GRUPPO RICICLA
Agricoltura, Ambiente, Biomasse ed Energie Rinnovabili

DiSAA

UdG lequia ECO-INNOVATIVE WATER SOLUTIONS

Production of polyhydroxybutyrate (PHB) by bio-electro recycling of carbon dioxide

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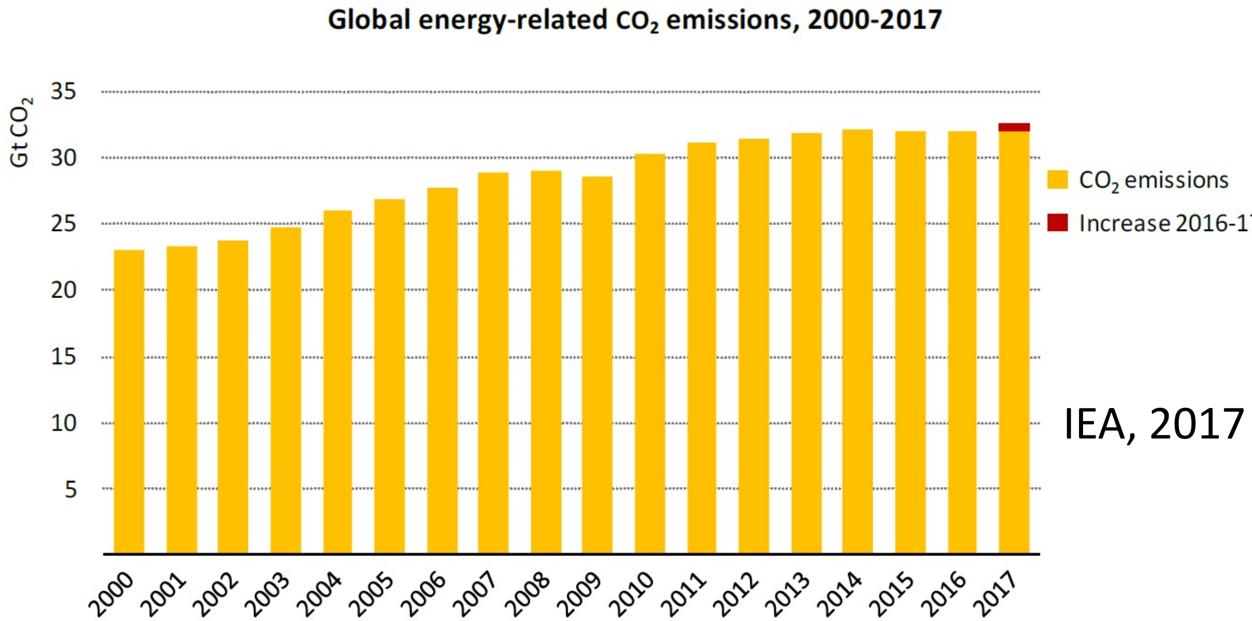
ECOMONDO

Sinergie tecnologiche e linee di sviluppo nel settore della bioeconomia in Italia
Mercoledì 7 Novembre 2018

Environmental crisis: CO_2 and GHG increase

Source:Google images

Fossil fuel combustion
(77% of the total emission)

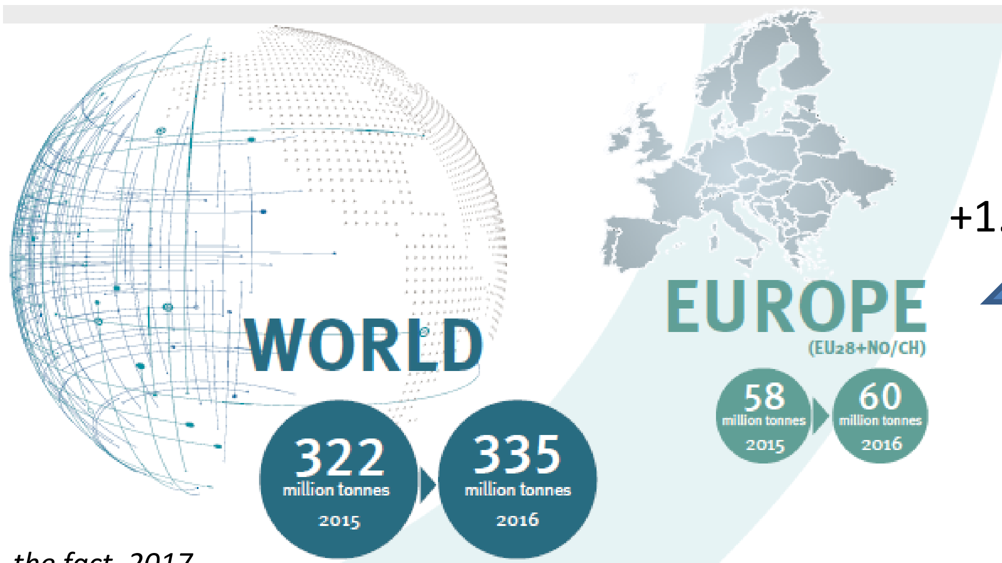


IEA, 2017

CO_2 emissions grew by 1.4% in 2017→historic total 32.5 Gt 2017

170 millions of additional cars

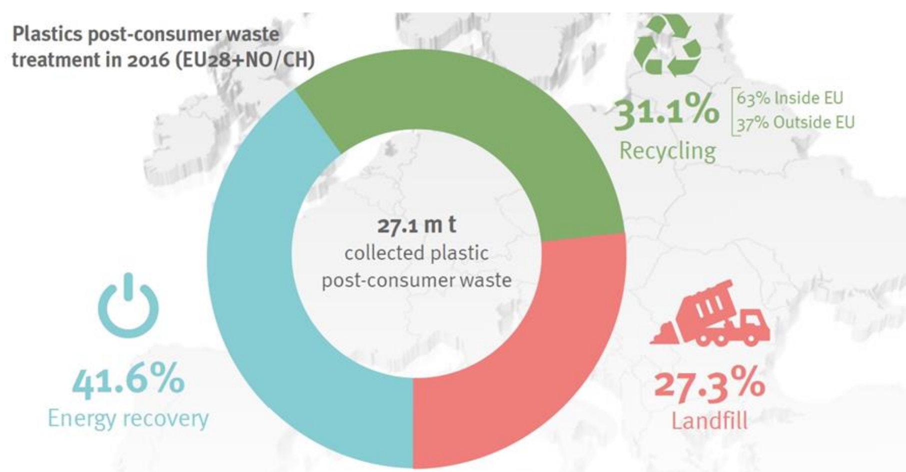
Environmental crisis: plastic production



