Not only Ethanol:
Bio-Based Chemical, building blocks and monomers from biorefineries

What chemistry needs

The chemical industry relies on six basic chemicals or chemical groups including ethylene, propylene, the C4 olefins (butadiene and butenes), the aromatics (benzene and toluene), the xylenes (ortho, meta and para) and methane.

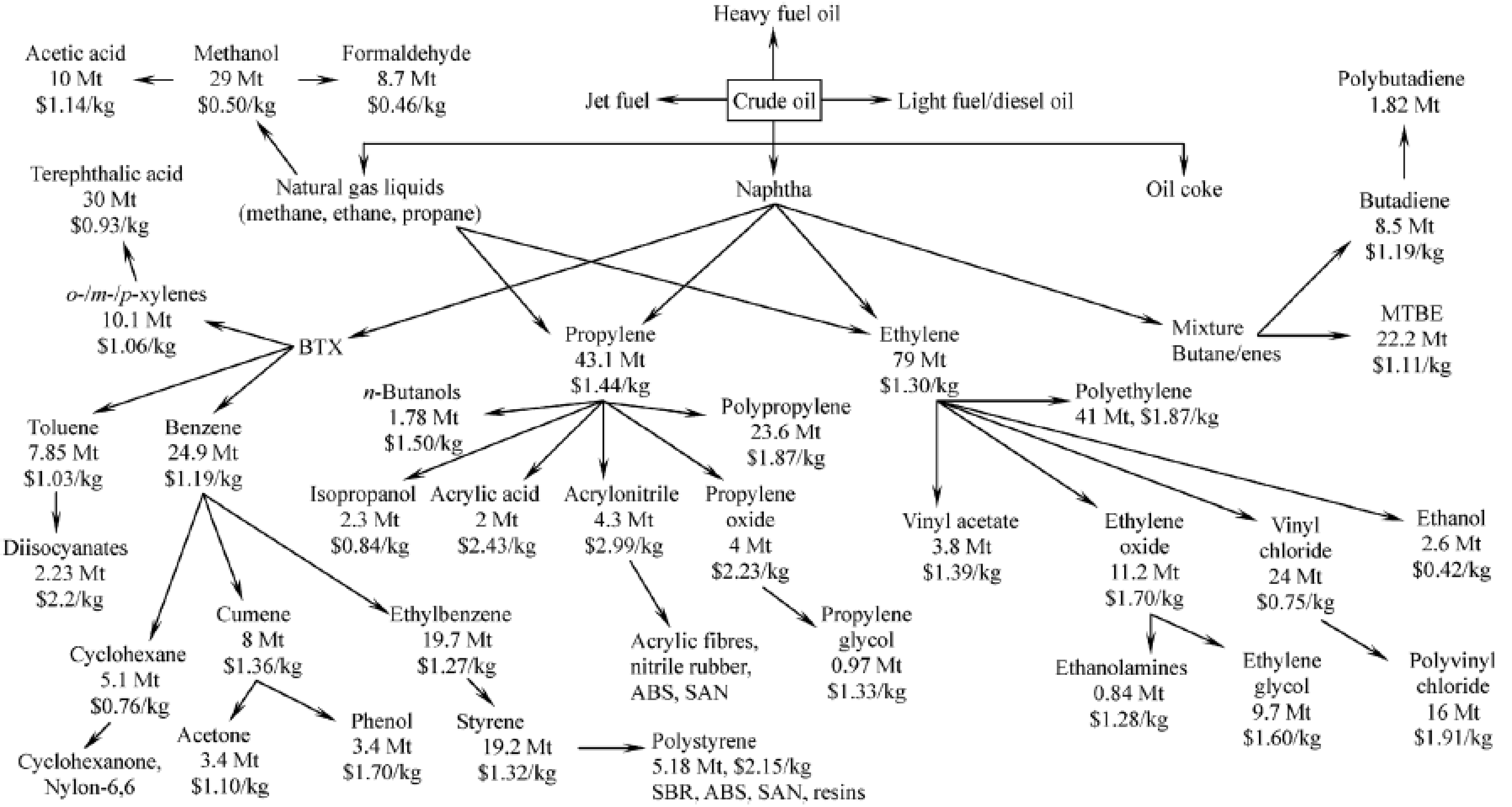
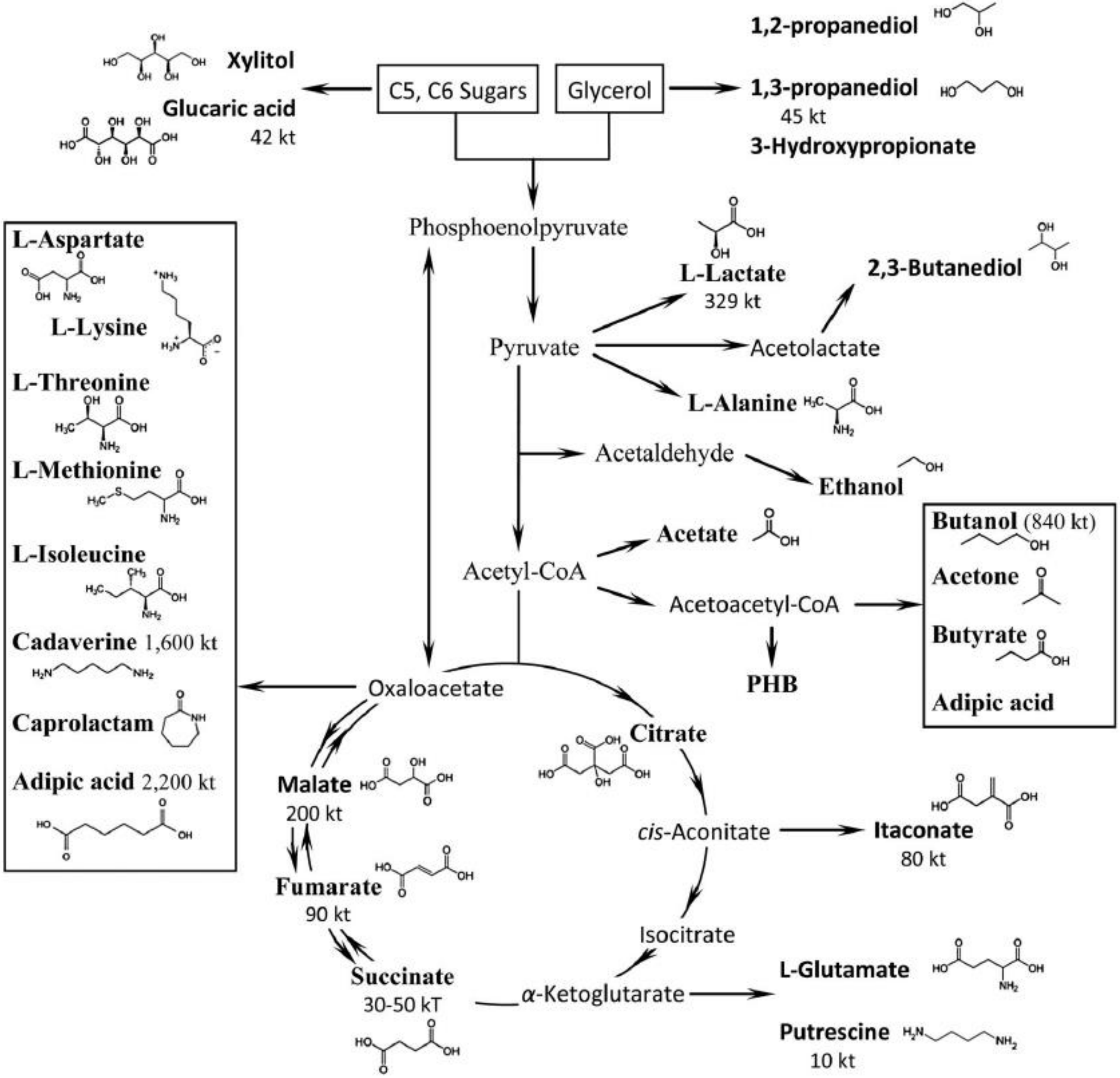
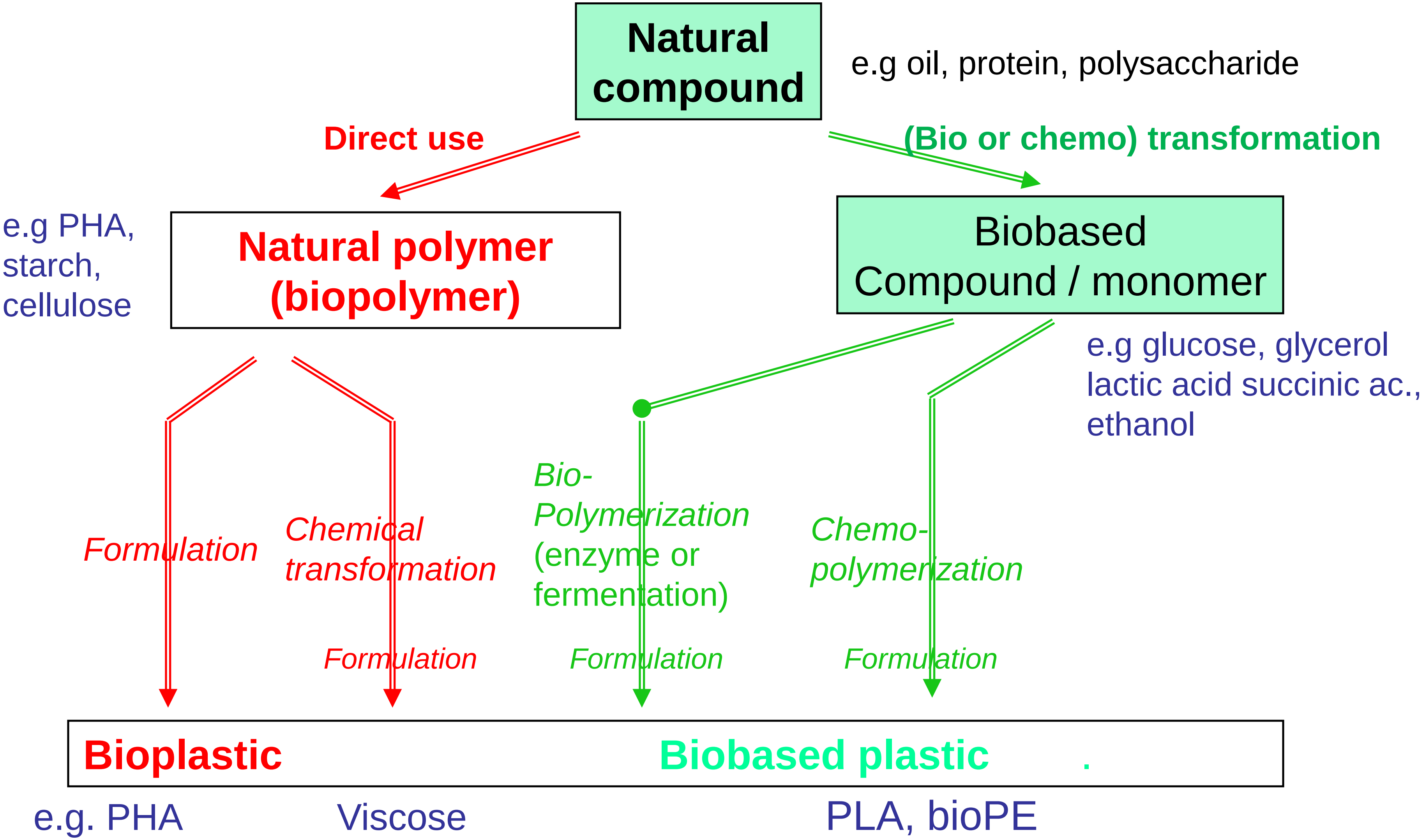


Fig. 1 Base chemicals and derivatives produced from petroleum (production capacities were taken from the journal *Chemical Engineering News*[4] and unit prices were taken from the *ICIS Indicative chemical prices*[5]).


Xylitol
Glucaric acid
42 kt
C5, C6 Sugars
Glycerol
1,2-propanediol
1,3-propanediol
45 kt
3-Hydroxypropionate
Phosphoenolpyruvate
Pyruvate
L-Lactate
329 kt
Acetolactate
2,3-Butanediol
L-Alanine
Acetaldehyde
Ethanol
Acetyl-CoA
Acetate
Acetoacetyl-CoA
PHB
Butanol (840 kt)
Acetone
Butyrate
Adipic acid
L-Aspartate
L-Lysine
L-Threonine
L-Methionine
L-Isoleucine
Cadaverine 1,600 kt
Caprolactam
Adipic acid 2,200 kt
Oxaloacetate
Citrate
Malate
200 kt
Fumarate
90 kt
Succinate
30-50 kT
cis-Aconitate
Itaconate
80 kt
Isocitrate
α-Ketoglutarate
L-Glutamate
Putrescine
10 kt

Strategies



Natural compound

e.g oil, protein, polysaccharide

Direct use

(Bio or chemo) transformation

e.g PHA, starch, cellulose

Natural polymer (biopolymer)

Biobased Compound / monomer

e.g glucose, glycerol lactic acid succinic ac., ethanol

Formulation

Chemical transformation

Formulation

Bio-Polymerization (enzyme or fermentation)

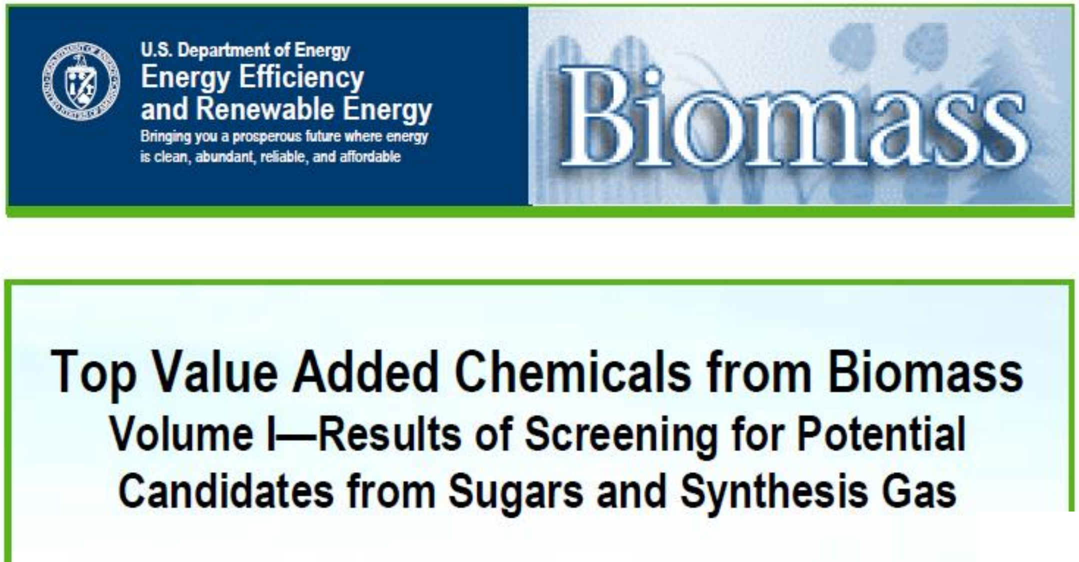
Formulation

Chemo-polymerization

Formulation

Bioplastic **Biobased plastic** .

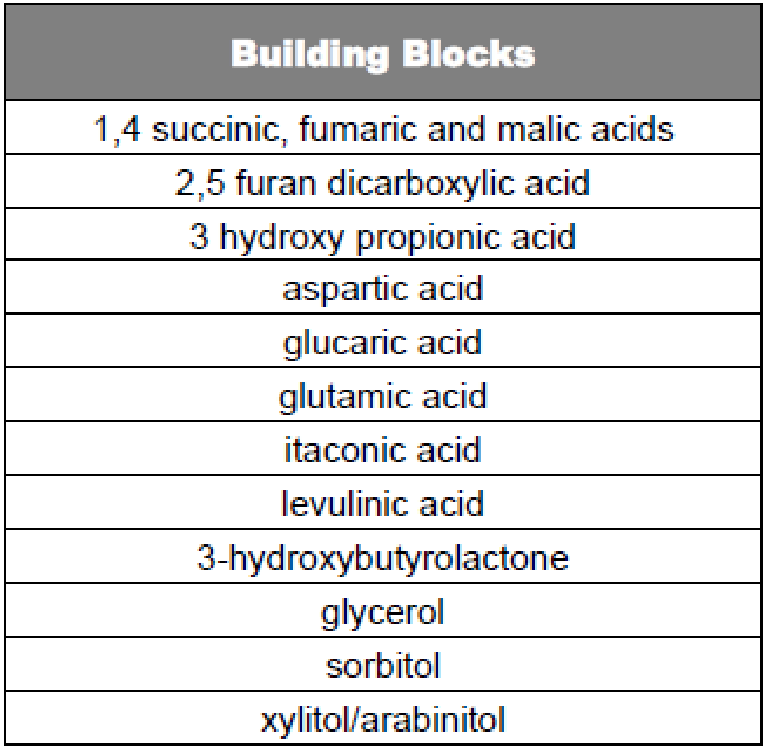
e.g. PHA Viscose PLA, bioPE



U.S. Department of Energy
Energy Efficiency
and Renewable Energy
Bringing you a prosperous future where energy
is clean, abundant, reliable, and affordable

Biomass

Top Value Added Chemicals from Biomass
Volume I—Results of Screening for Potential
Candidates from Sugars and Synthesis Gas



Building Blocks
1,4 succinic, fumaric and malic acids
2,5 furan dicarboxylic acid
3 hydroxy propionic acid
aspartic acid
glucaric acid
glutamic acid
itaconic acid
levulinic acid
3-hydroxybutyrolactone
glycerol
sorbitol
xylitol/arabinitol

By 2030, the market value of bio-based building blocks is expected to reach $3.2 billion, whereas the demand for fermentation-based chemical building blocks was less than s700 million in 2013.

Such building blocks could either be produced from renewable carbon through green chemical conversion routes or via microbial conversions.

The percentage of chemical production based on biotechnology is estimated to increase from less than 2% in 2005 to approximately a quarter of all chemical production by 2025.

