Mixed microbial chain elongation based on organic feedstock, H_2 and CO_2

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Pr. Patrick Gerin

Pr. Benoit Stenuit

Context: The biorefinery concept

Land / crops

Food and feed ingredients

Agro-industrial residues

Renewable organic feedstock

Platform molecules

Energy

Directly used

Traditional, green & bio-chemistry

Bio-based products

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Context: The biorefinery concept

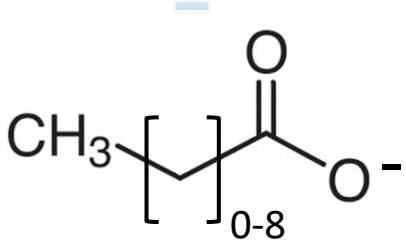
Land / crops

Food and feed ingredients

Agro-industrial residues

Renewable organic feedstock

Carboxylates as platform molecules



Directly used

Traditional, green & bio-chemistry

Bio-based products

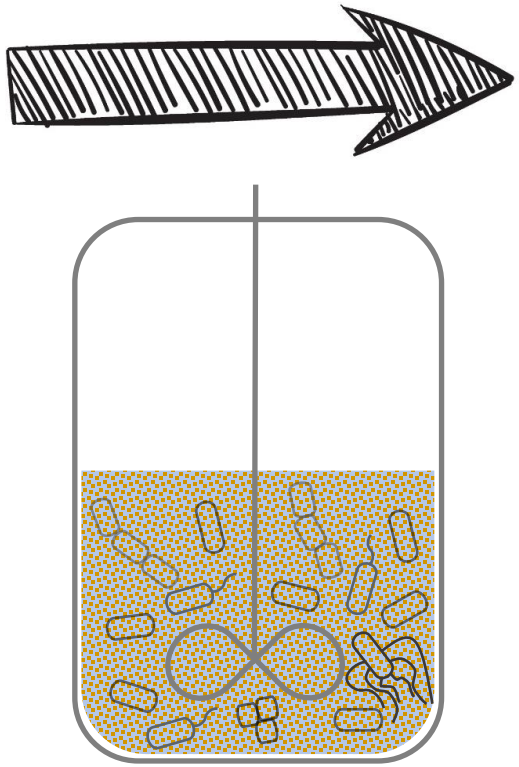
Energy

www.freepik.com

Context: Brewer's spent grain acidogenic fermentation

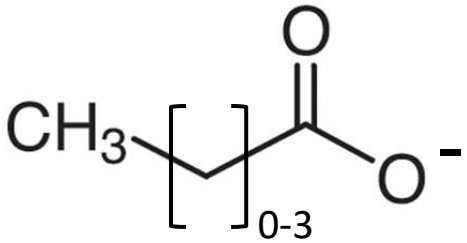
Brewer's spent grain (BSG)

Acidogenic fermentation



Microbial community

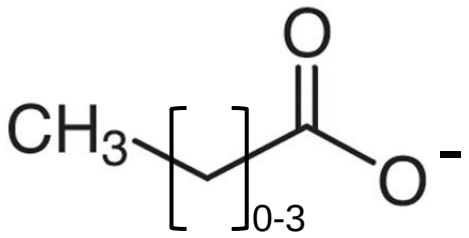
Short- chain carboxylates (SCC)



Acetate
Propionate
Butyrate
Pentanoate

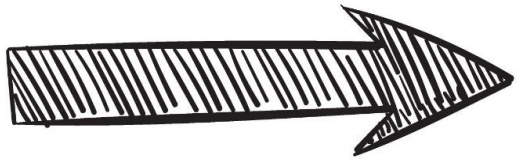
Context: Chain elongation

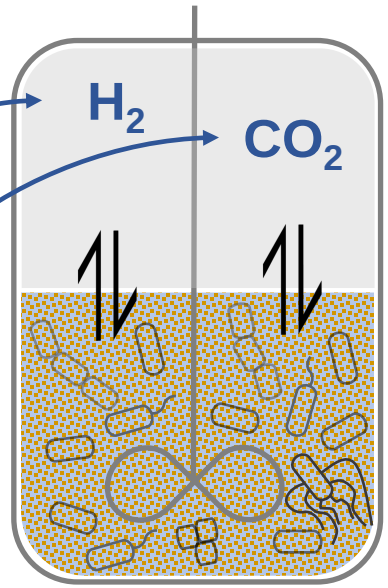
Short- chain carboxylates



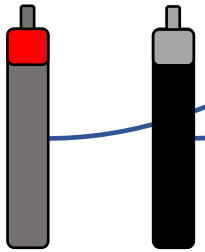
Acetate
Propionate
Butanoate
Pentanoate

Microbial chain elongation



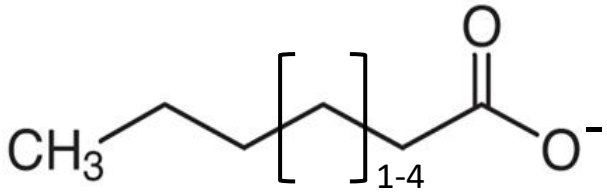


Exogeneous supply of
H_2 & CO_2
in the headspace



Specific physicochemical conditions triggered chain elongation pathways

Medium- chain carboxylates (MCC)

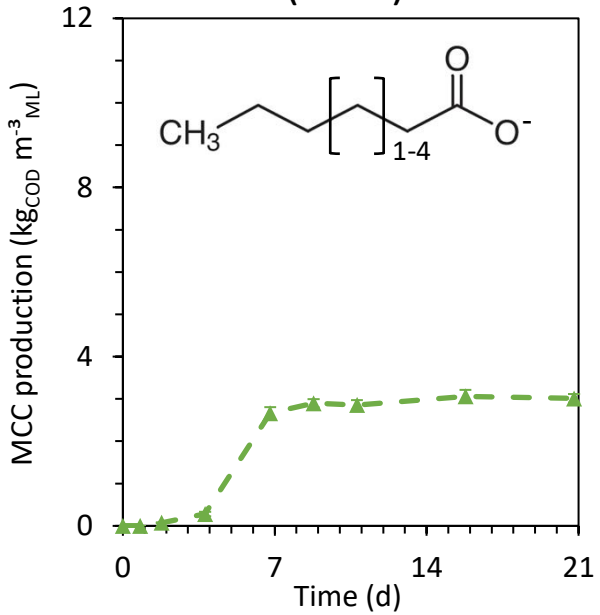


Hexanoate
Heptanoate
Octanoate
Nonanoate
Decanoate

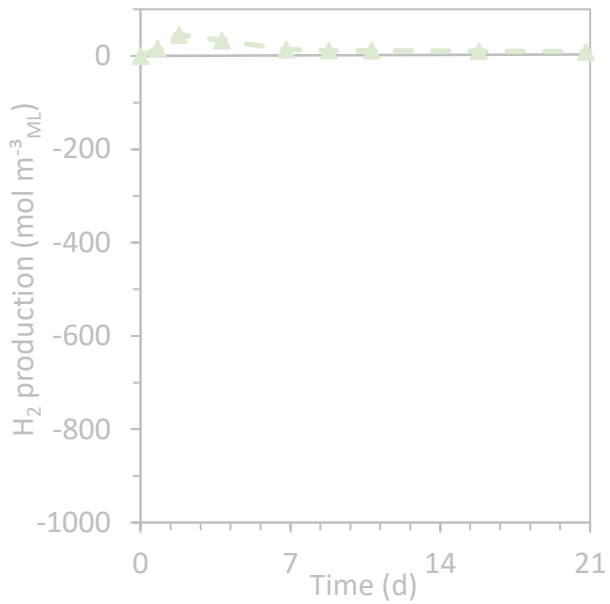
Easier to extract
Higher energy density
Higher market value

Context: Brewer's spent grain → MCC

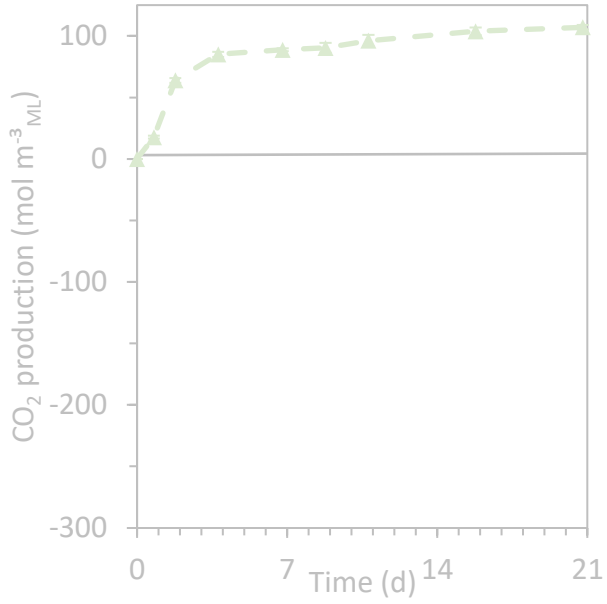
Medium chain carboxylates (MCC)

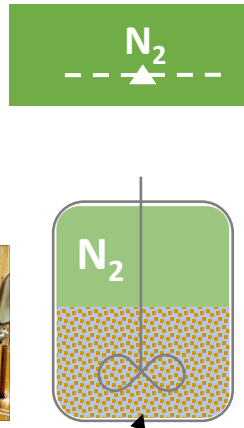


H_2



CO_2



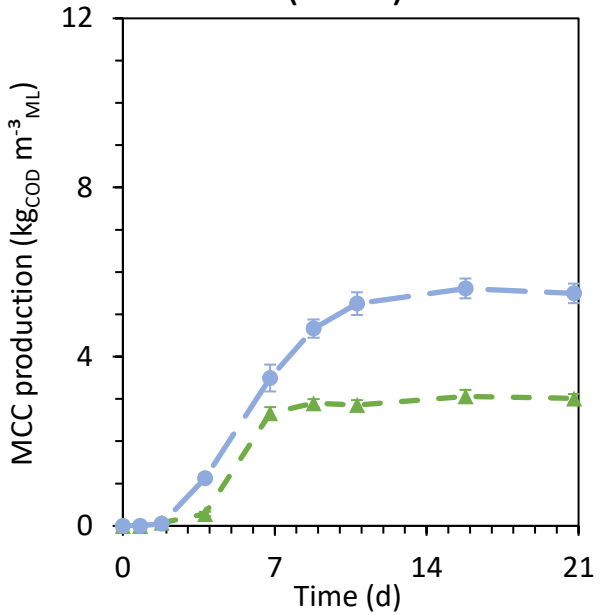


Reference condtiton

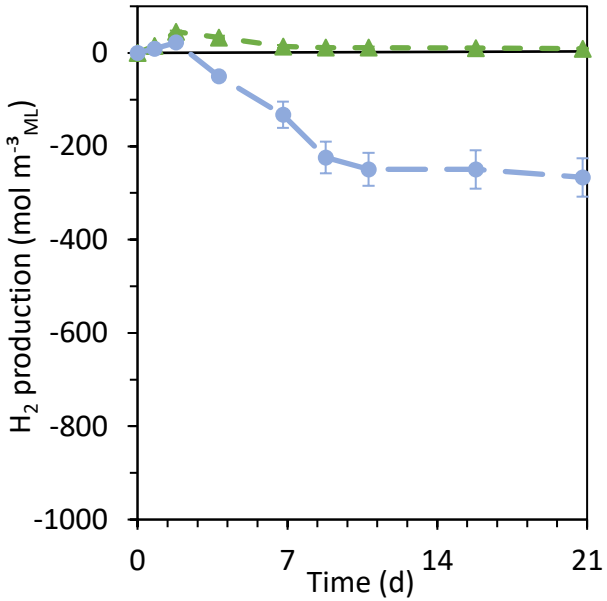
[Henry *et al.*, 2023]

Context: Brewer's spent grain & H_2 → MCC

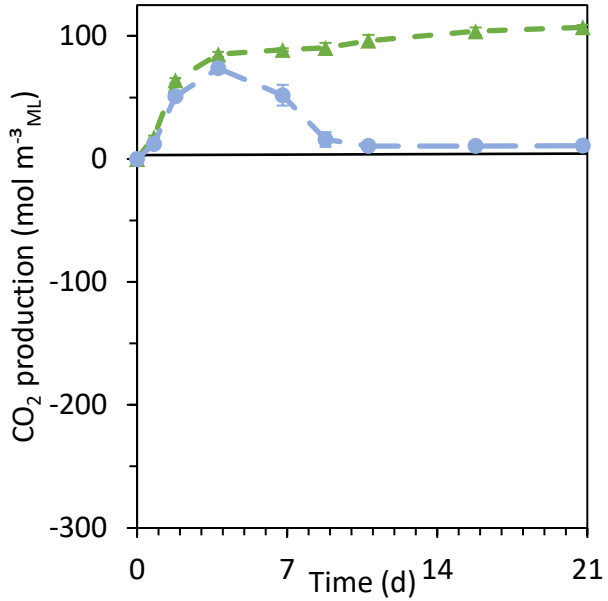
Medium chain carboxylates (MCC)



