

Current and future
ways to Closed
Life Support Systems

Joint Agrospace-MELISSA
Workshop



Rome
May 16 -18
2018

AGROSPACE

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eesa

MELISSA

UMONS
Université de Mons

ProtMic

**Polyhydroxyalkanoate production
by *Rhodospirillum rubrum* S1H :**

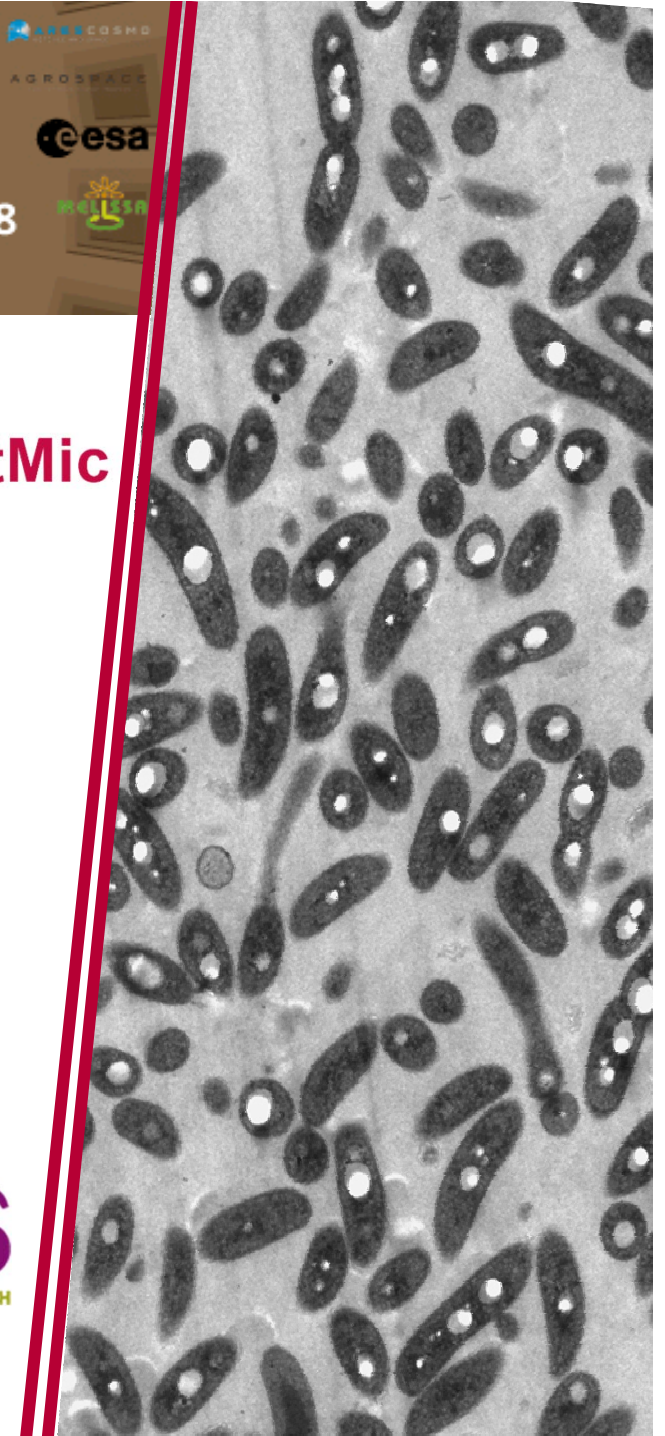
Carbon source and light intensity impact

biosciences
UMONS RESEARCH INSTITUTE
FOR BIOSCIENCES

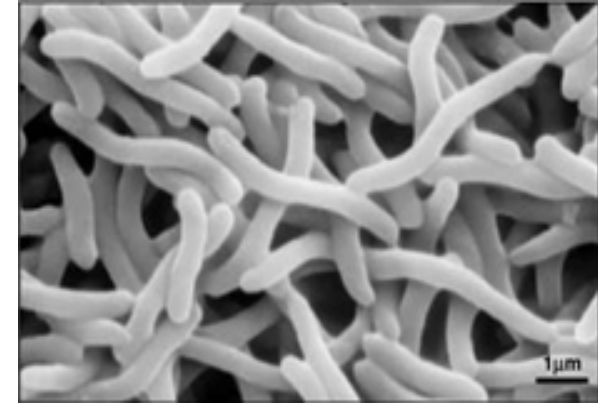
MELISSA

fnr's
FREEDOM TO RESEARCH

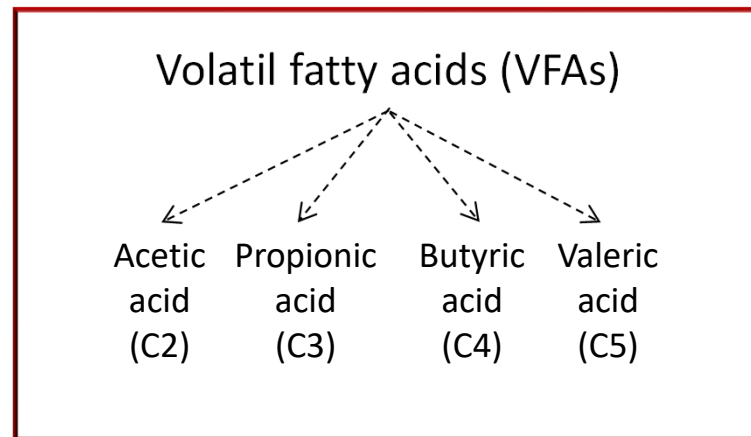
Guillaume Bayon-Vicente



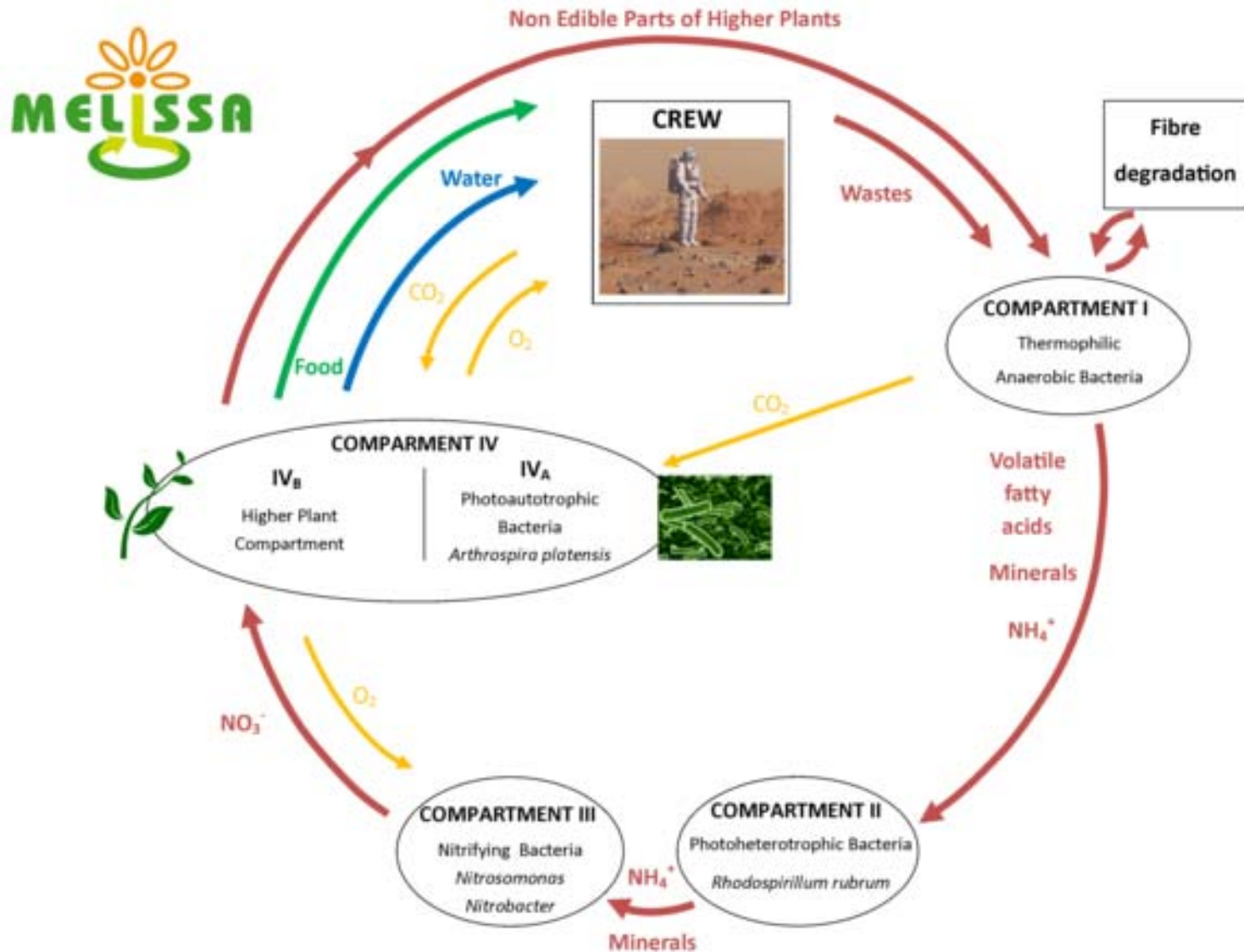
Rhodospirillum rubrum



- ✓ High metabolic versatility (chemoheterotrophy, photoautotrophy or **photoheterotrophy**)
- ✓ Assimilation of a wide range of carbon source