Plastics, the fact, 2017

+1.5%

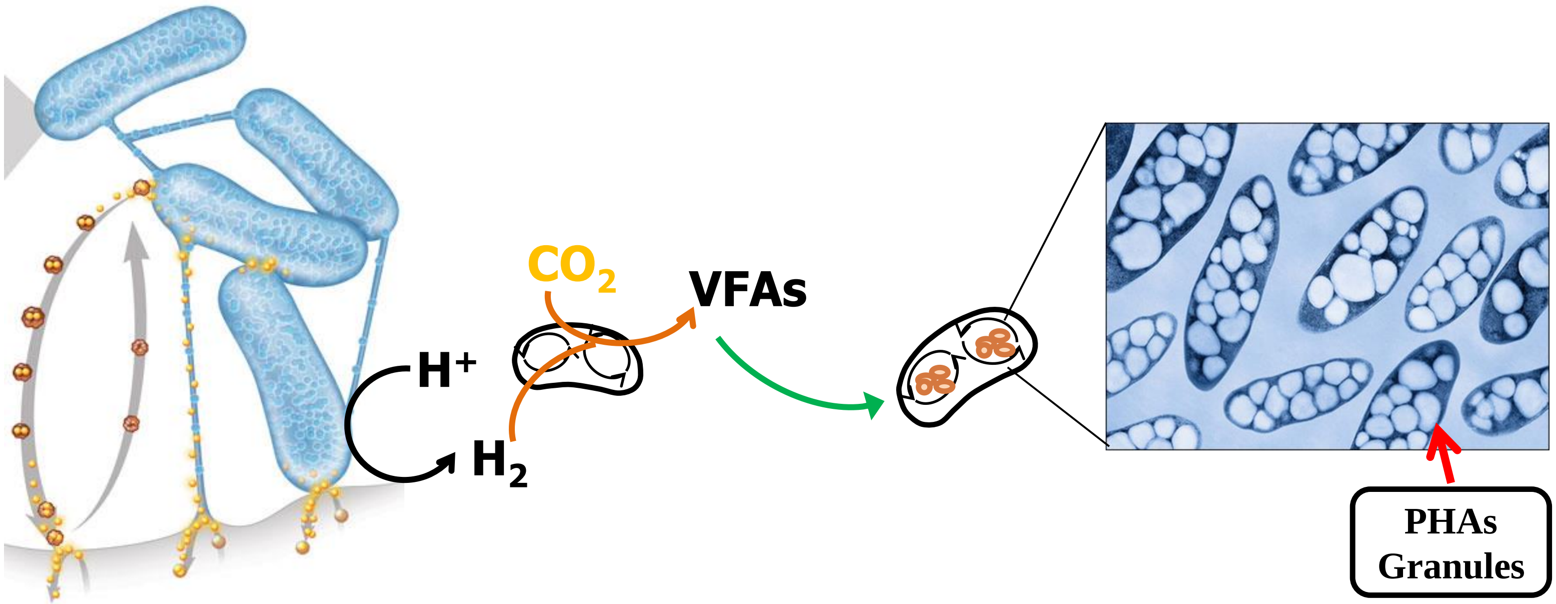
Source: Google images



Plastics, the fact, 2017

Possible solution:

culturing microorganism able to fix atmospheric CO_2 along with the microbial production of degradable biopolymers polydroxyalcanoates (PHAs)



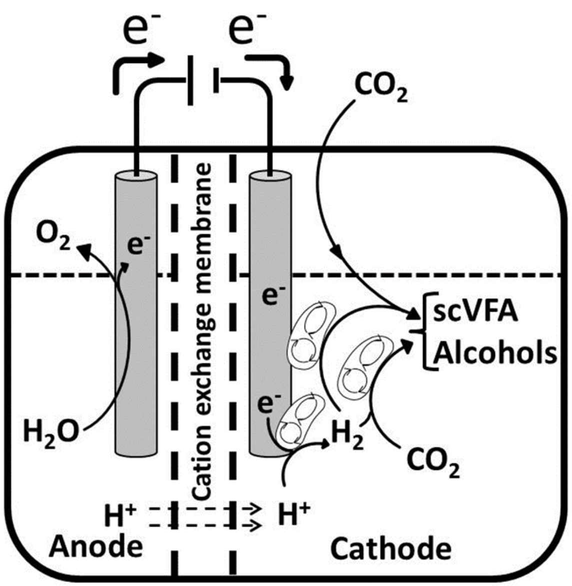
Source: www.microbiologiaitalia.it

Biological CO_2 fixation for biosynthesis by MES (Carboxydotrophic mixed cultures)

Biological VFAs conversion into P(3HB) by PHAs storing bacteria (MMC wwtp)

Microbial electrosynthesis:

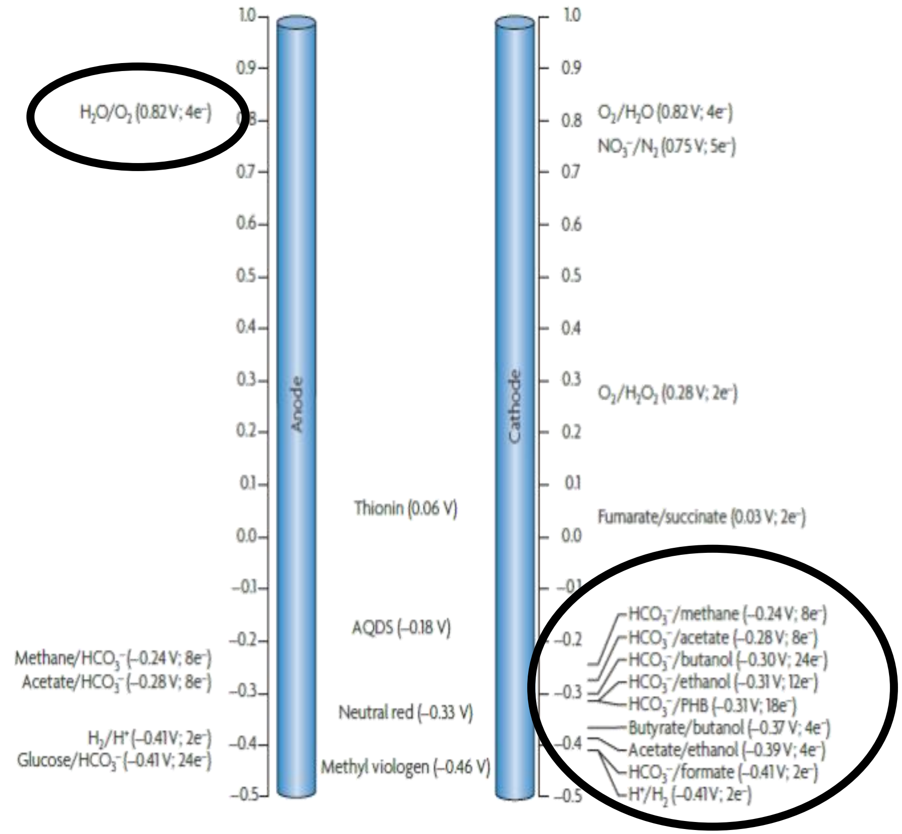
Electricity-driven reduction of CO_2 using microorganisms as electrocatalyst