H_2

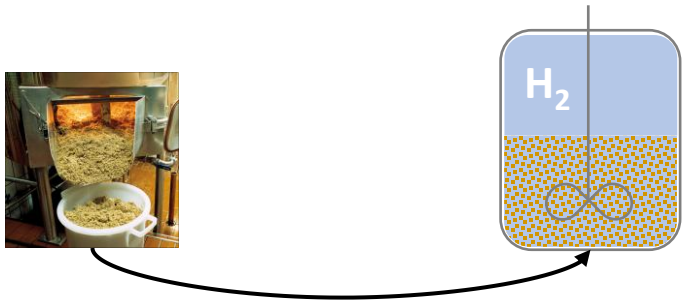


CO_2



MCC production increased by factor 2

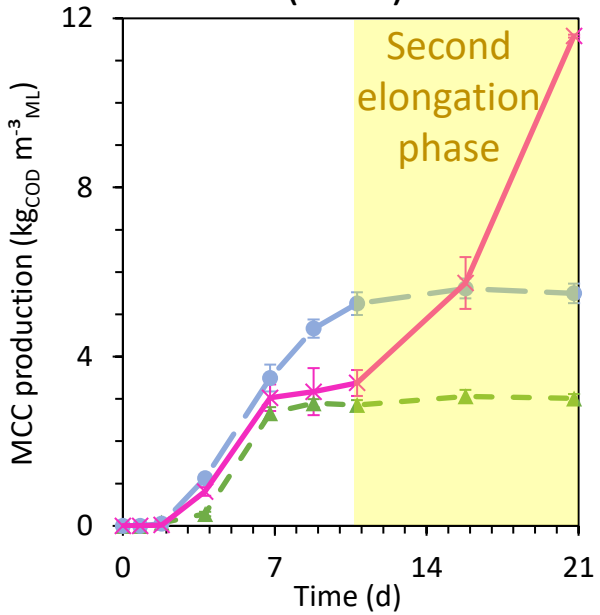
H_2 consumption stopped when acidogenic CO_2 is exhausted



[Henry *et al.*, 2023]

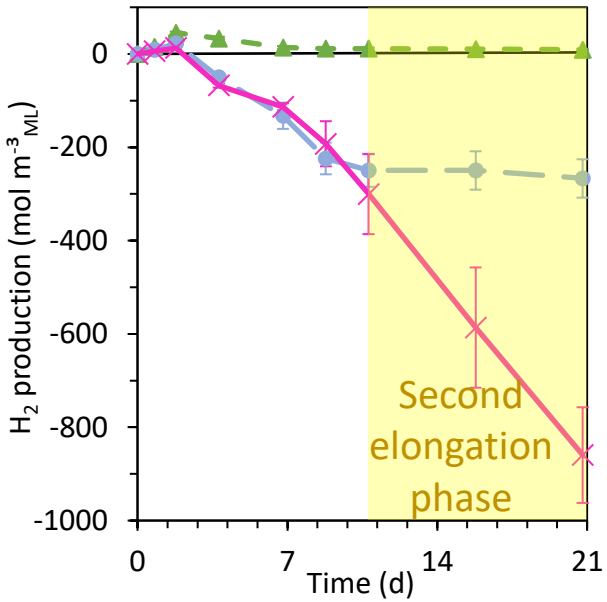
Context: Brewer's spent grain, H_2 & CO_2 → MCC

Medium chain carboxylates (MCC)

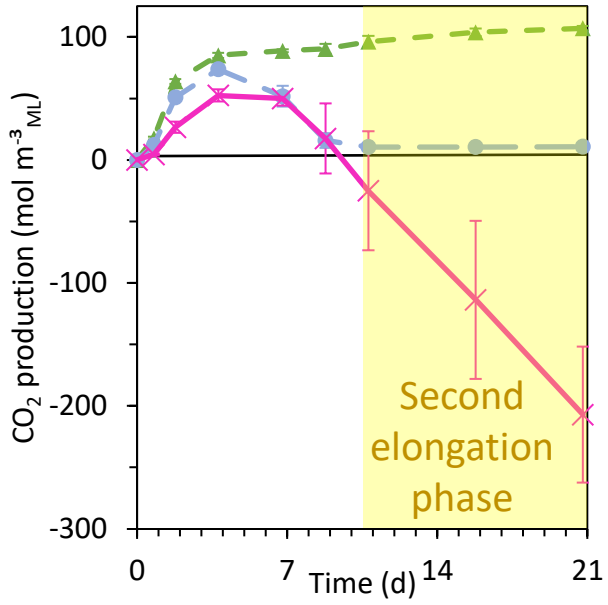


MCC production increased by factor 4

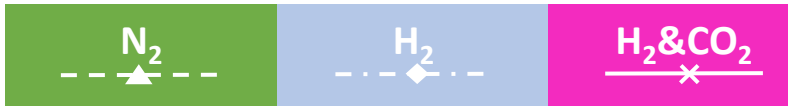
H_2

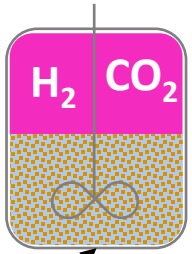


CO_2



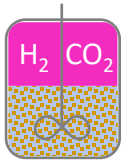
Supplied H_2 and CO_2 simultaneously consumed



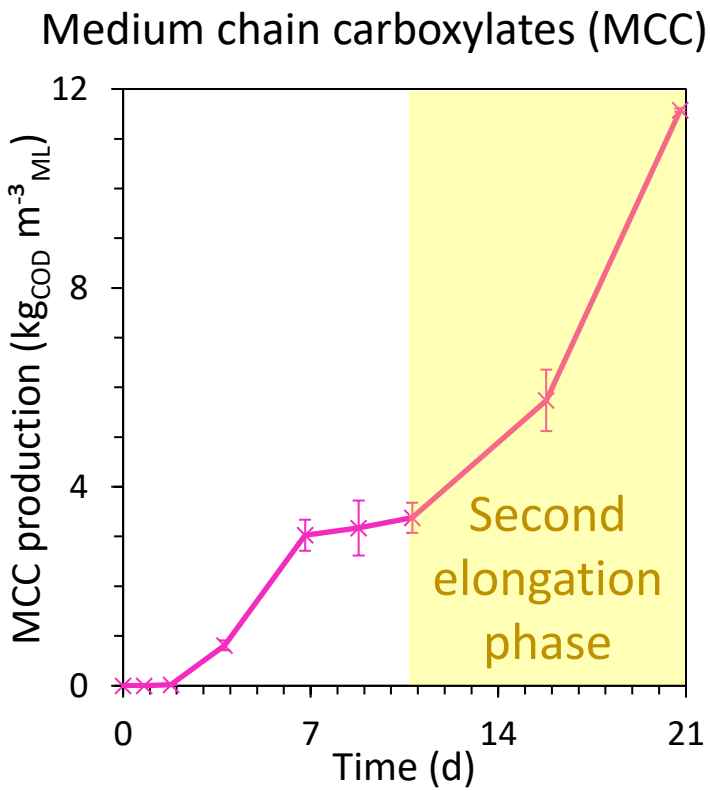


[Henry *et al.*, 2023]

Objective of the study

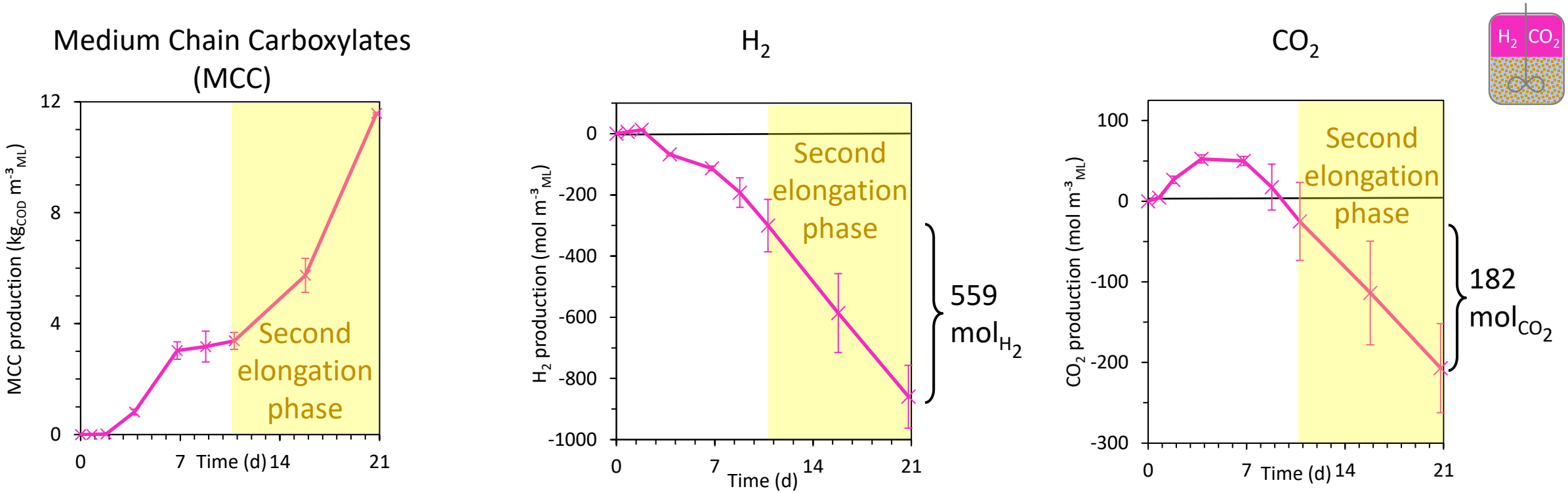


Identifying the chain elongation pathway
used by microorganisms
during this second elongation phase



[Henry *et al.*, 2023]

Second elongation phase is H_2 and CO_2 dependent

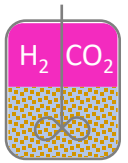


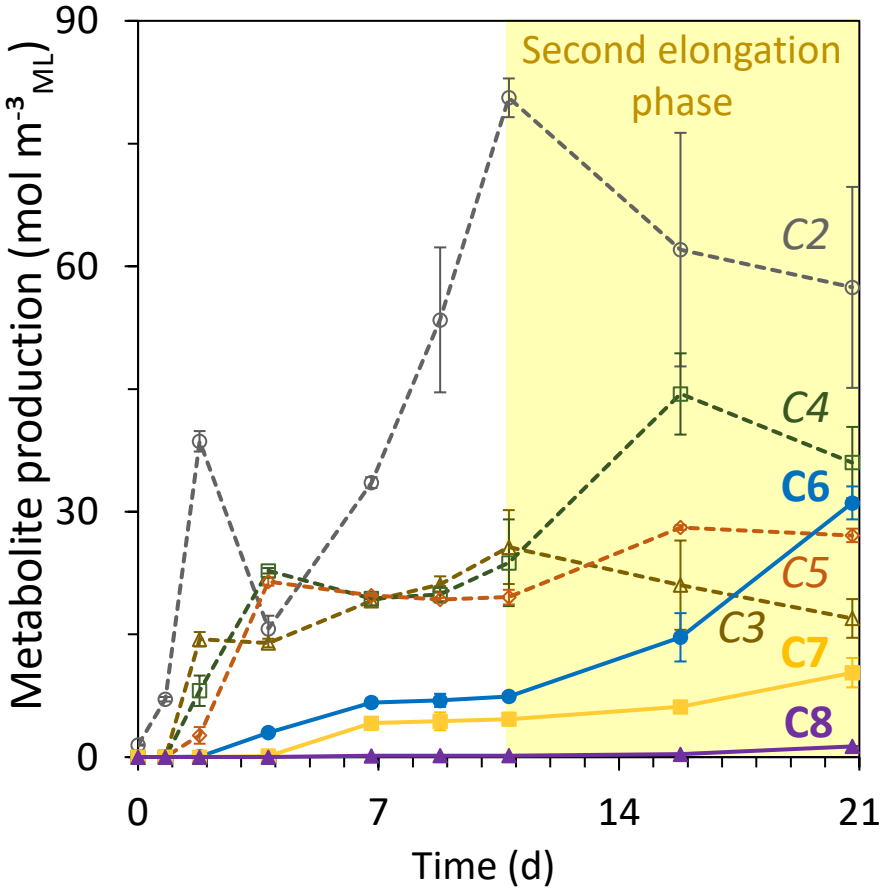
Experimentally measured H_2 / CO_2 consumption molar ratio:

$$\boldsymbol{R_{H_2/CO_2\_experiment}} = (559\ mol_{H_2} / 182\ mol_{CO_2}) = \mathbf{3.07}\ [mol_{H_2} / mol_{CO_2}]$$

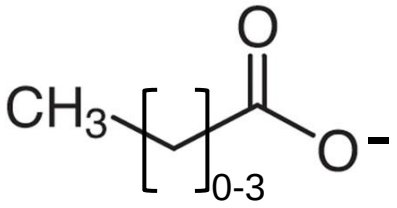
[Henry *et al.*, 2023]