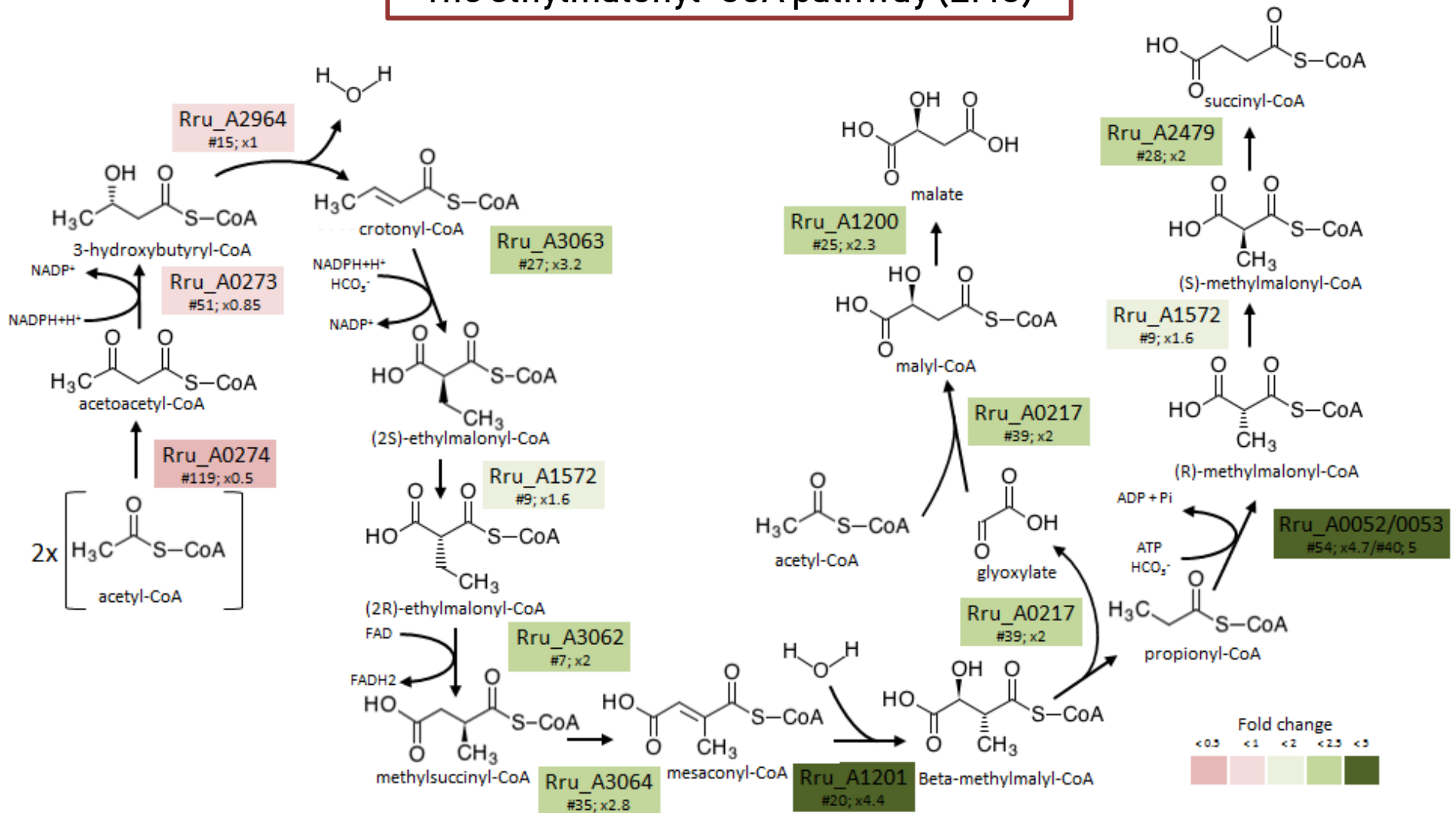


Implication in MELiSSA loop



Implication in MELiSSA loop

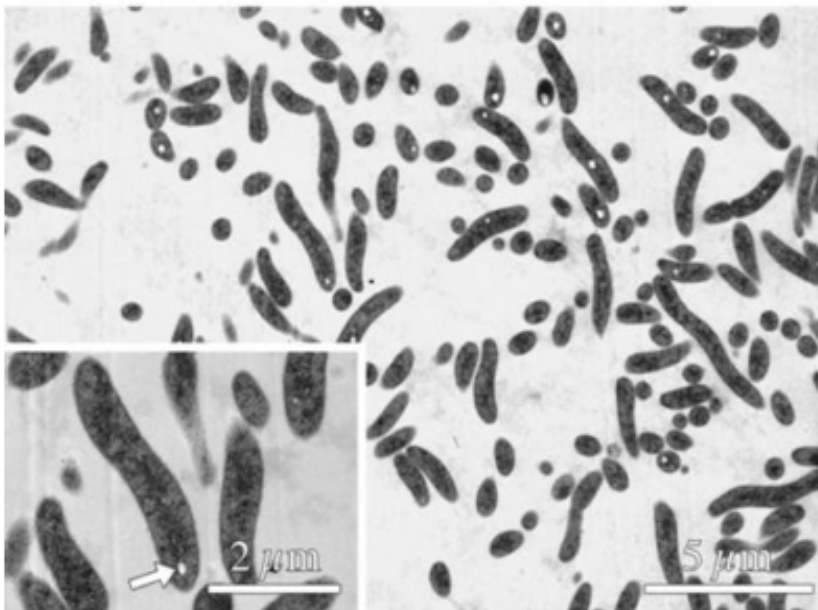
The ethylmalonyl-CoA pathway (EMC)



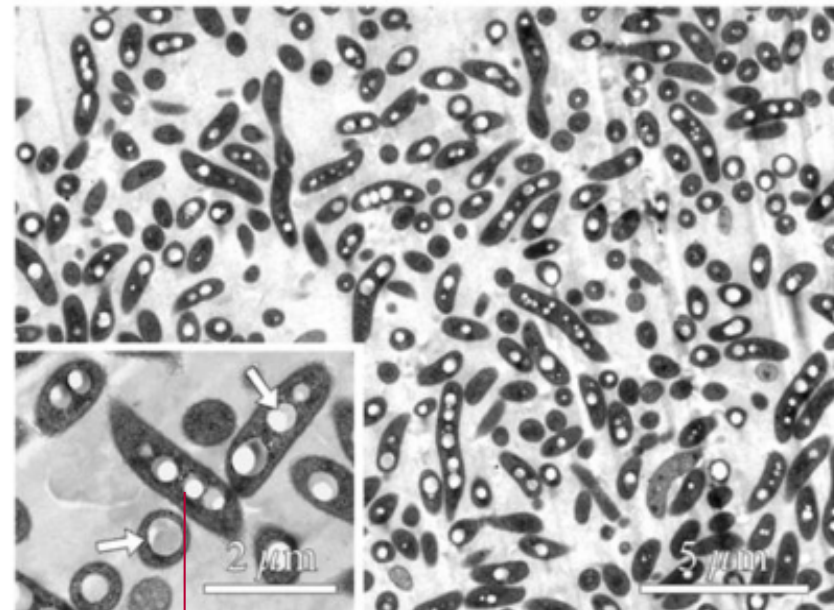
Leroy, B., De Meur, Q., Moulin, C., Wegria, G., & Wattiez, R. (2015). New insight into the photoheterotrophic growth of the isocitrate lyase-lacking purple bacterium *Rhodospirillum rubrum* on acetate. *Microbiology (Reading, England)*, 161, 1061–72.

