Polyhydroxybutyrate as a sustainable platform for the production of chemicals and bio-polymers

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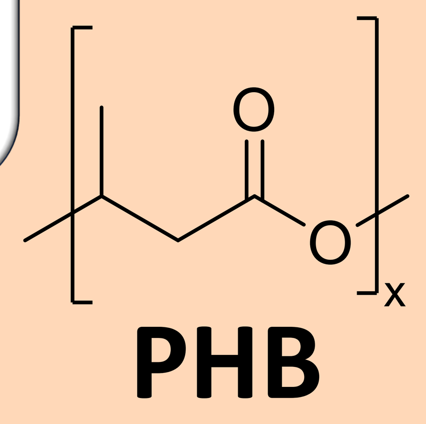
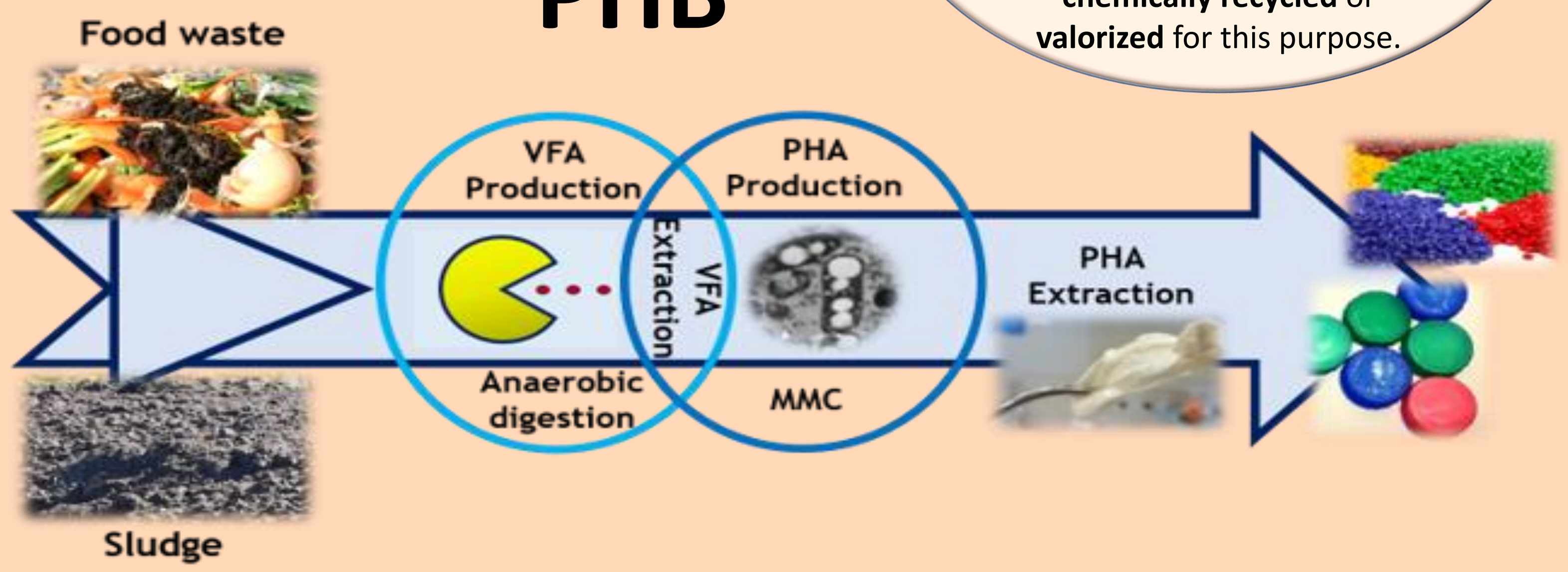


PHB is a very promising **platform chemical** for the production of C_4 building blocks, that can be used in the same PHB industrial cycle, or for different applications. **Wasted** or **low quality** PHB can be **chemically recycled** or **valorized** for this purpose.