Short- chain carboxylates are consumed during the second elongation phase



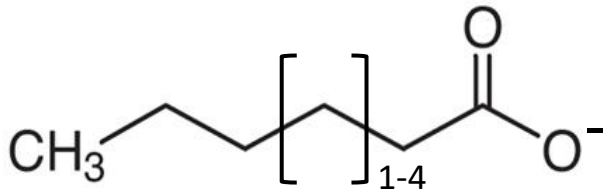


SCC: short-chain carboxylates



(*acetate*, *propionate*, *butanoate* and *pentanoate*) are consumed

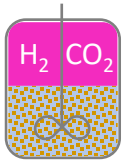
MCC : Medium- chain carboxylates



(hexanoate, heptanoate and octanoate) are produced

[Henry *et al.*, 2023]

R_{H_2/CO_2} as link between experimental and stoichiometry



Second elongation:

- Occurs only with a supply of H_2 AND CO_2
- H_2 & CO_2 simultaneously consumed
- Short- chain carboxylates consumption

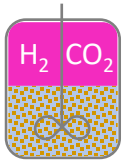
$$CH_3[CH_2]_mCOO^- + H_2 + CO_2 \longrightarrow CH_3[CH_2]_{m+2}COO^- + H_2O$$

Short- chain carboxylates
$m \geq 0$

Elongated carboxylates
***m* + 2**

[Henry *et al.*, 2023]

R_{H_2/CO_2} as link between experimental and stoichiometry



Second elongation:

- Occurs only with a supply of H_2 AND CO_2
- H_2 & CO_2 simultaneously consumed
- Short- chain carboxylates consumption

$$n\ CH_3[CH_2]_mCOO^- + 6n\ H_2 + 2n\ CO_2 \longrightarrow n\ CH_3[CH_2]_{m+2}COO^- + 4n\ H_2O$$

Short- chain carboxylates
$m \geq 0$

Elongated carboxylates
$\boldsymbol{m + 2}$

Stoichiometric H_2 / CO_2 consumption ratio

$\boldsymbol{R_{H_2/CO_2\_Stoichiometry}} = 6n / 2n = \mathbf{3}$

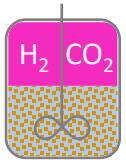
$\approx$

Experimental H_2 / CO_2 consumption ratio

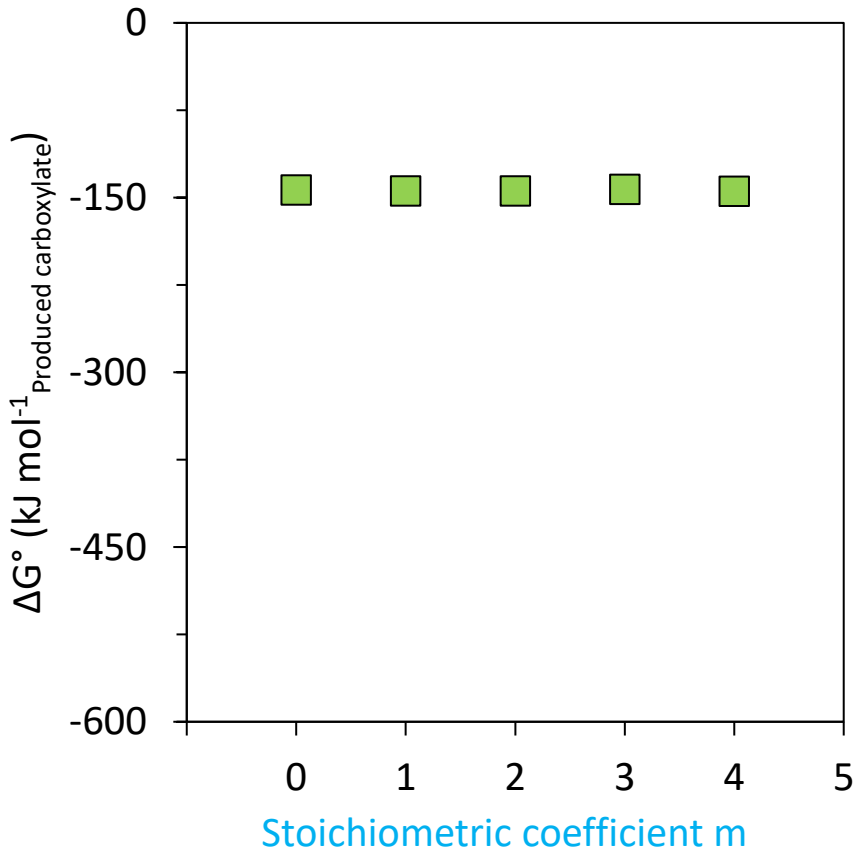
$\boldsymbol{R_{H_2/CO_2\_experiment}} = \mathbf{3.07}$ $[mol_{H_2} / mol_{CO_2}]$

[Henry *et al.*, 2023]

Is this chain elongation pathway thermodynamically valid ?



$$n\ CH_3[CH_2]_mCOO^- + 6n\ H_2 + 2n\ CO_2 \longrightarrow n\ CH_3[CH_2]_{m+2}COO^- + 4n\ H_2O$$



The standard Gibbs free energy of change FAVORABLE

$\Delta G° = -143.9 \pm 0.6$ kJ mol^{-1}

Independent of the stoichiometric coefficient m

[Henry *et al.*, 2023]

To conclude

Chain elongation pathway used by microorganisms based on carboxylates, H_2 and CO_2 consumption

$$n\ CH_3[CH_2]_mCOO^- + 6n\ H_2 + 2n\ CO_2 \longrightarrow n\ CH_3[CH_2]_{m+2}COO^- + 4n\ H_2O$$

Experimentally measured: $\boldsymbol{R_{H_2/CO_2\_experiment}}$ = **3.07** [mol_{H_2} / mol_{CO_2}]

Stoichiometrically confirmed: $\boldsymbol{R_{H_2/CO_2\_Stoichiometry}}$ = $6n$ / $2n$ = **3**

Thermodynamically validated: **$\Delta G° = -143.9 \pm 0.6$ kJ mol^{-1}**

Thank you for your attention

UCLouvain
EARTH & LIFE INSTITUTE
MOCA PLATFORM
Wallonie recherche CRA-W
FEDER
UNION EUROPÉENNE
LE FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL ET LA WALLONIE INVESTISSENT DANS VOTRE AVENIR
Wallonie
SPW
Service public de Wallonie

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Pr. Patrick Gerin


Pr. Benoit Stenuit

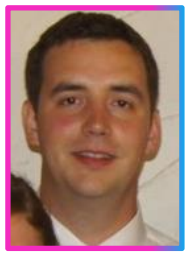

Dr. Lukas Braem


Michael Longin


Dr. Florent Awedem Wobiwo


Arnaud Isenborghs


Tanguy Depiesse


Thomas Nicolay


Hélène Dailly


Matthieu Leclercq

The Bertinchamps craft brewery in Gembloux is gratefully acknowledged for providing brewer's spent grain.

Publication

[Henry *et al.*, 2023]

Grégoire B.L. Henry* , Florent Awedem Wobiwo, Arnaud Isenborghs, Thomas Nicolay, Bruno Godin, Benoit A Stenuit and Patrick A. Gerin* (2023). A specific H_2/CO_2 consumption molar ratio of 3 as a signature for the chain elongation of carboxylates from brewer's spent grain acidogenesis. *Front. Bioeng. Biotechnol.* 9, 650631. doi: 10.3389/fbioe.2023.1165197

Microbial chain elongation based on organic feedstock, H_2 and CO_2

Grégoire B.L. Henry – Bioengineering and biorefining lab (ELIM - GEBI)

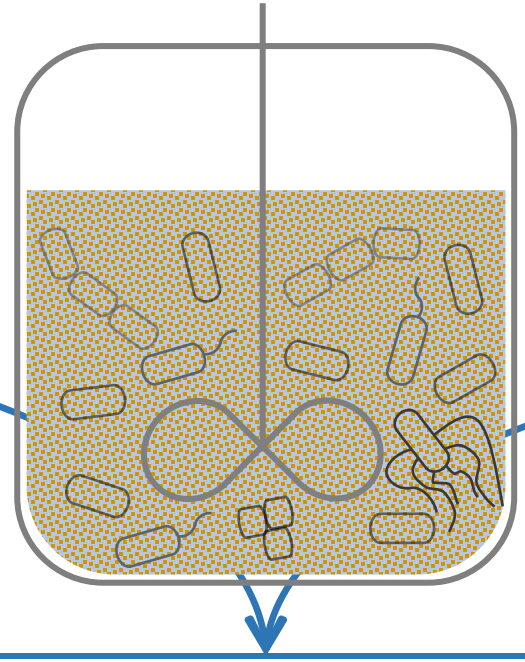
Complex organic feedstock valorization

Brewer's spent grain

Acidogenic fermentation

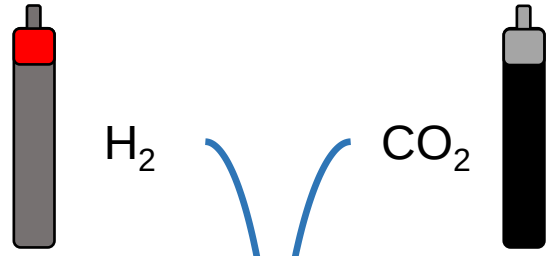
n $CH_3[CH_2]_mCOOH$
Short-chain carboxylates

Mixotrophic microbial community



n $CH_3[CH_2]_{(m+2)}COOH$ + $4n$ H_2O
Medium-chain carboxylates

Inorganic feedstock Supplementation



$6n$ H_2 + $2n$ CO_2

Short- & Medium-chain carboxylates
as platform molecules
for industrial production of
biofuels, bioplastics, biopesticides,
medicines

Take home message
Chain elongation pathway based on
a consumed H_2/CO_2 molar ratio of 3
experimentally measured,
stoichiometrically &
thermodynamically validated

From Belgian agro-industrial residues to bio-based chemicals;

Brewery residues -> Medium Chain Carboxylic Acids (C6 - C8)

Grégoire B.L. Henry

Barley crop

Fertilizer

Beer brewing

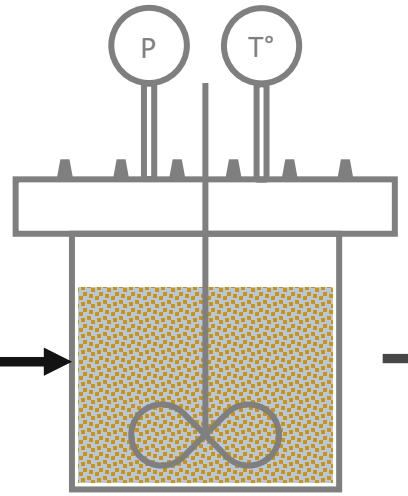
Barley malt

Water

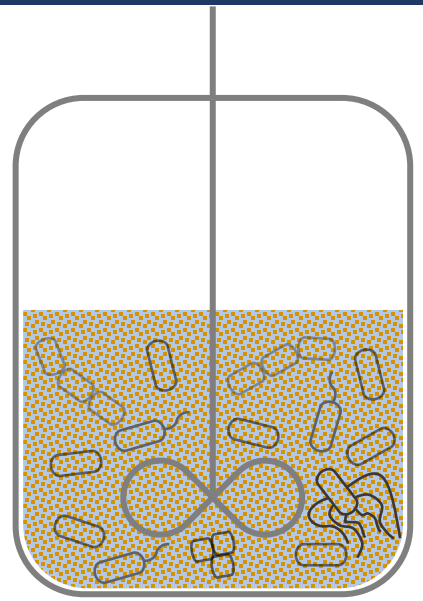
Brewer's spent grain

85% of the by-product of a brewery

Hot water pretreatment



Physico-chemical fermentation conditions



- **Complex feedstock**
- **Mixed Microbial Community**

Extract

Extraction

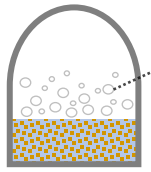
Raffinate

Liquid fraction

Solid fraction

Biomethanisation

Solid residues

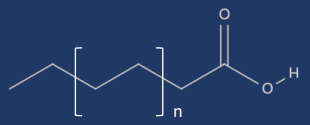


CH_4

Heat and Electricity

CNG

Medium Chain Carboxylic Acids



Bio-based Chemicals

Context : the biorefinery concept

Fossil resources

Refinery process

Platform molecules

Traditional chemistry

Petro-based products

Context : the biorefinery concept

Organic feedstock

Feed ingredients

Platform molecules

Energy

Traditional & green chemistry

Bio-based products

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Context : the biorefinery concept