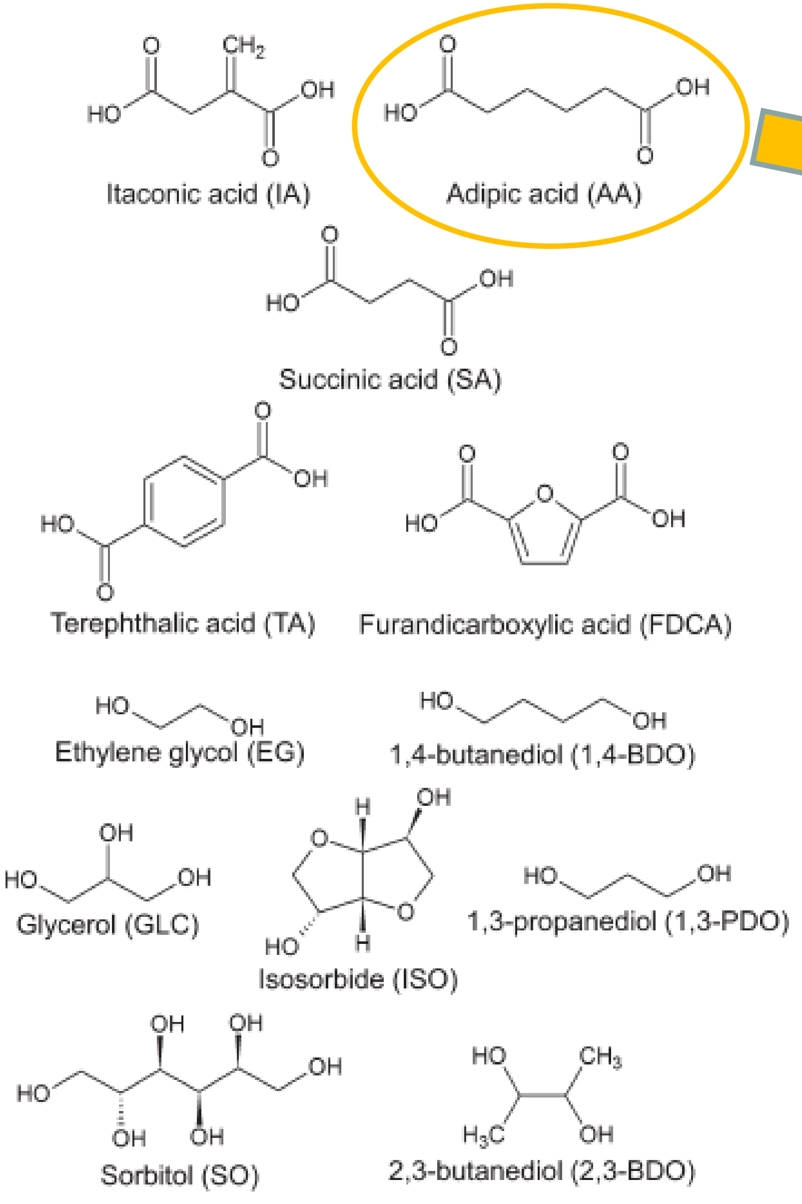
The largest contribution will come from the conversion of renewable carbon into chemicals via biotechnological routes.

The incorporation of fermentative production of basic building blocks as unit operations in integrated biorefineries is dependent on intense research activities ranging from microbial strain development and engineering to fermentation and down-stream processing optimization.

The EU demand in 2030 for biobased plastics:
5.2 BEUR

http://www.industrialbiotech-europe.eu/new/wp-content/uploads/2015/06/BIO-TIC-roadmap.pdf

Biotecnological production of monomers:



Scheme 1. Most important bio-based dicarboxylic acids and polyols currently available for the enzymatic synthesis of polyesters.

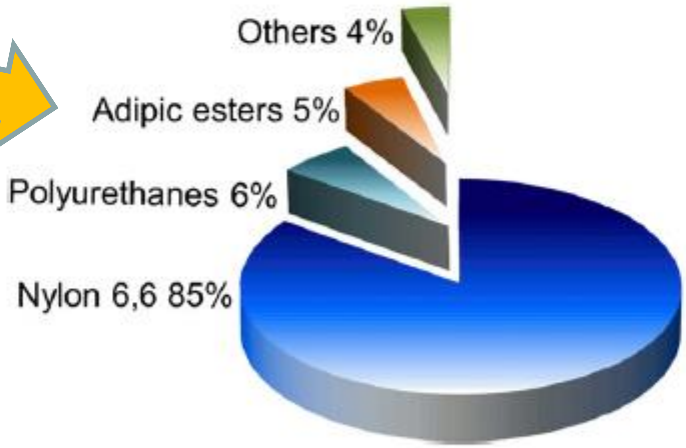
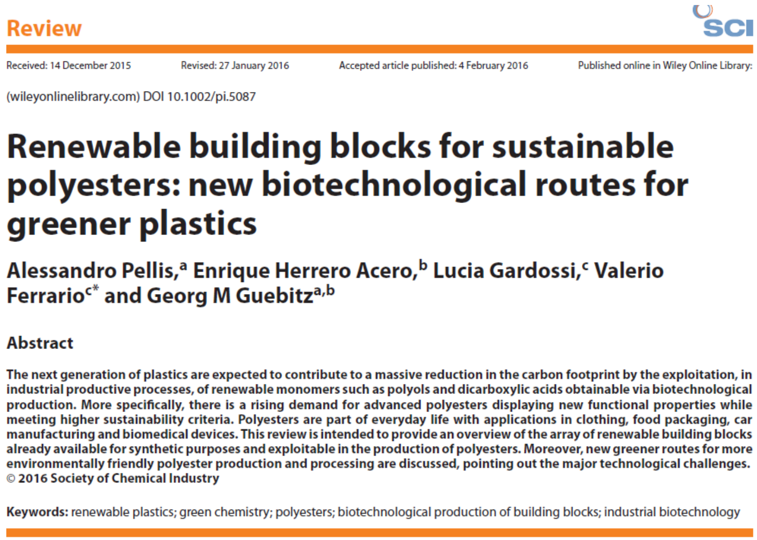


Figure 2. Most common commercial polymeric products derived from the bio-based monomer adipic acid.



Review

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Renewable building blocks for sustainable polyesters: new biotechnological routes for greener plastics

Alessandro Pellis,[a] Enrique Herrero Acero,[b] Lucia Gardossi,[c] Valerio Ferrario[c*] and Georg M Guebitz[a,b]

Abstract

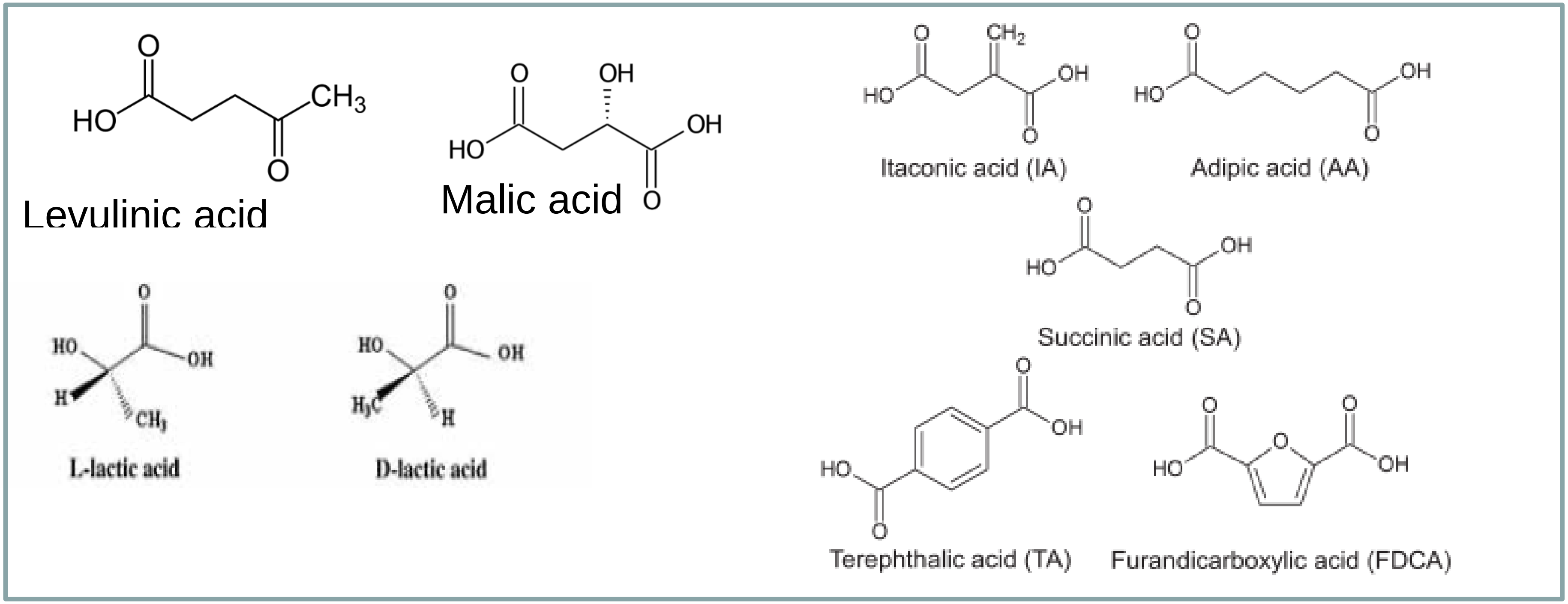
The next generation of plastics are expected to contribute to a massive reduction in the carbon footprint by the exploitation, in industrial productive processes, of renewable monomers such as polyols and dicarboxylic acids obtainable via biotechnological production. More specifically, there is a rising demand for advanced polyesters displaying new functional properties while meeting higher sustainability criteria. Polyesters are part of everyday life with applications in clothing, food packaging, car manufacturing and biomedical devices. This review is intended to provide an overview of the array of renewable building blocks already available for synthetic purposes and exploitable in the production of polyesters. Moreover, new greener routes for more environmentally friendly polyester production and processing are discussed, pointing out the major technological challenges.

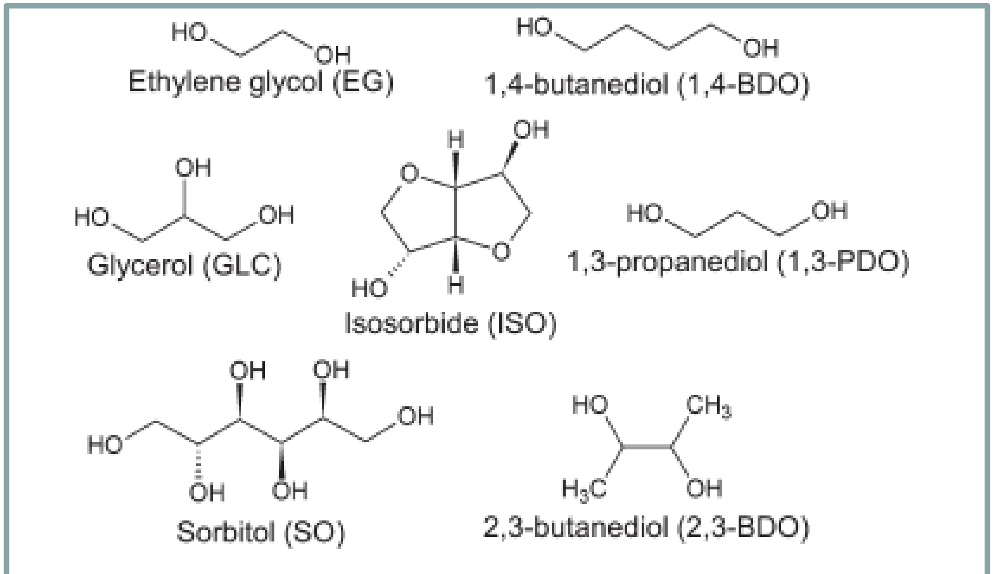
Keywords: renewable plastics; green chemistry; polyesters; biotechnological production of building blocks; industrial biotechnology

Some bio-based monomers for polymer production

Monomer	Biotechnological route		Company	Status	Application of the corresponding bio-based polyesters	.
Sorbitol	Fermentation + hydrogenation		Roquette, ADM	Market	Functional polyesters; coatings	
Isosorbide	Sorbitol dehydration		Roquette	Market	Thermosetting resins	
Ethylene glycol	Ethanol dehydration		India Glycols Ltd, Greencol Taiwan	Market	PET; PEF	
1,3-propanediol	Fermentation		Du Pont, Tate & Lyle, Metabolic Explorer	Market	PTT; fibers; elastomers; polyester-urethanes	
1,4-butanediol	Fermentation, succinic acid hydrogenation		BioAmber, Genomatica, Mitsubishi	Market	PBAT; PBS; PBT	
Adipic acid	Fermentation + hydrogenation		Celexion LLC, BioAmber, Rennovia, Verdezyme	Market	Resins; polyester-amines; polyester-urethanes	
Itaconic acid	Fermentation		Qingdao Kehai Biochemistry, Itaconix	Market	Photocurable precursors; plasticizers	
Lactic acid	Fermentation		Nature Works, BASF, Purac, Cargill, BBCA, Galactic	Market	PLA	
Succinic acid	Fermentation		BioAmber, Myriant, Reverdia, BASF, Purac, Succinity	Market	Textiles; coatings; PBS; PBT	
Terephthalic acid	Isobutylene oxidation, fermentation		Virient, Annellotech, Genomatica	Pilot plant	PET; coatings	
Levulinic acid	Fermentation, acid treatment of C6 sugars		GFBiochemicals, Bio-on, Biofine Renewables	Market	Coatings, hyperbranched dendrimeric polyesters	
Malic acid	Fermentation		Novozymes	Pilot plant	Functionalized chiral polyesters	
2,5-furandicarboxylic acid	Fermentation + dehydration + oxidation		Avantium	Pilot plant	PEF; polyester-urethanes	

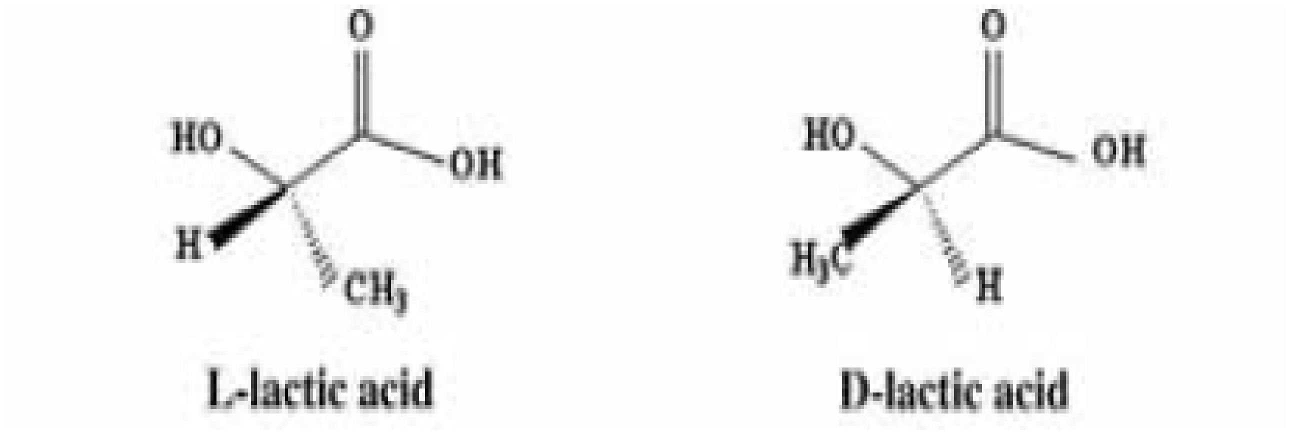
Some bio-based monomers for polymer production





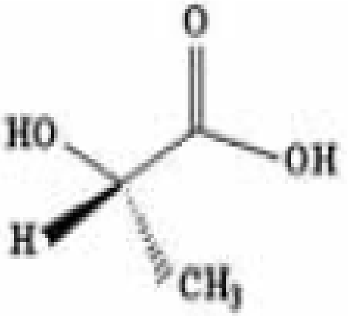
Scheme 1. Most important bio-based dicarboxylic acids and polyols currently available for the enzymatic synthesis of polyesters.

Bio-based monomers for polyesters plastics: PLA (polylactic acid)

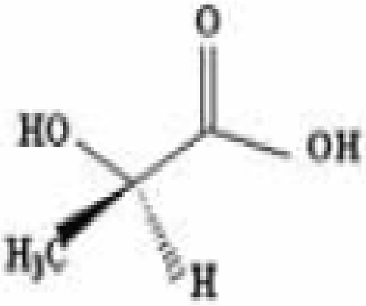


Among polymers, polyesters are a widely used class with applications ranging from clothing to food packaging and from the car industry to biomedical applications. The possibility to synthesize polyesters from bio-based monomers is demonstrated by PLA, currently the most important bio-based polyester in terms of volume, with a **capacity of approximately 180 000 tons/y.**

Lactic acid (2-hydroxypropionic acid), CH3–CHOHCOOH, is a simple chiral molecule which exists as two enantiomers.



L-lactic acid

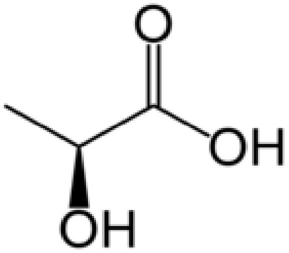


D-lactic acid

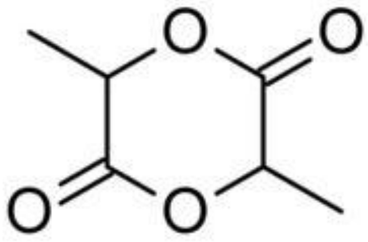
Chemical synthesis of lactic acid is mainly based on the hydrolysis of lactonitrile by strong acids, which provide only the racemic mixture of D- and L-lactic acid.

PLA was discovered in 1932 by Carothers (DuPont) who produced a low molecular weight product by heating lactic acid under vacuum. In 1954 Du Pont produced the polymer with a molecular weight greater and patented.

From lactic acid to PLA

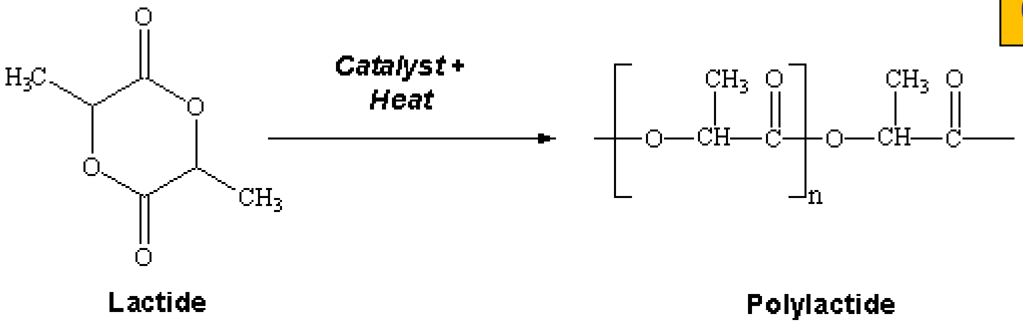


(S)-lactic acid



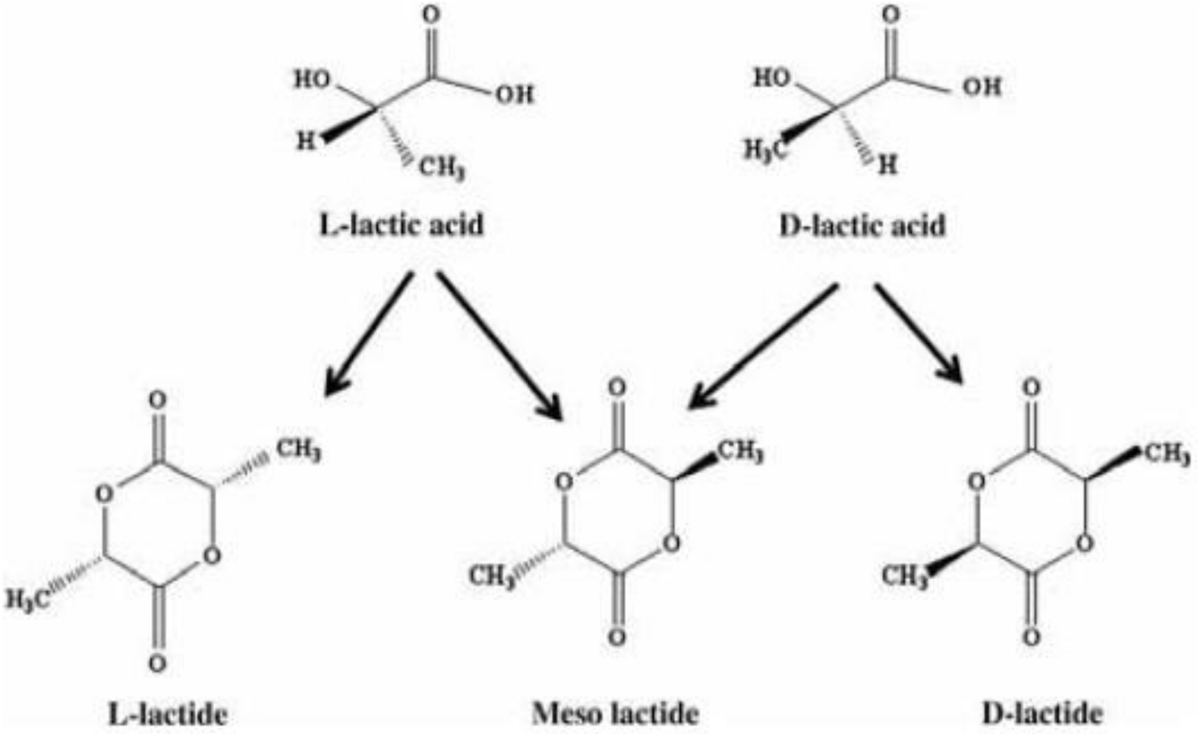
Lactide

Lactide is the cyclic di-ester of lactic acid, i.e., 2-hydroxypropionic acid.
Lactic acid can not form a lactone but first forms a dimer, which contains an hydroxy group at a convenient distance from the carboxylic group for the formation of a lactone. The dimer readily forms a six-membered cyclic diester known as lactide



Ring opening polymerization of lactide to polylactide

Since, lactic acid is a chiral molecule, PLA has stereoisomers, such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA).