Outcome of MELiSSA project collaboration

Succinate



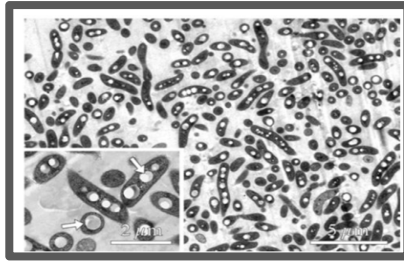
Acetate



→ Polyhydroxyalkanoates (PHA)

PHA a promising solution ?

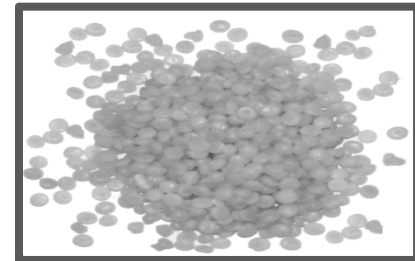
Pros



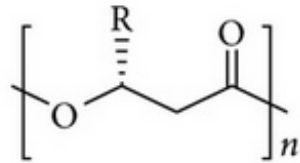
Bio-based



Biodegradable



Broad range of application

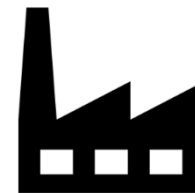


Poly(3-hydroxyalkanoates) [PHA]

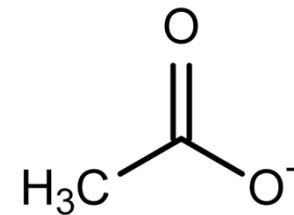
Limits



Final price

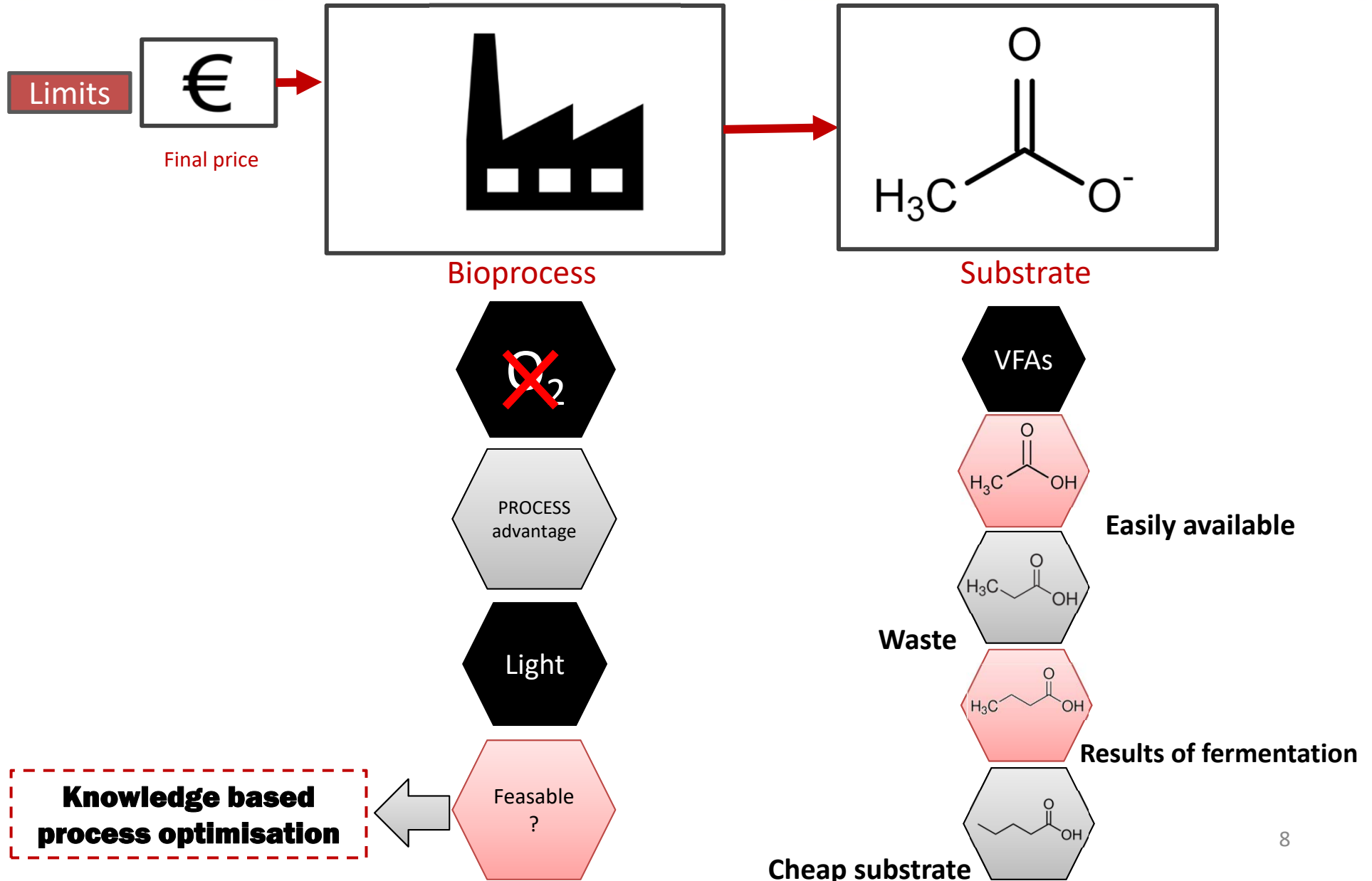


Bioprocess



Substrate

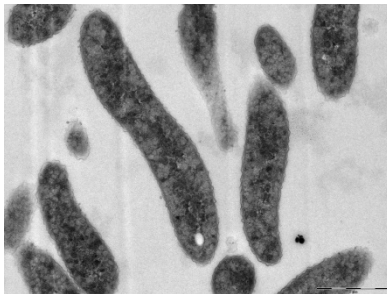
How to reduce production cost ?



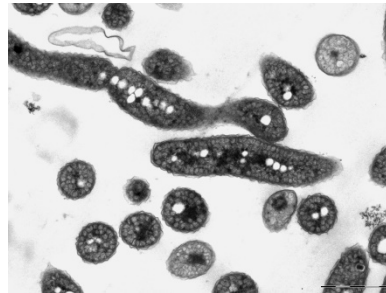
What are PHAs ?

- Accumulation inside granules
- Granules formed by phasins
- Another nutrient (N, S, P) is limiting
- Stock of energy, electron and carbon

