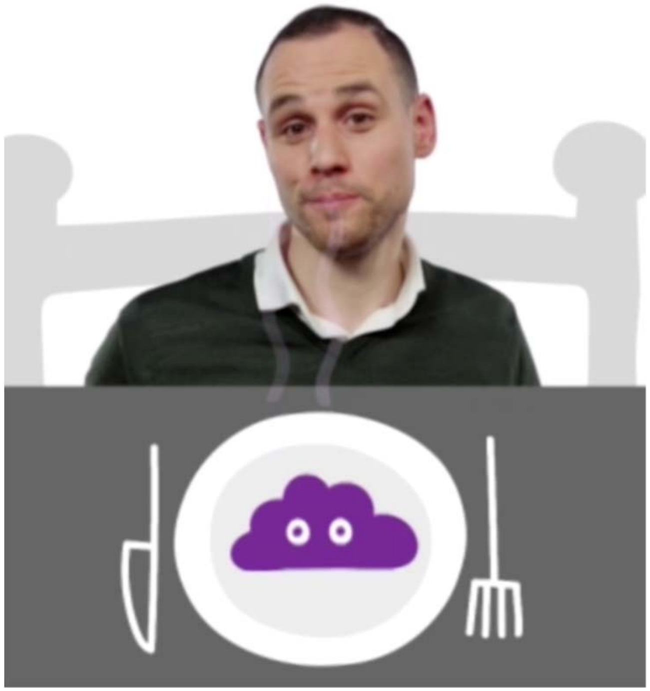


Screening **purple bacteria** for their growth kinetics on **volatile fatty acids**: paving the way for efficient production of **edible biomass** on fermented waste