Ecell < 0
ΔG > 0
Non spontaneous

Energy required

Rabaey and Rozendal, 2010. *Nature Rev Microbiol*

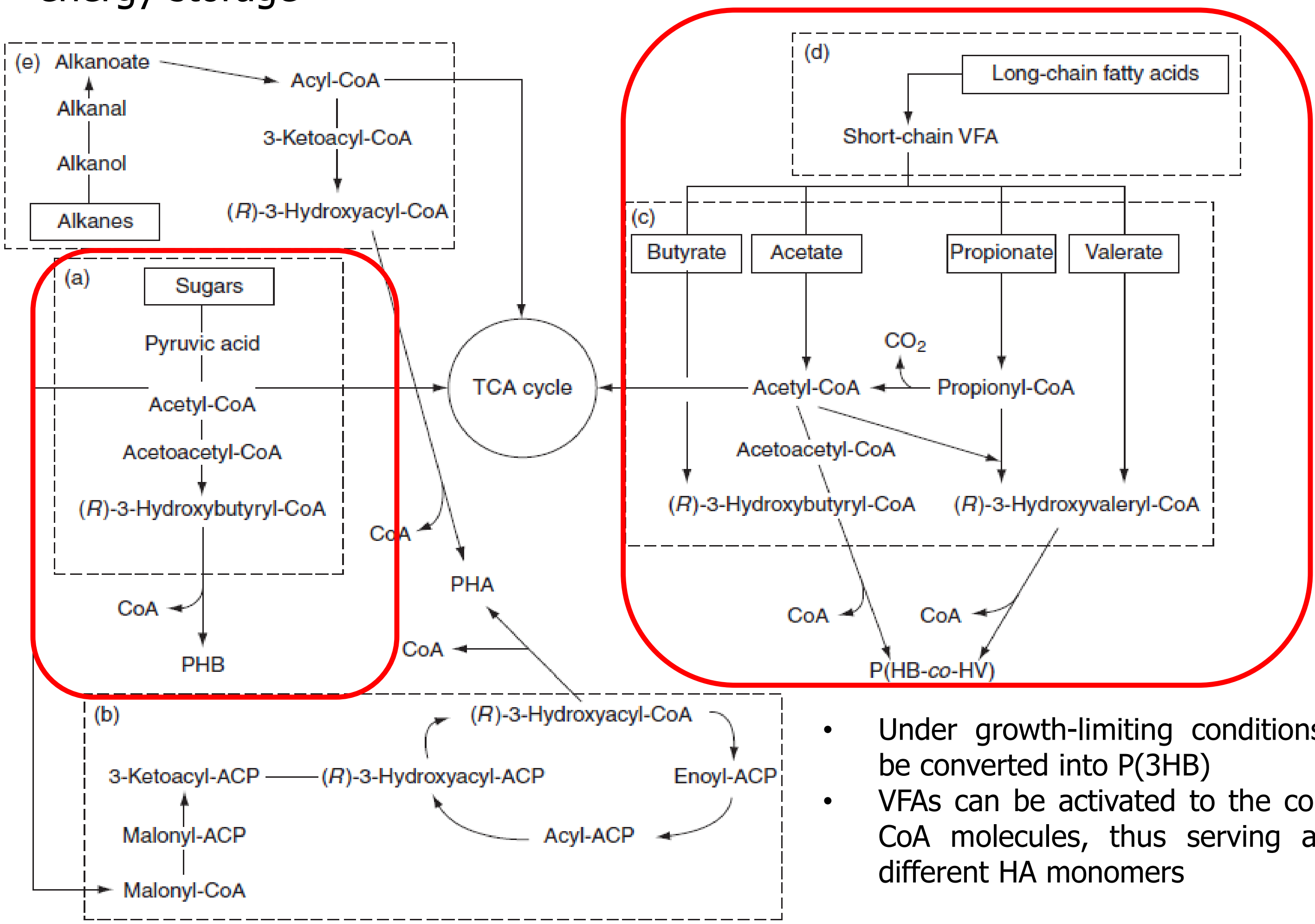


- Unlimited **reducing power**
- **Mitigation** and **valorisation of CO_2**
- Low **land usage**

Polyhydroxyalkanoates (PHAs)

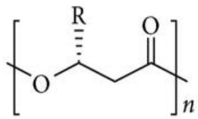
- Bio-based and biodegradable polyesters
- Synthesized by bacterial cells as carbon and energy storage

Reis et al., 2011. *Comprehensive biotechnology*



- Under growth-limiting conditions acetyl-CoA can be converted into P(3HB)
- VFAs can be activated to the corresponding acyl-CoA molecules, thus serving as precursors for different HA monomers

Polyhydroxyalkanoates (PHAs)



Poly(3-hydroxyalkanoates) [PHA]

R group		
$-CH_3$	Poly(3-hydroxyalkanoates)	PHA
$-CH_2$-CH_3	Poly(3-hydroxyvalerate)	PHV
$-(CH_2)_2-CH_3$	Poly(3-hydroxyhexanoate)	PHHex
$-(CH_2)_4-CH_3$	Poly(3-hydroxyoctanoate)	PHO
$-(CH_2)_6-CH_3$	Poly(3-hydroxydecanoate)	PHD
$-CH_2-$ 	Poly(3-hydroxy-5-phenylvalerate)	PHPV