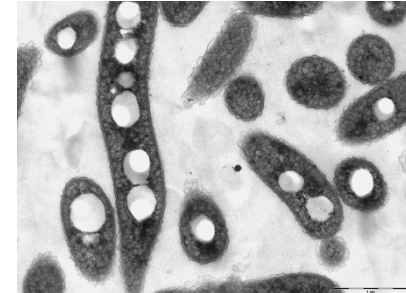
Succinic acid



Butyric acid



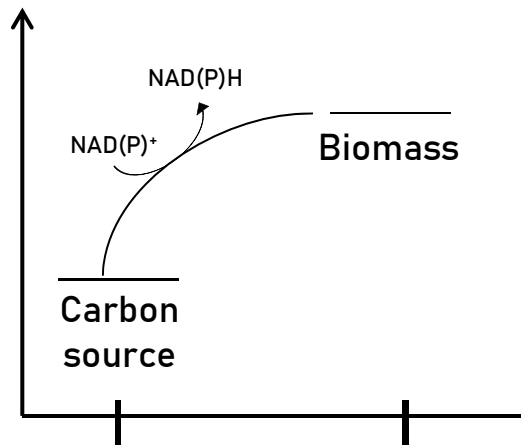
Acetic acid



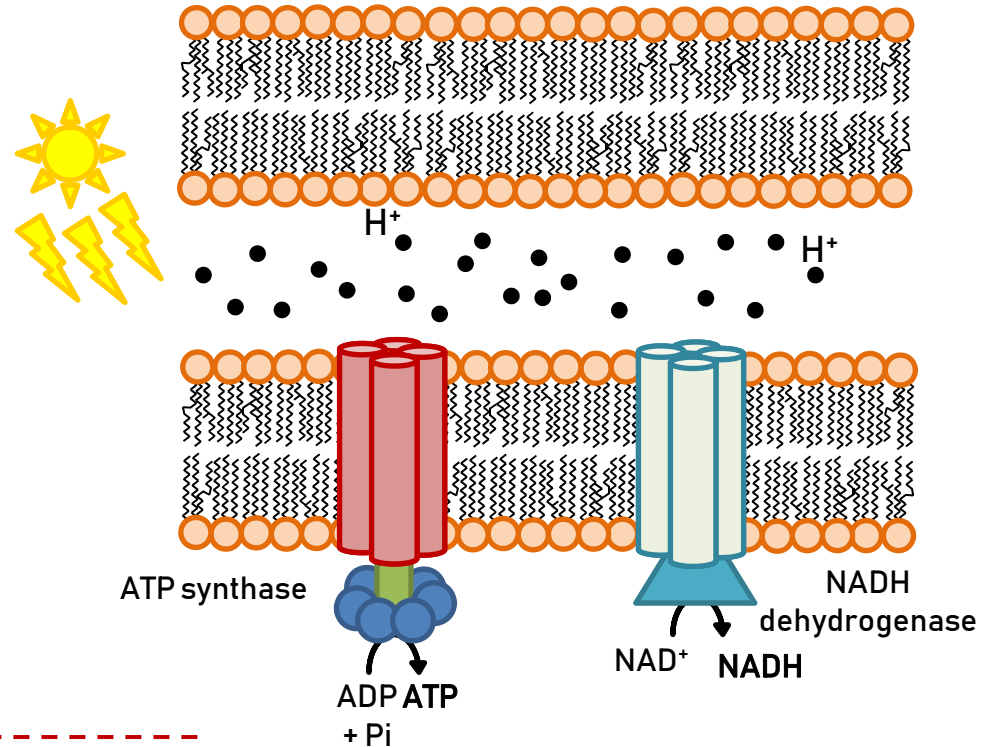
What drives the PHA accumulation ?