Siegfried E. Vlaeminck & Abbas Alloul

Sustainable Energy, Air & Water Technology
University of Antwerp

Life support in Space: from linear to circular

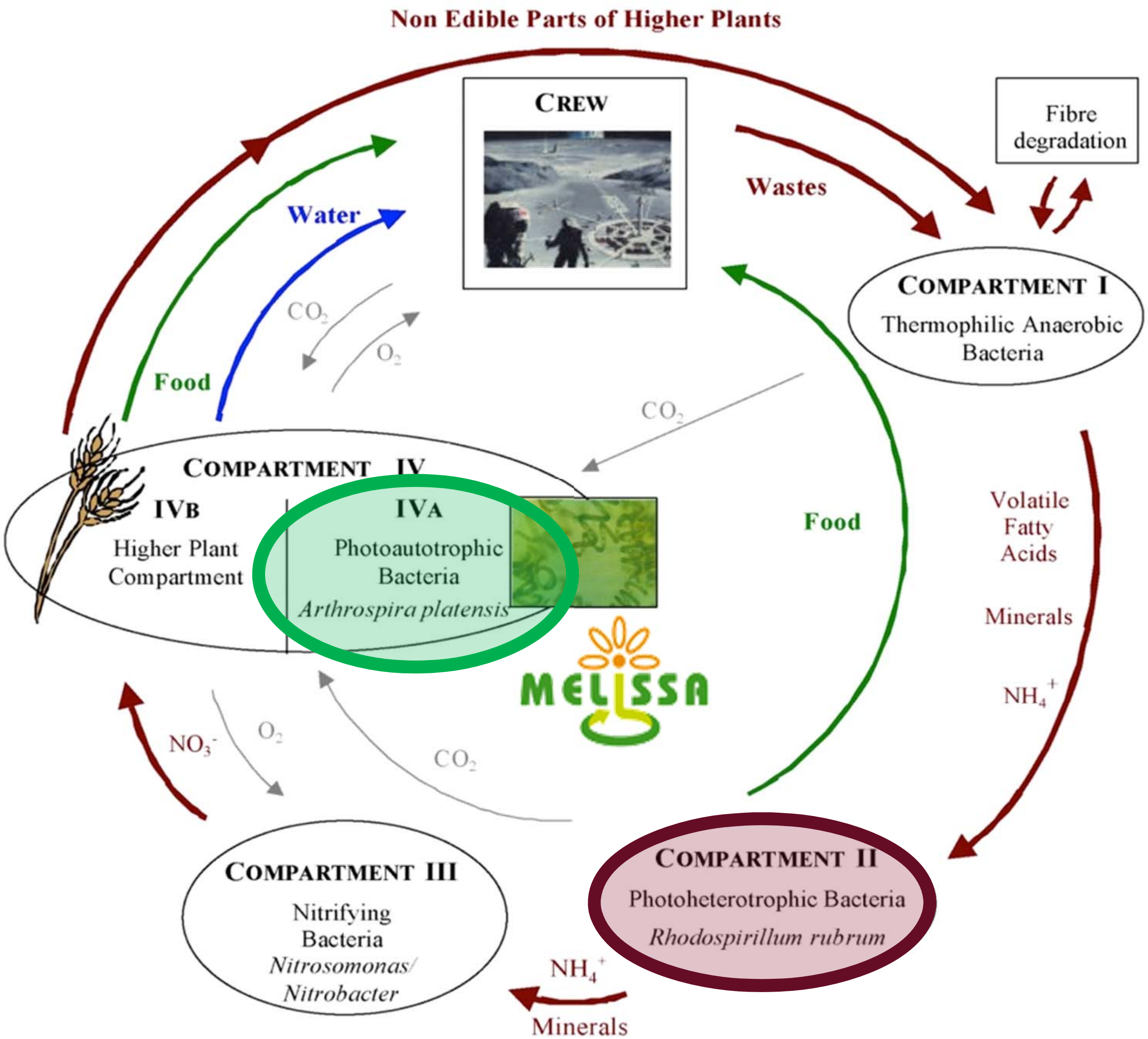
TODAY

~400 km

TOMORROW

(modified after Christophe Lasseur)

MELiSSA Cycle



Food production:

- Plants
- Microbes: single-cell protein
  - **CII: heterotrophic**
  - **CIVa: autotrophic**

(http://www.esa.int/Our_Activities/Space_Engineering_Technology/Melissa)

CII: Conversion of volatile fatty acids to …

- **Microbial biomass**
  - Single cell protein => Food for crew
- Inorganic carbon **CO_2**

Convert organic carbon by heterotrophic microorganisms

O_2 → CO_2

Photoheterotrophic
- Purple non-sulfur bacteria (PNSB)
- Photo-assimilation of carbon
- Biomass yield >1 $gC_{biomass}/gC_{removed}$

Chemoheterotrophic
- Aerobic heterotrophic bacteria
- Oxidation of carbon
- Biomass yield 0.3-0.5 $gC_{biomass}/gC_{removed}$

Rhodospirillum rubrum: historic choice for CII

- ***Rsp. rubrum*** consumes **broad spectrum of organics**
- What about the **ALiSSE criteria** the multi-parametric approach to evaluate and compare RLSS:
  - **High efficiency**
  - **Low mass**
  - Low energy
  - High safety
  - Few crew time