Optically active PLLA and PDLA are crystalline, whereas optically inactive PDLLA is amorphous

Polylactic acid

a.k.a. polylactide

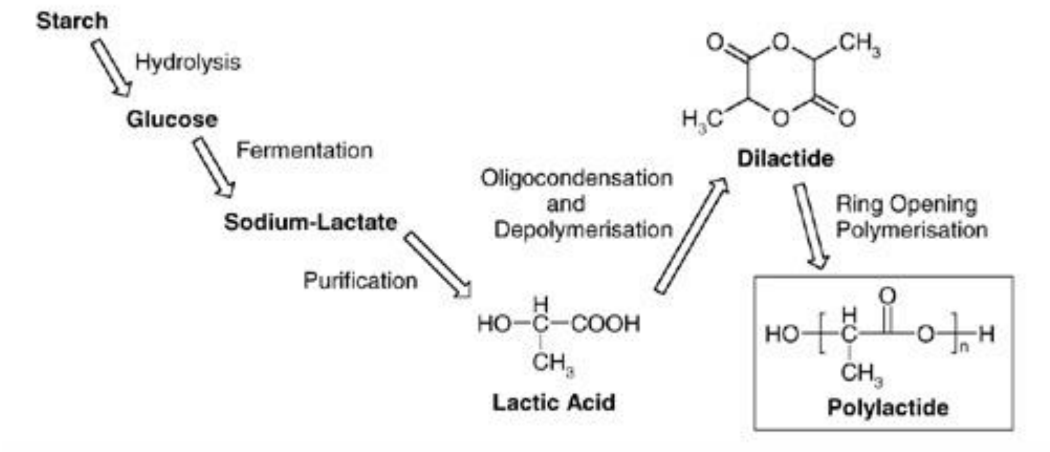
Aliphatic polyester

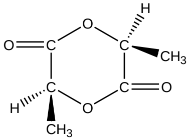
Monomer produced in fermentation

Chemical polymerization
- copolymers

Natureworks (Cargill/Teijin)
140.000 t/a cap.

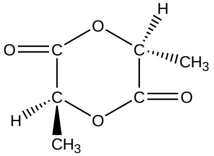
Biodegradable





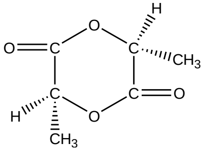
LL-Lactide

(mp 97 C)



LD-Lactide

(mp 52 C)



DD-Lactide

(mp 97C)

Companies, e.g. Cargill Dow Polymer LLC, Shimadzu Corp, Mitsui Chemicals, Musashino Co. Are now producing PLA-targeting markets for **packaging materials**, **films, textile fibers**, along with **pharmaceutical** products. The US Food and Drug Administration (FDA) and European regulatory authorities have approved the PLA resins for all food type applications and some **chirurgical** applications such as **drug releasing systems**

PLLA has gained great attention because of its excellent biocompatibility and mechanical properties. It has extensive applications in **biomedical** fields, including suture, bone fixation material, drug delivery microsphere, and tissue engineering.

However, its long degradation times coupled with the high crystallinity of its fragments can cause inflammatory reactions in the body. In order to overcome this, PLLA can be used as a material combination of L-lactic and D, L-lactic acid monomers, being the latter rapidly degraded without formation of crystalline fragments during this process.

Approximately 90% of the total lactic acid produced worldwide is made by bacterial fermentation and the remaining portion is produced synthetically by the hydrolysis of lactonitrile.

The fermentation processes to obtain lactic acid can be classified according to the type of bacteria used. The carbon source for microbial production of lactic acid can be either sugar in pure form such as glucose, sucrose, lactose or sugar containing materials such as molasses, whey, sugarcane bagasse, cassava bagasse, and starchy materials from potato, tapioca, wheat and barley. Sucrose-containing materials such as molasses are commonly exploited raw materials for lactic acid production because they represent cheaper alternatives. Sugarcane bagasse is reported to be used as support for lactic acid production by **Rhizopus oryzae** and **Lactobacillus** in solid-state fermentation by supplementing sugars or starch hydrolysates as carbon source.

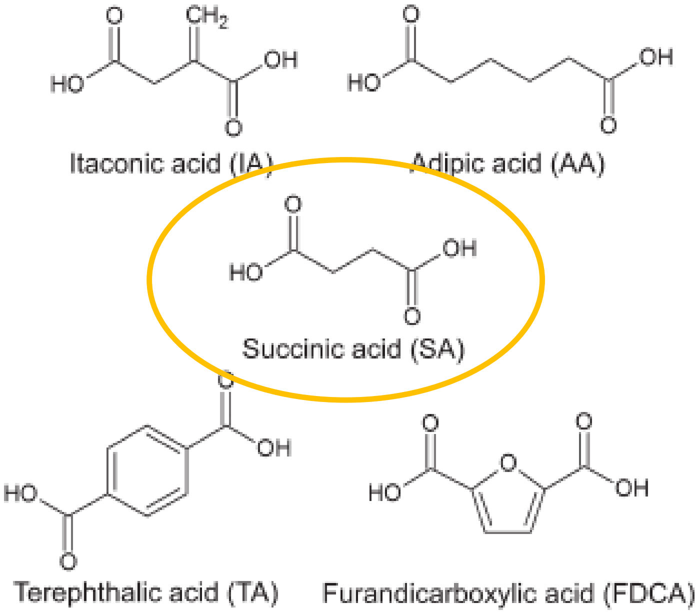
More on PLA: http://www.galateabiotech.com/it/

Besides high product specificity, as it produces a desired optically pure L-(+)- or D-(-)-lactic acid, the biotechnological production of lactic acid offers several advantages compared to chemical synthesis like low cost of substrates, low production temperature, and low energy consumption

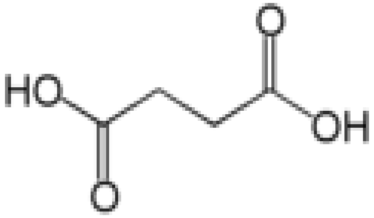
PLA-based products had already been developed by the 1940s and 1950s, but their production became economically viable only 70 years later. This demonstrates the importance of optimizing the productivity and robustness of bioconversions to achieve cost-effective production.

The success of bio-based polyesters does not rely solely on their capacity to replace fossil-based polymers while being economically competitive. Rather, the next generation of bio-based polyesters should bring entirely new advanced chemical and functional properties to the polymer scenario.

Biotecnological production of di-carboxylic acids:

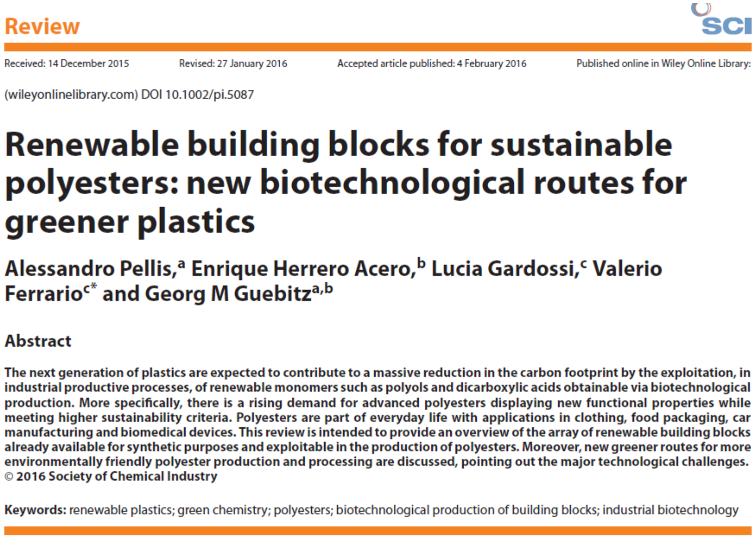


The high interest in SA is because of the fact that this dicarboxylic acid is a key component/intermediate in the production of several solvents, adhesives, printing inks, magnetic tapes, coating resins, plasticizers, emulsifiers, deicing compounds and chemical and pharmaceutical intermediates.



Succinic acid

Microbial strain able to convert raw hydrolysates from biomass to succinate (US Patent 6,743,610).



Review

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Renewable building blocks for sustainable polyesters: new biotechnological routes for greener plastics

Alessandro Pellis,[a] Enrique Herrero Acero,[b] Lucia Gardossi,[c] Valerio Ferrario[c*] and Georg M Guebitz[a,b]

Abstract

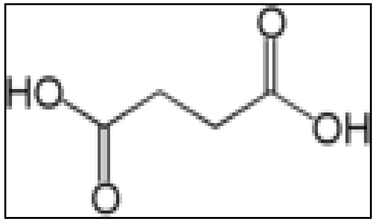
The next generation of plastics are expected to contribute to a massive reduction in the carbon footprint by the exploitation, in industrial productive processes, of renewable monomers such as polyols and dicarboxylic acids obtainable via biotechnological production. More specifically, there is a rising demand for advanced polyesters displaying new functional properties while meeting higher sustainability criteria. Polyesters are part of everyday life with applications in clothing, food packaging, car manufacturing and biomedical devices. This review is intended to provide an overview of the array of renewable building blocks already available for synthetic purposes and exploitable in the production of polyesters. Moreover, new greener routes for more environmentally friendly polyester production and processing are discussed, pointing out the major technological challenges.

Keywords: renewable plastics; green chemistry; polyesters; biotechnological production of building blocks; industrial biotechnology

Succinic acid (SA)

Since 2008, various companies (such as DSM, BASF and Purac) have shown an interest in the production of bio-based SA at an industrial scale.

For SA the most important production process from renewable feedstock is microbial fermentation of various glucose sources by a variety of microorganisms such as genetically engineered microorganisms:



Escherichia coli,
Actinobacillus succiniproducens and
Anaerobiospirillum succiniproducens

The processes are in use by two companies: the Myriant SA biorefinery in Lake Providence (Louisiana, USA) that employs grain sorghum grits as its saccharificable starting material32 and the Reverdia process (used by DSM+Roquette) where ethanol and SA are co-produced through glucose fermentation.

Both processes run with genetically modified anaerobic bacteria, in such a way that alcoholic fermentation sustains the SA production.Theoretical calculations performed by Pinazo *et al.* concluded that, despite having a lower material efficiency, fermentative SA production is attracting attention due to its very competitive cost and market position close to competitiveness with an important petrochemical feedstock such as maleic anhydride.

From succinic acid (SA) to 1,4-butandiol and to adipic acid (AA)

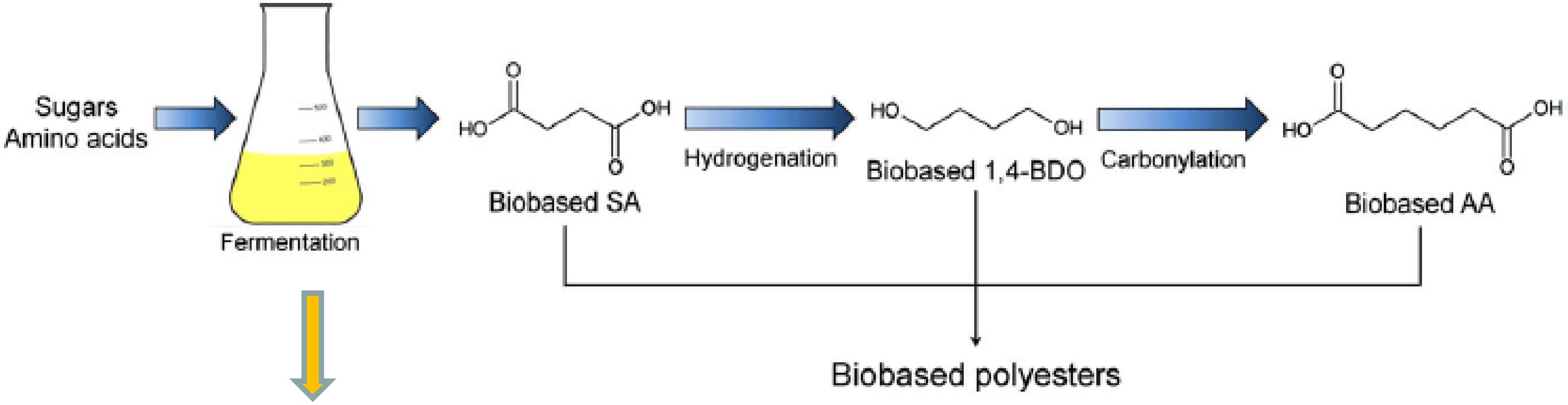
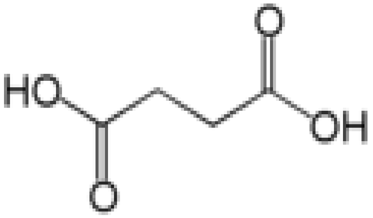
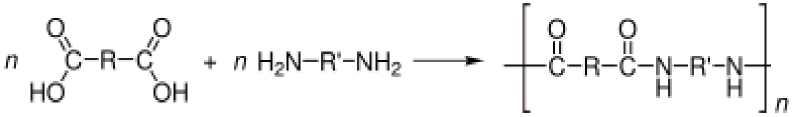


Figure 3. Biotechnological process for the production of bio-based succinic acid (SA) and its derivatives 1,4-butanediol (1,4-BDO) and adipic acid (AA).



Succinic acid



Adipic acid

nylon

Microbial strain able to convert raw hydrolysates from biomass to succinate (US Patent 6,743,610).

1,4-Butanediol (1,4-BDO)

1,4-BDO is an important chemical that is used for the manufacture of over 2.5 million tons of polymers annually. Nowadays its production is almost entirely based on fossil carbon resources (production via the Reppe process in which acetylene is reacted with formaldehyde) with the exception of BASF and Bioamber that started production via hydrogenation of SA which is accessible from biogenic sources as described below.

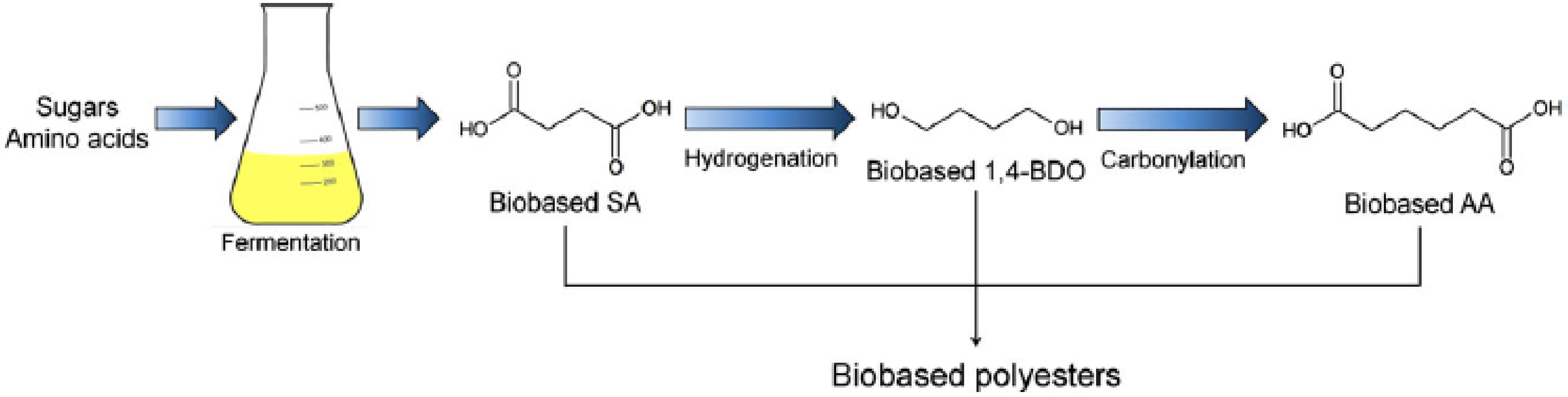


Figure 3. Biotechnological process for the production of bio-based succinic acid (SA) and its derivatives 1,4-butanediol (1,4-BDO) and adipic acid (AA).