Use of reduced carbon sources

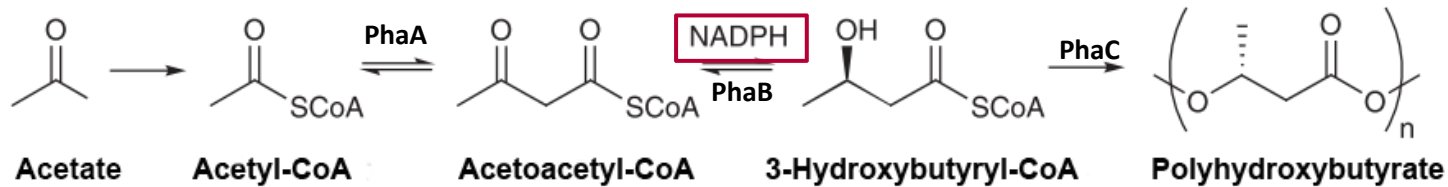
Redox state



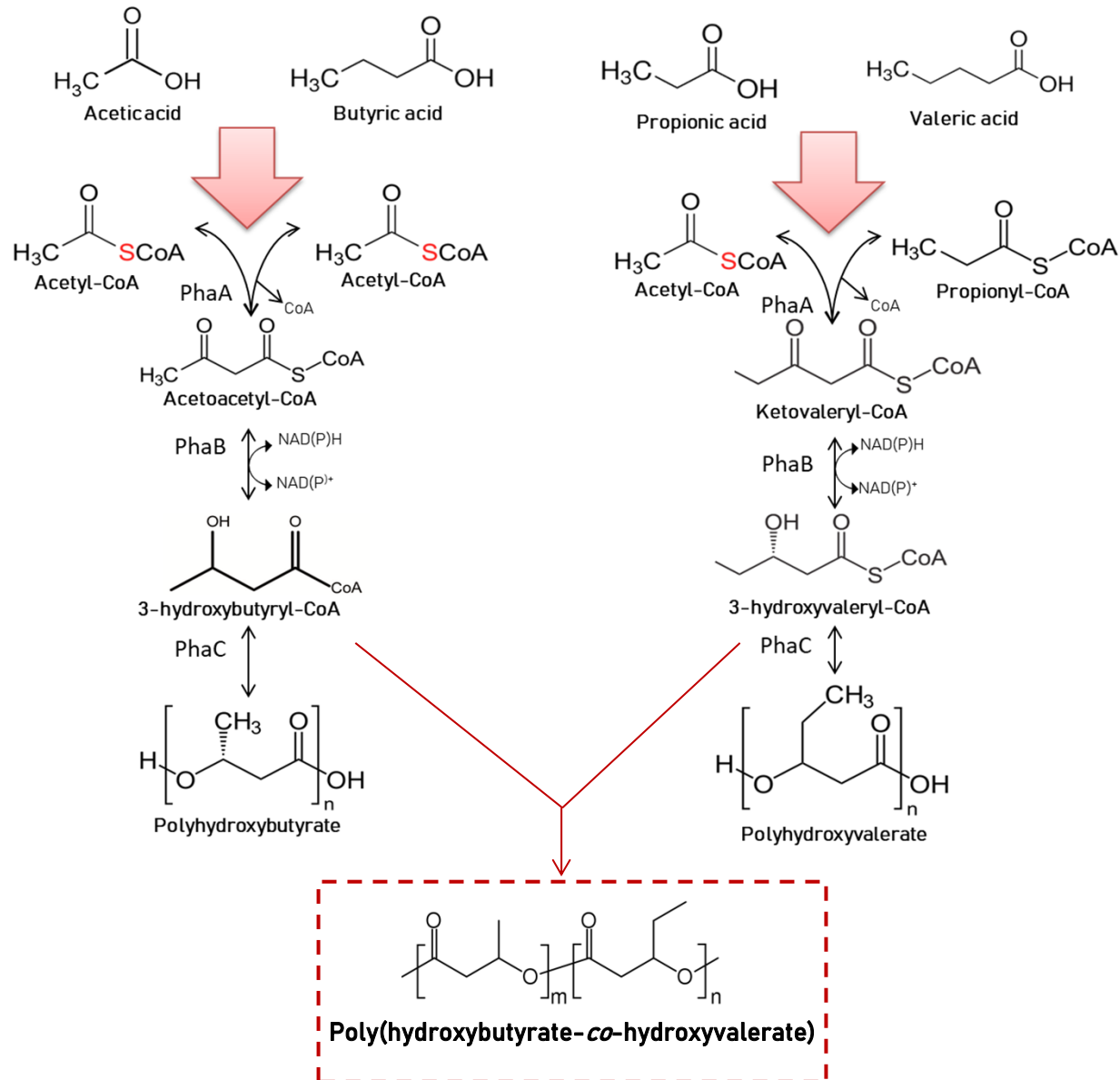
Photoreduction of NAD⁺



Electron sink

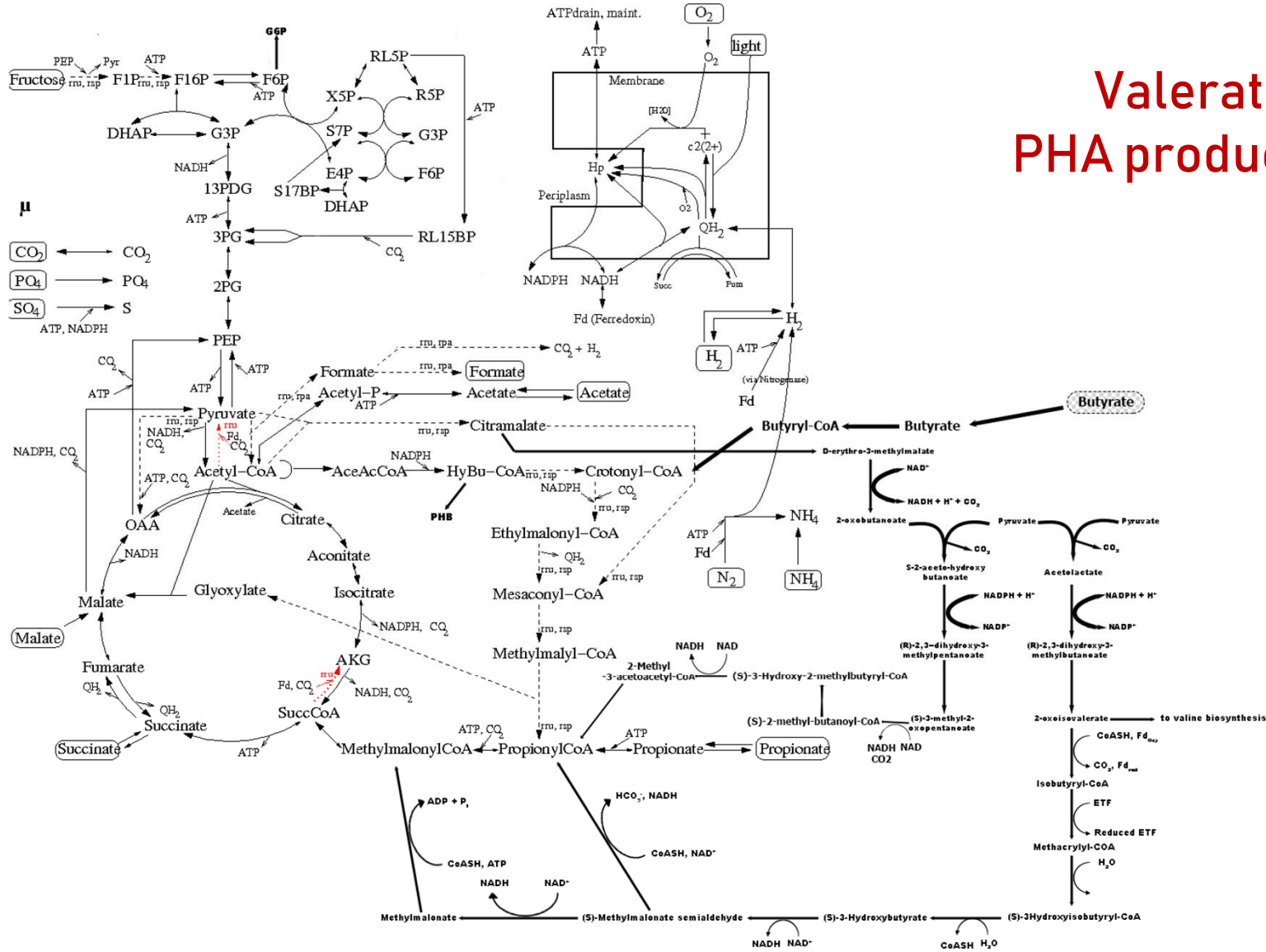


What drives the PHA composition ?



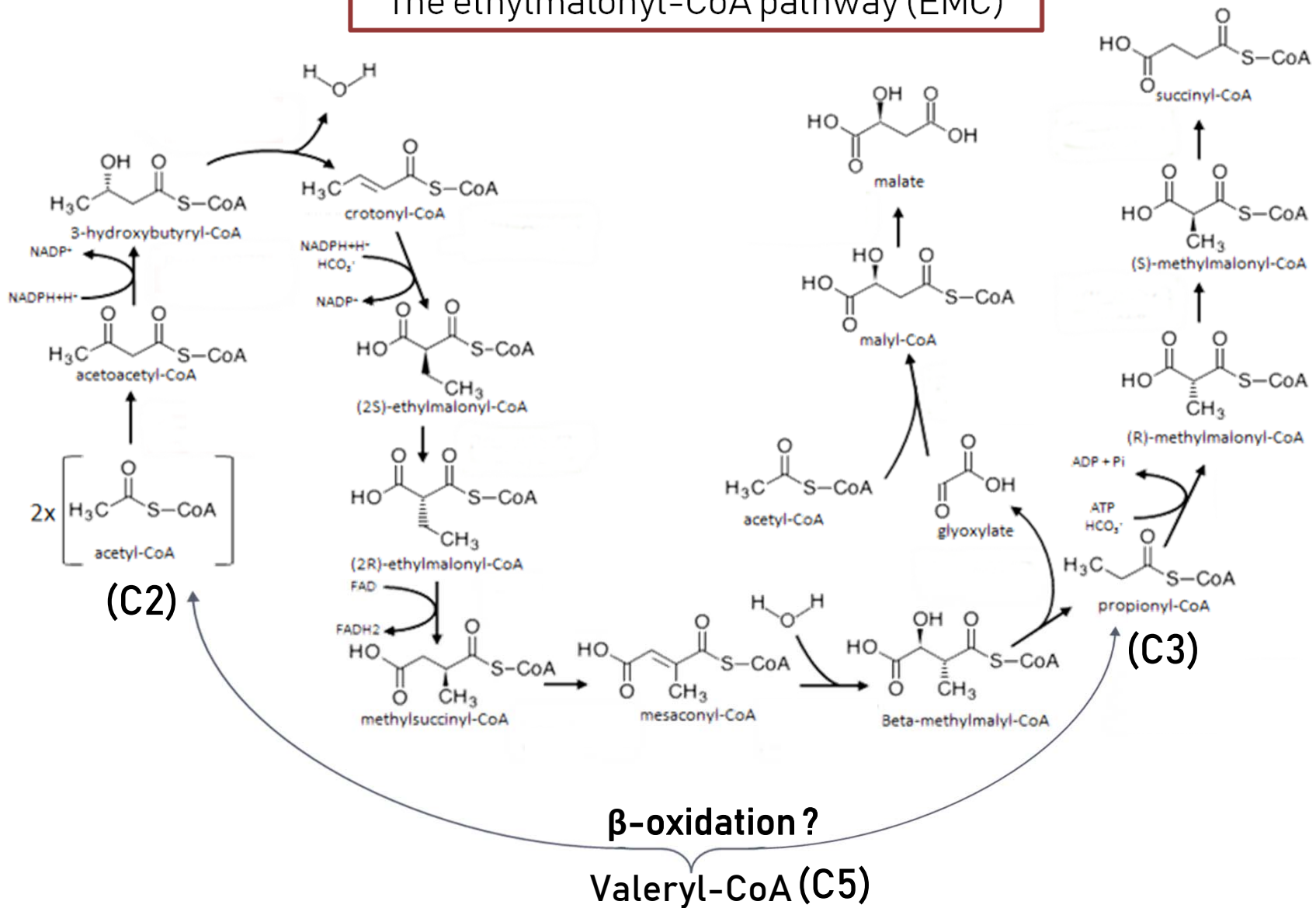
Rhodospirillum rubrum metabolism

Valerate ?
PHA production ?

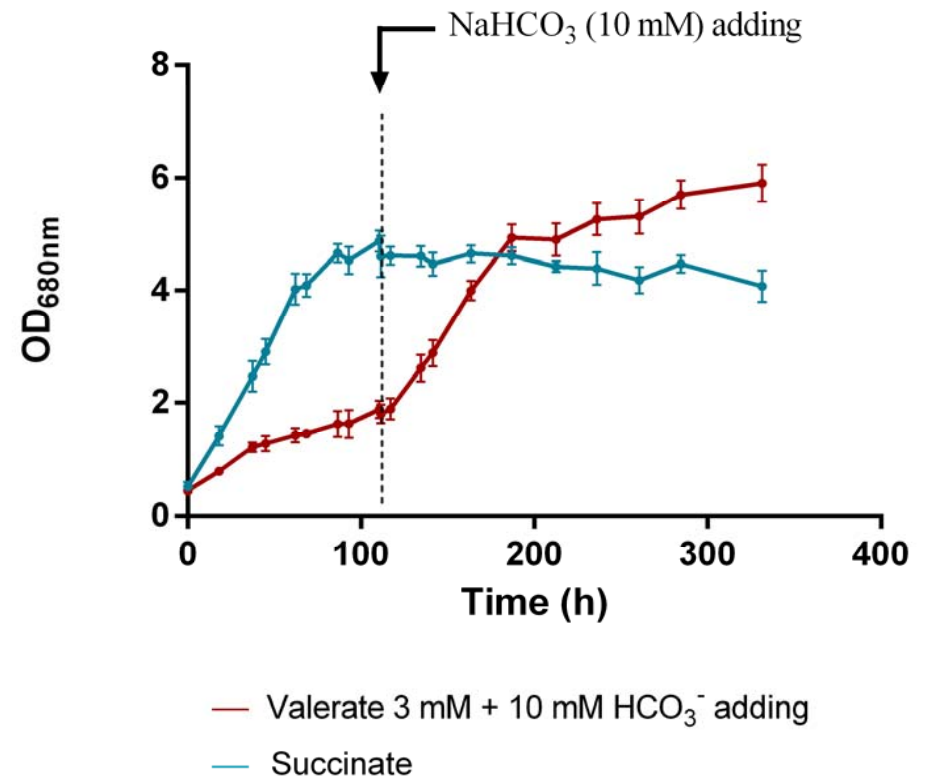
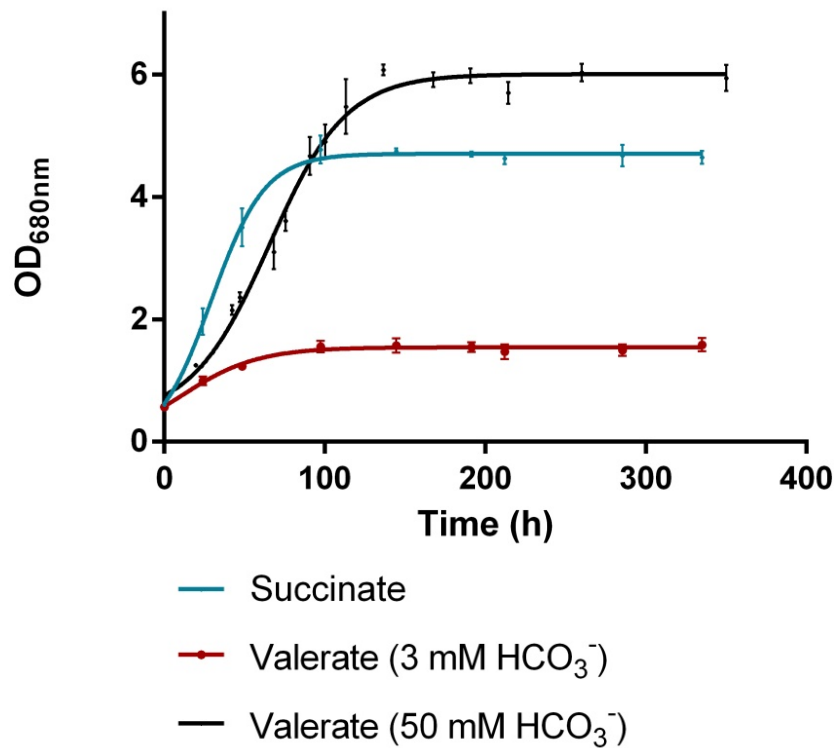