Carbon sources:	*Alphaproteobacteria* – *Rhodospirillales*: ***Rhodospirillum rubrum***	*Rhodospirillum photometricum*	*Phaeospirillum fulvum*	*Rhodopila globiformis*	*Roseospira mediosalina*	*Rhizobiales*: *Rhodomicrobium vannielii*	*Rhodobium orientis*	*Rhodoplanes roseus*	***Rhodopseudomonas palustris***	*Rhodopseudomonas julia*	*Rhodoblastus acidophilus*	*Blastochloris viridis*	*Rhodobacterales*: ***Rhodobacter capsulatus***	*Rhodobacter azotoformans*	*Rhodobacter blasticus*	***Rhodobacter sphaeroides***	*Rhodobacter veldkampii*	*Betaproteobacteria* – *Rhodocyclales and Burkholderiales*: *Rhodocyclus purpureus*	*Rubrivivax gelatinosus*	*Rhodocyclus tenuis*	*Rhodoferax fermentans*	*Rhodoferax antarcticus*
Acetate	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspartate	+	±	±	-	+	-	nd	-	±	+	-	nd	±	nd	nd	nd	+	-	-	+	+	+
Benzoate	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
Butyrate	+	+	+	-	+	+	+	+	+	+	±	-	+	+	+	+	+	+	+	±	+	+
Caproate	+	-	+	-	nd	+	+	-	+	-	±	-	+	nd	nd	+	+	+	+	nd	-	-
Caprylate	nd	-	+	-	-	+	-	-	+	-	-	nd	+	-	nd	+	+	-	±	nd	-	-
Citrate	-	-	-	-	-	-	-	+	±	-	±	-	±	nd	+	+	-	-	-	+	-	+
Ethanol	+	+	+	+	-	+	-	-	±	-	+	+	-	nd	-	+	-	-	±	+	±	-
Formate	-	-	nd	-	-	±	-	-	+	+	±	-	+	+	-	-	-	-	-	±	-	-
Fructose	±	+	-	+	-	-	+	-	±	+	-	-	+	+	+	+	-	-	-	+	+	+
Fumarate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	-	+	±	+	-	-	+	-	±	+	±	±	+	+	+	+	+	-	-	+	+	+
Glutamate	+	-	nd	-	+	-	±	-	+	-	-	+	+	+	+	+	+	-	-	+	+	-
Glycerol	-	+	-	-	-	±	-	-	+	+	±	-	-	+	+	+	-	-	-	-	-	-
Lactate	+	+	-	-	+	+	+	+	+	nd	+	±	+	+	+	+	+	-	+	+	±	+
Malate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	-	+	-	-	±	-	±	+	-	±	±	+	+	+	-	-	-	-	+	-
Methanol	±	-	±	-	-	±	-	-	±	-	±	-	-	-	-	±	-	-	-	±	-	-
Propionate	+	±	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-	±	±	-	-
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Succinate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Tartrate	-	-	nd	+	-	-	-	+	-	-	±	±	-	-	-	+	-	-	-	±	-	nd
Valerate	+	+	+	-	-	+	+	+	+	+	+	-	+	nd	nd	+	+	-	+	+	nd	-

(Bergey's Manual of Systematic Bacteriology Volume Two)

Study objectives

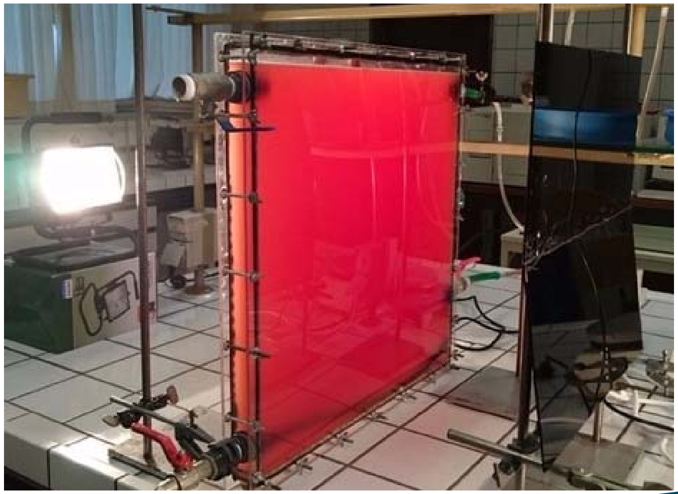
Part 1: Batch growth

- Map the effect of VFA on growth rate and uptake profile for purple bacteria
- Select strain or community for microbial protein production

Part 2: Continuous growth

- Optimize operational conditions of a photobioreactor to maximize protein production

Part 3: Terrestrial valorization of PNSB



Batch growth: Experimental variations

Carbon source: Volatile fatty acids (VFA)