	PHB	PHBHV$_{20\ \%}$	PP
Density (g/cm^3)	-	1.25	0.9
Glass transition T [°C]	+15	-1	-10
Melting T[°C]	175	145	176
Crystallinity [%]	80	42	70
Tensile strength [MPa]	40	30	38
Elongation at break [%]	8	50	40
Flexural strength [GPa]	3,5	1	-
Resistance to acids	1	1	4
Resistance to alkalis	1	1	4
Resistance to alcohols	2	2	4
Resistance to oil and fats	3	3	2/3
Resistance to UV	2	2	1

Chemical and UV resistance: 1= low, 2= medium, 3= good, 4= very good;
PHB= polyhydroxibutyrate; PHBHV= polyhydroxybutyrate-co-valerate; PP= polypropylene

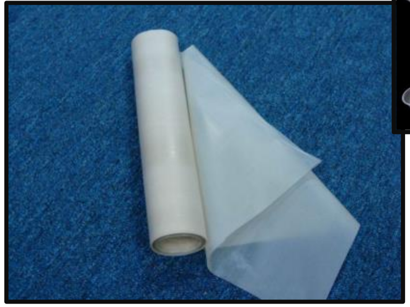
Valera, 2001 *Chimica & Industria*

Properties

- Thermoplastic polymers (145-175 °C)
- Water resistant
- Low oxygen permeability

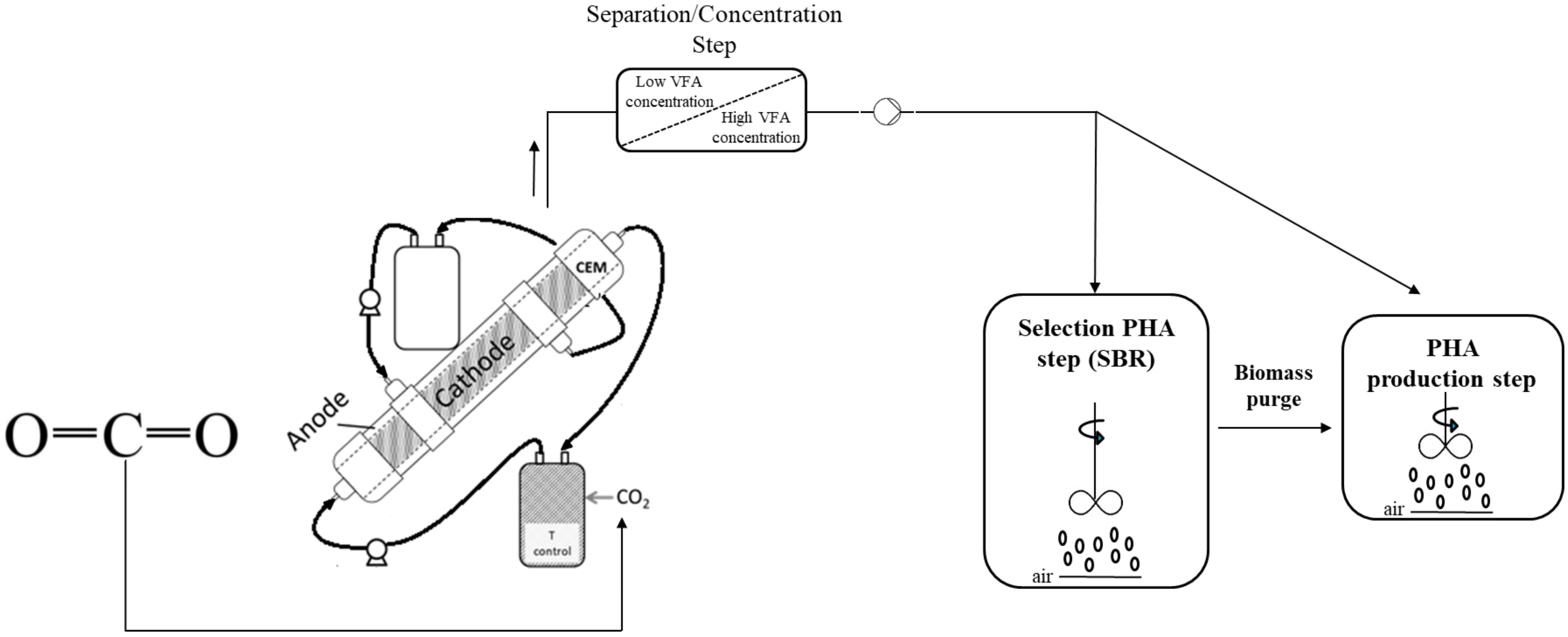
Applications

- Packaging and items
- Medical devices and drugs delivery carriers
- Environmental applications



Aims

- Coupling MES VFAs to PHAs production
- Stable PHA polymer production
- Evaluate CO_2 conversion efficiency and C balance



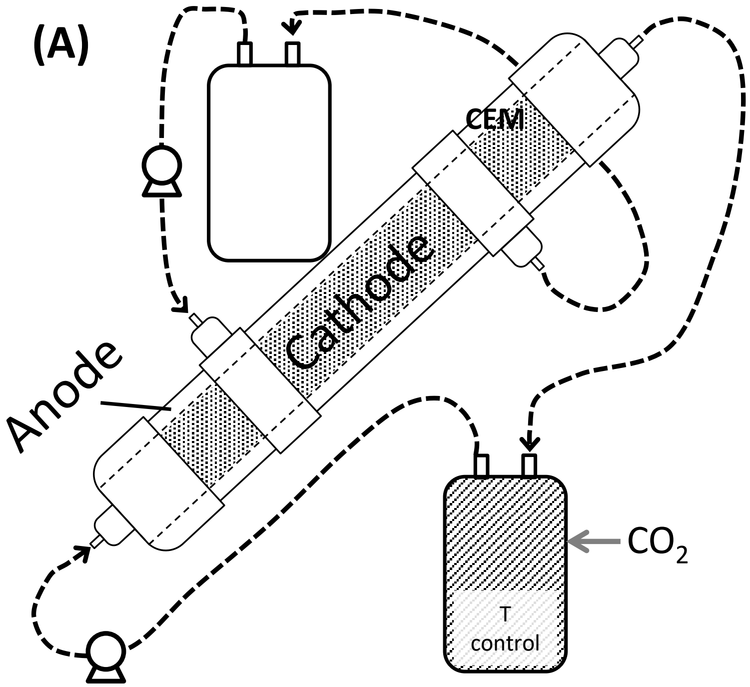
Pepè Sciarria et al., 2018. *Green Chem. 20, 4058-4066*

Setup and operation

MES: BES reactor and operation

Inoculum sources: Syngas fermentor
Cathode potential: -0,8 V vs SHE
Batch operation

Batlle-Vilanova et al. 2017, *Bioelectrochemistry*



anode & cathode connected to buffer tanks (recirculation 5.8 L h^{-1})

Cathode V=1.30 L
Anode V= 1.49 L
T=38 ± 1 °C.

