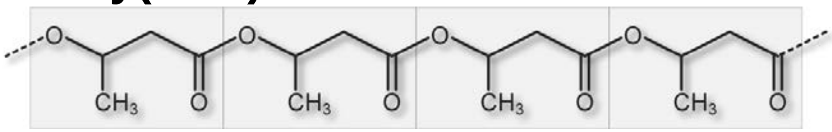




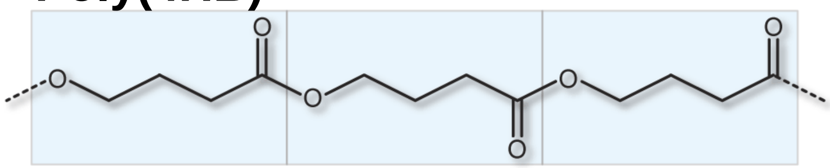
Examples of PHAs (synthesized by PHA synthases)

Poly(HA_{SCL}) *Ralstonia eutropha*

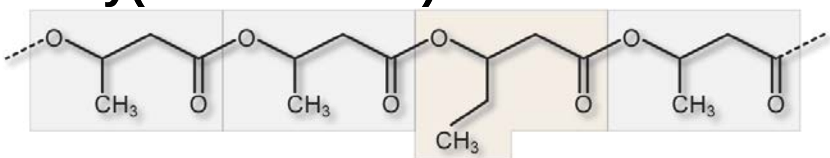
Poly(3HB)



Poly(4HB)

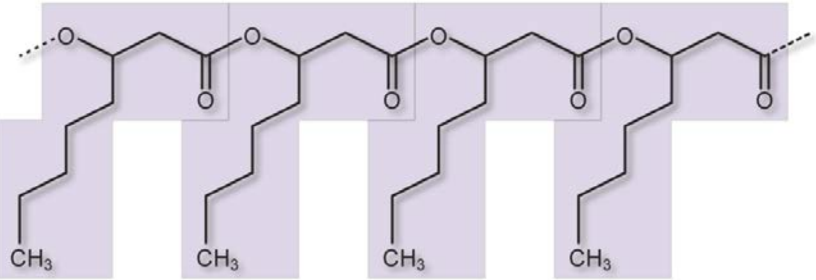


Poly(3HB-*co*-3HV)



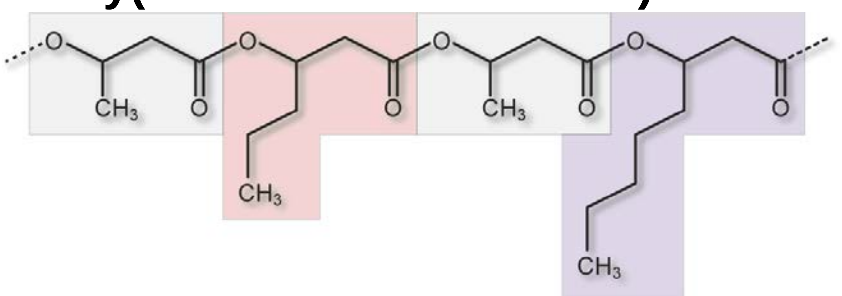
Poly($3HA_{MCL}$) *Pseudomonas putida*

Poly(3HO)



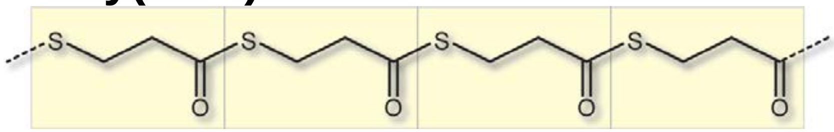
Poly(3HB-*co*-$3HA_{MCL}$) *Pseudomonas sp.*

Poly(3HB-*co*-3HHx-*co*-3HO)