Valeric acid assimilation - Hypothesis

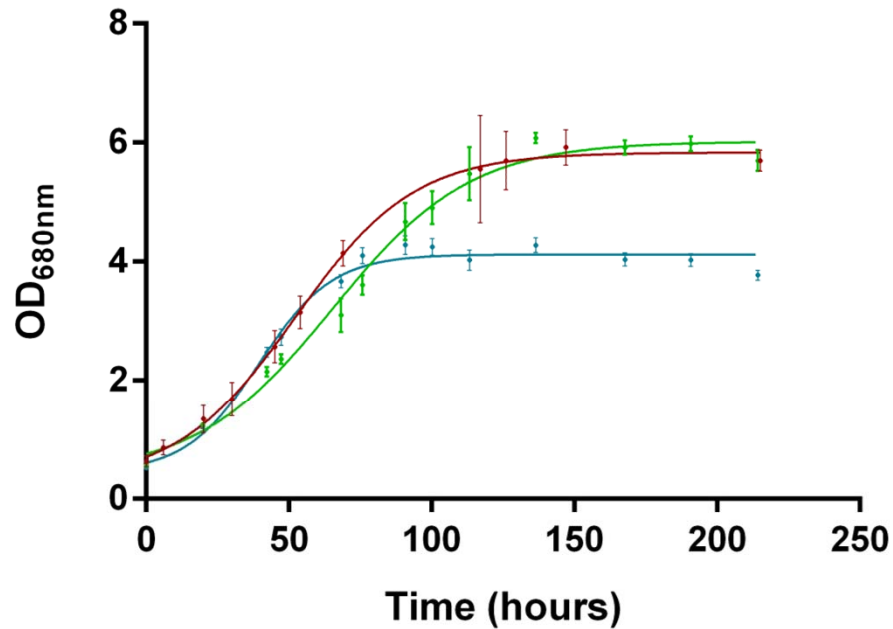
The ethylmalonyl-CoA pathway (EMC)



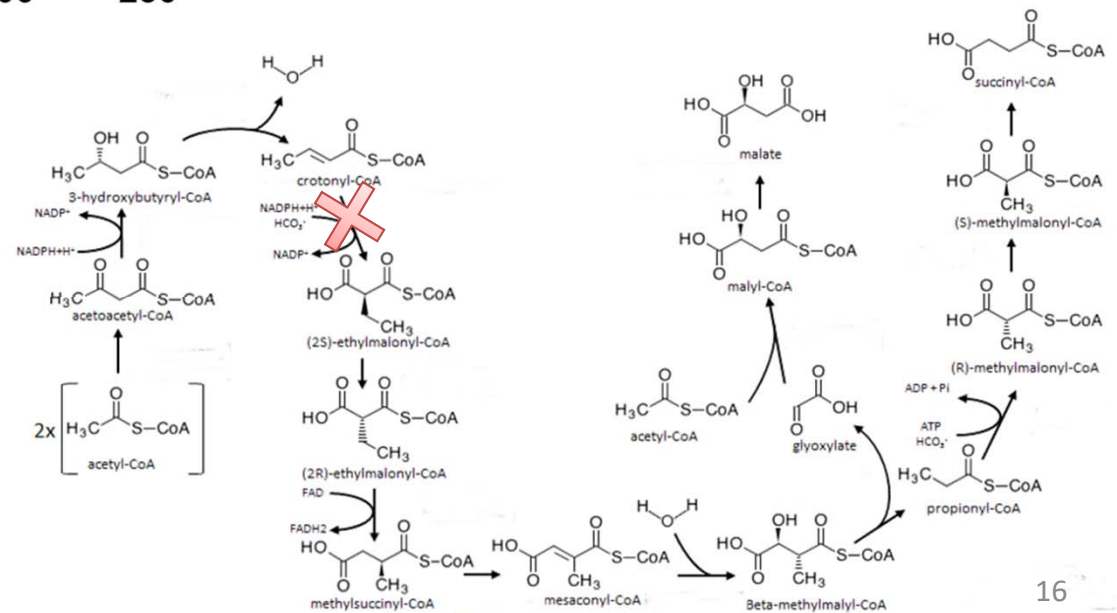
Valeric acid assimilation – First characterisation