MES: membrane extraction

Selective separation of Butyrate

Impregnated Hollow fibre membrane:
3M-S6/2 (Membrana, DE)
$Ø_{in}$= 180 µm; $Ø_{ext}$ = 980 µm
Porous: 0,2 µm

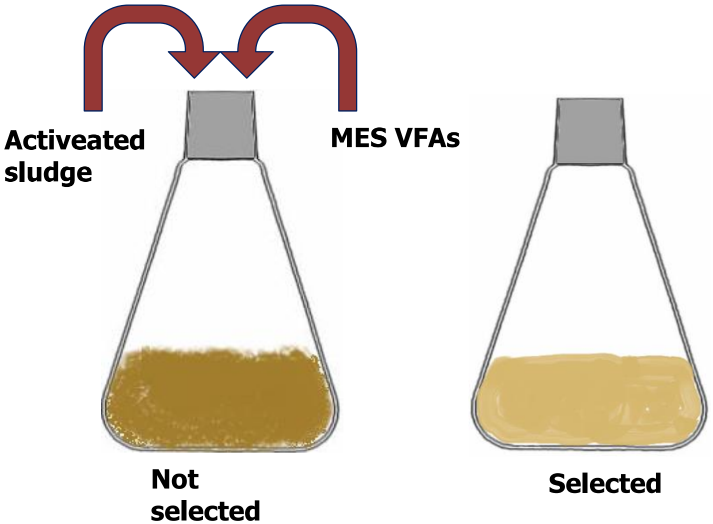
Impregnated with:
dodecane/dodecanol (6%, v/v)

Extracting phase: NaOH, 0.1M

Batlle-Vilanova et al. 2017, *Bioelectrochemistry*

PHA: MMC selection process

Select PHA storing bacteria from a mixed microbial culture (WWTP)



Operating conditions

-Cycle length 12 h

-HRT 1day

-C:N:P 100:10:1

-25 °C

-pH 8

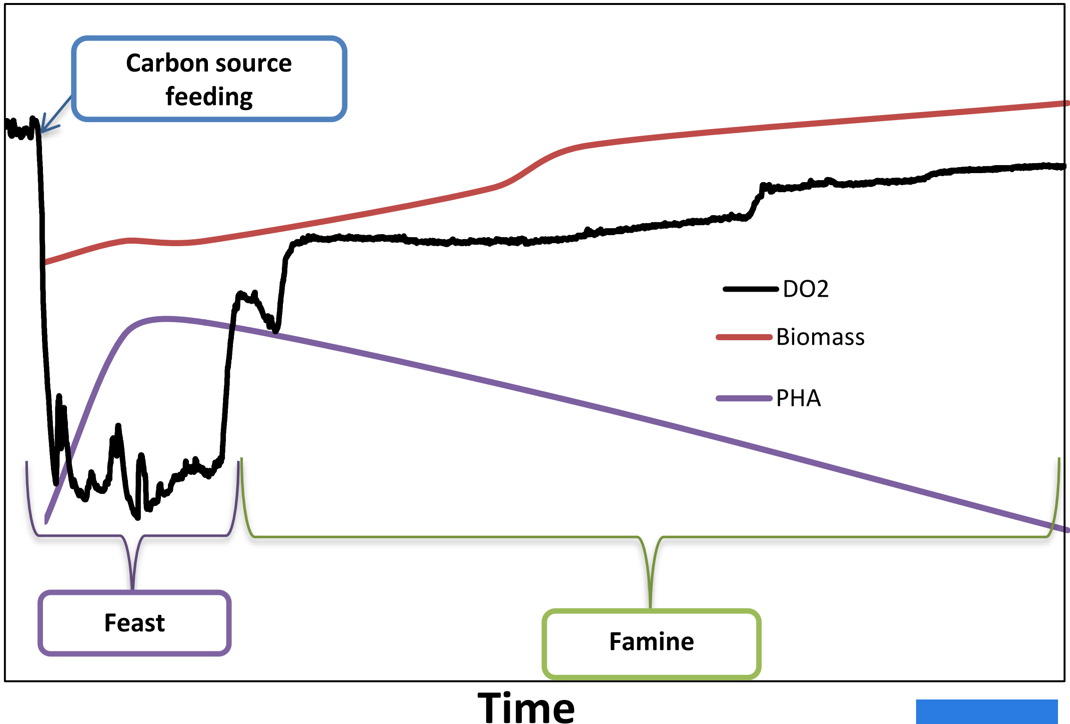
-Mechanical stirring

-Aeration

Method: aerobic dynamic feeding (feast famine alternation) controlled by Dissolved Oxygen (DO)