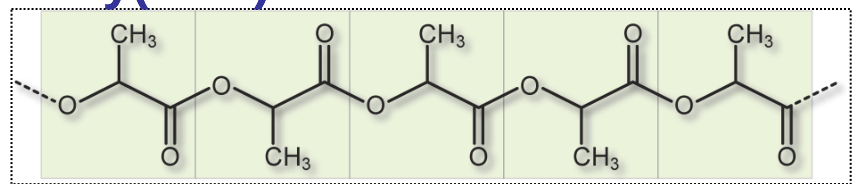


PTEs recombinant *E. coli*

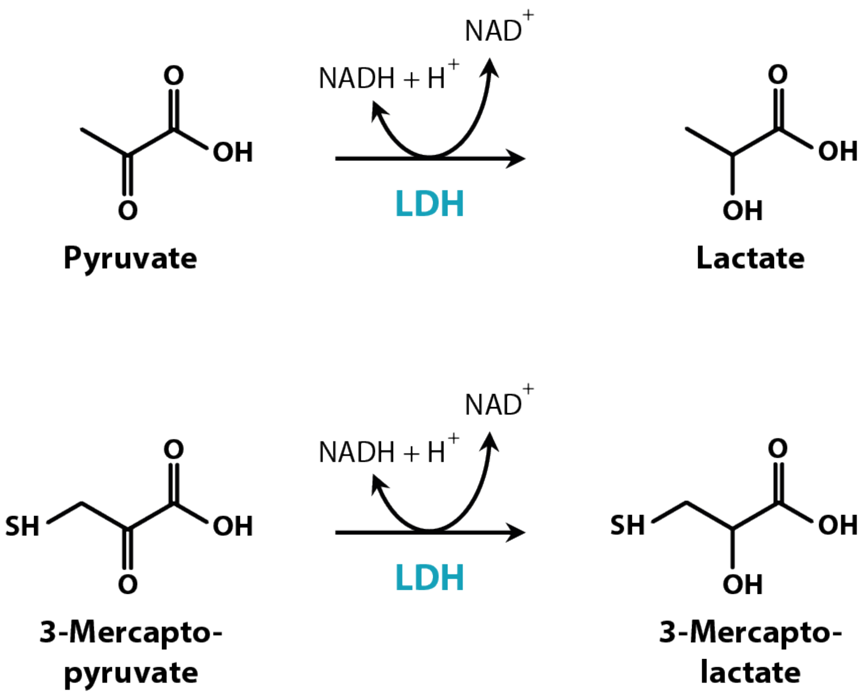
Poly(3MP)