PHB is a biobased and biodegradable polyester of hydroxybutyric acid bio-synthesized by different kinds of bacteria through the aerobic conversion of various feedstock:

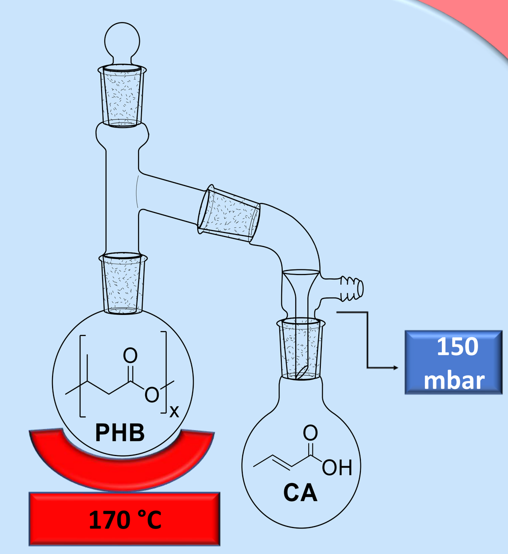
- Ad hoc substrates (sugar, starch, alcohols, lipids) using Single Strain Cultures (**SSC**)
- Wastes (fermented food, molasses, wastewaters) using Mixed Microbial Cultures (**MMC**)

High quality PHB can be used as plastic and is potentially capable to replace fossil-based plastics thanks to similar mechanical and physical proprieties.

PHB and **PHB inclusions** used in this work is produced from sewadge sludge and has been supplied by **B-plas**.

Bio-based crotonic acid…

A novel **thermolytic distillation** process was developed to depolymerize polyhydroxybutyrate (PHB) for the selective production of crotonic acid (**CA**).



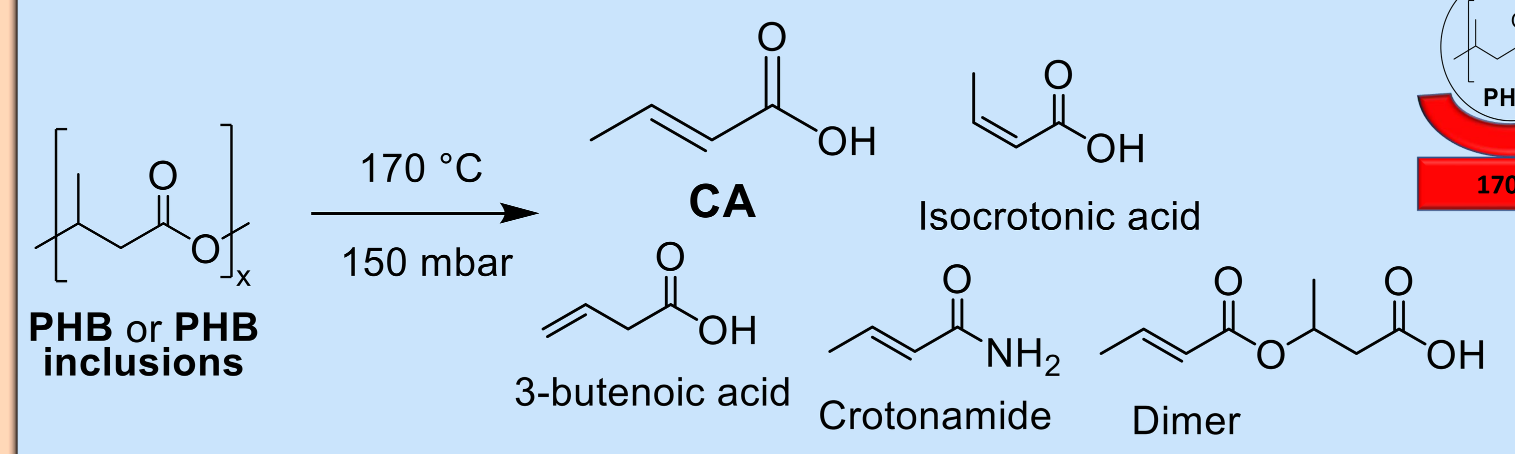


Table 1. CA yields and purities starting from PHB or PHB inclusions (30-60% PHB content)

Label	Sample	Volatiles Yield (on total biomass)	CA purity	Detectable impurities			
				Isocrotonic acid	3-butenoic acid	Dimers	Crotonamide
CA_{PHB}	PHB	94% (94%)	98.4%	0,10%	/	/	/
CA_{60}	60% PHB	82% (49%)	96.3%	0.50%	/	/	/
CA_{30}	30% PHB	63% (19%)	93%	0.90%	0.20%	/	0.50%

CA and its esters find **application** in the coating, paint, textile, binder, adhesive, flocculant, ceramics, and agrochemical industries.