1. C2: Acetic acid
2. C3: Propionic acid
3. C4: Butyric acid
4. C2/C3/C4: VFA mix (1/1/1 C-ratio)

Purple bacteria species/communities

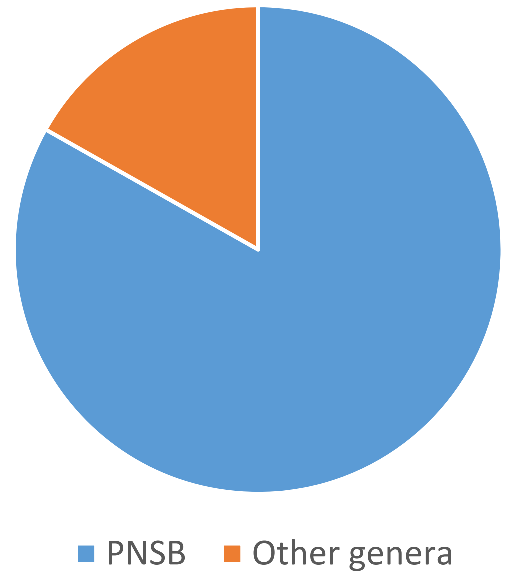
1. *Rhodospirillum rubrum*
2. *Rhodopseudomonas palustris*
3. *Rhodobacter sphaeroides*
4. Synthetic community, SynC (1/1/1 VSS ratio of *Rsp. rubrum*, *Rps. palustris* and *Rba. sphaeroides*)
5. Purple bacteria enrichment community:
   - 1/1/1 VSS ratio of sewage activated sludge (Aquafin), sediment of local pond and dairy activated sludge
   - Enriched under IR light (filter) with VFA mix (1/1/1 C-ratio)

Credits to Maarten Muys UAntwerpen

Batch growth: Microbial composition of purple bacteria enrichment Community

MiSeq Illumina

- V4 region 16S rDNA
- Amplification 233 bp

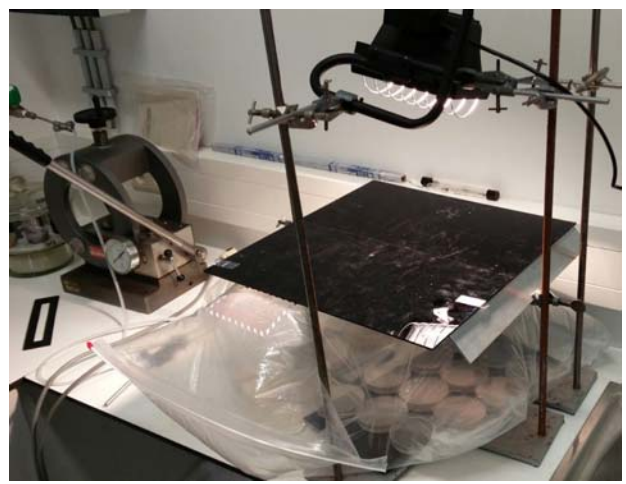


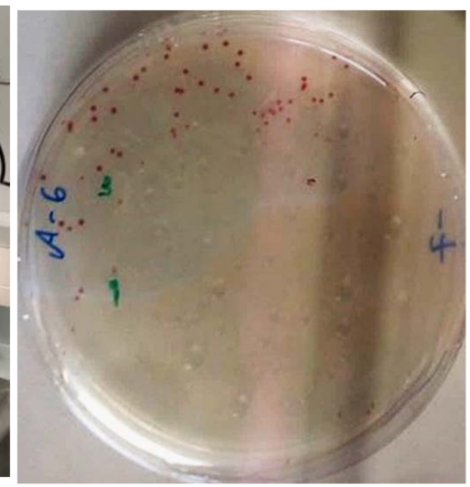
Enrichment success!