In September 2016 Novamont opened the first plant at commercial scale in the world for the direct fermentation of sugar to produce 1,4-butandiol.

nature chemical biology

ARTICLE

PUBLISHED ONLINE: 22 MAY 2011 | DOI: 10.1038/NCHEMBIO.580

Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol

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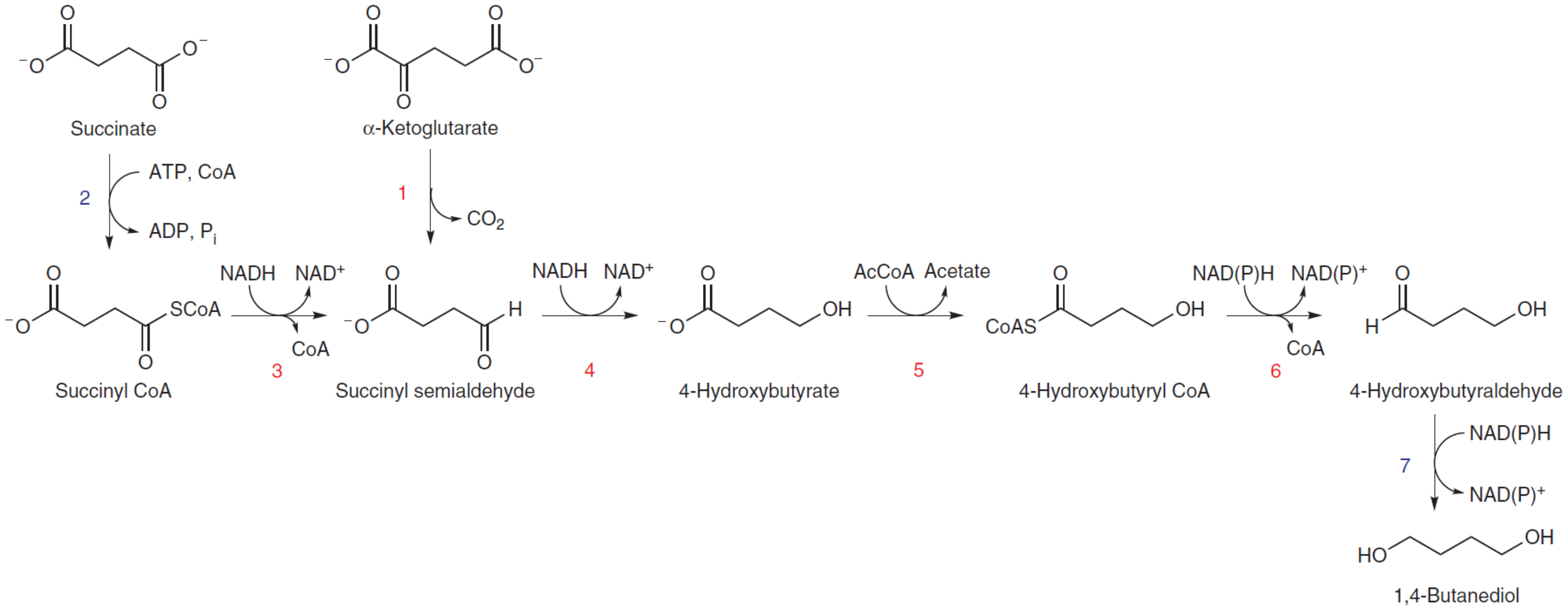
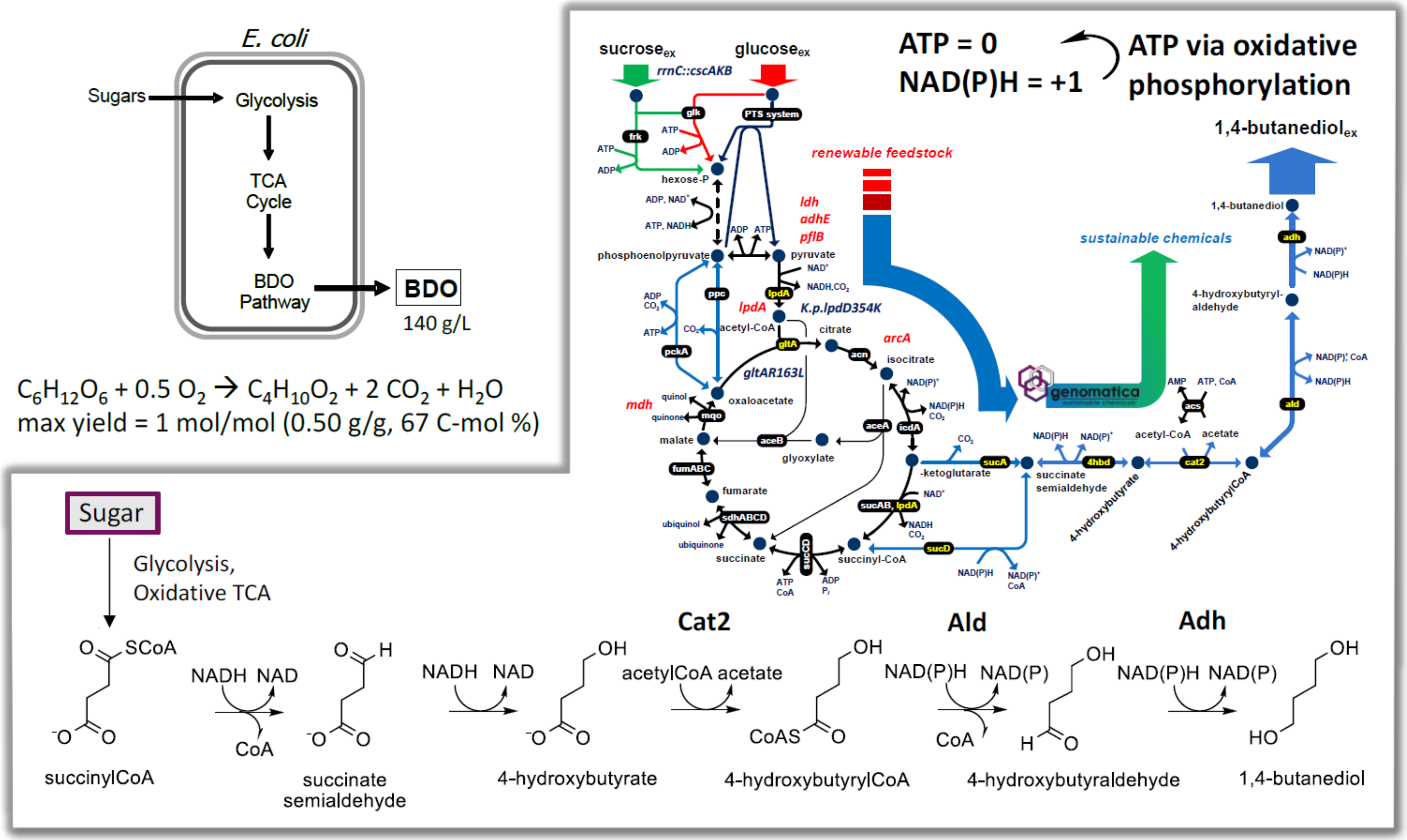
1,4-Butanediol (BDO) is an important commodity chemical used to manufacture over 2.5 million tons annually of valuable polymers, and it is currently produced exclusively through feedstocks derived from oil and natural gas. Herein we report what are to our knowledge the first direct biocatalytic routes to BDO from renewable carbohydrate feedstocks, leading to a strain of *Escherichia coli* capable of producing 18 g l^{-1} of this highly reduced, non-natural chemical. A pathway-identification algorithm elucidated multiple pathways for the biosynthesis of BDO from common metabolic intermediates. Guided by a genome-scale metabolic model, we engineered the *E. coli* host to enhance anaerobic operation of the oxidative tricarboxylic acid cycle, thereby generating reducing power to drive the BDO pathway. The organism produced BDO from glucose, xylose, sucrose and biomass-derived mixed sugar streams. This work demonstrates a systems-based metabolic engineering approach to strain design and development that can enable new bioprocesses for commodity chemicals that are not naturally produced by living cells.



Scheme 1 | BDO biosynthetic pathways introduced into *E. coli*. Enzymes for each numbered step are as follows: (1) 2-oxoglutarate decarboxylase; (2) succinyl-CoA synthetase; (3) CoA-dependent succinate semialdehyde dehydrogenase; (4) 4-hydroxybutyrate dehydrogenase; (5) 4-hydroxybutyryl-CoA transferase; (6) 4-hydroxybutyryl-CoA reductase; (7) alcohol dehydrogenase. Steps 2 and 7 occur naturally in *E. coli*, whereas the others are encoded by heterologous genes introduced in this work.



$C_6H_{12}O_6 + 0.5\ O_2 \rightarrow C_4H_{10}O_2 + 2\ CO_2 + H_2O$

max yield = 1 mol/mol (0.50 g/g, 67 C-mol %)

Figure 1. Producing BDO through microbial metabolism. Upper left, basic stoichiometry of bio-BDO, the 0.50 g/g theoretical yield is based on the pathways shown in the rest of the figure and this does omit the needs for biomass production in growing cells and energy conservation. Bottom, the published 1,4-BDO pathway from succinyl-CoA. Middle and right, the BDO pathway incorporated into *E. coli* cellular metabolism. Other pathways to produce BDO have also been identified and tested.

TCA: tricarboxylic acid cycle or citric acidcycle or Krebs cycle

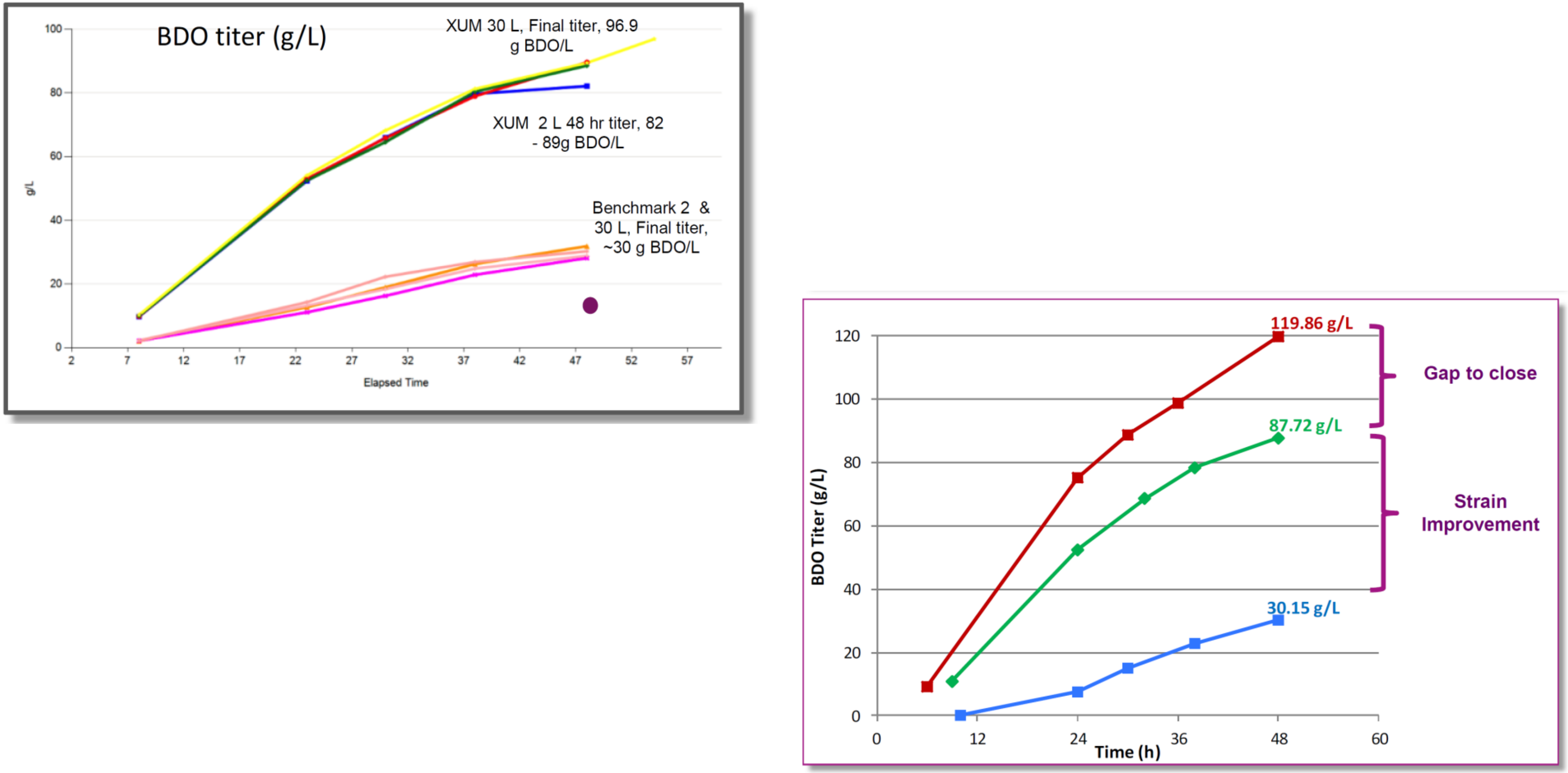


Figure 6. Comparison of BDO titer improvements due to strain engineering and hydrolysate composition. **A.** The large dark magenta filled circle is the 48 hr. BDO titer in early pre-lot 001 B2 hydrolysate (~180 g monomeric sugar/L) with the original benchmark strain, "H". The other curves are BDO titer values over 48 hrs. in both 2 L and 30 L fermenters. All used B2 lot 014/12 and two different strains, "H" (lower curves) and "Super G" (upper curves). **B.** Some of the same data from 6A for H (blue curve) vs. Super G on B2 lot 014/12 lignocellulosic hydrolysate (green curve) combined with Super G on C hydrolysate (hardwood feedstock treated with HCl) (red curve).

1,4-BDO is an important chemical that is used for the manufacture of over 2.5 million tons of polymers annually1,4-BDO and its derivatives represent a market ripe for the introduction of a competitive bio-based route.

derivatives:

- tetrahydrofuran
- γ-butyrolactone
- *N*-methylpyrrolidone
- 2-pyrrolidone.

Thanks to an investment of 100 million euro, Novamont has managed to revive an abandoned manufactory site of Bioitalia, former Ajinomoto, who was acquired in 2012 by Novamont, safeguarding 27 jobs, which later became 51 at the end of 2015.
The plant of Bottrighe di Adria is the first facility in the world capable of producing butanediol (BDO) directly from sugars (30 thousand tons yearly).

BDO produced by the plant enables Novamont to deliver its fourth-generation of Mater-Bi bioplastics with greater sustainability (e.g. renewable components).

The products made with this new BDO will save an estimated 56 percent of greenhouse gas emissions compared to the use of conventional BDO.

https://www.youtube.com/watch?v=cWPcKil4z4M

https://www.youtube.com/watch?v=awxsW2nzsN8


ATTENZIONE
GC BioStream

Chemical platform for 3-hydroxypropionic acid

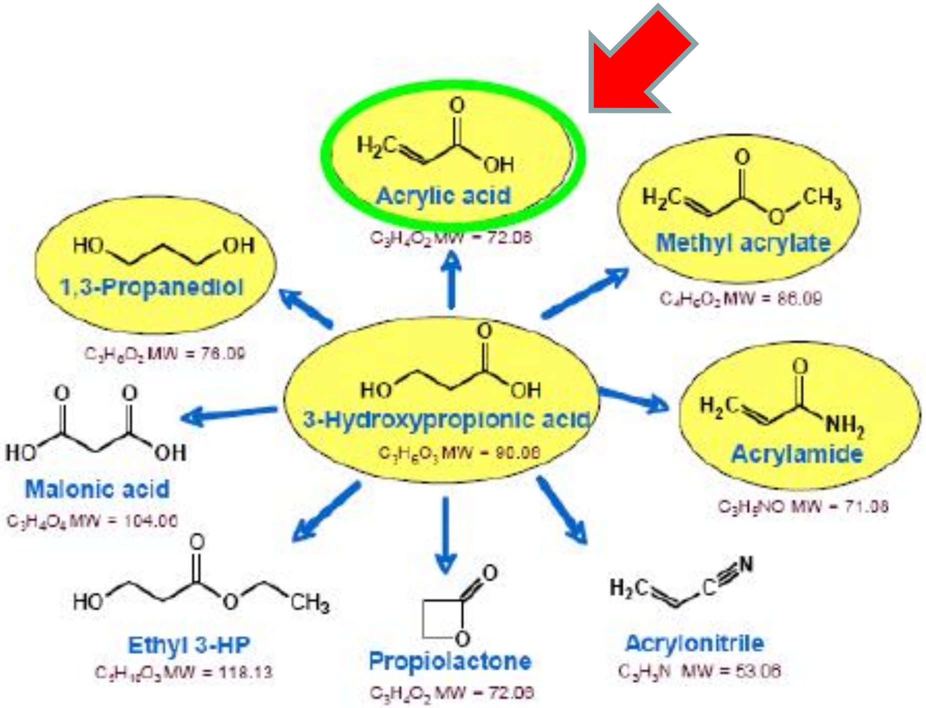
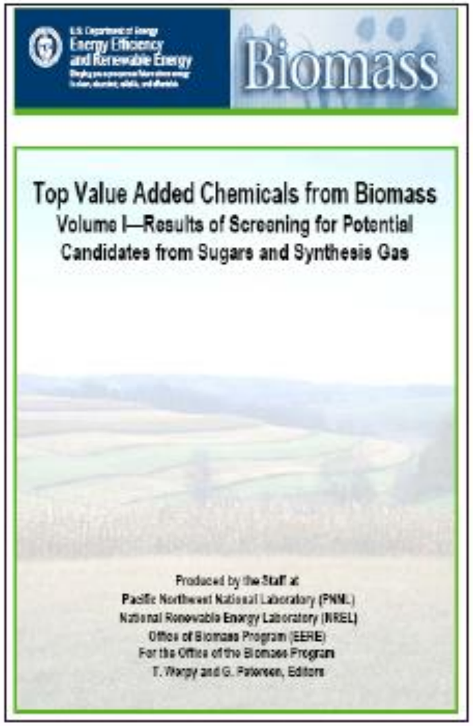


Figure 8 – Derivatives of 3-HPA



U.S. Department of Energy
Energy Efficiency and Renewable Energy

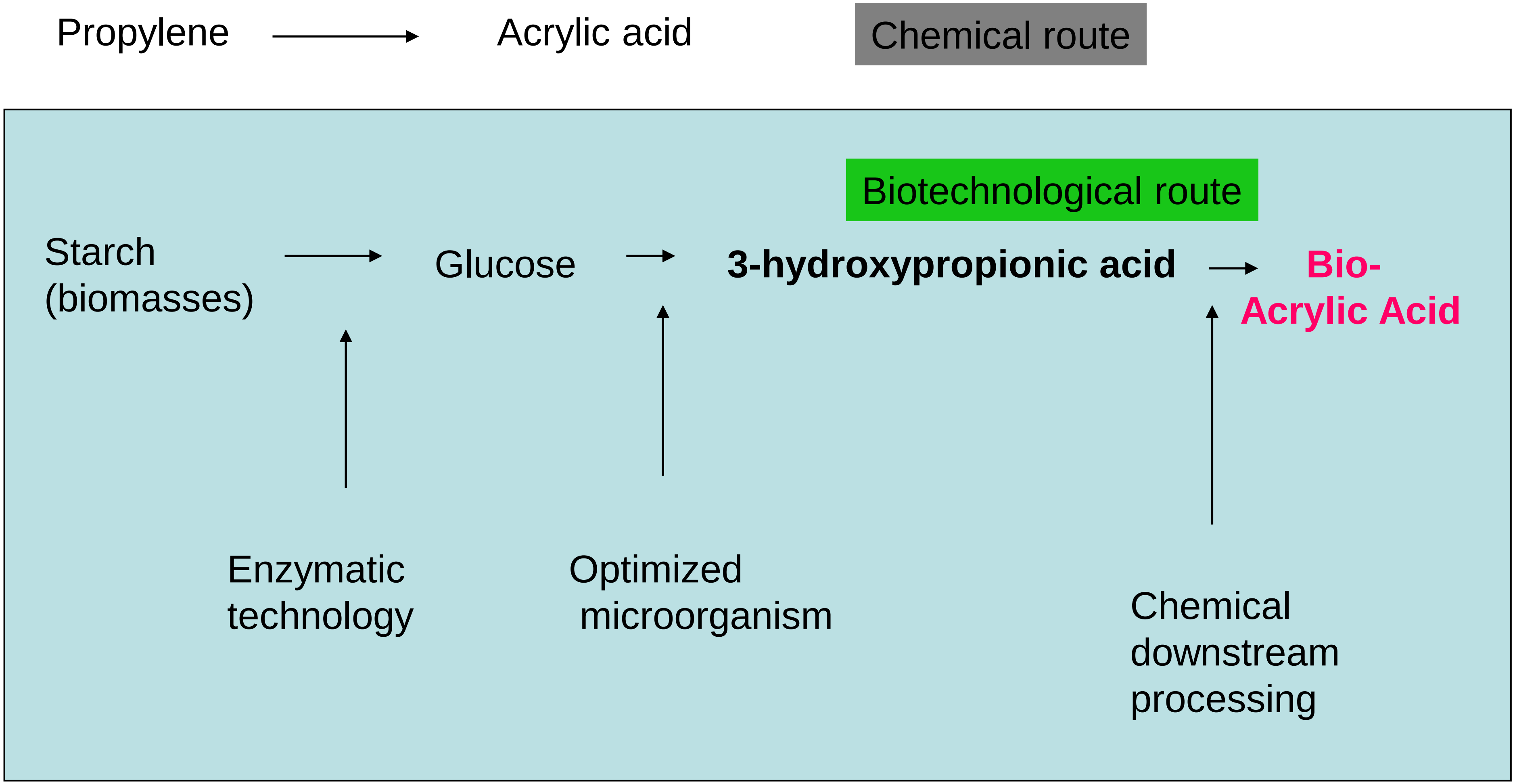
Biomass

Top Value Added Chemicals from Biomass
Volume I—Results of Screening for Potential Candidates from Sugars and Synthesis Gas

Produced by the Staff at
Pacific Northwest National Laboratory (PNNL)
National Renewable Energy Laboratory (NREL)
Office of Biomass Program (EERE)
For the Office of the Biomass Program
T. Werpy and G. Petersen, Editors

http://www1.eere.energy.gov/biomass/pdfs/35523.pdf

Production of Bio-acrylic acid



Propylene → Acrylic acid

Chemical route

Biotechnological route

Starch (biomasses) → Glucose → **3-hydroxypropionic acid** → **Bio-Acrylic Acid**

Enzymatic technology

Optimized microorganism

Chemical downstream processing

(source Novozymes communication)

Bio Polyethylene

- Equivalent to fossil based PE

- 100 % biobased (ASTM 6866)

- Not biodegradable

- Braskem 2009, 200.000 t/a
- Dow 2011, 350,000 t/a
- Solvay PVC

Sugar Cane
↓ fermentation, distillation
Ethanol
↓ dehydration
Ethylene
↓ polymerization
PE

- Ethanol fermentation carbon efficiency?