The conditions adopted (170 °C, 150 mbar) were applied to pure PHB and **PHB-enriched bacteria** containing 60 and 30% of PHB, giving a recovery of crotonic acid of 92, 78 and 58%, respectively, with purities up to 98%.

Industrially CA is produced through the oxidation of crotonaldehyde, which involves the use of fossil resources.

…and its photocatalized hydroacylation

In collaboration with **Prof. D. Ravelli** Pavia's Photo Green Lab, the versatility of bio-based **CA** was investigated through a photochemical approach. The photocatalytic addition (promoted by tetrabutylammonium decatungstate – **TBADT**) of aliphatic and aromatic aldehydes to crotonic acid took place under solar-simulated light irradiation. The functionalization took place in a satisfying yield quite independently from the sample of crotonic acid (whether commercial or produced via thermolytic distillation).

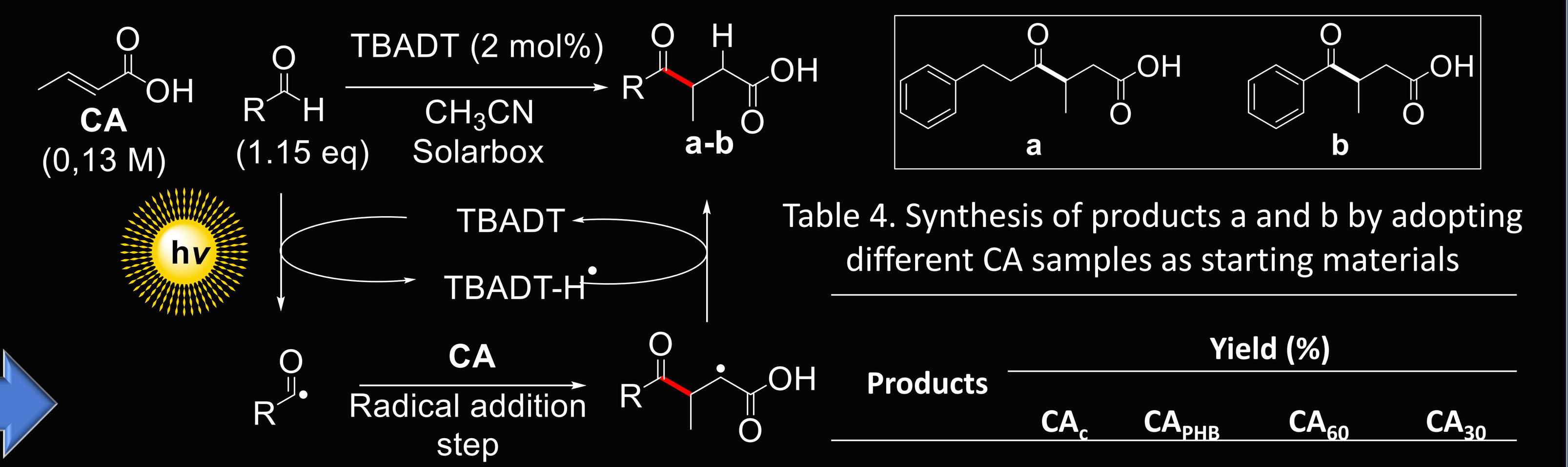


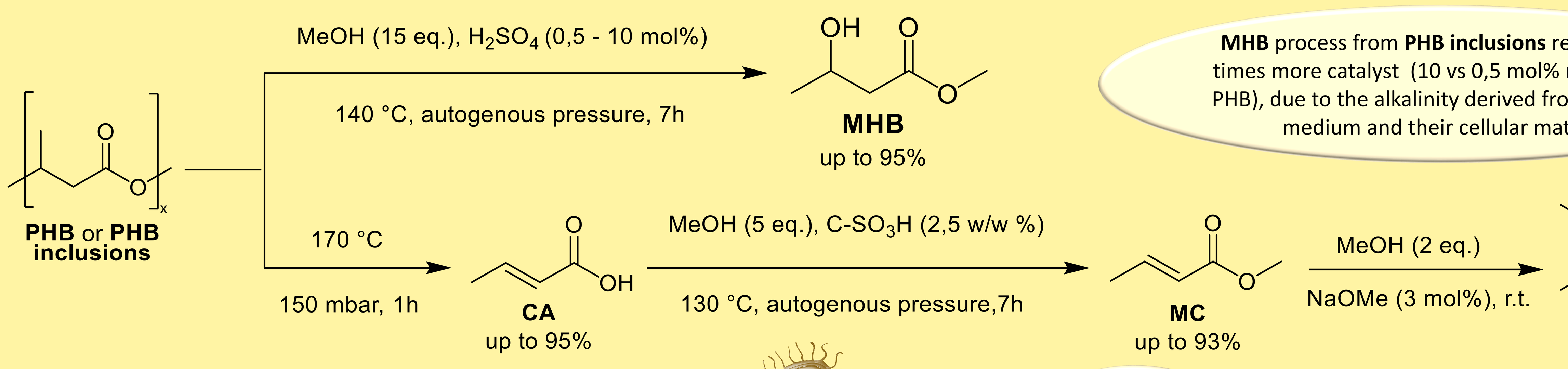
Table 4. Synthesis of products a and b by adopting different CA samples as starting materials

Products	Yield (%)			