Organic feedstock

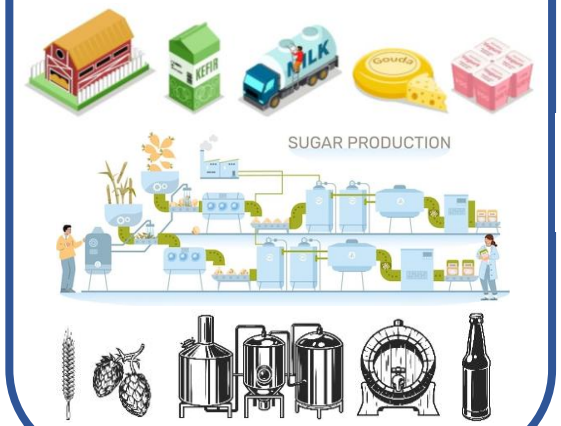
Biorefinery processes

Platform molecules

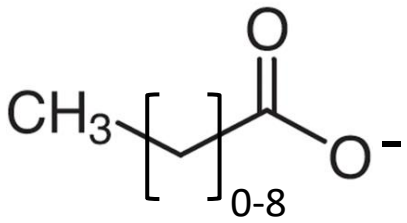
Traditional & green chemistry

Bio-based products

Agro-industrial residues



The carboxylate platform



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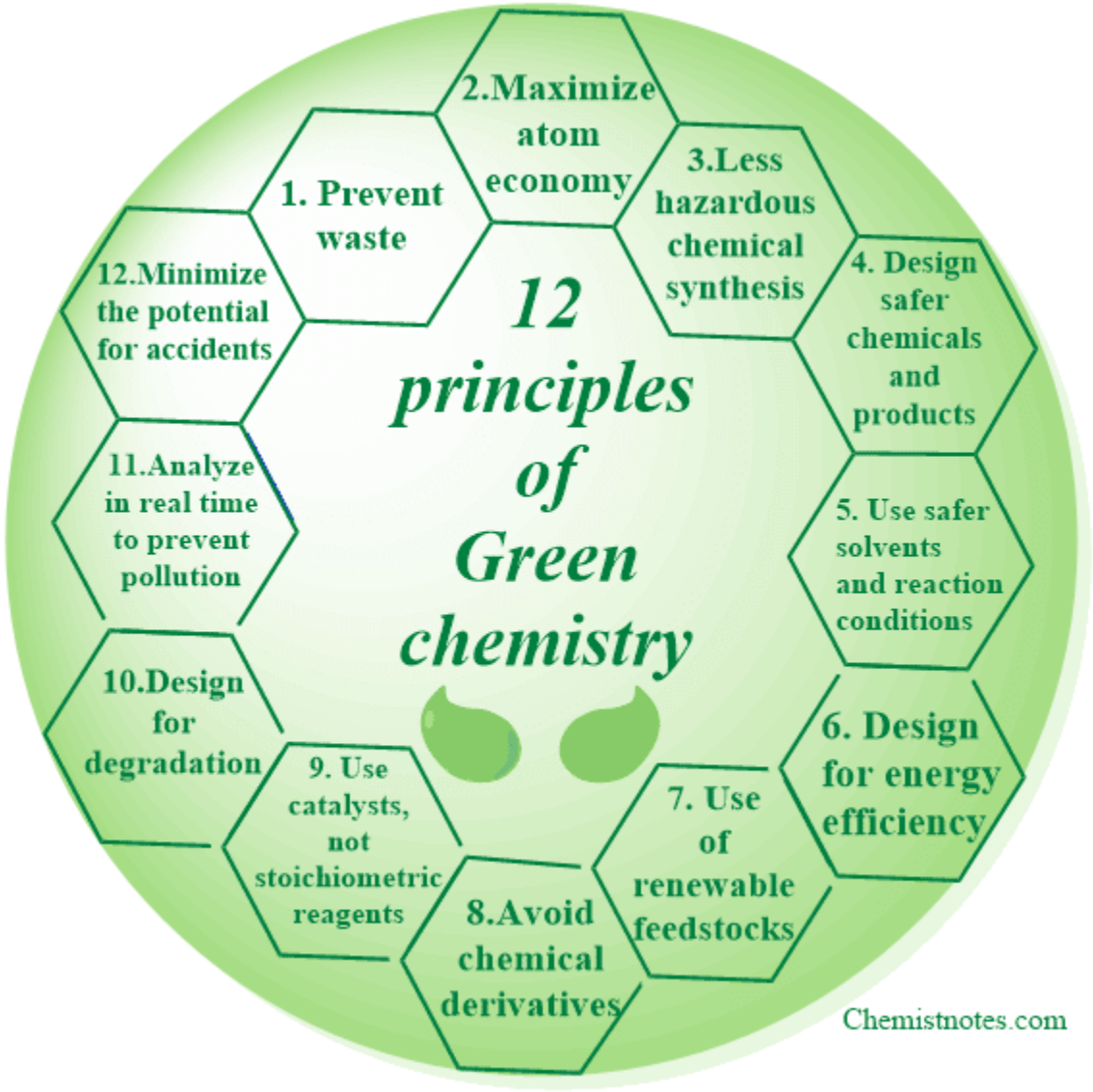
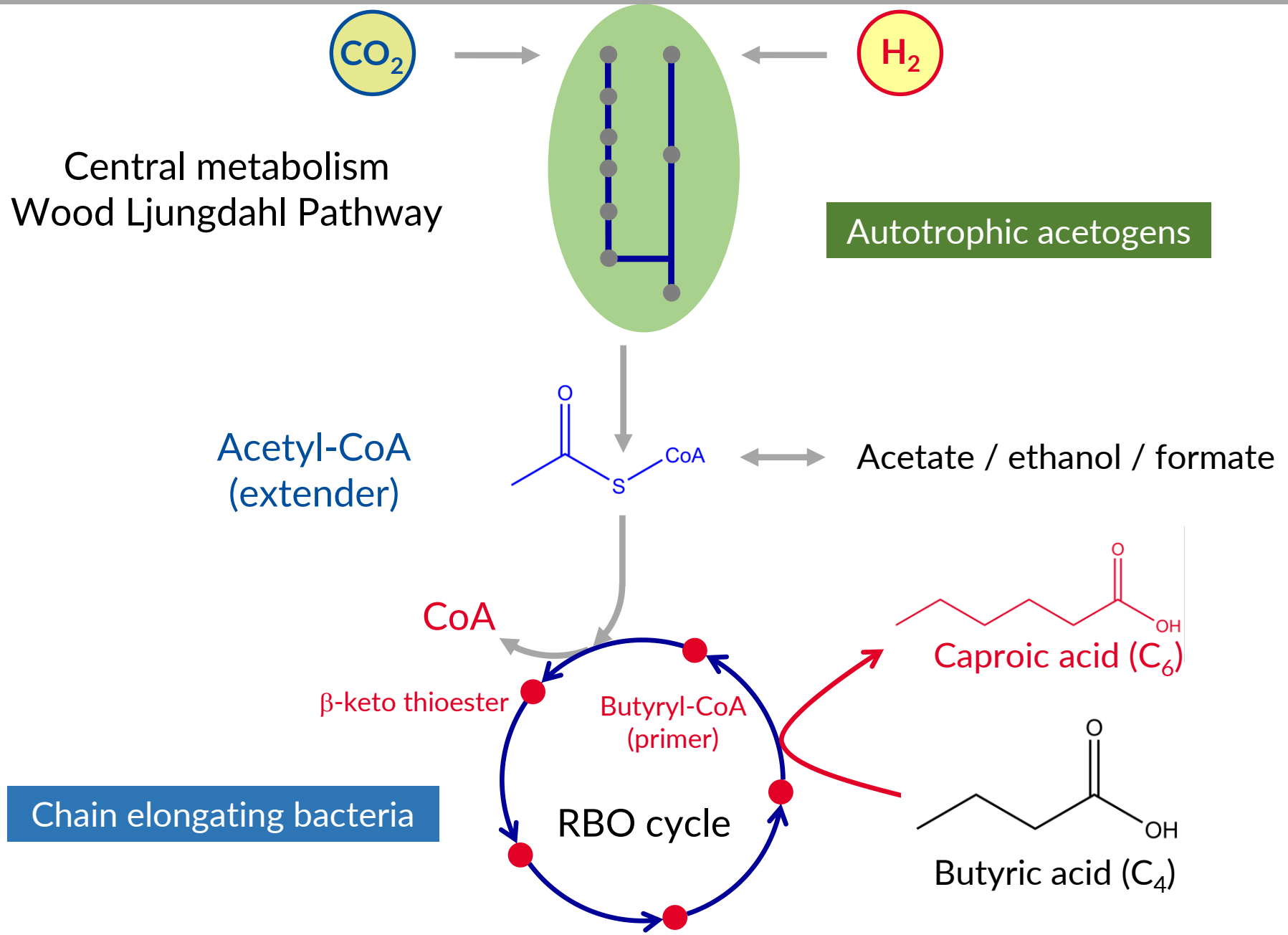
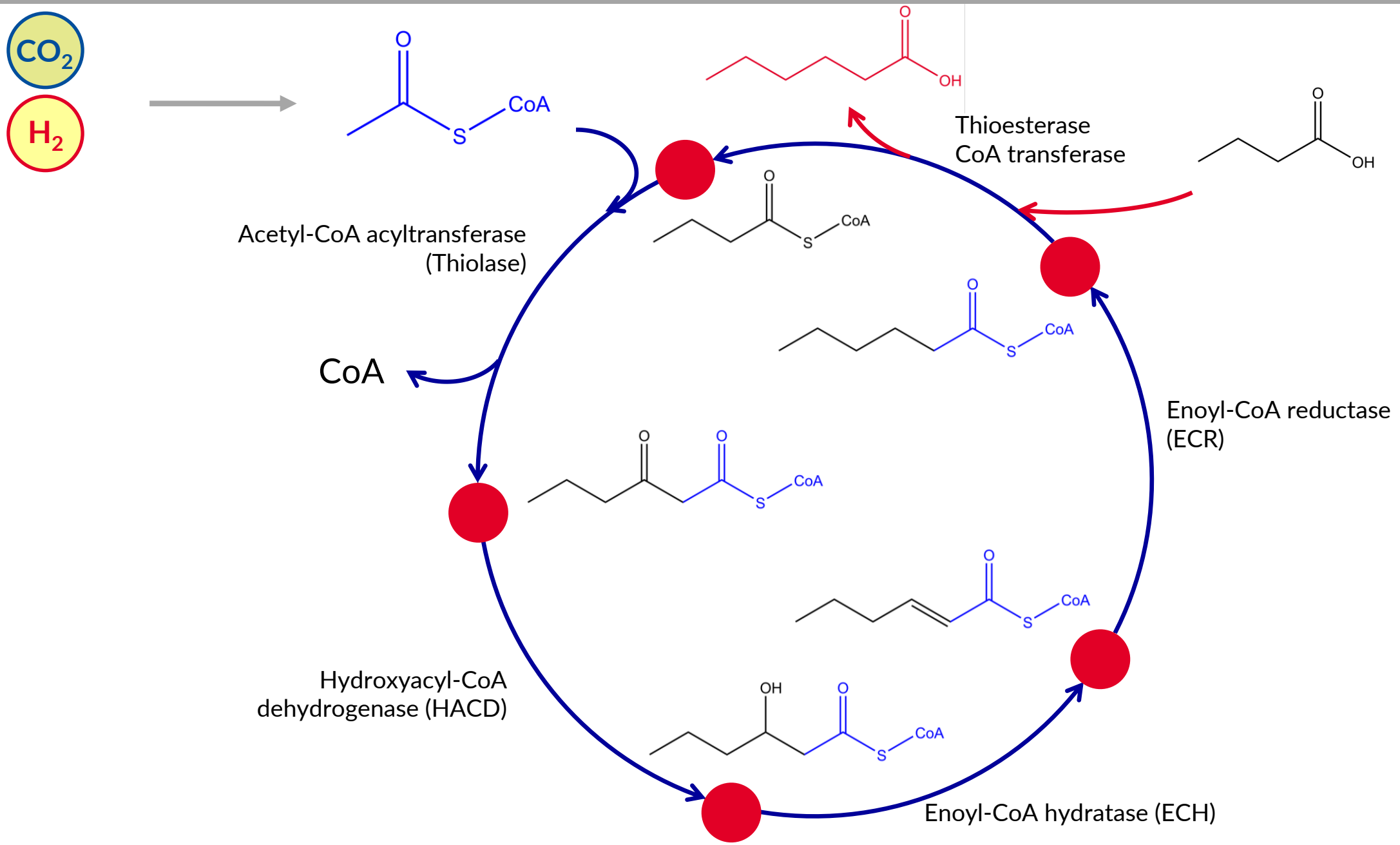
The 12 principles of green chemistry