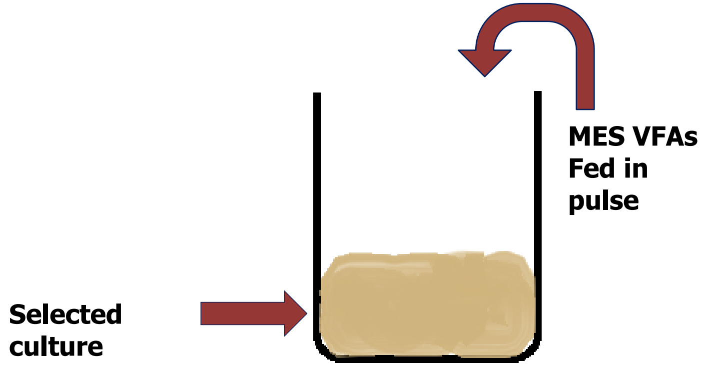
Duque et al., 2013

F/F <0.3

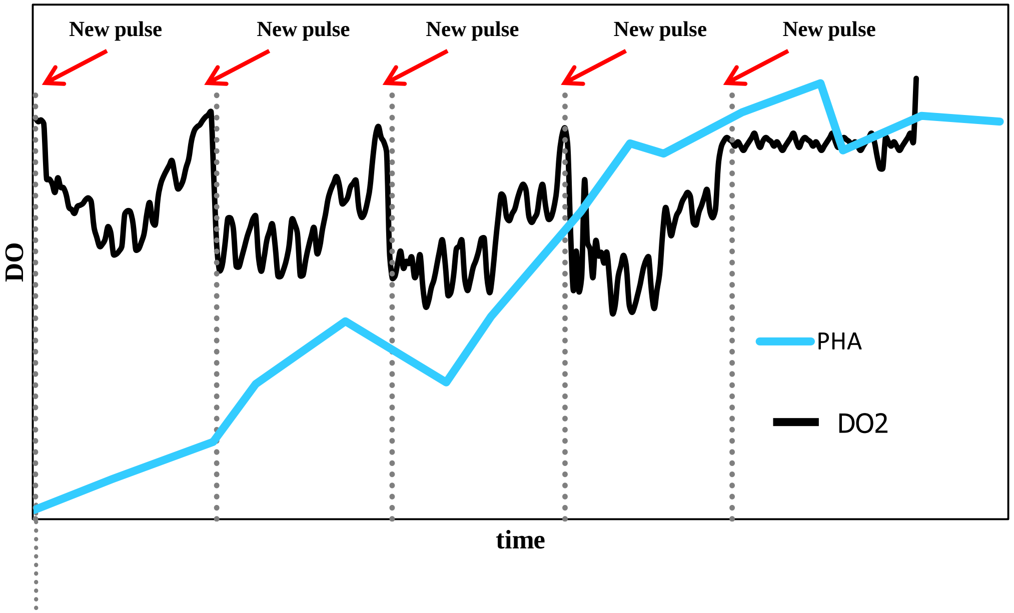


PHA:Accumulation process

Evaluate the maximum PHA storing capacity of the selected culture



Method: pulse wise feeding controlled by Dissolved Oxygen (DO)
Duque et al., 2013