	CA_c	CA_{PHB}	CA_{60}	CA_{30}
a	74	66	65	62
b	79	75	73	50

Parodi, A.; Jorea, A.; Fagnoni, M.; Ravelli, D.; Samori, C.; Torri, C.; Galletti, P. Green Chem. **2021**, 3420–3427.

Bio-based solvents for the circular extraction of PHB

Two novel protocols for the chemical recycle and valorization of PHB were developed, aiming at the production of two bio-based molecules: MHB and MMB. **MHB** was synthesized through a single step catalytic methanolysis under autogenous pressure, **MMB** was synthesized through a three-steps process: **thermolytic distillation** to give CA_{PHB}, esterification catalysed by **C-SO_3H** (a previously reported acidic heterogenous catalyst)[1] to give methyl crotonate (**MC**), and oxa-Michael addition of MeOH. Optimized reaction conditions were applied to pure **PHB** and **PHB inclusions** inside bacterial cells as starting materials.



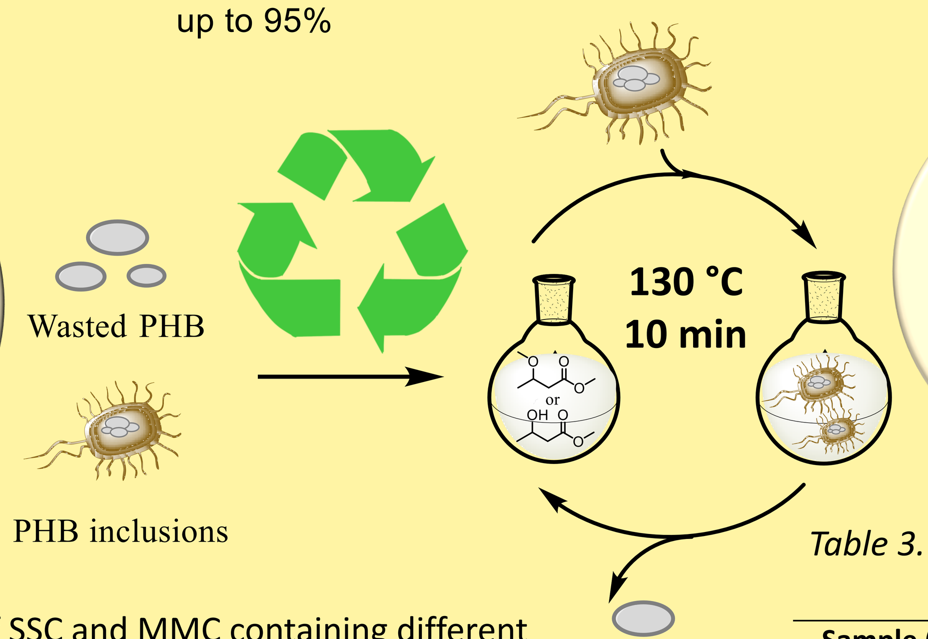
MHB process from **PHB inclusions** required 20 times more catalyst (10 vs 0,5 mol% needed for PHB), due to the alkalinity derived from bacteria medium and their cellular matrix.