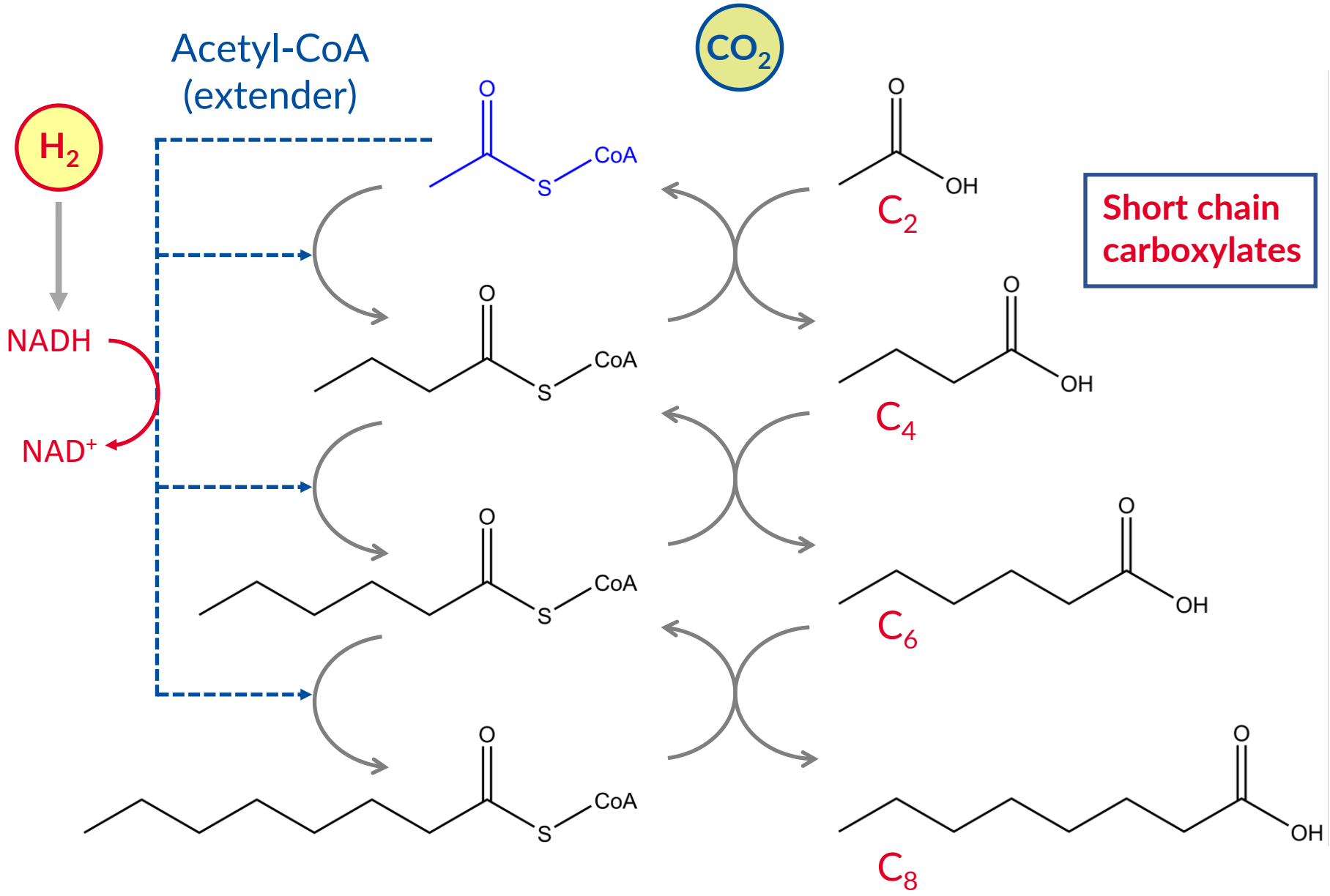
Chain elongation based on carboxylates, H_2 and CO_2



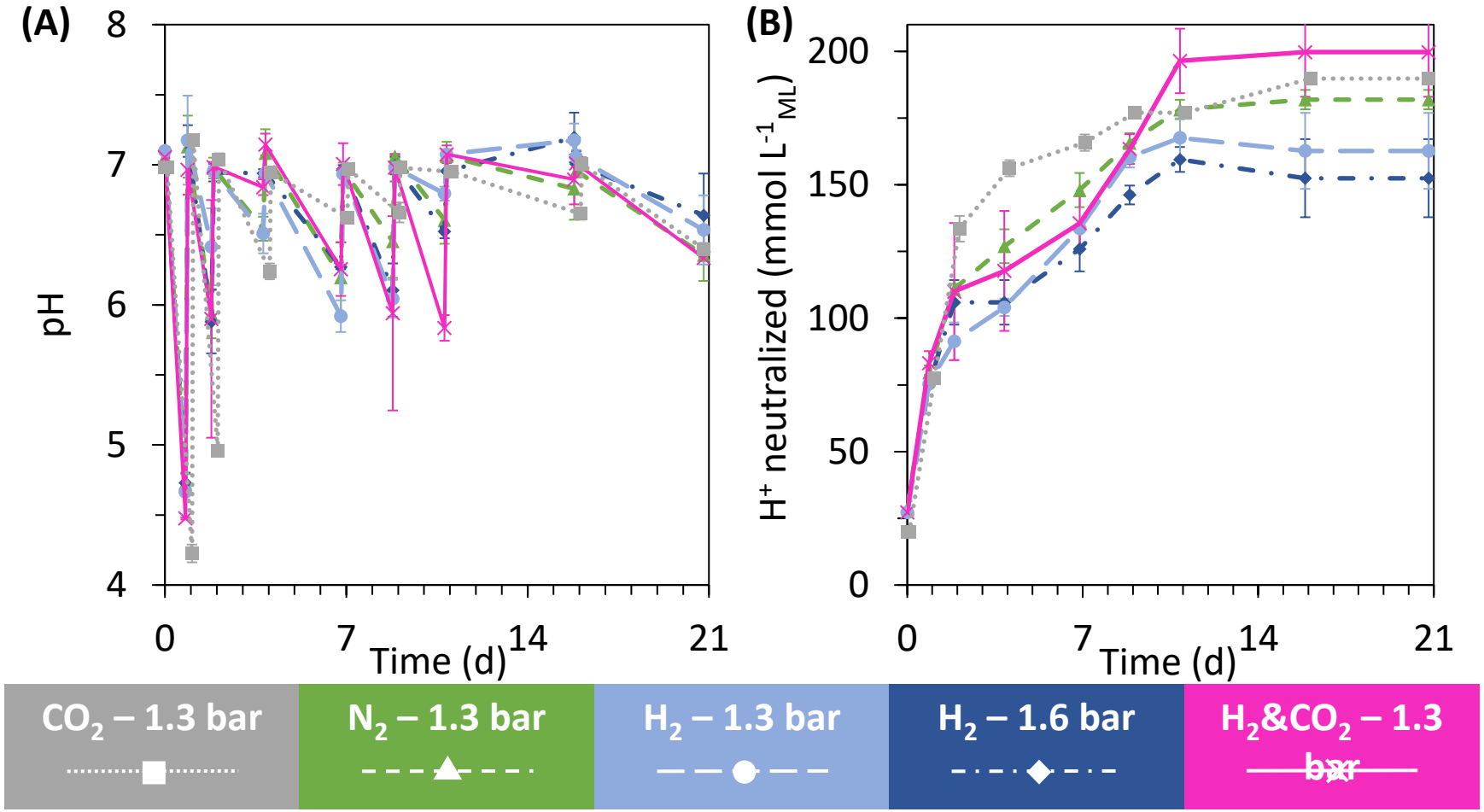
Chain elongation based on carboxylates, H_2 and CO_2



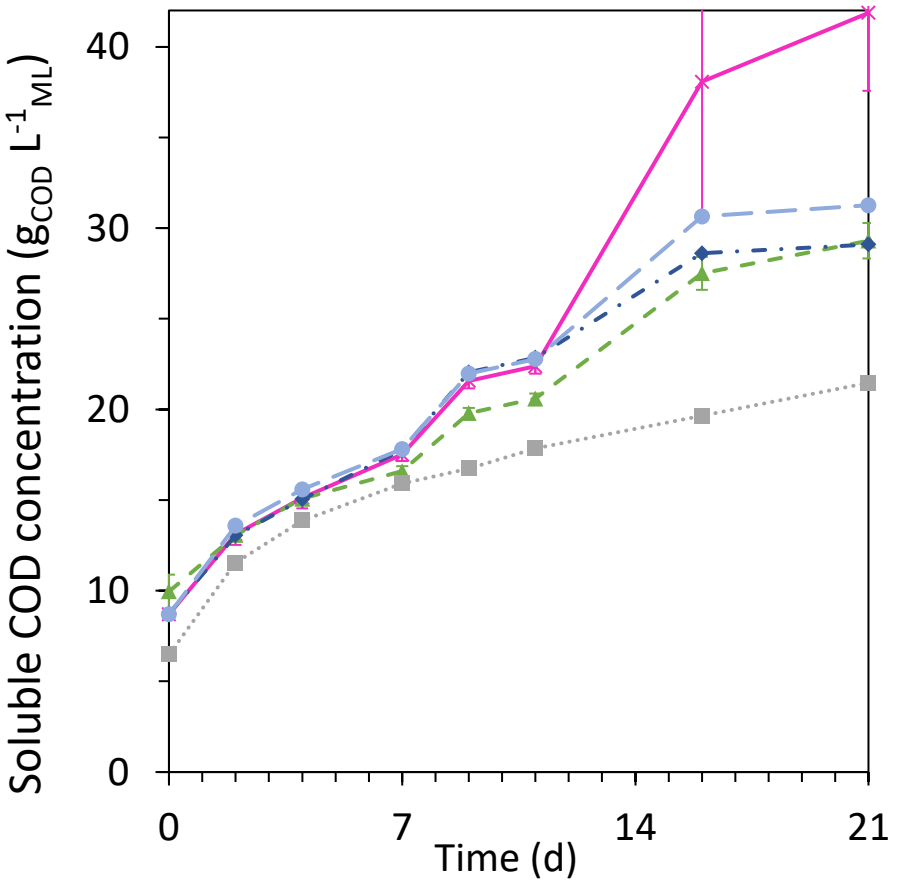
Chain elongation based on carboxylates, H_2 and CO_2