Valeric acid assimilation

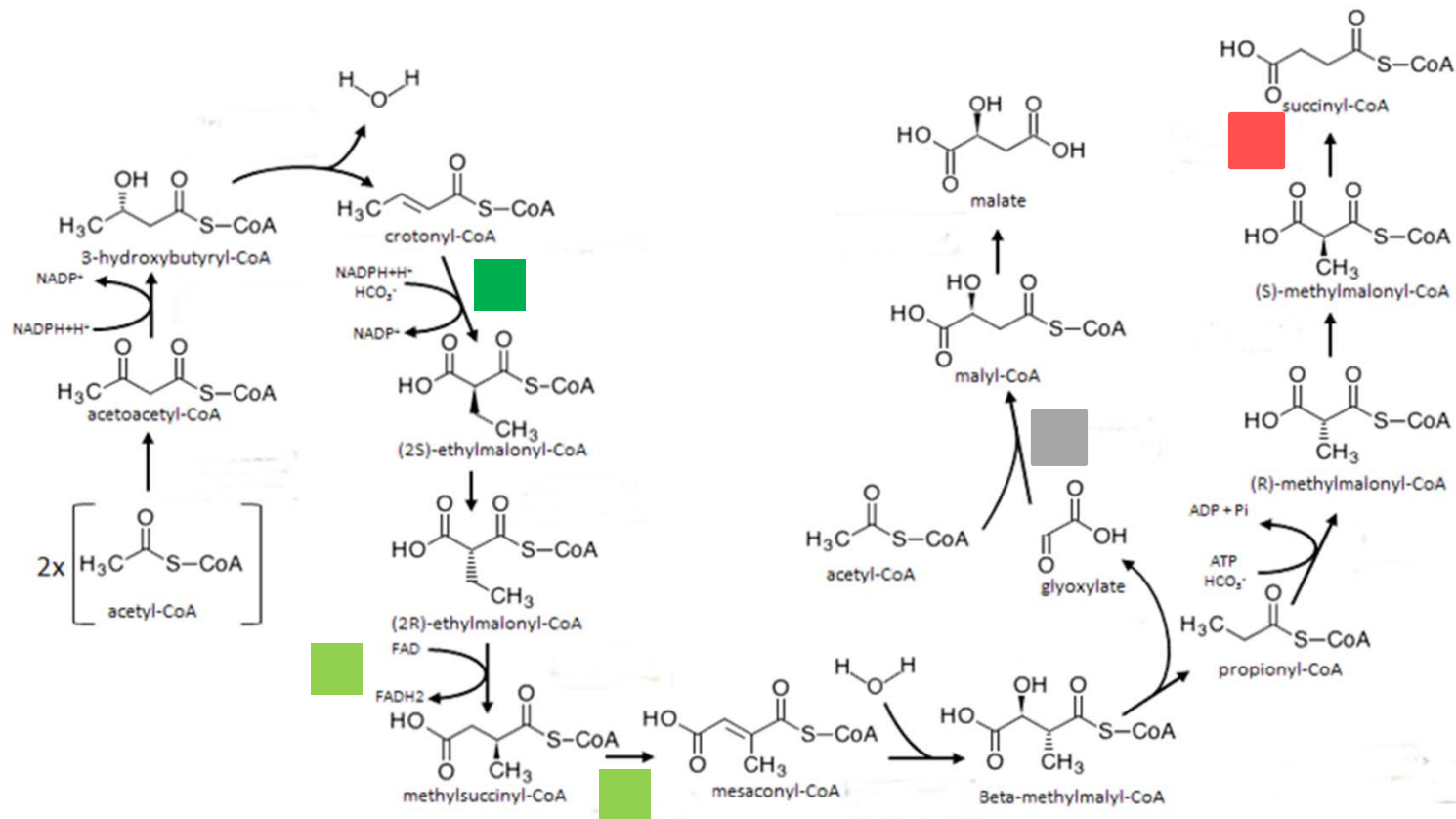


- Valerate WT
- Valerate Δ Ccr
- Succinate Δ Ccr



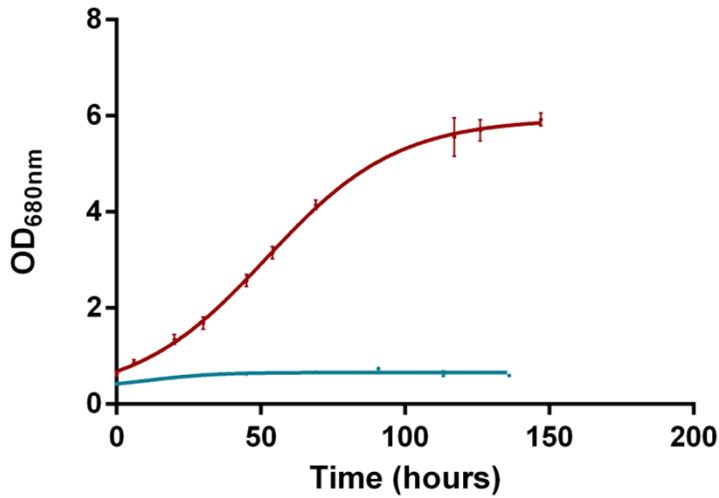
Valeric acid assimilation – Pathway used

	Peak name	Enzymes	RefSeq Locus Tag	p-value	Fold change Val/Succ	Identified peptides
EMC pathway	Q2RPT7	Crotonyl-CoA reductase	Rru_A3063	0.00012	6.35020217	26
	Q2RPT8	Methylmalonyl-CoA mutase	Rru_A3062	0.05	1.648625809	9
	Q2RPT6	Isovaleryl-CoA dehydrogenase	Rru_A3064	0.01151	2.527293212	42
	Q2RXX3	Citrate lyase	Rru_A0217	0.03587	0.849474298	25
	Q2RRG6	Methylmalonyl-CoA mutase	Rru_A2479	0.01698	0.596213911	37

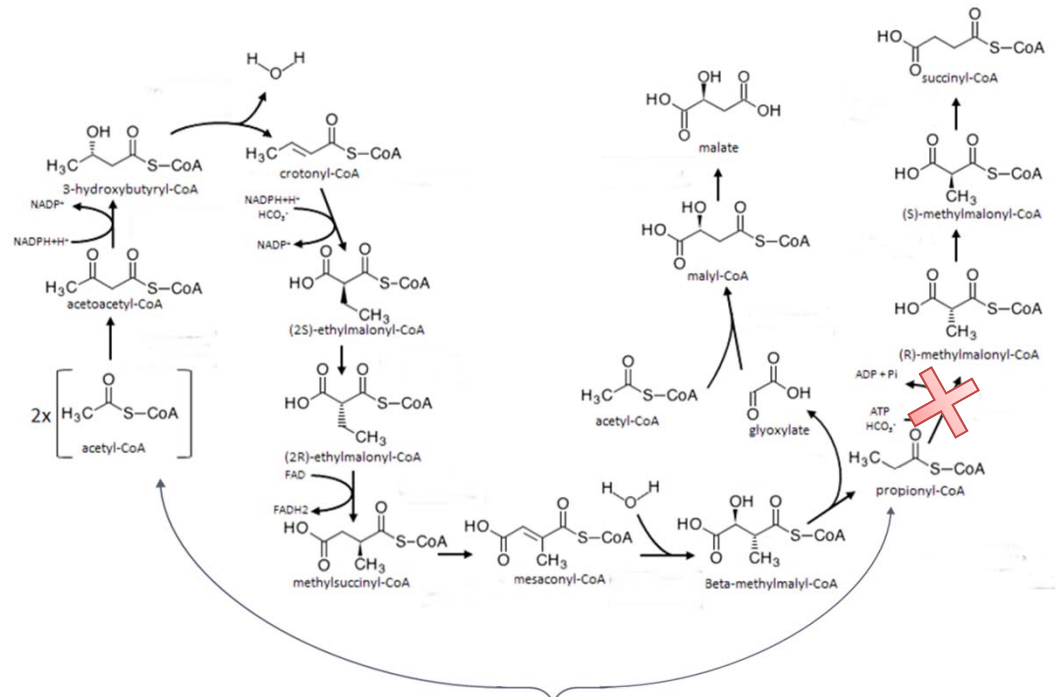


Valeric acid assimilation – Pathway used

Use of itaconic acid

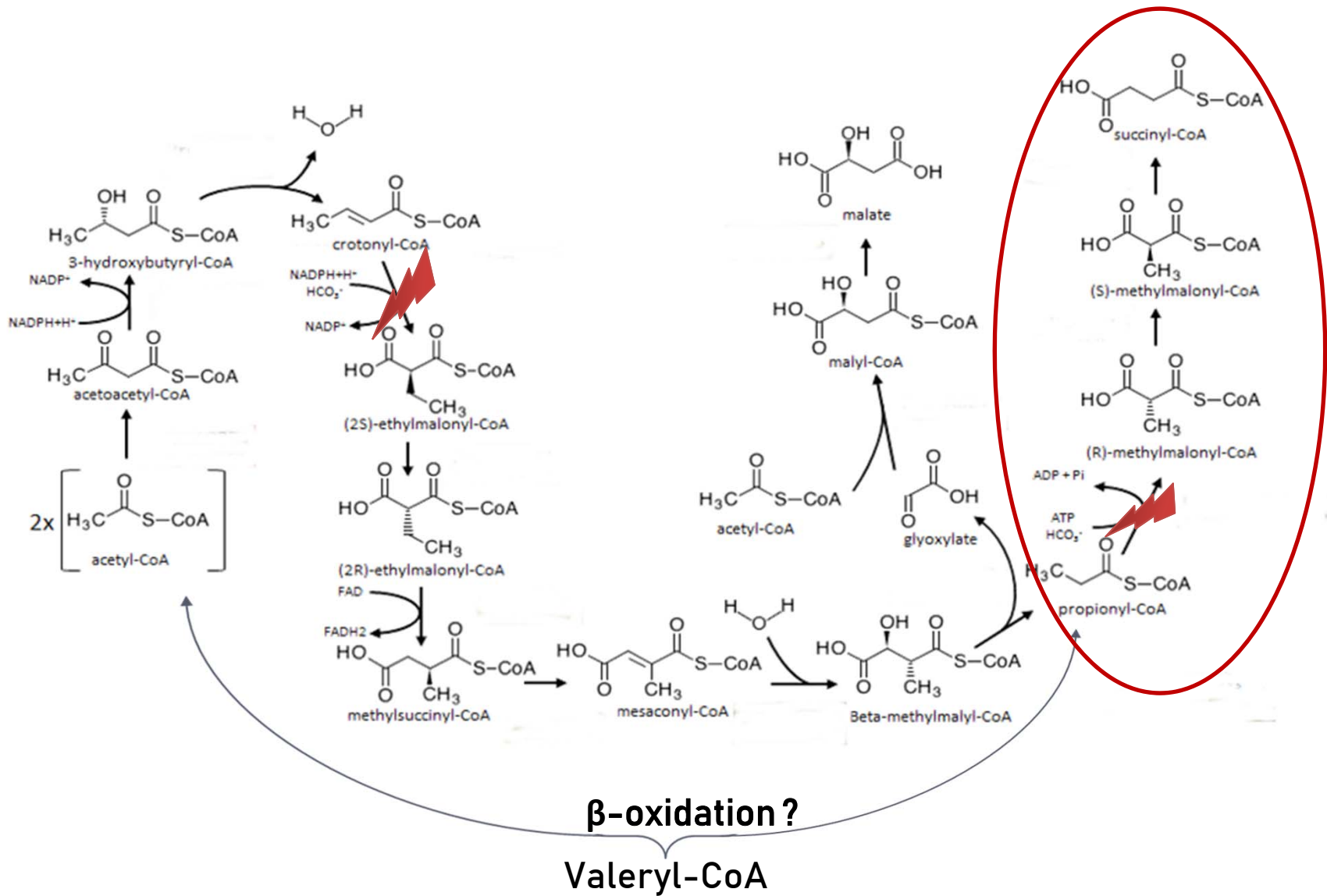


- Valerate WT
- Valerate WT + Itaonate (20mM)