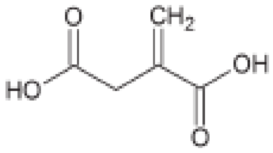
$$C_6H_{12}O_{6(l)} + H_2O_{(l)} \rightarrow 2C_2H_5OH_{(l)} + 2CO_{2(g)} + H_2O_{(l)} + heat$$

1,3-Propanediol (1,3-PDO)

The microbial production of 1,3-PDO is one of the oldest processes reported in the literature.

This diol has a wide range of possible applications, e.g. composites, adhesives, solvents, monomers for aliphatic polyesters, and as an anti-freezing agent. In addition, 1,3-PDO is used for the production of **poly(trimethylene terephthalate),** a polymer with remarkable ‘stretch–recovery’ properties that is used in specialty resins and other applications.

Various bacteria including *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Lactobacillus brevis* and *Clostridium butyricum* have been reported to produce 1,3-PDO during anaerobic growth on glycerol.45 The highest concentration of 1,3-PDO was obtained using a *K. pneumonia* strain that led to a concentration of 73.3 g L−1.



Itaconic acid (IA)

Itaconic acid (IA)

IA has been known since 1837 when Baup first described the thermal decomposition of citric acid, leading to IA.

Neither thermal decomposition nor alternative chemical methods are used for commercial production since fermentation by fungi is economically more profitable.

Biosynthesis of IA was first described by Kinoshita in 1932 who isolated the product from cultivation media of the osmophile eukaryotic *Aspergillus itaconicus*.

Various *Aspergillus terreus* strains were found more suitable for the fermentation process.

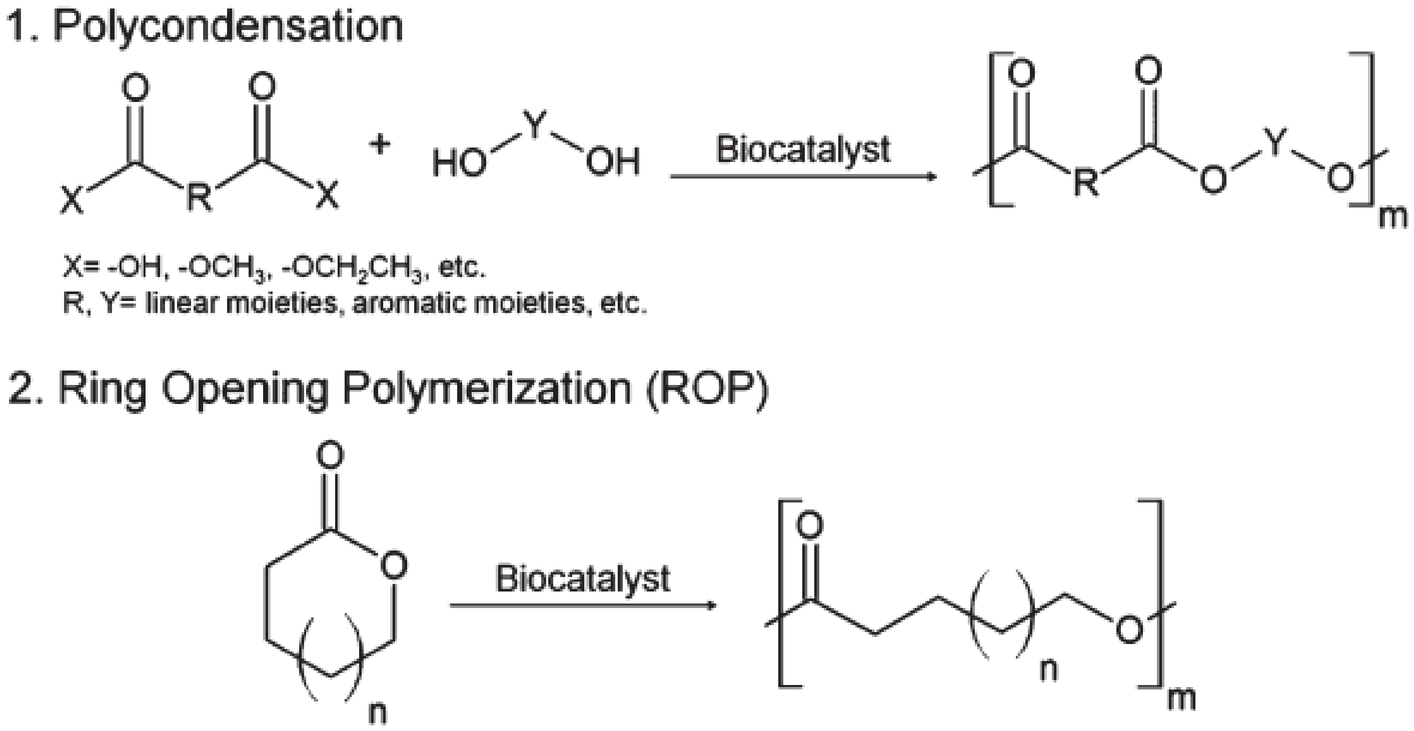
The best yields of IA production were achieved using glucose or sucrose as substrates, but for the economic sustainability of the process, complex carbon sources like starch, molasses and hydrolysates of corn syrup or wood were also tested and found to be suitable.
During the fermentation process, the pH drops below 2 and IA becomes the main fermentation product.

For an optimal reaction setup the temperature is usually maintained at around 37 ◦C. An adequate oxygen supply is essential since anaerobic conditions will irreversibly kill the cells.

Economically speaking, the most productive process was established by Pfizer which involves a submerged fermentation process using suspended *A. terreus* biomass, inoculated as spores on pretreated molasses.

IA is currently used in paper-coating and carpet-backing, which are the primary consumers at the industrial scale. Some IA derivatives are used in medicines, cosmetics, lubricants and herbicides.

Enzymatic synthesis of polesters



X= -OH, $-OCH_3$, $-OCH_2CH_3$, etc.
R, Y= linear moieties, aromatic moieties, etc.

Scheme 3. Routes for the enzymatic synthesis of bio-based polyesters. (1) Polycondensation reaction of diacids (or their diesters) with polyols. (2) Ring-opening polymerization of lactones.

Trends for polyester synthesis and functionalyzation

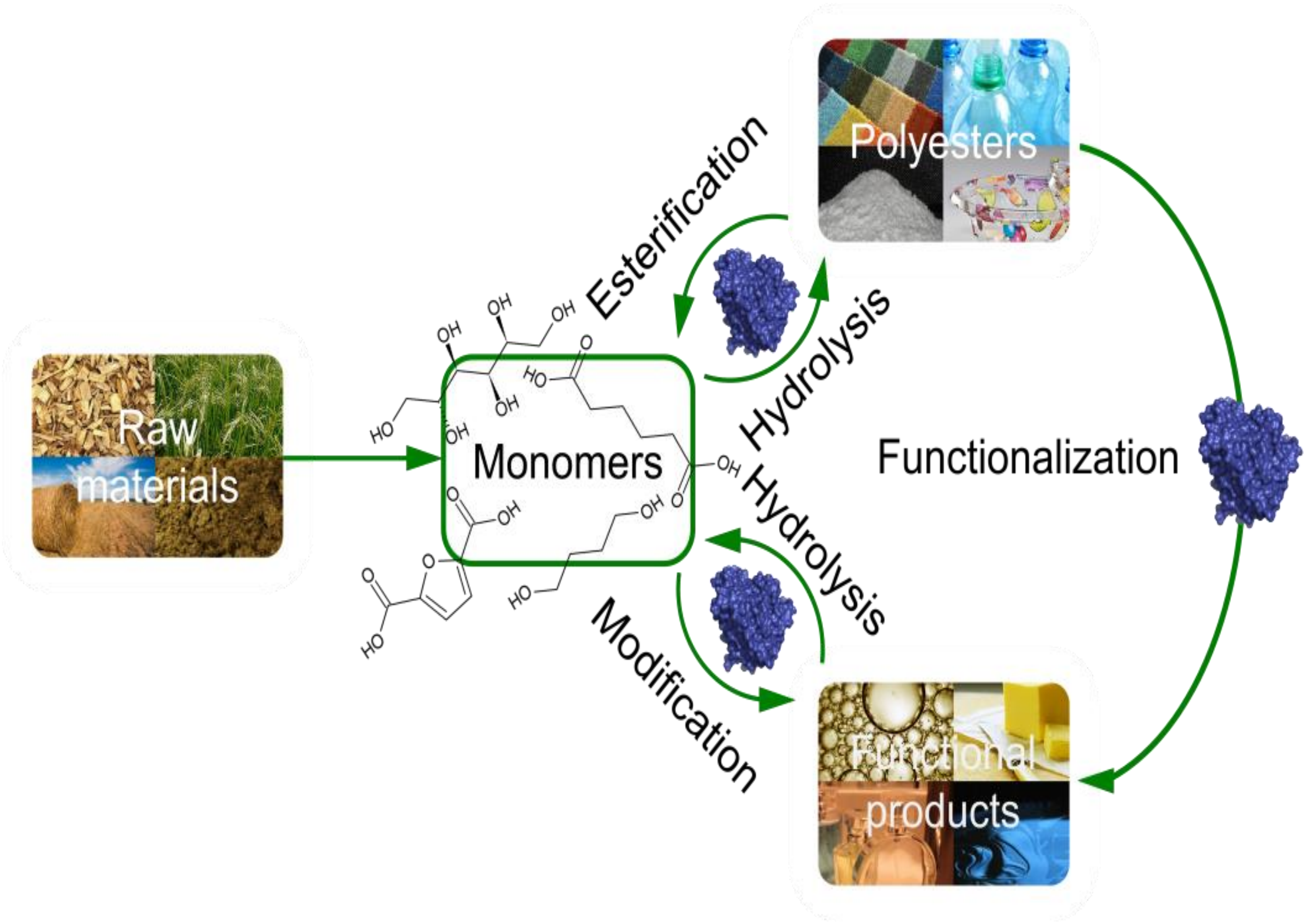


Table 2. Most recent enzymatic processes for synthesis and functionalization/hydrolysis of bio-based polyesters.

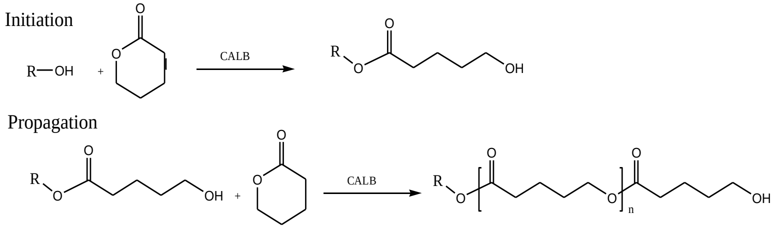
Biocatalyst	**Material class**	**Goal**
CaLB	Lactic-acid based	ROP of D,D-lactide
	Terephthalic acid-based Ethylene glycol-based 2,5-furandicarboxylic acid-based	Synthesis of aromatic/aliphatic polyesters
	Sorbitol-based	Synthesis of hydroxy-functional polyesters
	Adipic acid-based 1,4-butanediol-based Isosorbide-based	Synthesis of aliphatic polyesters
	Itaconic acid-based 1,4-butanediol-based	Synthesis of vinyl-functional polyesters
	Glycerol-based Itaconic acid-based	Branched-controlled polyesters, epoxide-containing polyesters
	Malic acid-based Adipic acid-based	Copolymers of L-malic acid, adipic acid and 1,8-octanediol
HiC	Adipic acid-based 1,4-butanediol-based	Synthesis of aliphatic polyesters
	Terephthalic acid-based Ethylene glycol-based	PET hydrolysis
	Lactic-acid based	Surface functionalization of PLA films
Thermobifida cellulosilytica cutinase 1	Terephthalic acid-based Ethylene glycol-based	Surface functionalization of PET films
Thermobifida fusca cutinase	Terephthalic acid-based Ethylene glycol-based	Degradation of PET nanoparticles
Thermobifida halotolerans esterase	Terephthalic acid-based Ethylene glycol-based	PET hydrolysis
	Lactic-acid based	PLA hydrolysis
Thielavia terrestris cutinase	Succinic acid-based 1,4-butanediol-based	PBS hydrolysis
	Terephthalic acid-based Ethylene glycol-based	PET hydrolysis

Why enzymes in polyester synthesis?

- ➢ Selective and efficient under mild conditions
- ➢ No need of toxic metal catalysts
- ➢ Solvent-free processes feasible

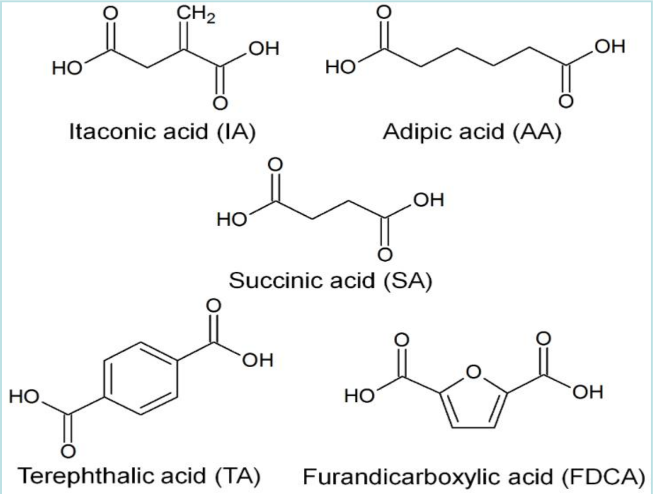
(Polycondensation)

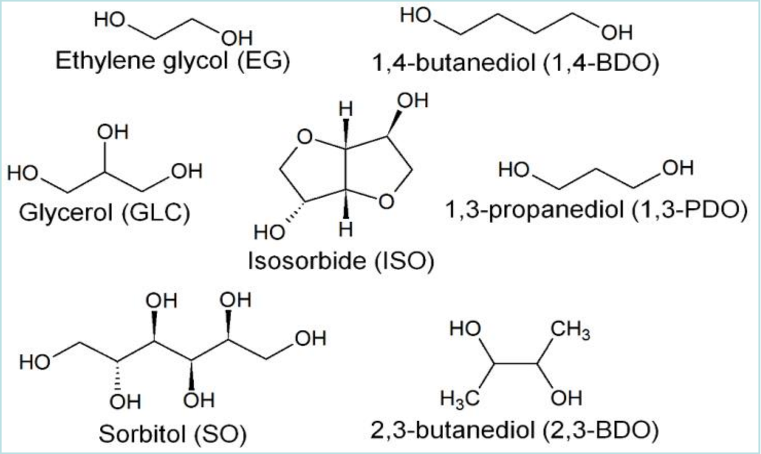
(ROP)



Mn < 20000

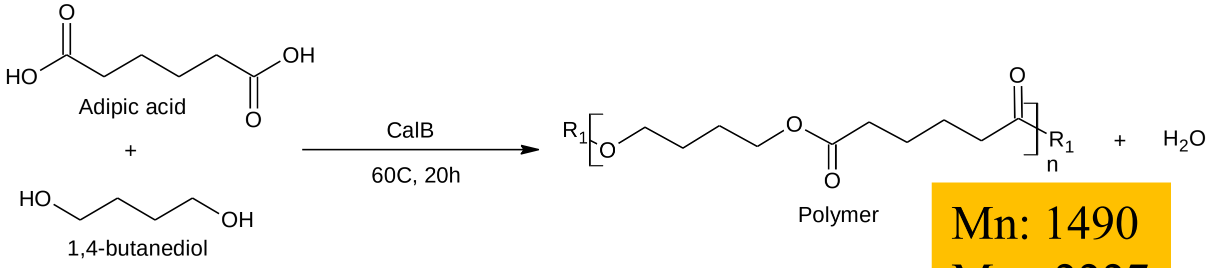
- ➢ **Tailored pre-polymers for advanced functionalities**



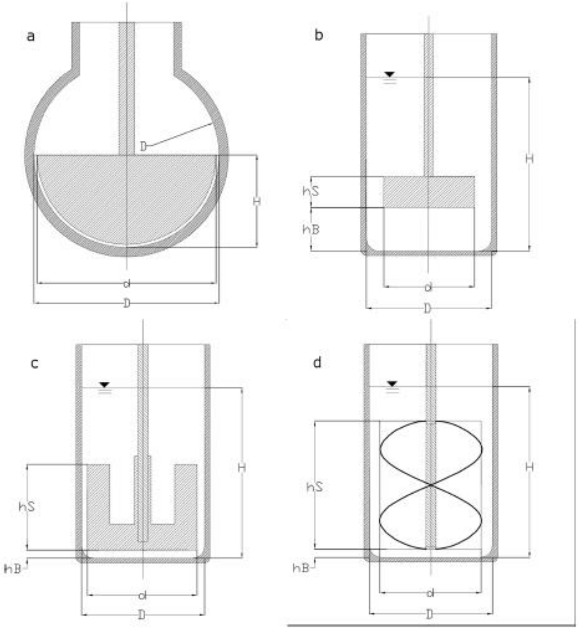


.... industrial applications?

Challenges for the solvent-free enzymatic synthesis of polyesters in general

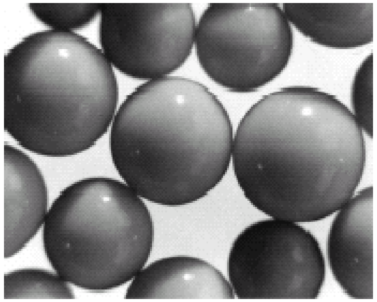


Mn: 1490
Mw: 2227



Adsorption

Leaching



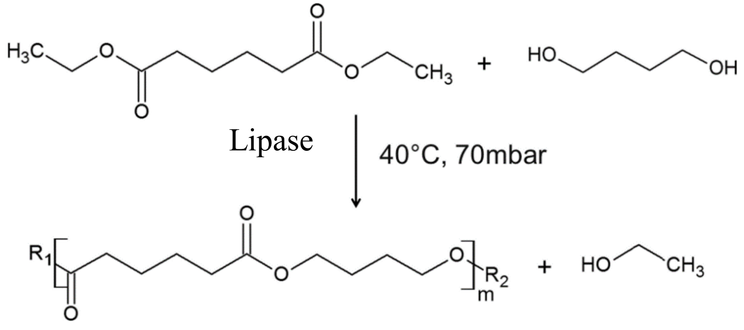
Novozyme 435: lipase B from *Candida antarctica* adsorbed on acrylic resin

Biocatalysts damaged under operational conditio

C. Korupp, R. Weberskirch, J. J. Müller, A. Liese and L. Hilterhaus, *Org. Process Res. Dev.*, 2010, 14, 1118–1124.
F. Binns , S. M. Roberts , A.Taylor and C. F. Williams. *J. Chem. Soc., Perkin Trans*. 1, 1993, 899.