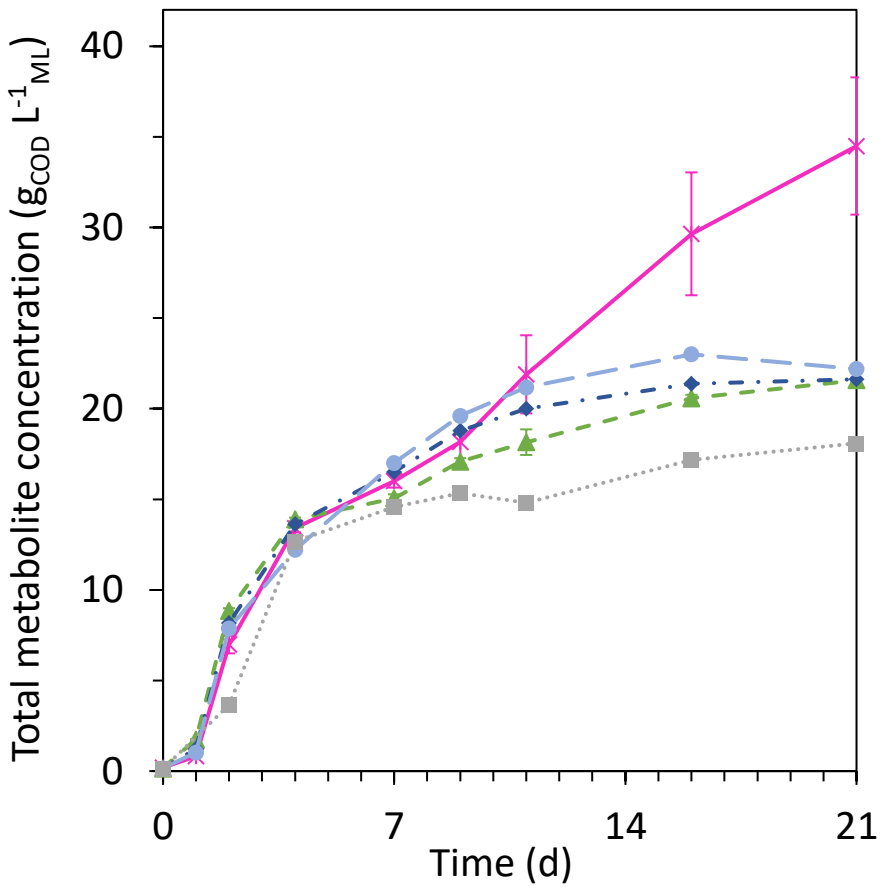


pH evolution during fermentation



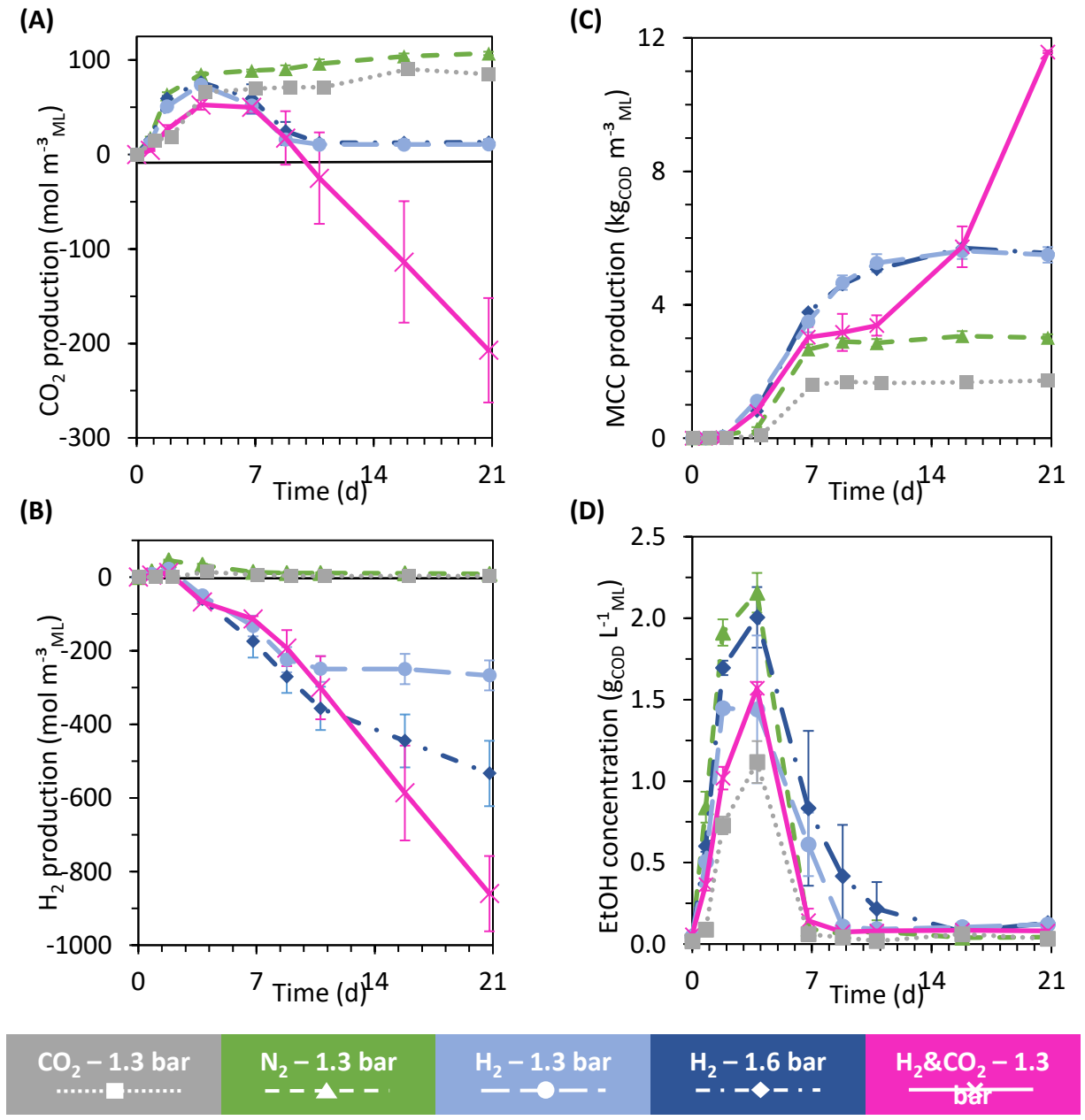
Soluble COD and total metabolites concentration





CO_2 – 1.3 bar	N_2 – 1.3 bar	H_2 – 1.3 bar	H_2 – 1.6 bar	H_2&CO_2 – 1.3 bar

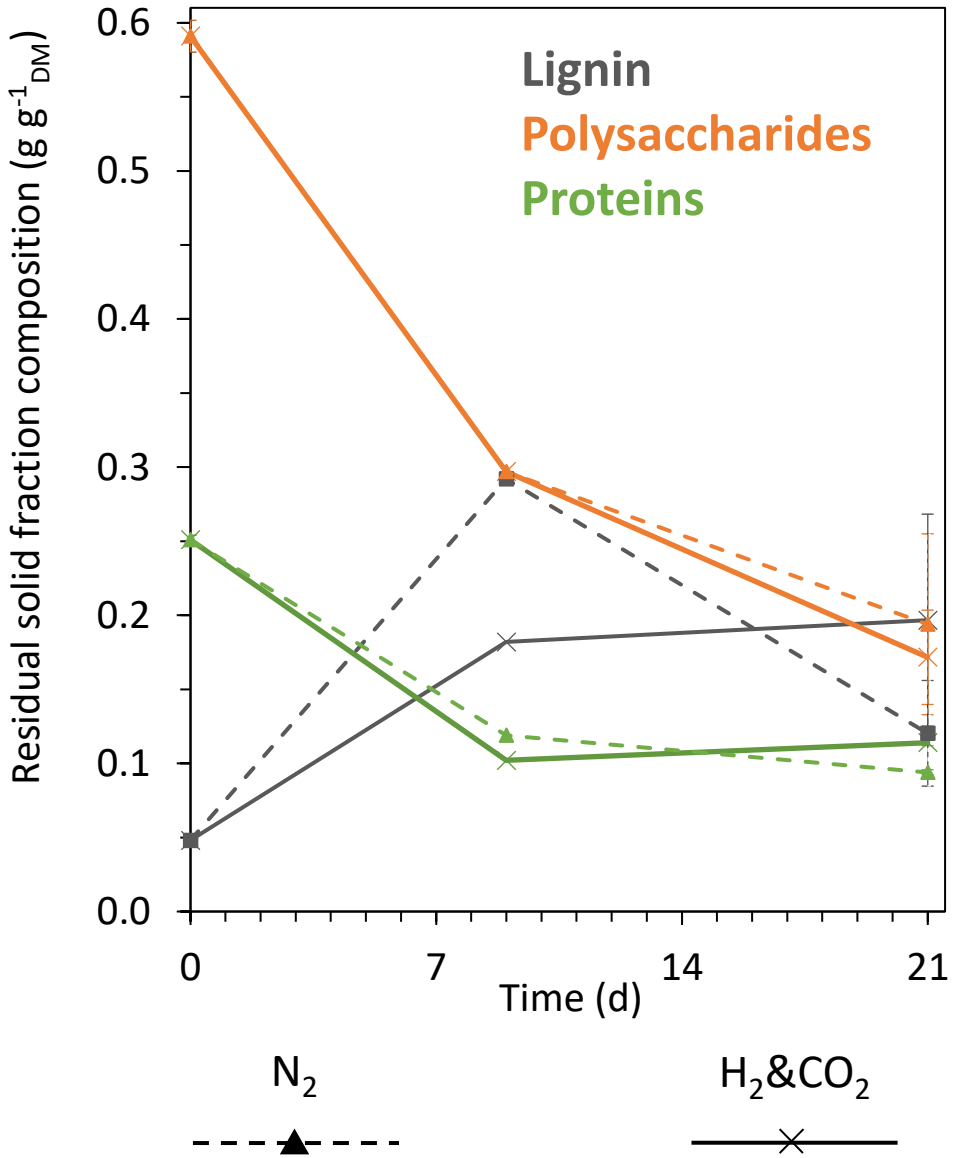
CO_2, H_2, MCC production and ethanol concentration



Metabolite productions according to the condition tested

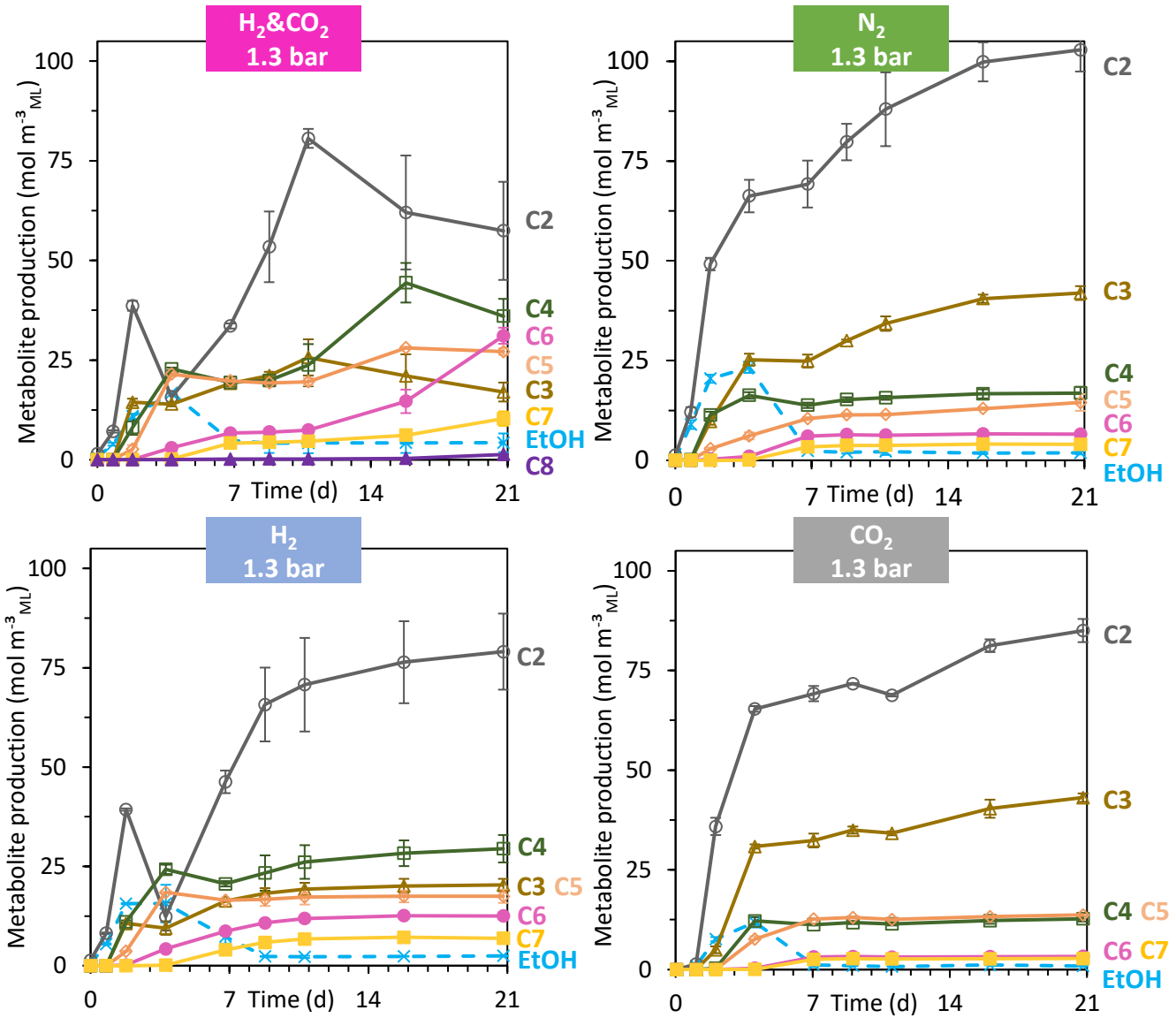
Matter composition of the initial BSG

Dry Matter$_{105\,°C}$	23.2 %$_{FM}$
Volatile Solid$_{550\,°C}$	95.9 %$_{DM}$
Lignin	4.8 %$_{DM}$
Hemicelluloses	33.4 %$_{DM}$
Cellulose	17.9 %$_{DM}$
Starch	7.0 %$_{DM}$
Soluble sugars	0.8 %$_{DM}$
Proteins	25.1 %$_{DM}$
Minerals	4.1 %$_{DM}$
Other	7.0 %$_{DM}$



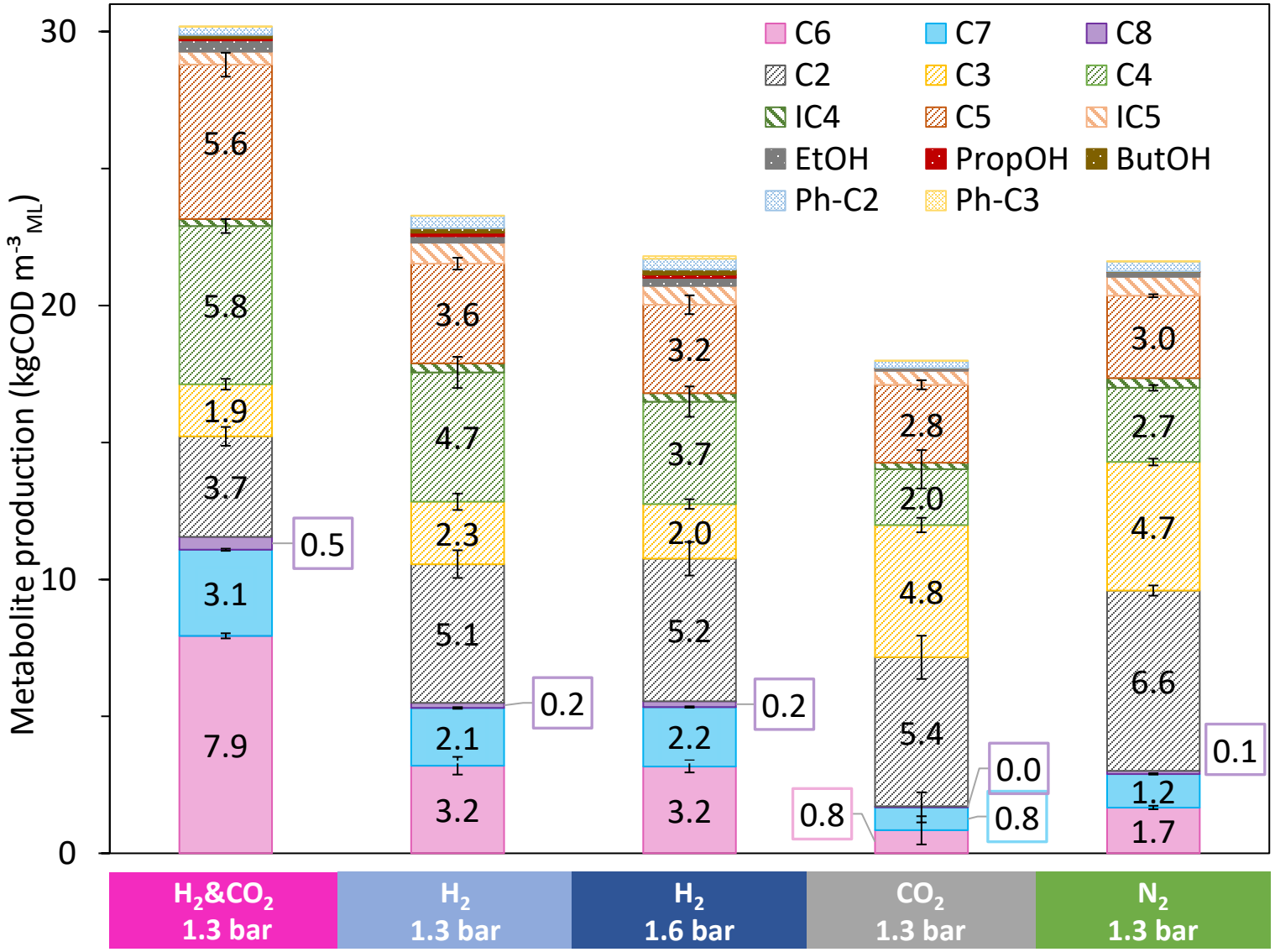
[Henry *et al.*, 2023]