MHB and **MMB** were characterized in terms of solubility in H_2O, logK_{ow} and biodegradability.

Compound	Solubility in H_2O (g/L)	logK_{ow}	Biodegradability (%)
MHB	miscible	0.83	72
MMB	145	1.43	73.5

. Biodegradability test, revealed that both **MHB** and **MMB** are readily biodegradable.

MHB and **MMB** were tested as solvents for the recovery of PHB itself both from freeze-dried **SSC** and **MMC** with low to medium content of PHB (22-57 wt%). PHB-to-solvent ration maintained constant = 26 mg/mL.



Extraction from **MMC slurry** (with a PHB content of 39 % on dry weight) was also performed, in order to avoid the high energy demanding freeze-drying step.

Both solvents were reused more than **10 times** in the extraction procedure with no loss in activity, thus demonstrating the **high recyclability** potential of both solvents.

Table 2. Extraction of SSC and MMC containing different amounts of PHB with MMB, MHB, MC and DCM

Solvent	PHB recovery (%)				
	SSC-57	SSC-35	MMC-22	MMC-39	MMC-39 slurry
MMB (10 min, 130°C)	98 ± 1	97 ± 2	98 ± 1	98 ± 2	92 ± 2
MHB (10 min, 130°C)	95 ± 1	96 ± 1	94 ± 2	96 ± 3	77 ± 2
MC (60 min, 118 °C)	54 ± 2	/	/	/	/
DCM (10 min, 60°C)	97 ± 3	/	95 ± 1	/	/

[1] *Samorì et al., Journal of Analytical and Applied Pyrolysis 155 (2021) 105030*

Table 3. Physical characteristics of PHB obtained with MMB and MHB, in comparison to commercial PHB and PHB extracted with CH_2Cl_2 (10 min, 60 °C).

Sample (%PHB)	Purity (%)			$\overline{M_w}$ (MDa)			PDI		
Commercial PHB	98 ± 2			0.8			5.9		
	MMB	MHB	DCM	MMB	MHB	DCM	MMB	MHB	DCM
SSC (freeze dried)	98 ± 2	95 ± 2	94 ± 3	2.3	1.1	2.1	3.9	2.8	3.3
MMC (freeze dried)	98 ± 1	96 ± 1	94 ± 2	3.3	1.6	3.5	4.5	4.6	3.1
MMC (slurry)	97 ± 1	94 ± 2	/	3.2	1.4	/	4.7	4.8	/

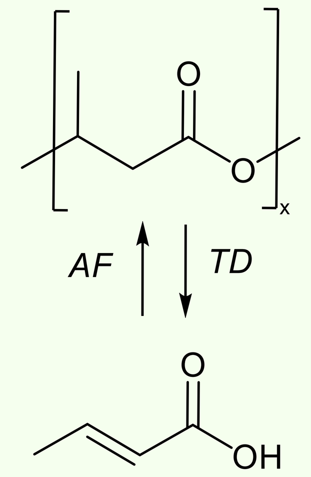
MHB halved the molecular weight and this evidence was observed independently on the bacteria type (SSC or MMC) and their water content.

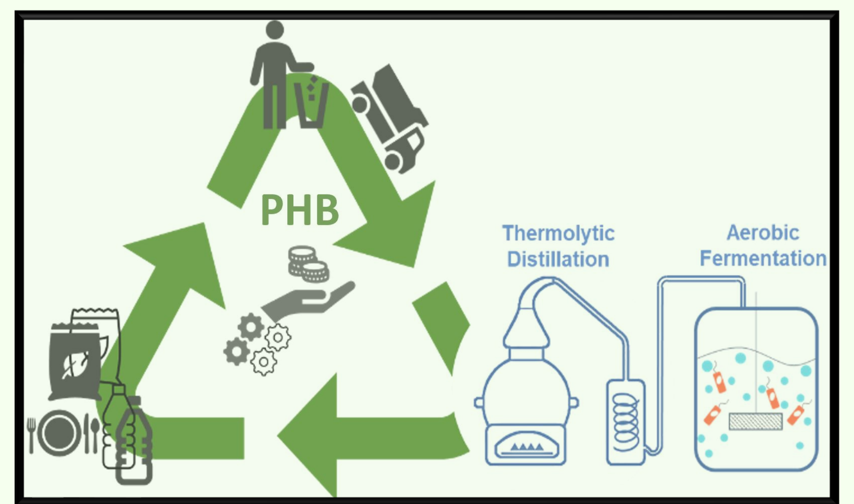
Thanks to **Prof. L. Mazzocchetti** (unibo) for mean molecular weight ($\overline{M_w}$) and PDI analysis.