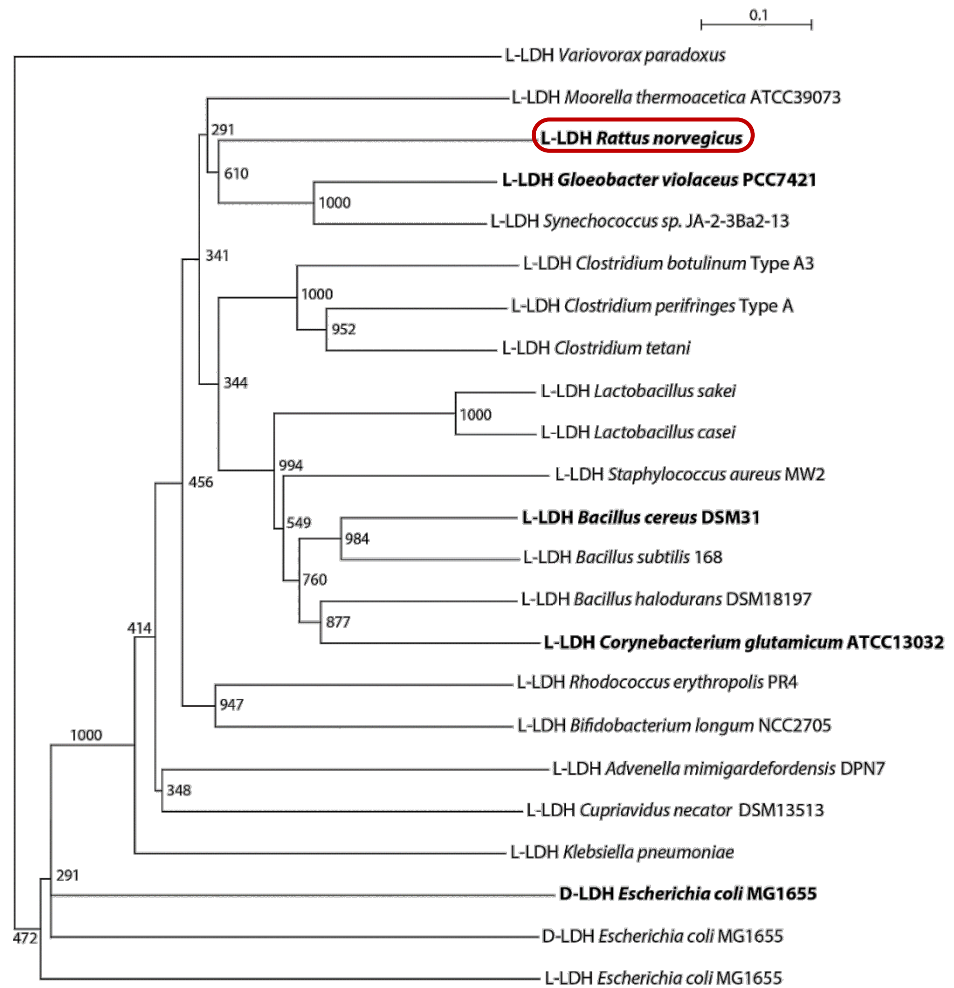


Poly(2HP) = PLA

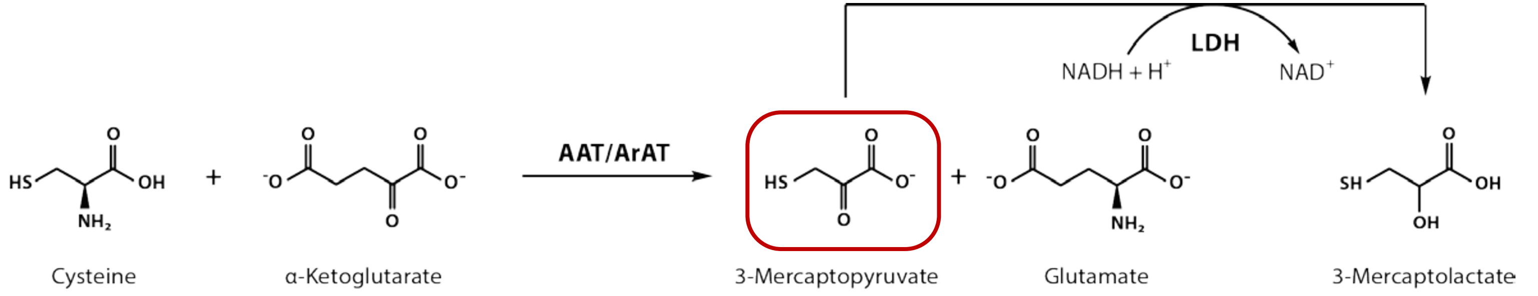


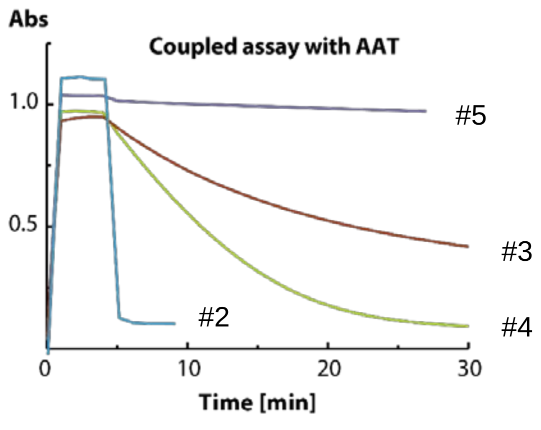
Studies on LDHs capable of reducing 3-Mercaptopyruvate

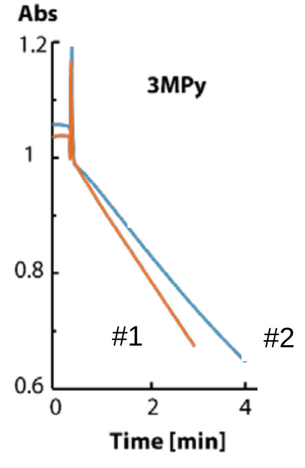


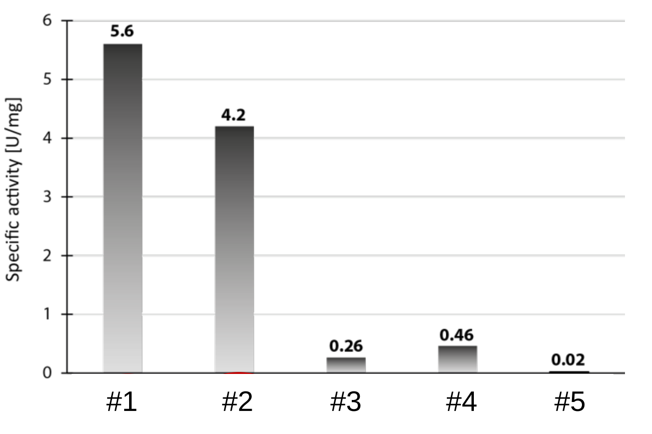


Studies on LDHs capable of reducing 3-mercaptopyruvate









How are compounds degraded, which are not available in natural habitats ?

by unspezific enzymes

assembled

in patchwork pathways

Persistence of Poly(3MP) homopolymer

PMP bar

<u>Soil microcosm</u> → Incubation for 2-8 months at 30°C

Persistence of Poly(3MP) Homopolymer

aerobic ← → **anaerobic**

SRB

After drying

(for 24 h)