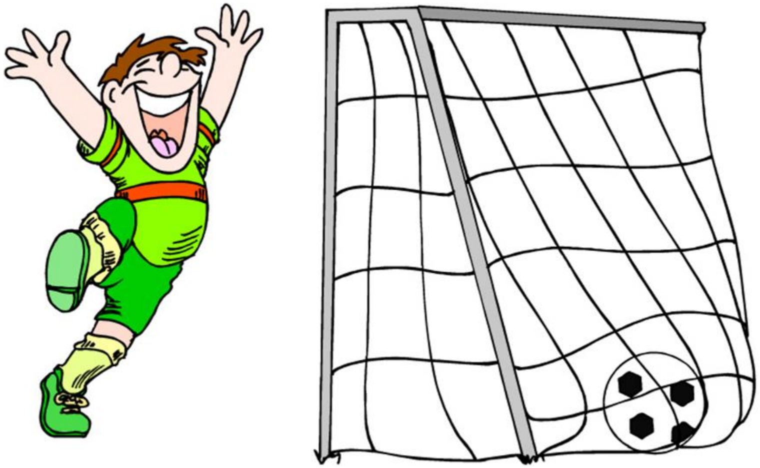


- **Operating conditions**

- 25 °C
- No Nitrogen source
- Mechanical stirring
- Aeration

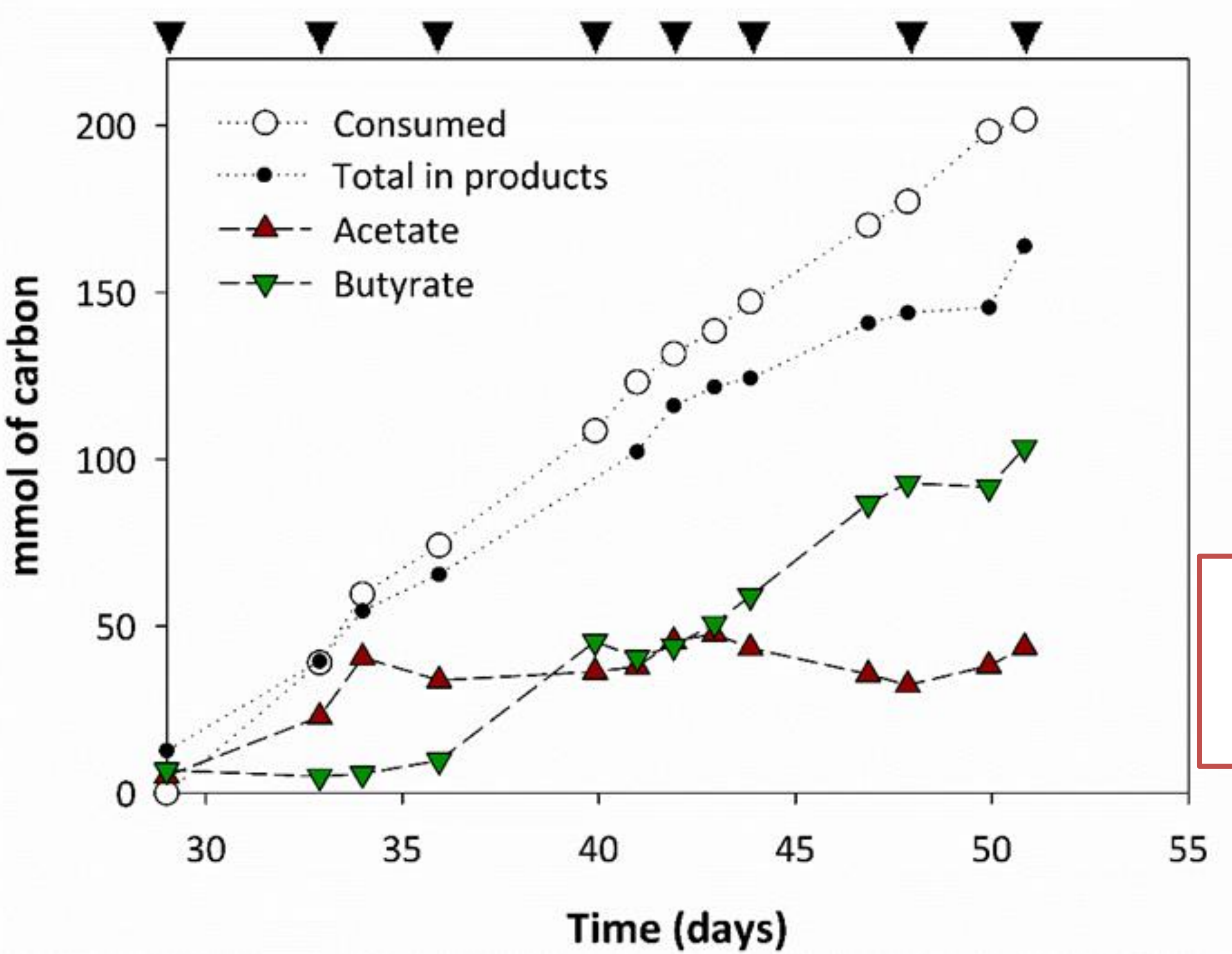
Selected culture

Accumulation



Results

MES: Butyrate and Acetate production



103 mmol $C_{Butyrate}$

39.5 mmol $C_{Acetate}$

73% on C_{cons}
51% on C_{in}

After membrane separation
400 mmol C L^{-1}
But:Ac→16:4

Pepè Sciarria et al., 2018. *Green Chem. 20, 4058-4066*

PHA: selection process results

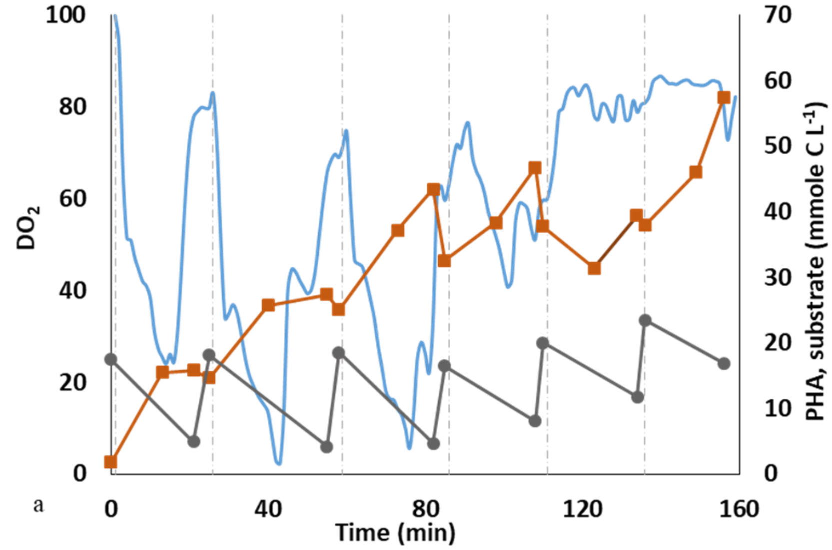
F/F < 0.14

Substrate	PHA content[a] (g PHA 100 g^{-1} VSS)	Polymer composition (ΔHB/ΔHV) (% w/w)	PHA Yield[a] (mmole C_{PHA} $mmole^{-1}$ $C_{cons.}$)	Growth Yield[b] (mmole C_X $mmole^{-1}$ $C_{cons.}$)	References
Acetate +Butyrate	19.3	100/0	0.79	0.2	This study
Acetate	42 ± 3	100/0	0.4 ± 0.0	0.25 ± 0.01	Colombo et al., 2016
Acetate	53	100/0	-	-	Johnson et al., 2009
Butyrate	14.5	100/0	0.45	0.3	Lemos et al., 2006
Acetate	17.5	100/0	0.49	0.3	Serafim et al., 2004

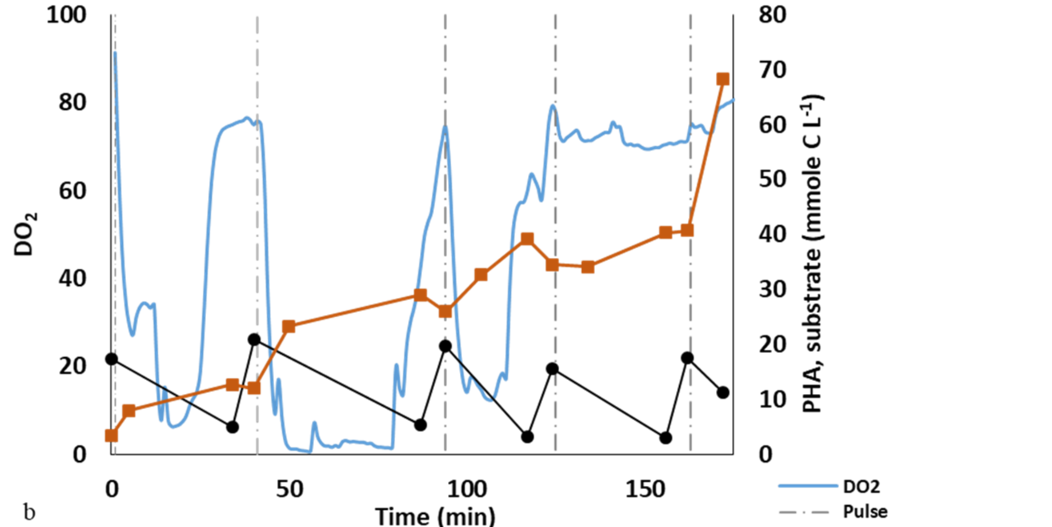
[a]PHA storage yield expressed on substrate consumed.
[b]Growth yield expressed on substrate consumed.