Metabolite productions according to the condition tested

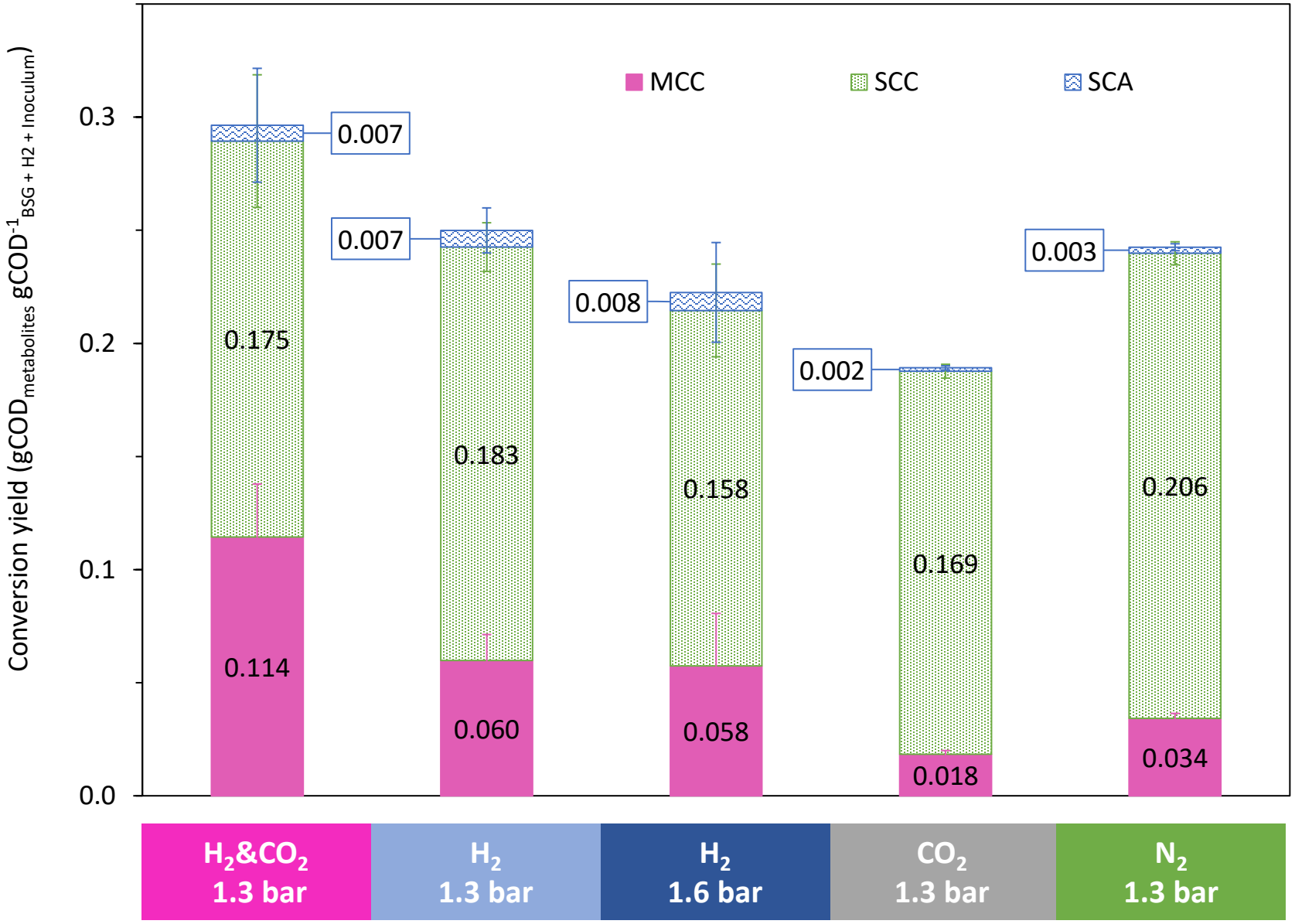


[Henry *et al.*, 2023]

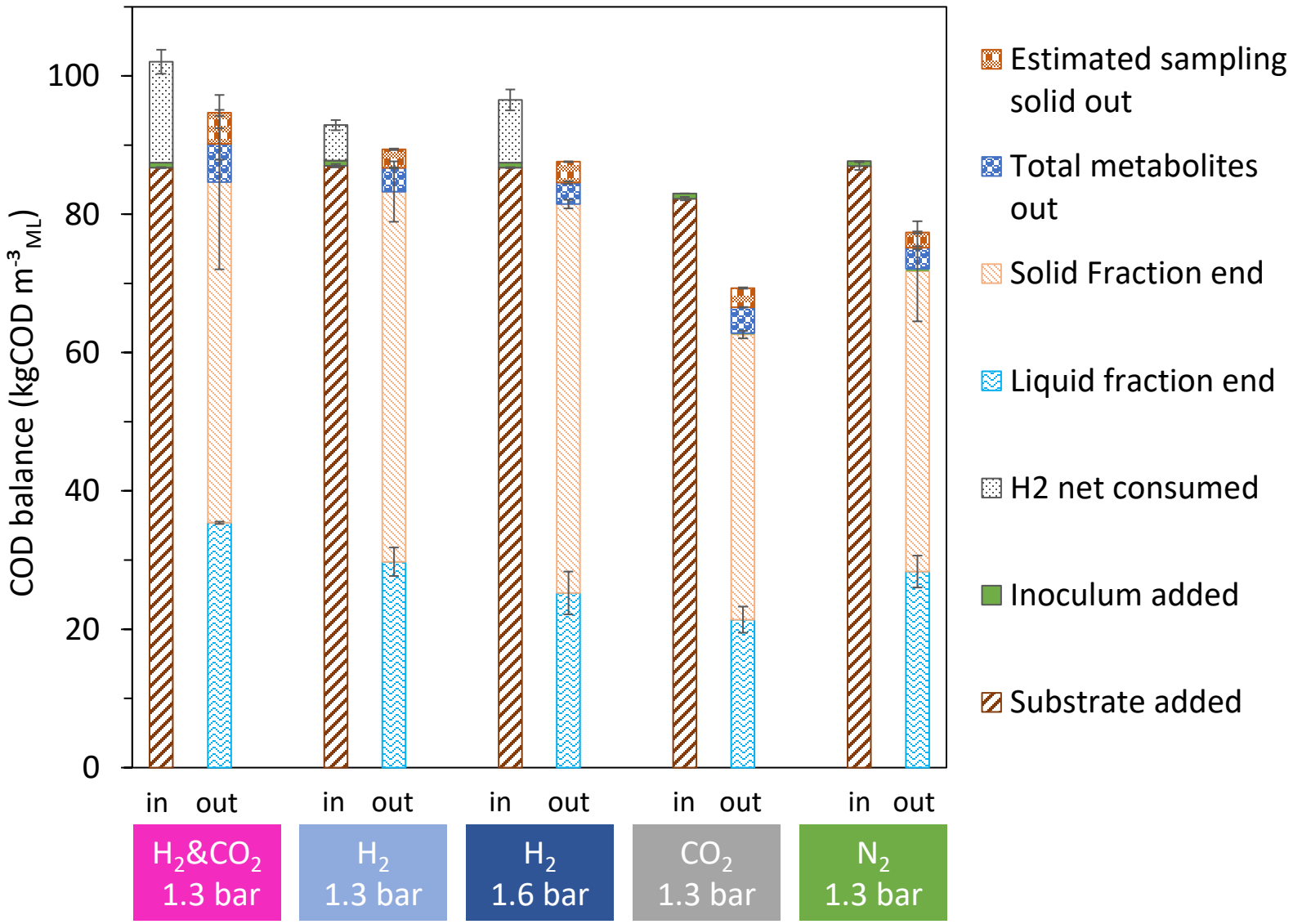
Final metabolite productions



Conversion yield

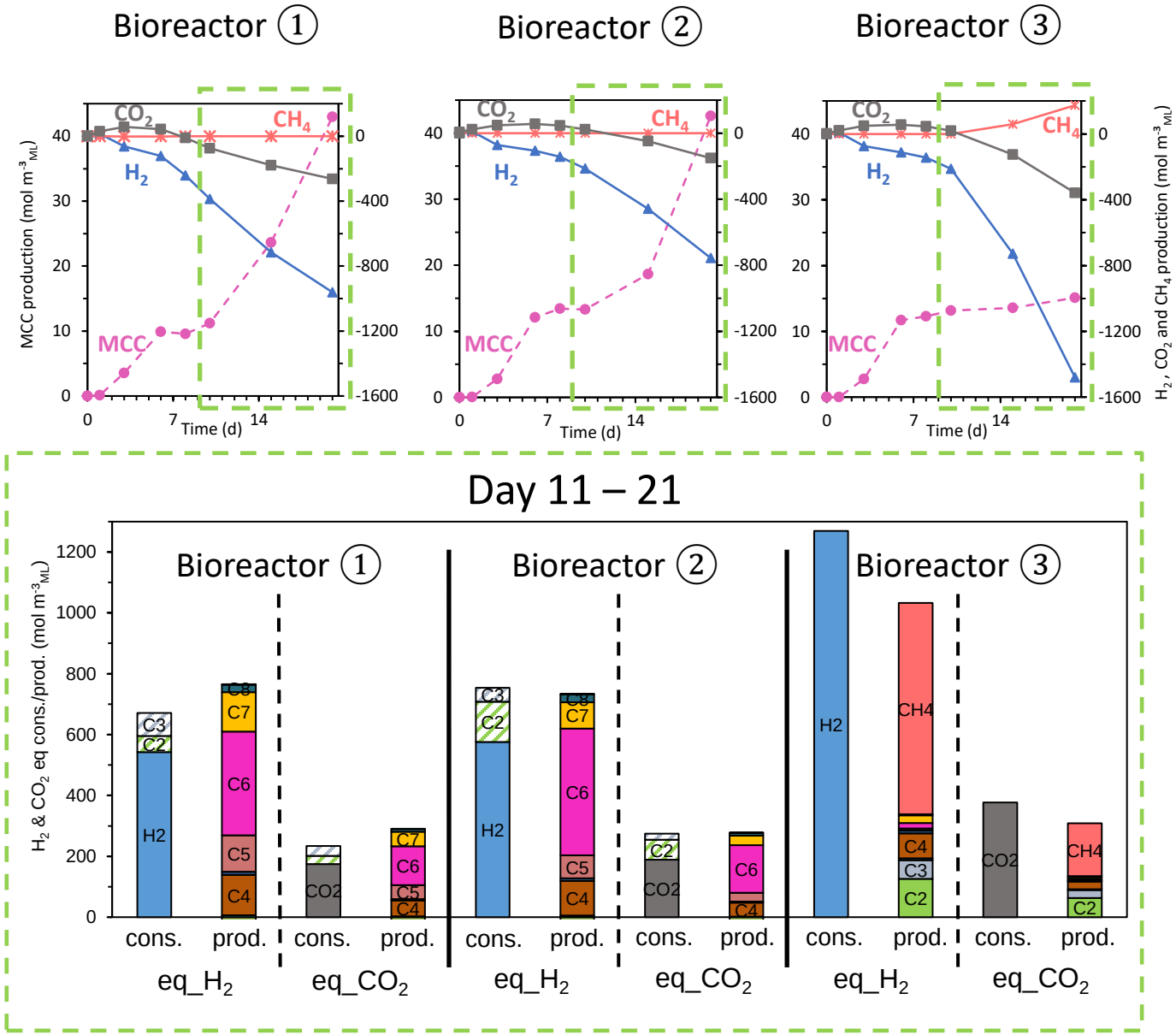


COD balance (start – end)