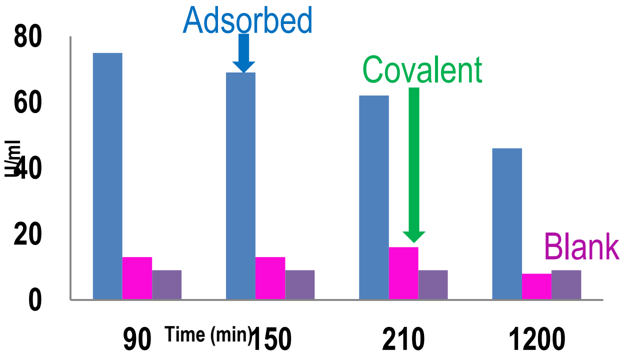
Making lipase robust and recyclable: covalent immobilization

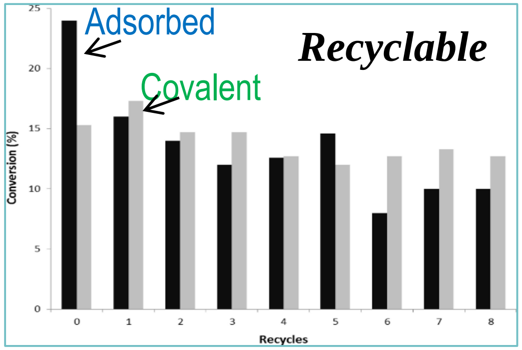
Synthetic activity: 2400 U $^{g-1}$ (PLU)



No solvent

Leaching prevented





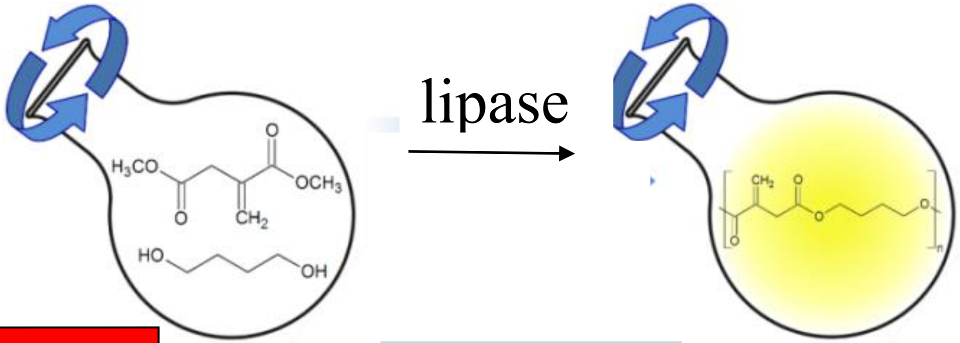
Ezymatic activity detected in the final product after biocatalyst filtration

Gardossi et al., WO2012085206 A1

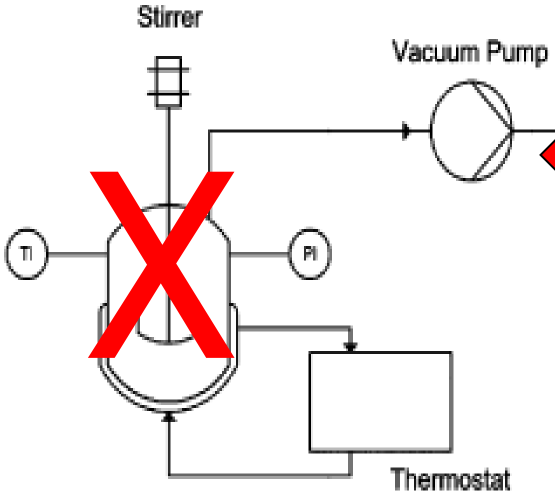
A. Pellis et al.*Green Chem.* **2015**, 17, 1756–1766.

V. Ferrario, et al, *Biomolecules* **2013**, 3, 514-534

Combining robust biocatalysts with new process configuration for preserving biocatalyst integrity



Thin film



New alternative process configuration

Scalable?

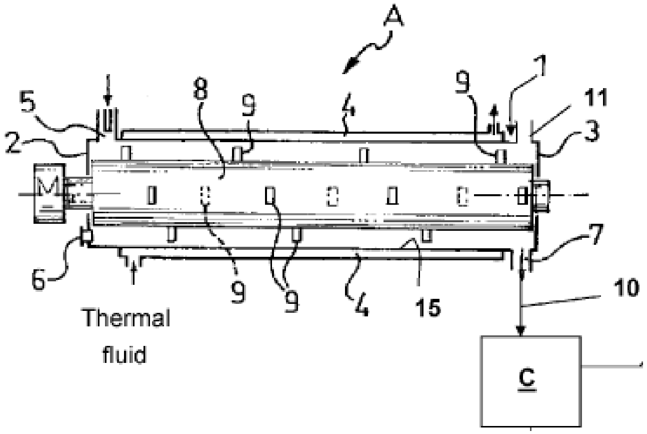
A. Pellis et al.*Green Chem.* **2015**, 17, 1756–1766.

The process:
covalent immobilised enzyme + turbo reactors

THIN LAYER TECHNOLOGY

- ✓ **High efficiency in overcoming viscosity (solvent-free conditions)**
- ✓ **Easy water/alcohol removal**
- ✓ **Mechanical integrity of the biocatalyst**
- ✓ **No protein leaching**

OPTIMAL HEAT AND MASS TRANSFER

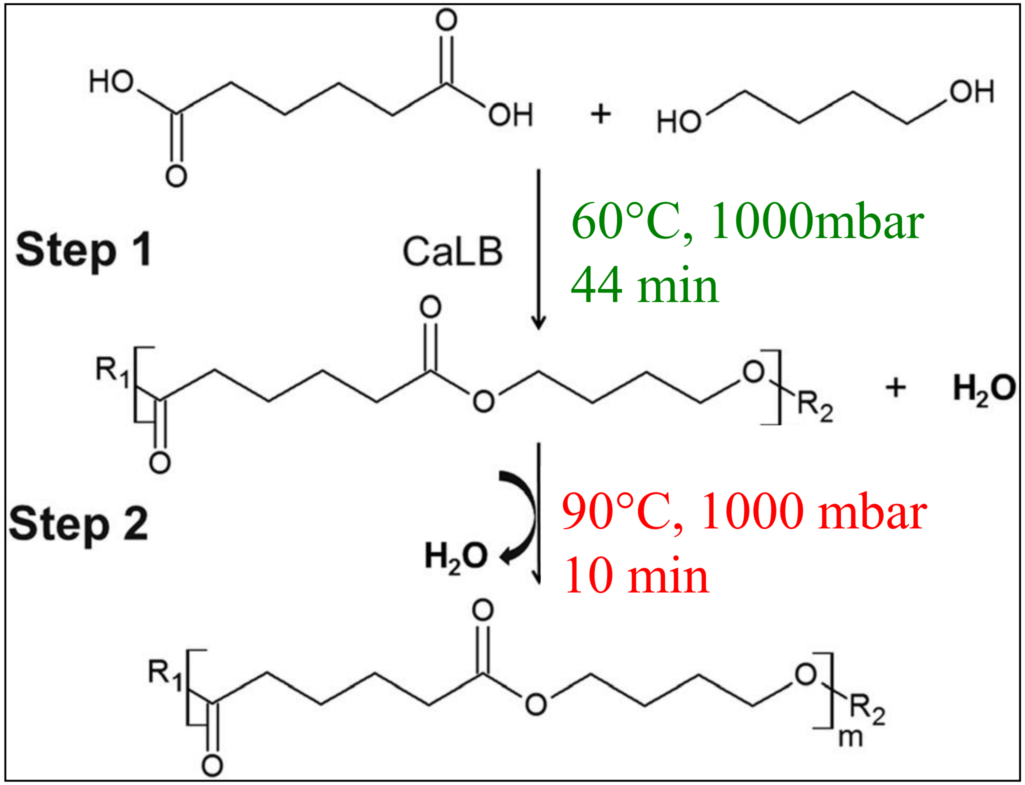


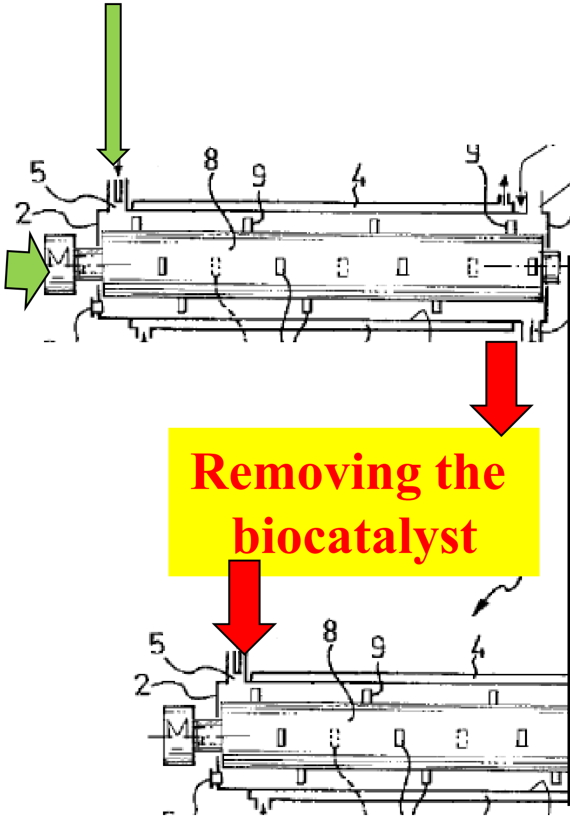

In collaboration with

VOMM

Gardossi L., et al.. Patent EP 2 620 462 A1.

Preserving biocatalyst activity: two-step process





Removing the biocatalyst

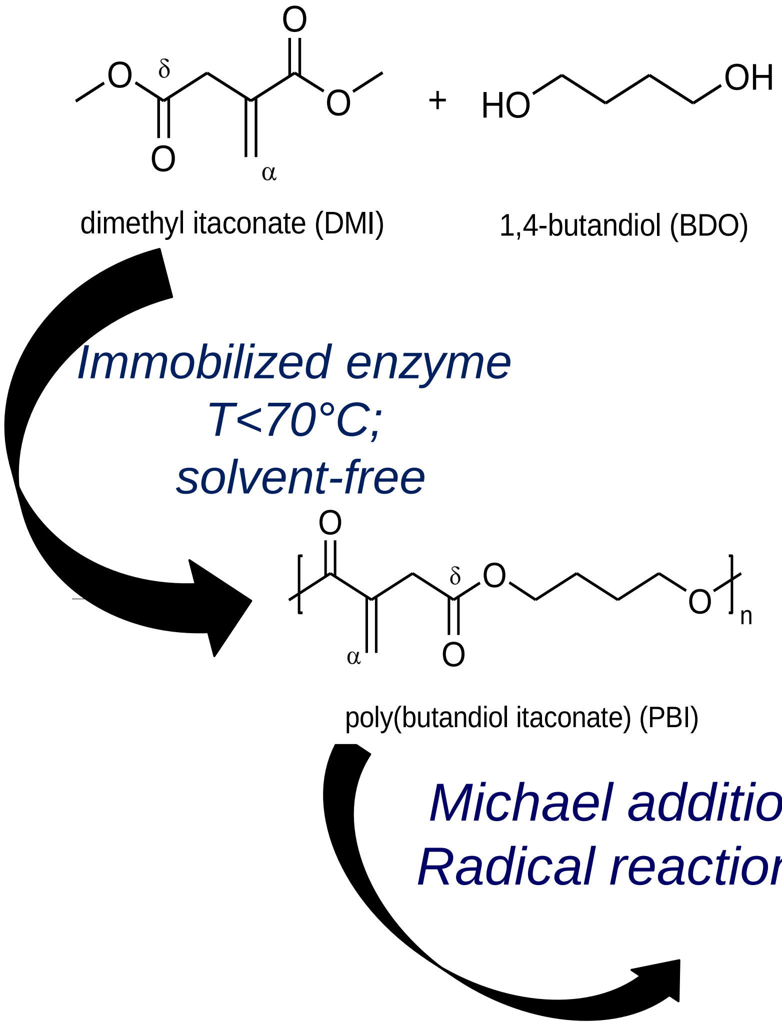
Mn (PDI)
1800 (1.33)
2900 (1.34)

Biocatalyst: 9% w^{-1} : 30 U g^{-1} monomers

Specific activity: 300 U g^{-1}

A. Pellis et al.*Green Chem.* **2015**, 17, 1756–1766

The case study:
fully renewable polyesters of itaconic acid



dimethyl itaconate (DMI)

1,4-butandiol (BDO)

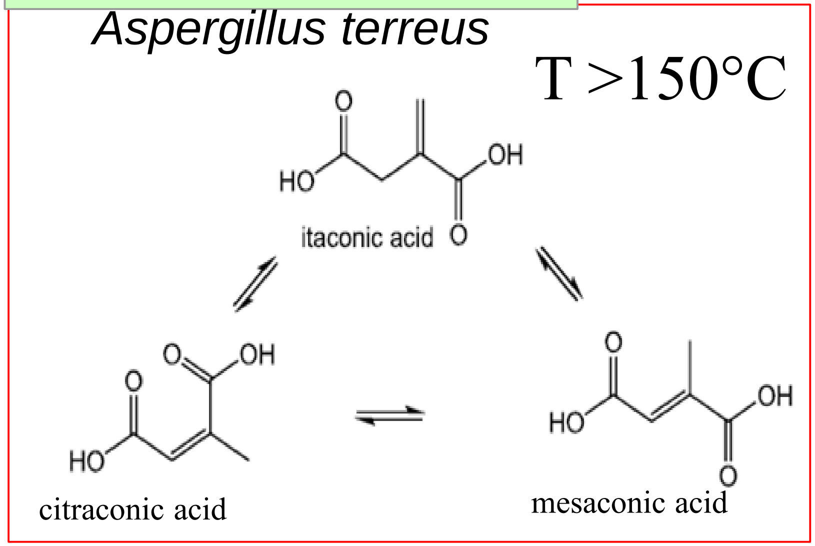
Immobilized enzyme
T<70°C;
solvent-free

poly(butandiol itaconate) (PBI)

Michael addition
Radical reactions

Grafting Biomolecules

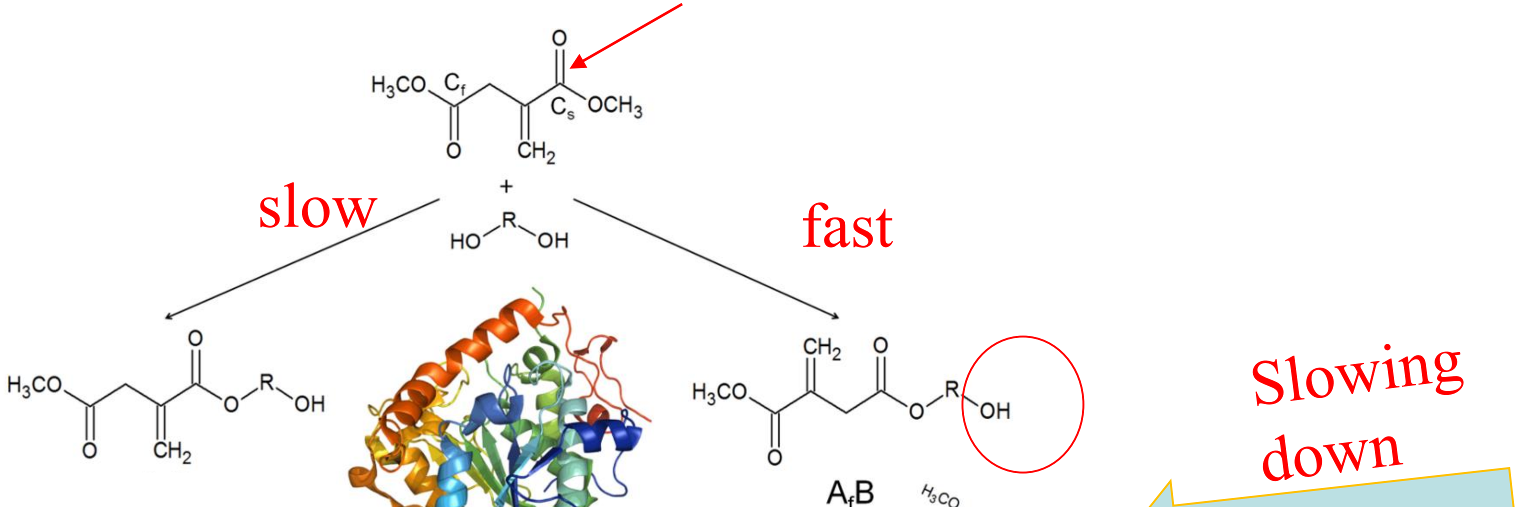
Renewable monomer:
produced by
fermentation of
Aspergillus terreus



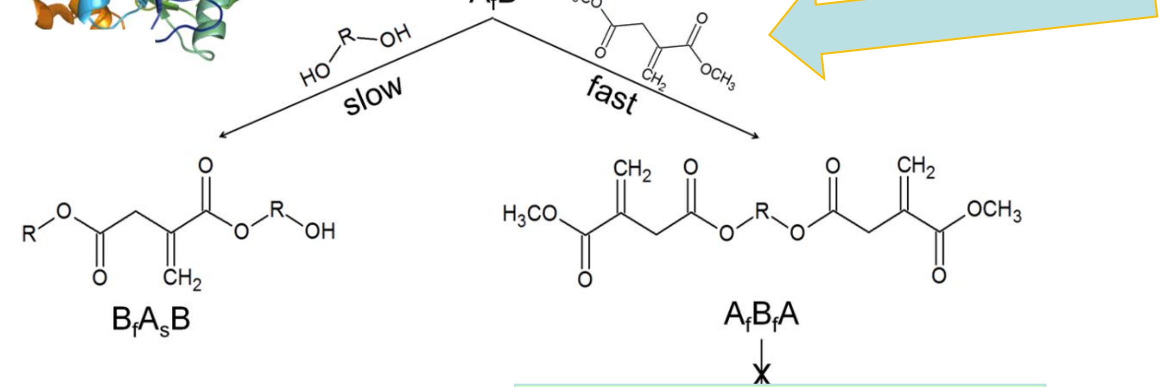
L. Corici, et al., *Adv. Synth. Catal.*, 2015, 357, 1763–1774.

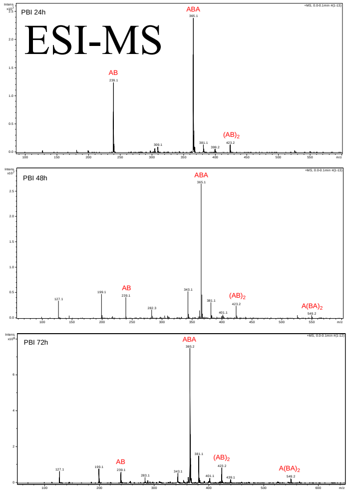
A. Pellis et al., *Polym. Int.*, 2016, 65, 861–871.

Itaconic acid: low reactivity of conjugated acyl carbon



Slowing down





ESI-MS

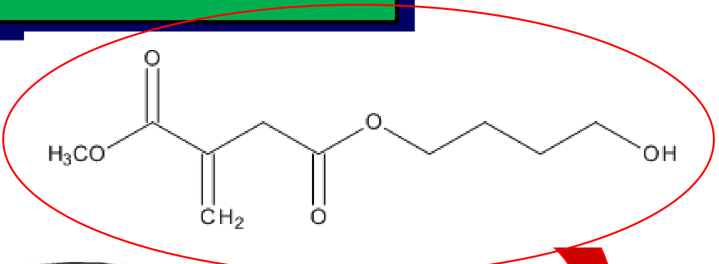
10% w w^{-1}
Cov-CaLB

No elongation!