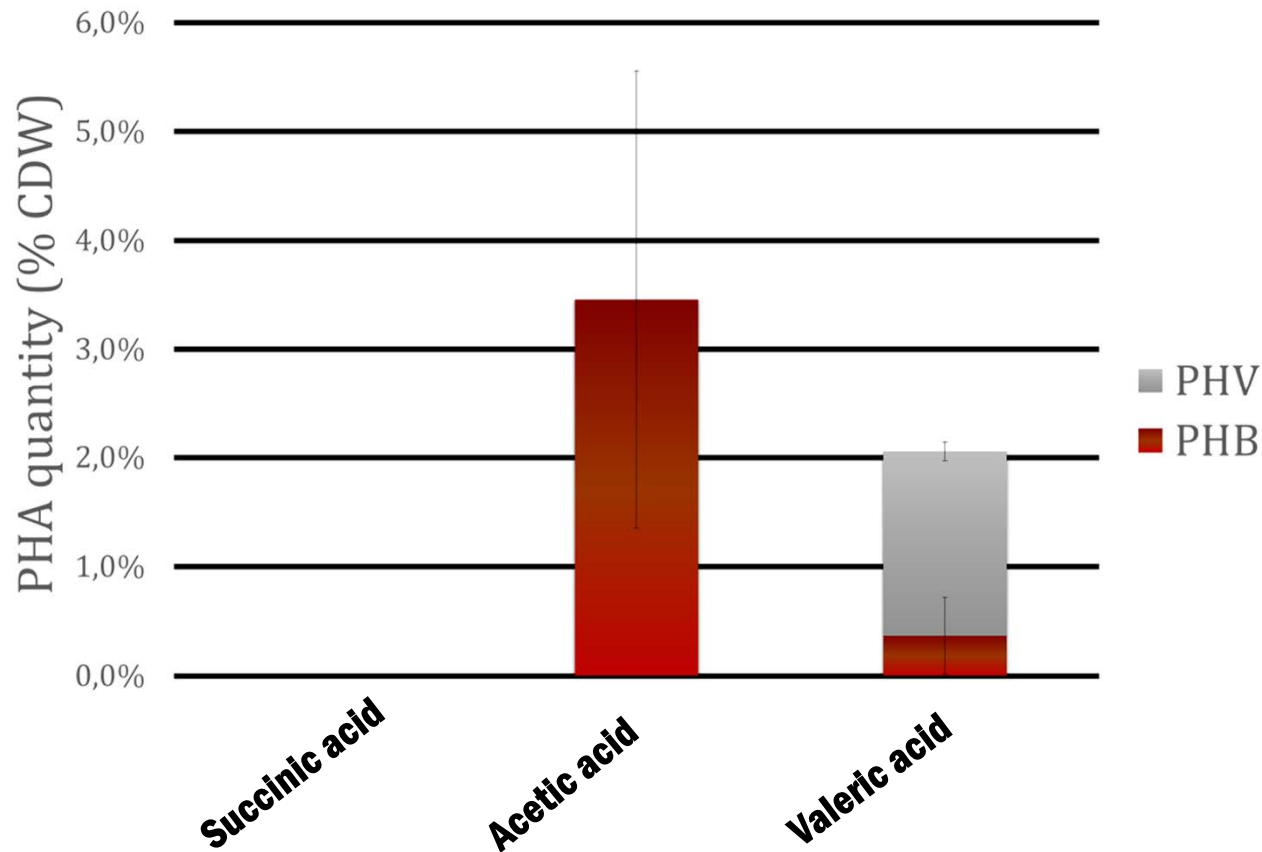
**β-oxidation?
Valeryl-CoA**

Valeric acid assimilation – Pathway used

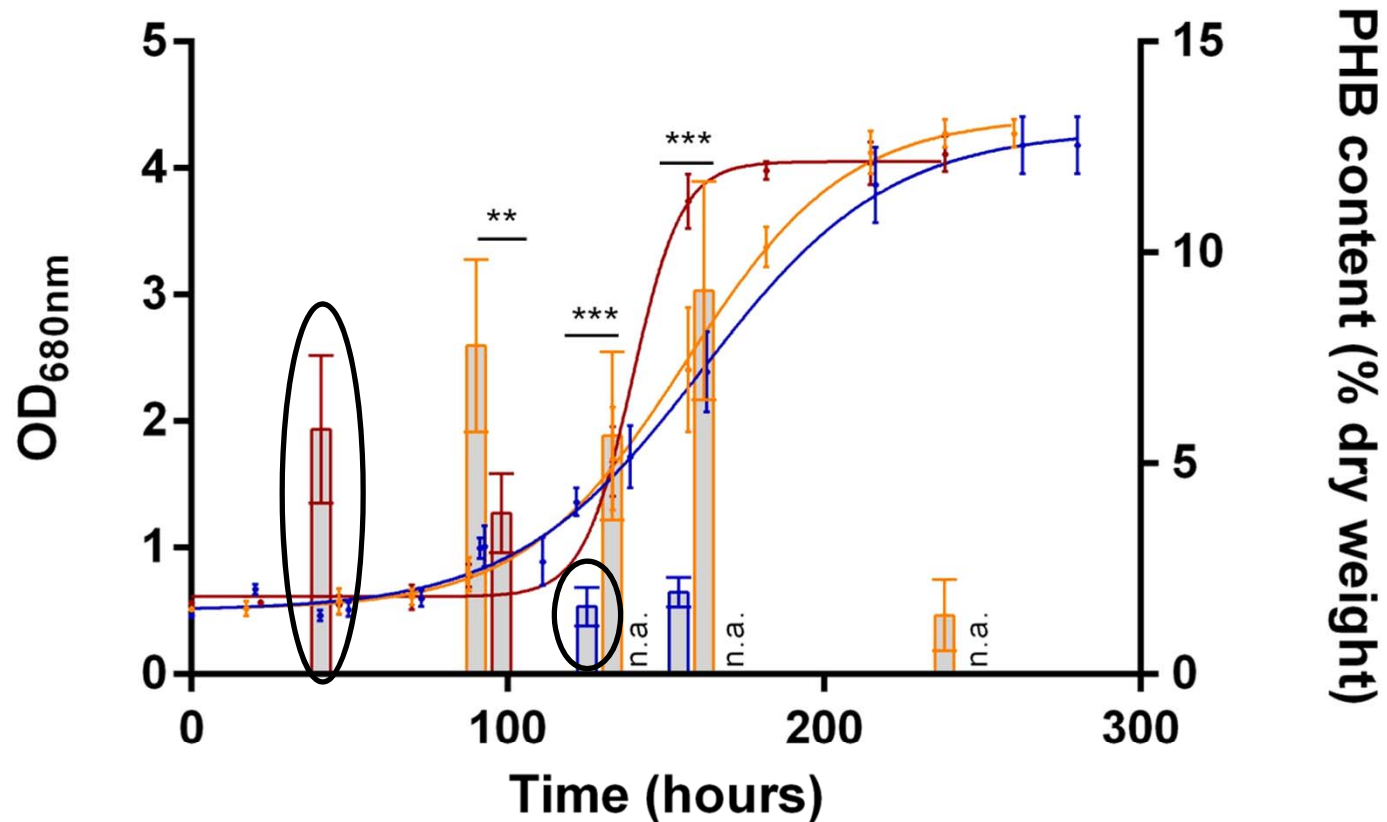


Valeric acid assimilation – P(HB-*co*-HV) production

	Peak name	Enzymes	RefSeq Locus Tag	p-value	Fold change Val/Succ	Identified peptides
PHA production	Q2RNZ5	Polyhydroxyalkanoate depolymerase	Rru_A3356	0.00414	0.581772011	3
	Q2RQI1	Phasin	Rru_A2817	0.00038	48.24069362	24
	Q2RP67	Phasin	Rru_A3283	0.02786	2.056047044	80



Impact of light intensity on PHB production in presence of acetic acid



- 10 μmol photon/m² s
- 50 μmol photons/m² s
- 150 μmol photon/m² s

Thank you for your attention