Pepè Sciarria et al., 2018. *Green Chem. 20, 4058-4066*

PHA: accumulation process trends



a



b

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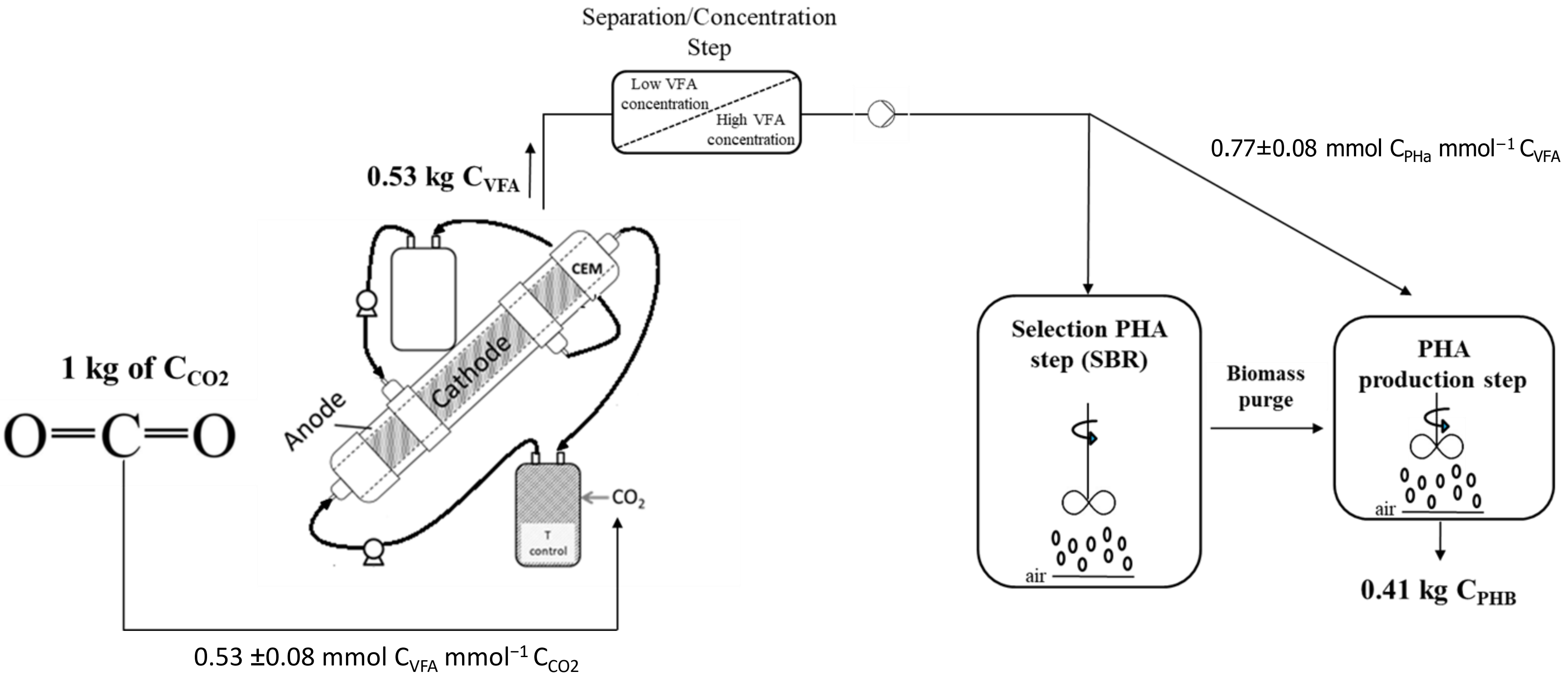
PHA: accumulation process results

Test	Substrate	PHA content[a] (g PHA 100 g^{-1} VSS)	Polymer composition (ΔHB/ΔHV) (% w/w)	PHA Yield[b] (mmole C_{PHA} $mmole^{-1}$ $C_{cons.}$)	PHA Yield[c] (mmole C_{PHA} $mmole^{-1}$ C_{in})	PHA Productivity (g PHA L^{-1} d^{-1})	Total Biomass (gVSS L^{-1})	References
Accumulation 1	Ac+But	74.44 ± 6	100/0	0.87 ± 0.19	0.66 ± 0.17	15.51 ± 4.55	1.51±0.05	This study
Accumulation 2	Ac +But	69.01 ± 4.1	100/0	1.12 ± 0.20	0.77 ± 0.18	11.35± 4.03	1.37±0.04	This study
Accumulation	Ac	82.4 ± 5.8	100/0	0.68 ± 0.04	-	0.9	0.54	Colombo et al.,2016
Accumulation	Ac	89 (g PHA 100 g^{-1} TSS)	100/0	-	0.6	-	-	Johnson et al.,2009

Comparison between PHA accumulation trials performed in this study and tests reported in literature
[a]PHA accumulated at the end of the test referred to VSS.
[b]PHA storage yield expressed on substrate consumed.
[c]PHA storage yield expressed on substrate fed.

Carbon balance



Pepè Sciarria et al., 2018. *Green Chem. 20, 4058-4066*

Conclusions

- Stable production of Butyrate and Acetate
- Stable PHA polymer production (100% P-(3-hydroxybutirate))
- High C recovery
- New possible bio-refinery concept

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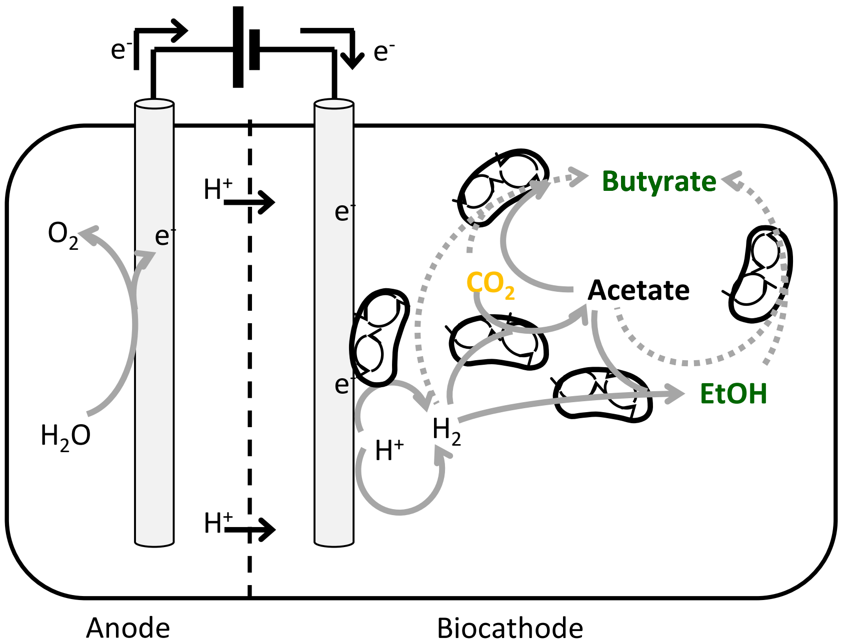
Thank you for your attention!

myepicideas.wordpress.com

MES: VFAs production steps

***Clostridium kluyveri*:**

Chain elongation (acetate + ethanol)



Bioelectrochemical H_2 trigger for the subsequent reactions where CO_2 and H_2 would initiate a series of reactions, consisting of:

1. homoacetogenesis of CO_2 and H_2 to acetate
2. reduction of acetate to ethanol at the expenses of H_2
3. chain elongation (reverse β-oxidation) of acetate and ethanol into butyrate

Ganigué et al., 2015. *Chem Comm*
Agler et al., 2011. *Trends Biotechnol*
Batlle-Vilanova et al., 2017. *Bioelectrochemistry*