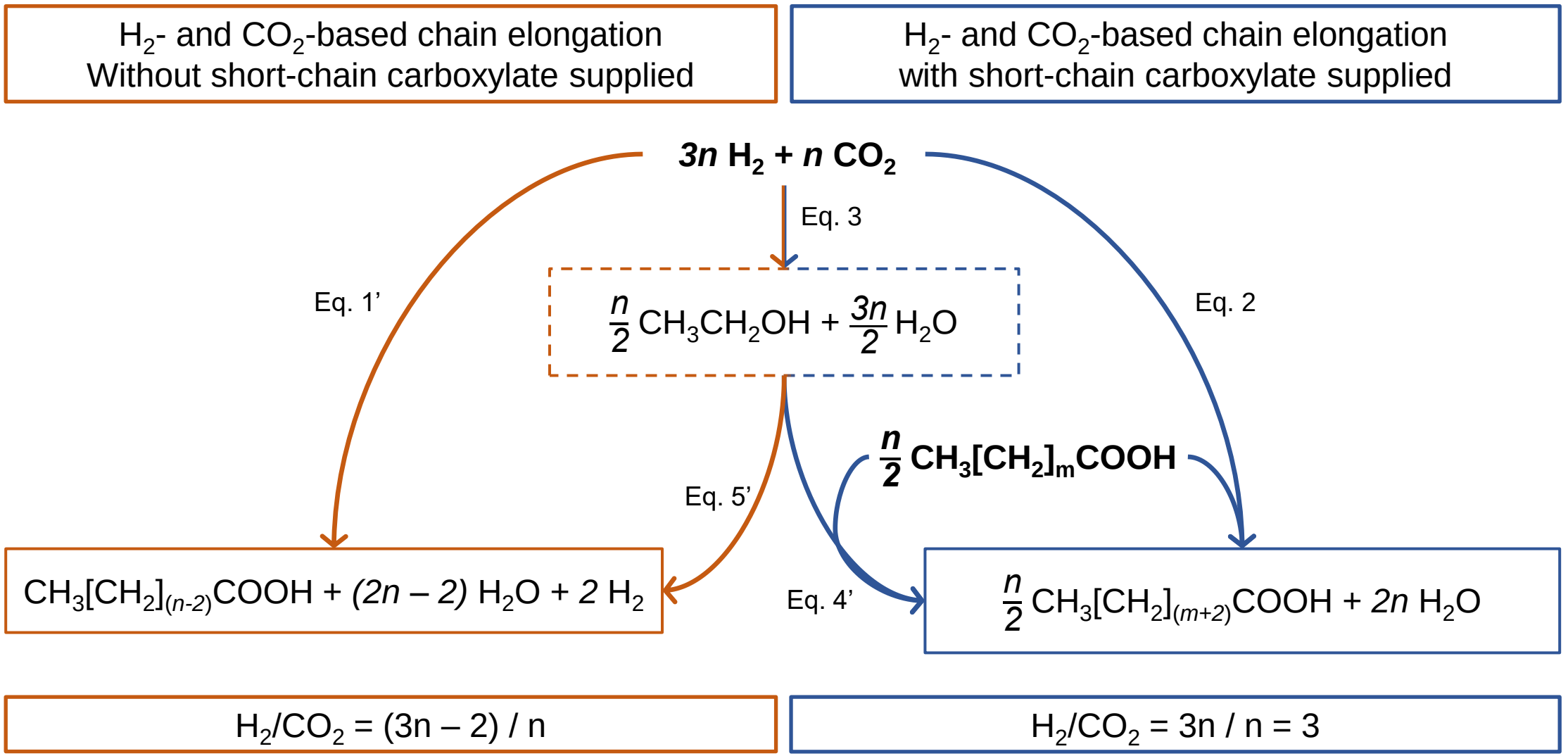


Carbon and electron balances in term of eq_H_2 and eq_CO_2

$(3n\text{-}2)\ H_2 + nCO_2 \rightarrow CH_3[CH2]_{n\text{-}2}COOH + (2n\text{-}2)H_2O$

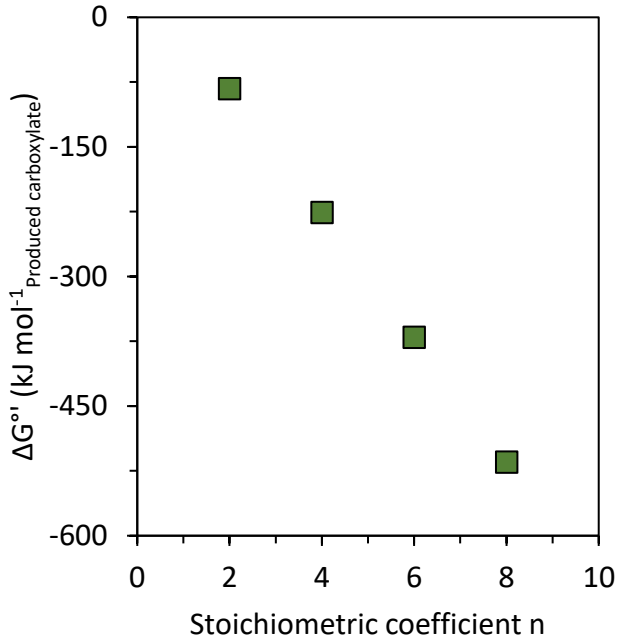


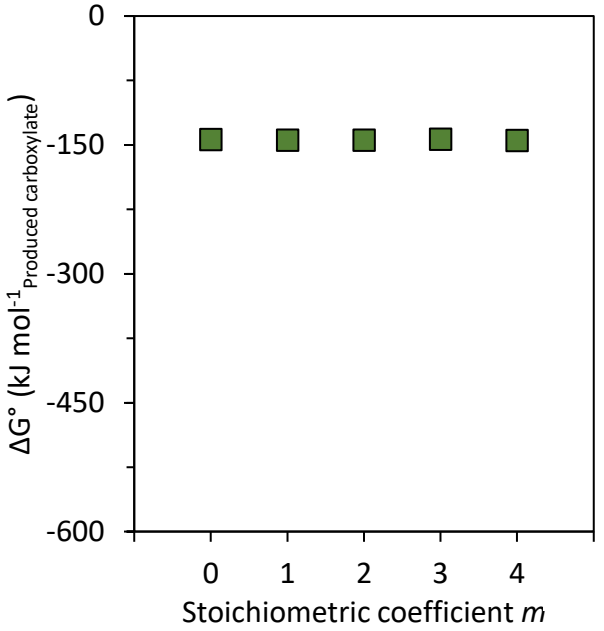
Two pathways : carboxylates based or not

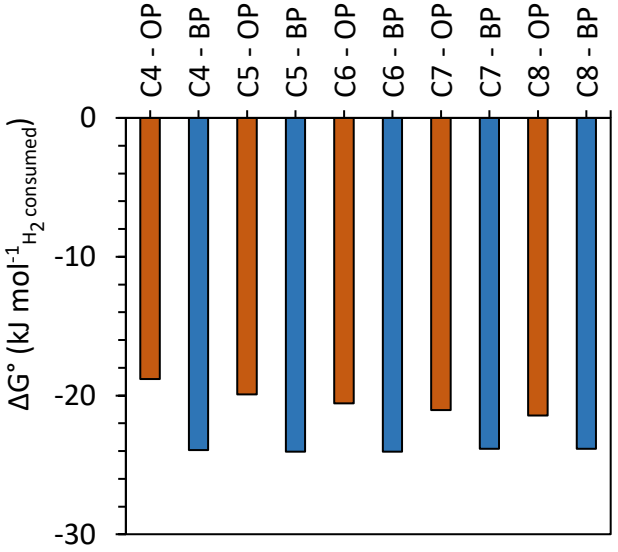


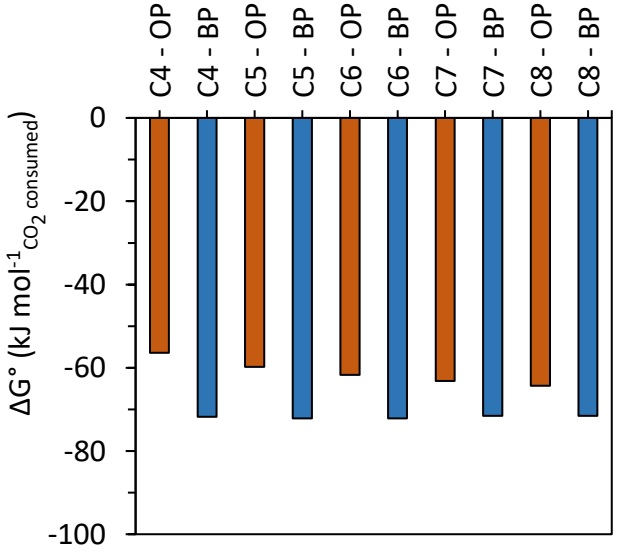
[Henry *et al.*, 2023]

Thermodynamic of the pathways



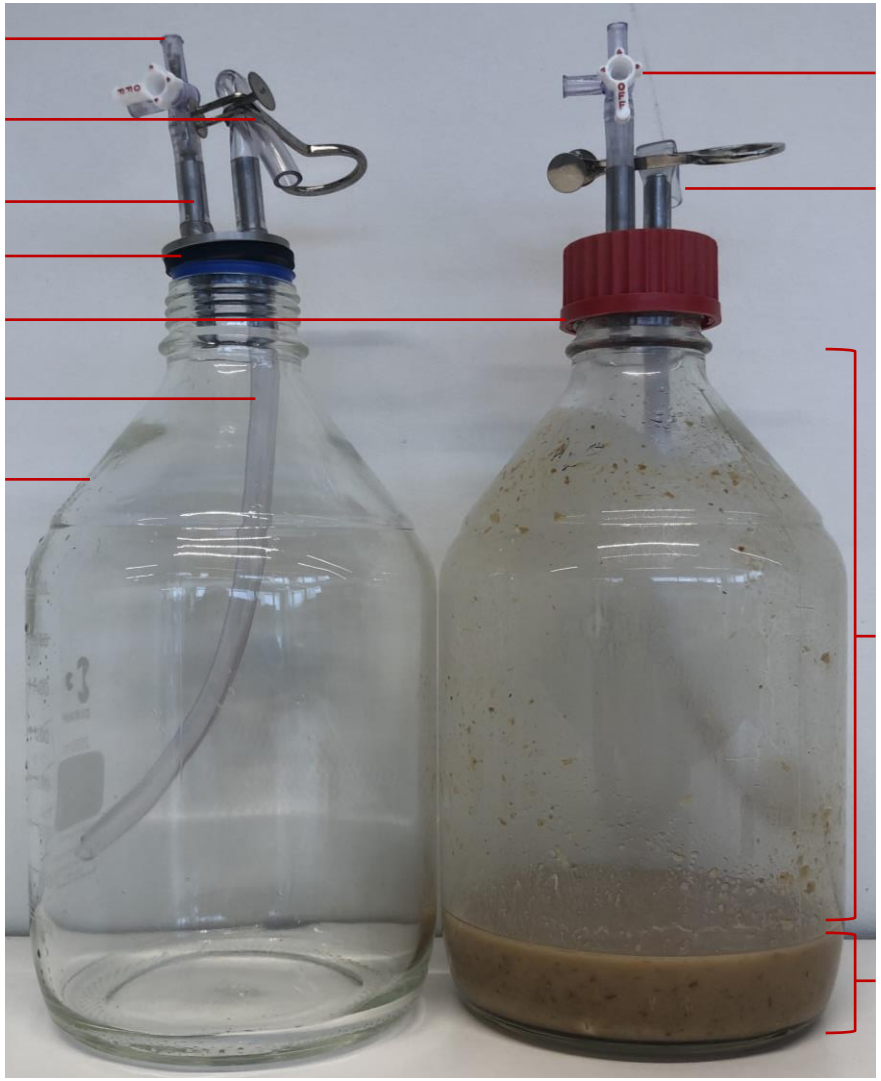






[Henry *et al.*, 2023]

Assembled and filled bioreactors



[Henry *et al.*, 2023]


ORGANIC
Potting Soil
Fertilizer
Fertilizer
IS
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KEFIR
wheat
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SUGAR
BERTINCHAMPS
BIERES DE TERROIR BELGES
BRASSEES A LA FERME
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Spirulina products
SUGAR PRODUCTION

Context: The biorefinery concept

Renewable organic feedstock

Platform molecules

Directly used

Traditional, green & bio-chemistry

Bio-based products

Energy

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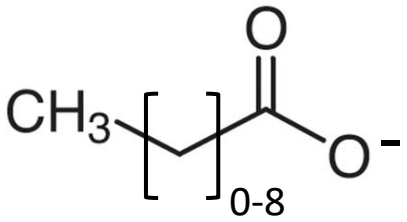
Context: Carboxylates as platform molecule

Renewable organic feedstock

Carboxylates as platform molecules



Directly used

Traditional, green & bio-chemistry

Bio-based products

Context: The biorefinery concept

Renewable organic feedstock

Food and feed ingredients

Platform molecules

Energy

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Bio-based products

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