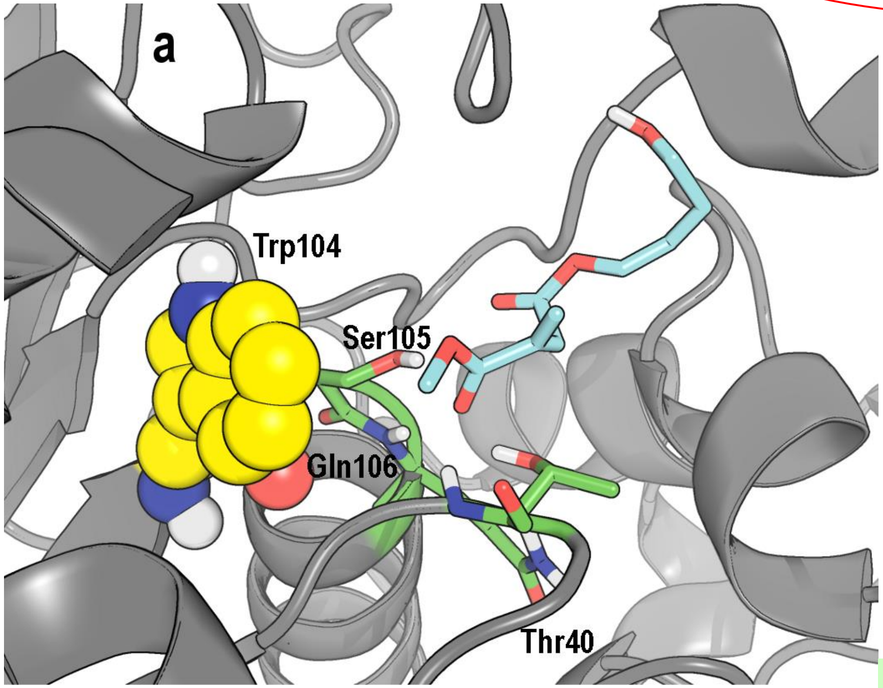
Fast acylation of second hydroxyl group leads to **dead-end product ABA**

L. Corici, et al., *Adv. Synth. Catal.,* 2015, 357, 1763–1774.

Docking the AB intermediate



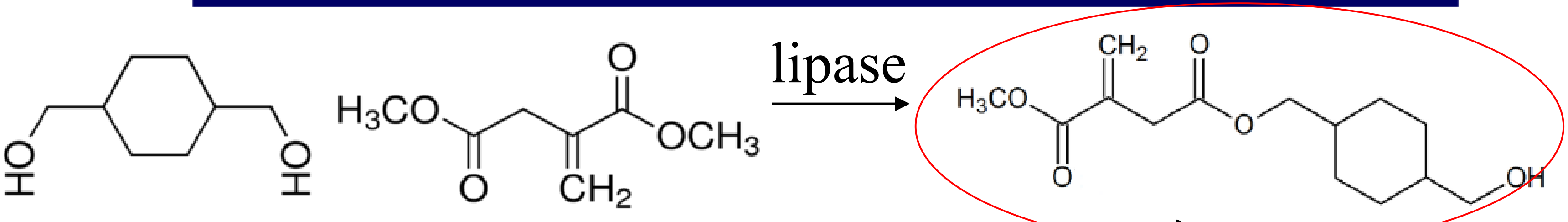
Docking



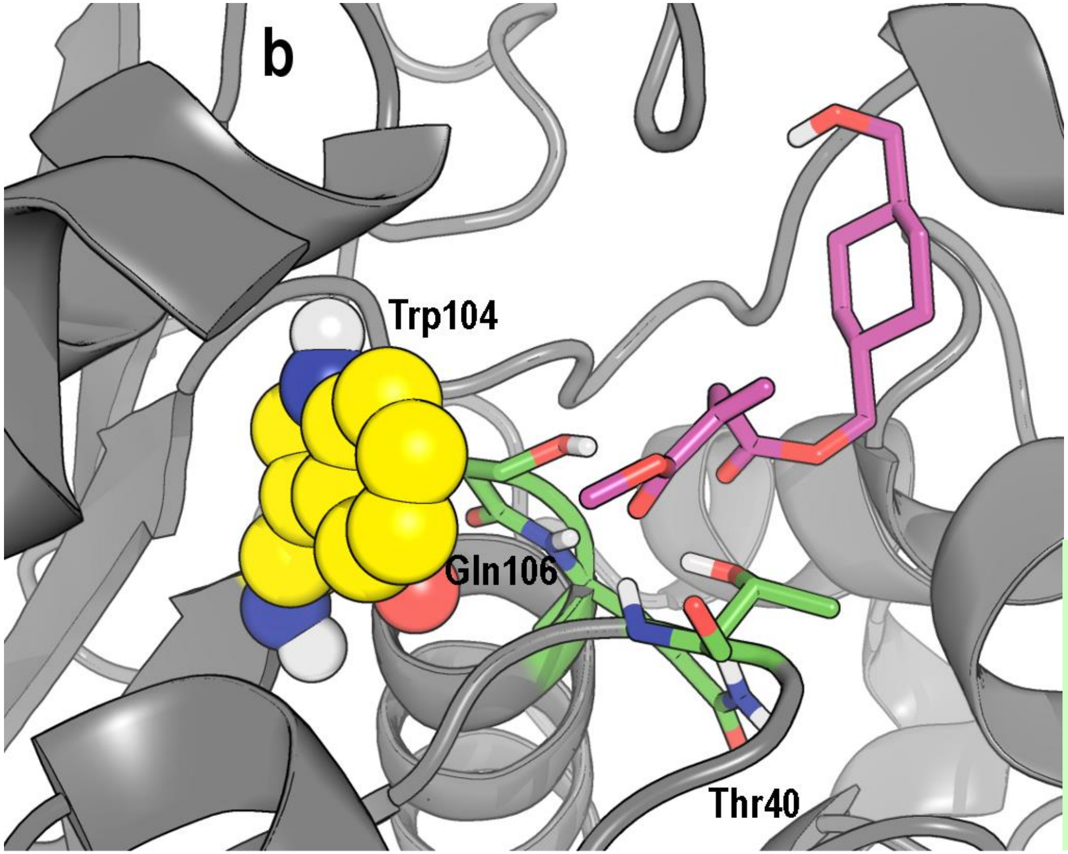
Only 2% of productive poses leads to elongation on the slow reacting acyl group

L. Corici, et al., *Adv. Synth. Catal.,* 2015, 357, 1763–1774.

Introducing rigidity in the diol: CHDM



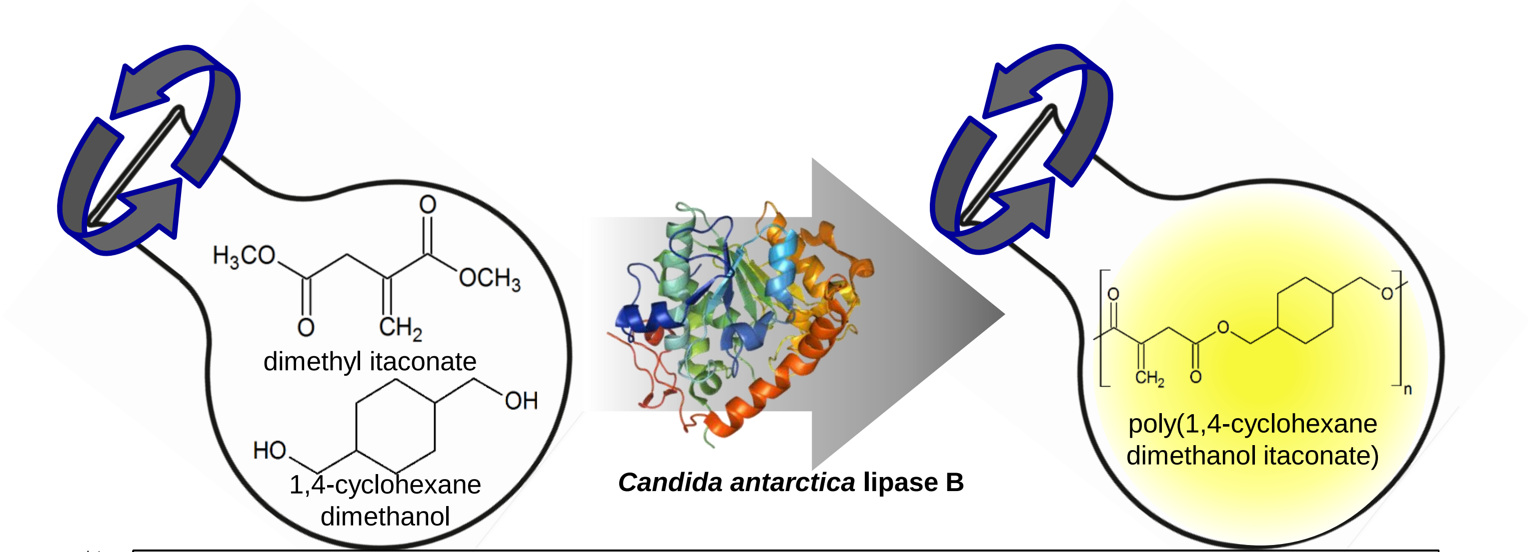
Docking



- The bulky and rigid diol does not fit in the alcoholic pocket
- 9% of productive poses leads to elongation on slow reactive acyl group

L. Corici, et al., *Adv. Synth. Catal.*, 2015, 357, 1763–1774.

Enzymatic polycondensation of DMI + CHDM

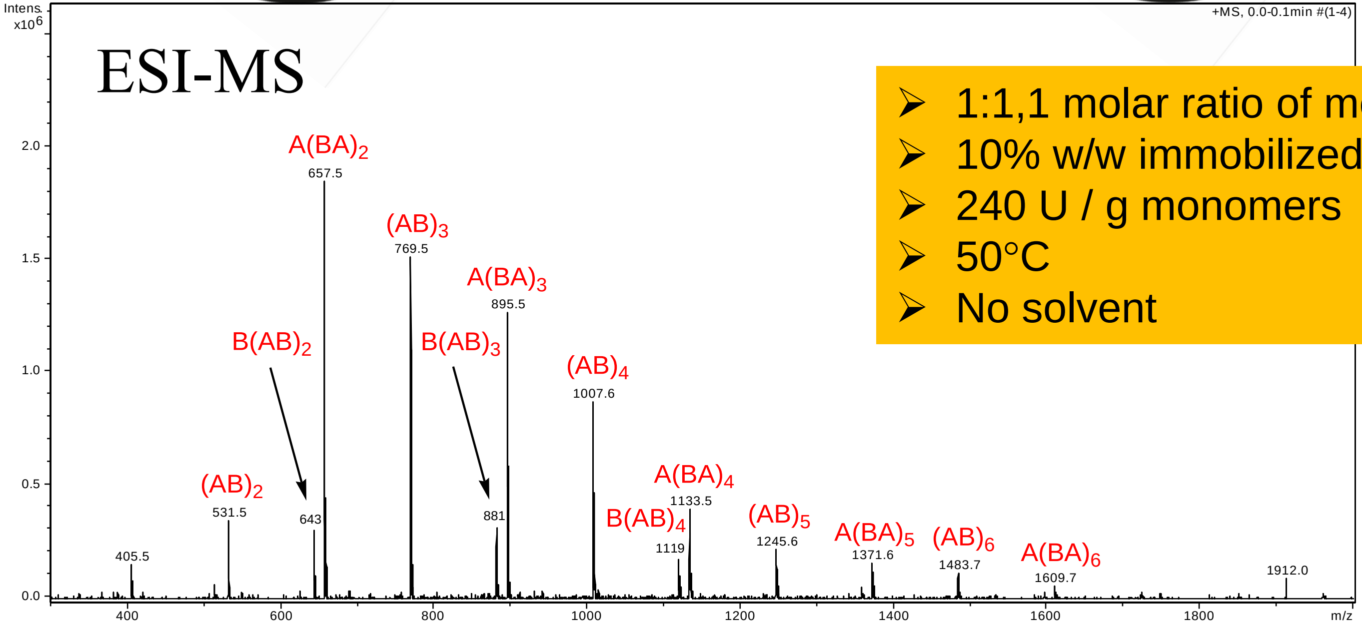


dimethyl itaconate

1,4-cyclohexane dimethanol

***Candida antarctica* lipase B**

poly(1,4-cyclohexane dimethanol itaconate)

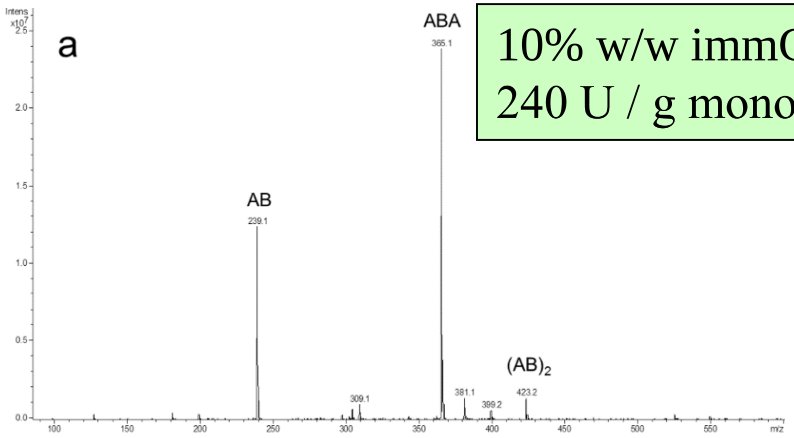


- ➢ 1:1,1 molar ratio of monomers
- ➢ 10% w/w immobilized CaLB
- ➢ 240 U / g monomers
- ➢ 50°C
- ➢ No solvent

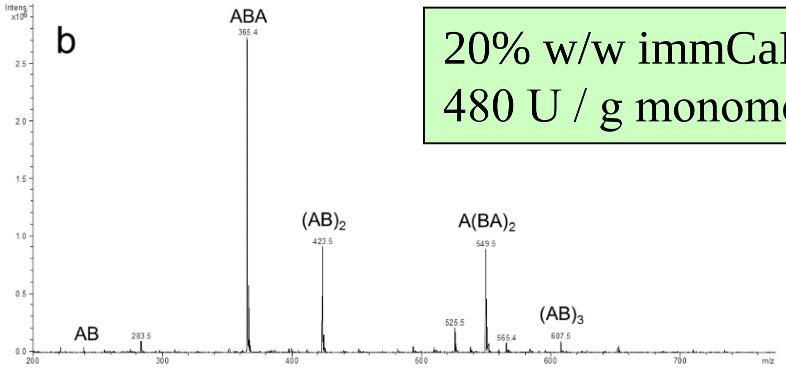
L. Corici, et al., *Adv. Synth. Catal.*, 2015, 357, 1763–1774.

Enzyme distribution and accessibility is crucial in viscous systems: working with less enzyme distributed on a larger amount of carrier

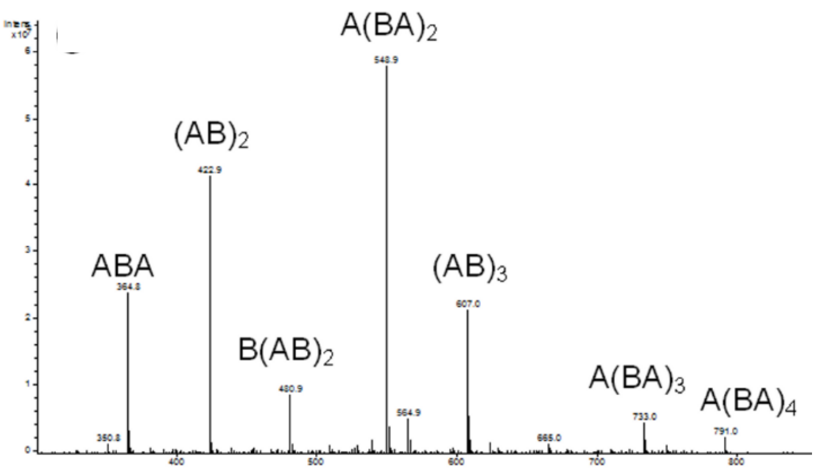
30% w/w immCaLB
70 U/g

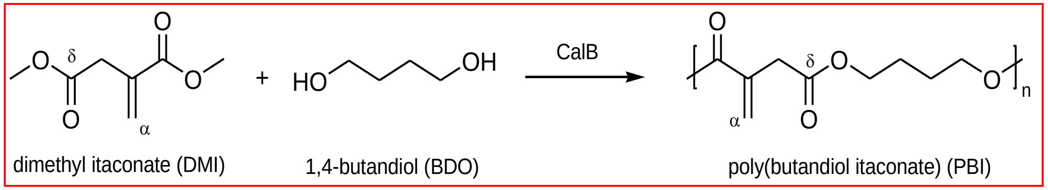


10% w/w immCaLB
240 U / g monomers



20% w/w immCaLB
480 U / g monomers





L. Corici, et al., *Adv. Synth. Catal.*, 2015, 357, 1763–1774.

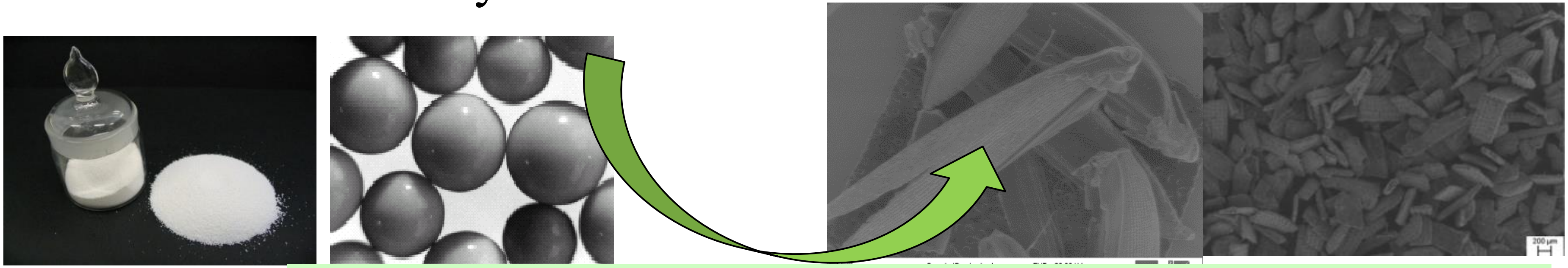
Sustainability of immobilized enzymes?