From PHB to PHB: a circular recycling

In collaboration with **Dr. G.A. Martinez** (unibo), **CA** obtained from **thermolytic distillation** (**TD**) of wasted PHB was used to produce back the PHB through an aerobic fermentation (**AF**), performing a **circular recycling**.

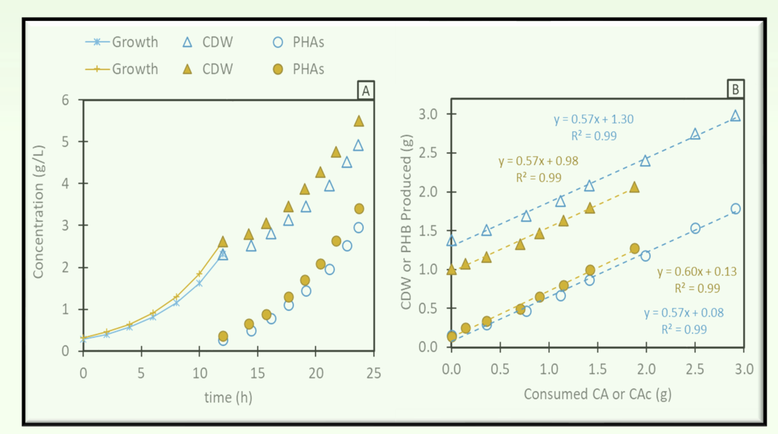
Commercial CA (**CAc**) was used too and it behaved almost identical to CA_{PHB} confirming the equivalency of the two carbon sources.





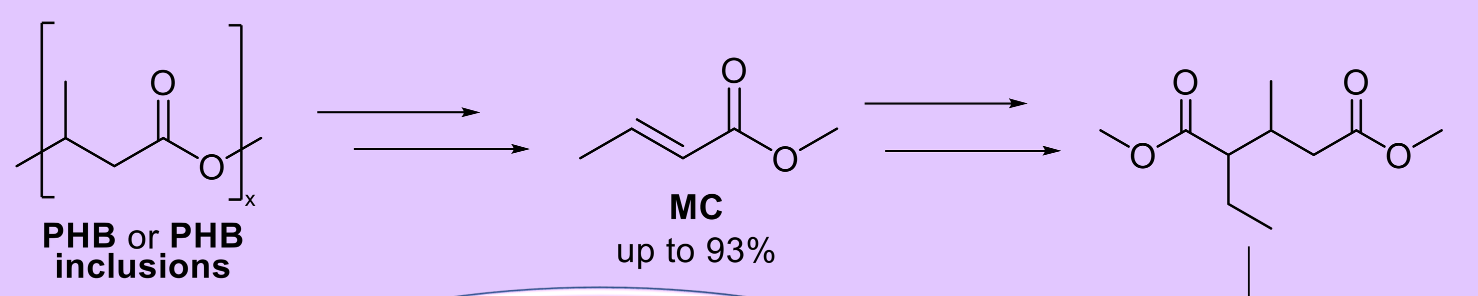
Cupravidus necator was used for the fermentation step and was able to accumulate a PHB content of **63 wt%**, producing **0.6 g PHB/g CA**.

Cellular dry weight (**CDW**) and PHB content of feeded bacterias increased lineary with the consumption of CA_{PHB} or **CAc** over time.



From PHB to new bio-polymers

As previously reported,[2] **MC** can be converted in it's dimer through a base catalyzed Michael reaction.



MC dimer is a branched di-ester and can be used as a bulkier analogous to **Methyl Adipate**.

Polymers obtainable from this PHB derived building block include **polyesters** and **polyamide**: analogous to **nylon 6,6** and polybutylen-adipate-co-terephtalate (**PBAT**) could be synthetized.

[2] *J. Flanagan et al., ACS Catal. 2015, 5, 5328–5332*