LCA: epoxy activated methacrylic resins represents the primary greenhouse gas emission source for immobilized enzymes because of the fossil based raw materials (glycidyl methacrylate, ethylene dimethyl acrylate)

S. Kim, *Int. J. Life Cycle* Assess., 2009, 14, 392–400.

Fossil based methacrylic resin

Rice husk: cellulose, lignin, silica

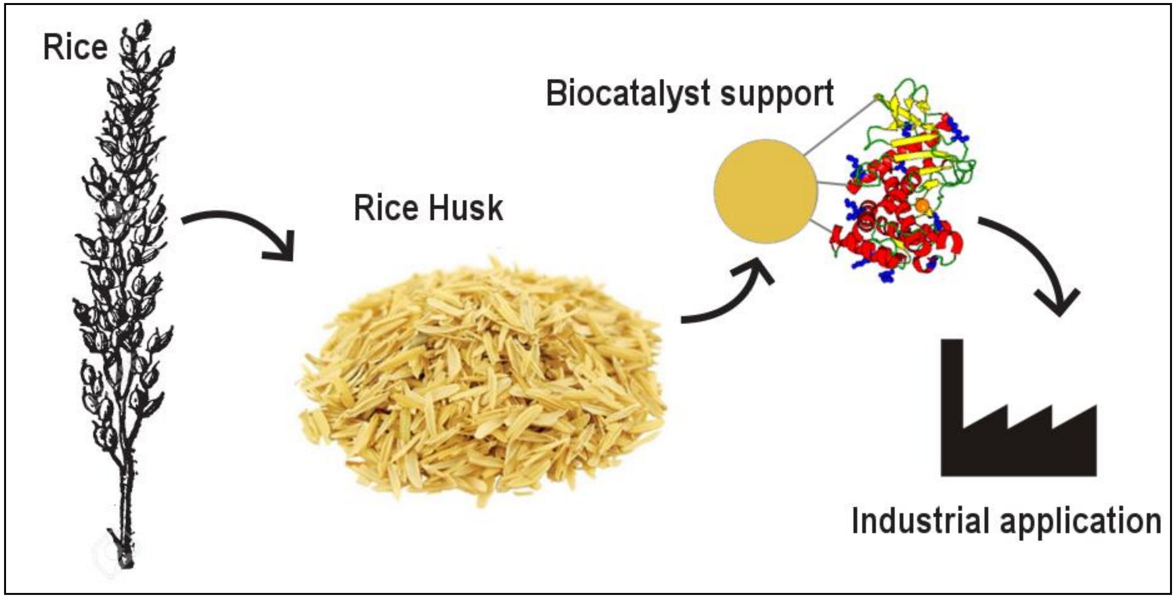


The cost contribution of biocatalysts per kg product:

- Can varies from few hundred € (for pharmaceuticals)
- Down to few cents (for bulk chemicals)
- Depends on the number of recycles of the enzyme

M. C. R. Franssen, et al., *Chem. Soc. Rev.,* 2013, **42**, 6491-6533.

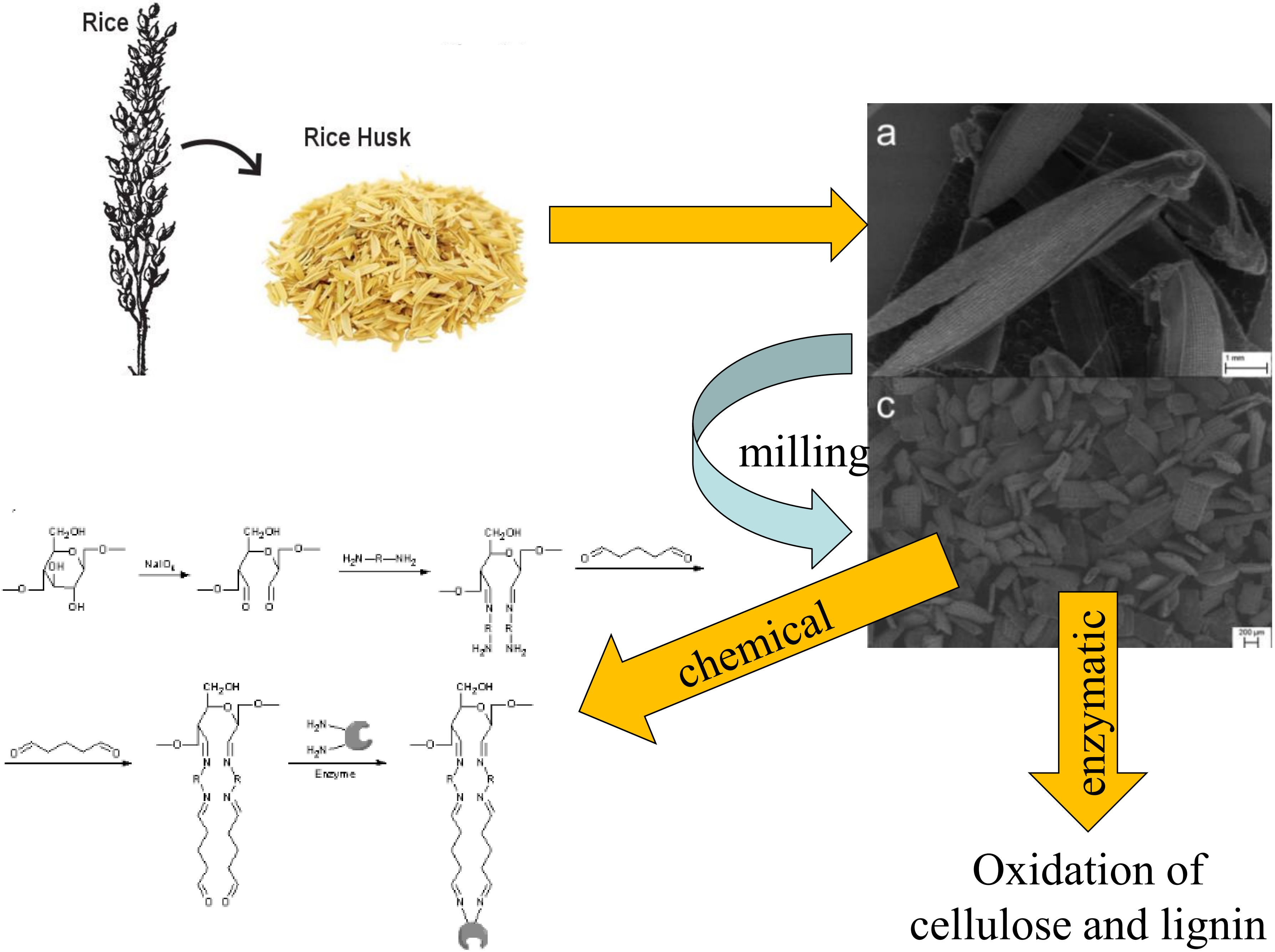
Less enzyme, more immobilization carrier: need of renewable and inexpensive carrier



4 t → 1 t

Around 100 Mt accessible at large scale and with **continuous supply**.

L. Corici et al. *RSC Adv.*, 2016, 6, 63256



milling

chemical

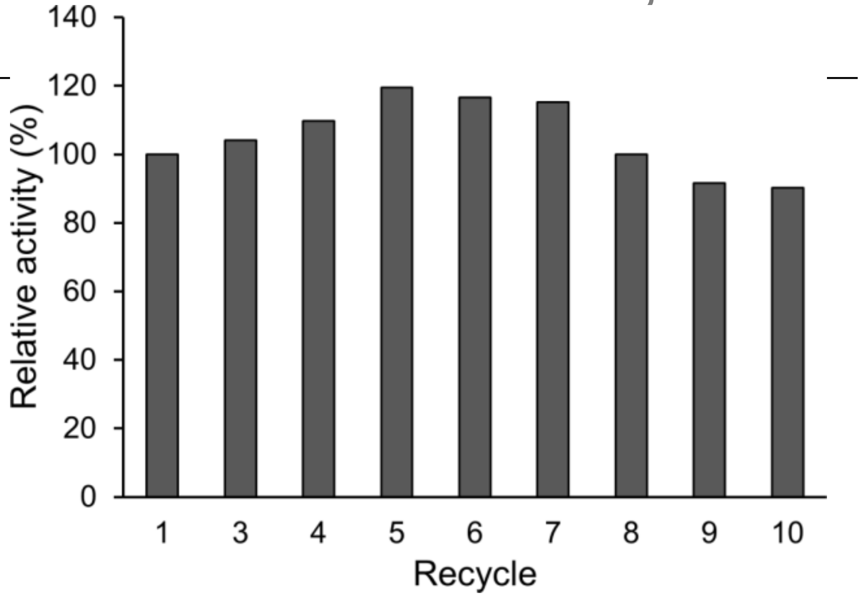
enzymatic

Oxidation of
cellulose and lignin

L. Corici et al. *RSC Adv.,* 2016, 6, 63256

Comparing CaLB on rice-husk and on epoxy acrylic resin

Functionalization	Loading (U gdry^{-1})*	Protein loaded (%)	Synthetic activity (U g dry^{-1})**
Oxidized RH + HMDA	10,000	70	2,090
Epoxy (EC-EP)	27,000	>90	2,000

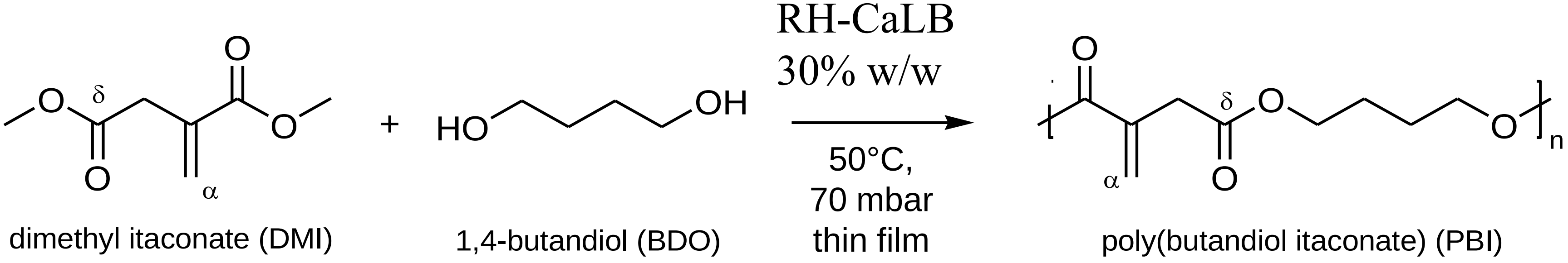


*Tributyrin hydrolysis

**Propyl laurate synthesis

L. Corici et al. *RSC Adv.,* 2016, 6, 63256

Fully renewable solvent free synthesis of (poly)esters catalyzed by RH-CaLB on thin-film

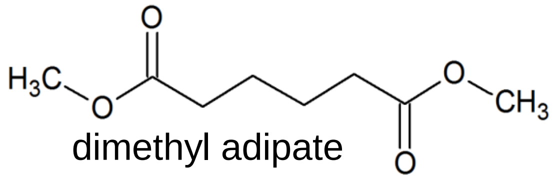


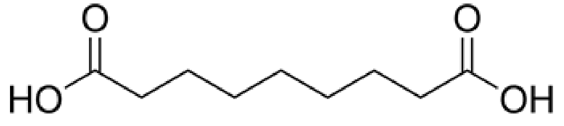
dimethyl itaconate (DMI)

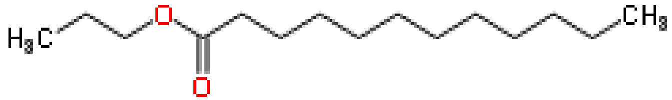
1,4-butandiol (BDO)

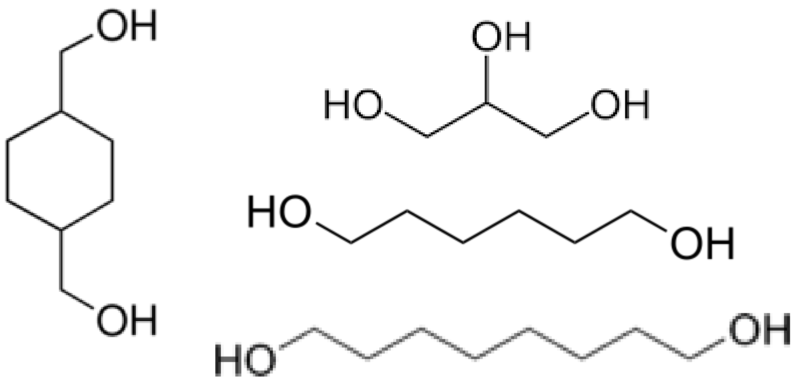
poly(butandiol itaconate) (PBI)

92% conv., Mw 970 Da


dimethyl adipate


azelaic acid


Propyl laurate

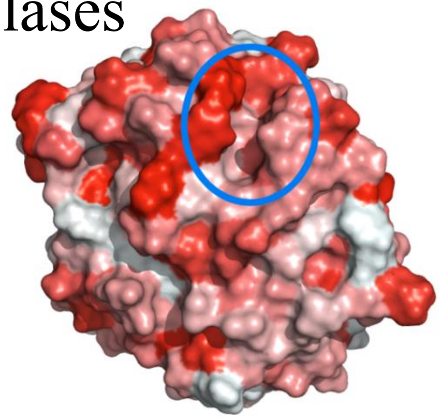


Green Chem., 2017, 2017,**19**, 490-502. DOI: 10.1039/C6GC02142E

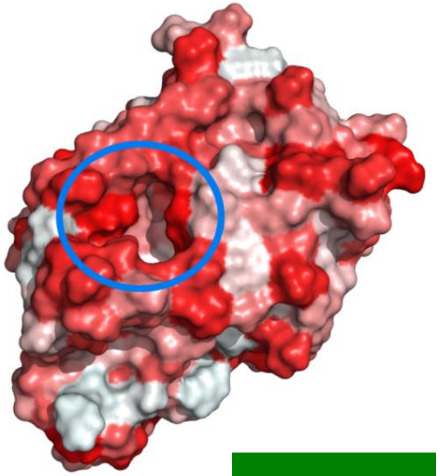
Enlarging the tools for polycondensation: cutinase from *Thermobifida cellulosilytica*

In collaboration
with G. Guebitz

Ser hydrolases

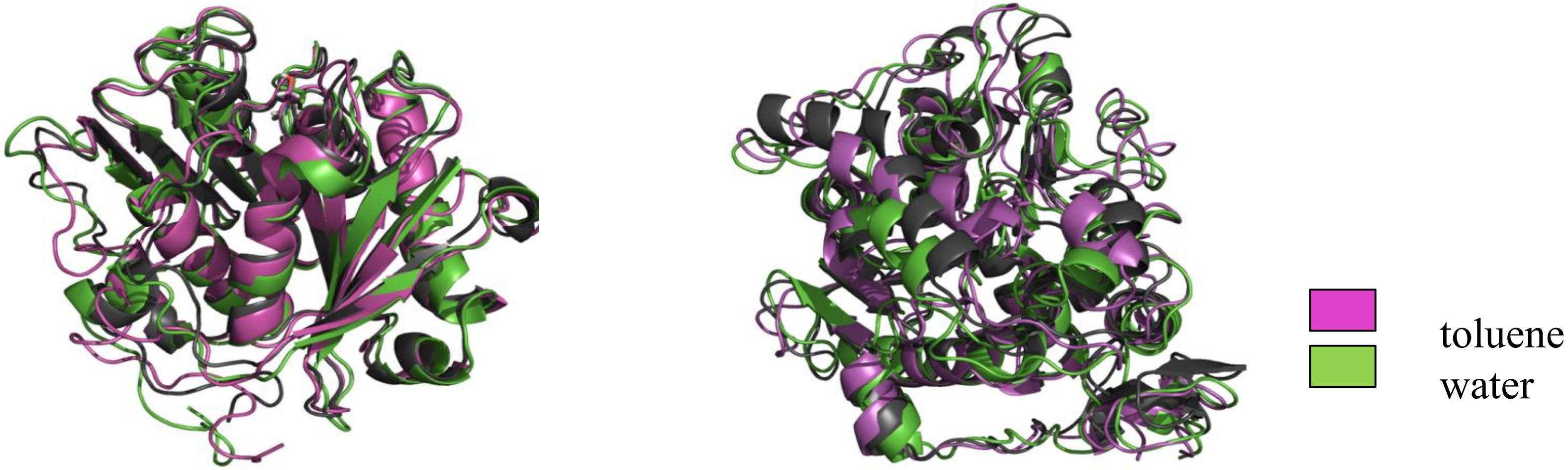


Cutinase1



CaLB

🟥 hydrophobic

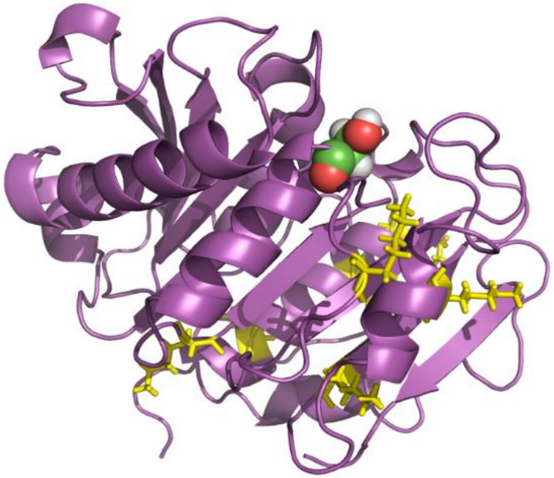


A. Pellis, et al., *Catal. Sci. Technol.*, 2016, 6, 3430–3442.

Enlarging the tools for polycondensation: cutinase from *Thermobifida cellulosilytica*

In collaboration with

Thc_cut1

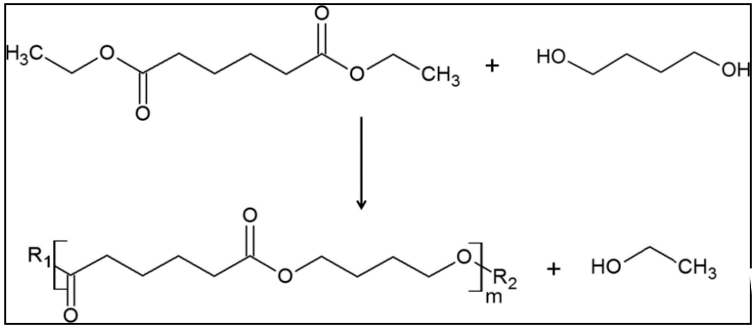


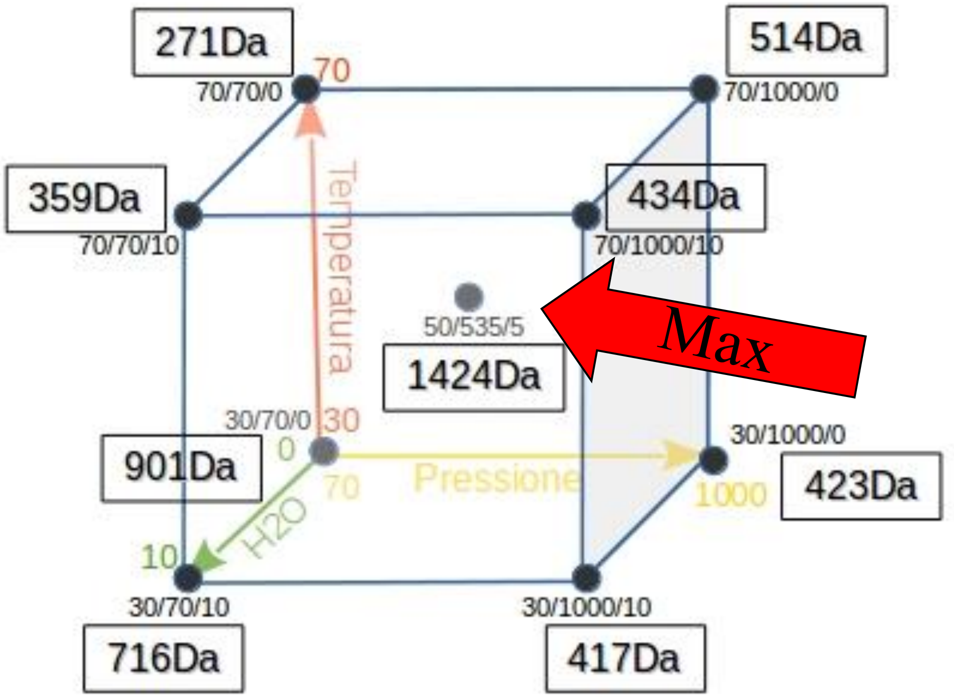
Factors:

T

p

a_w





Mn product

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