- Enrichment conditions selective for PNSB
- OTU of PNSB dominated the microbial community

Dilution to extinction

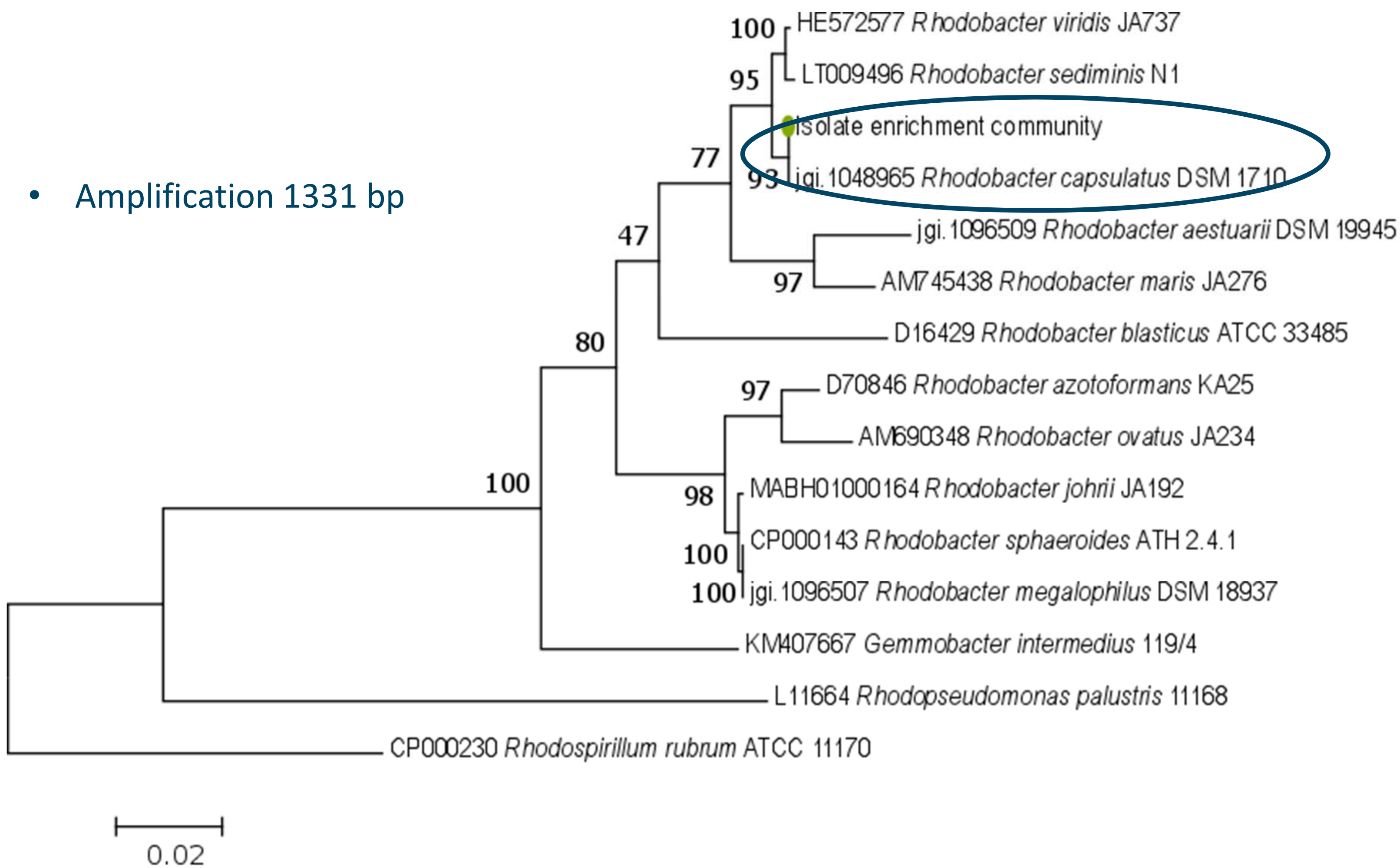
Isolation dominant species =>





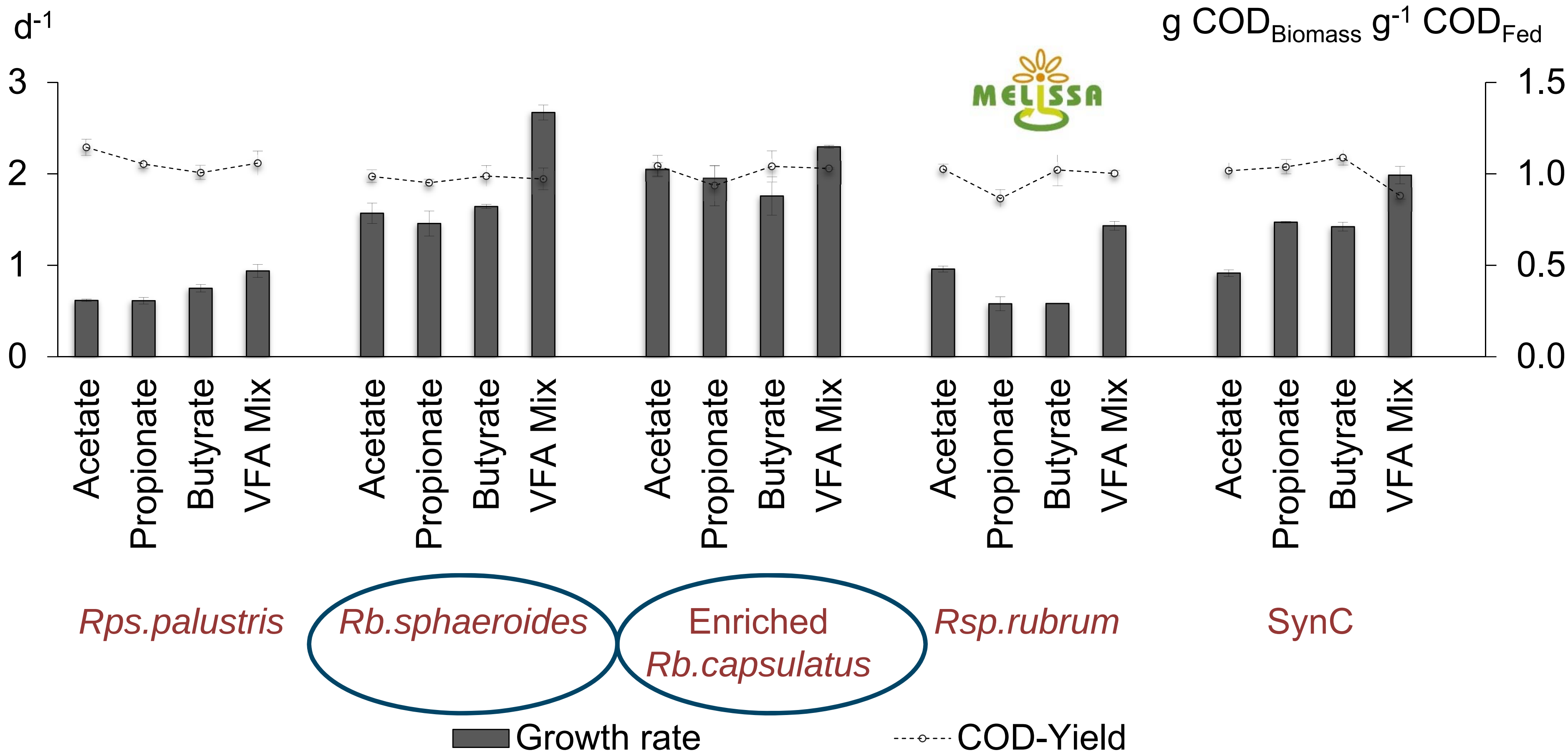
Batch growth: Characterization of isolate - Sanger sequencing and Phylogenetic tree

- Amplification 1331 bp



Rhodobacter capsulatus is most dominant species

Batch growth: Effect of carbon on growth rate and biomass yield

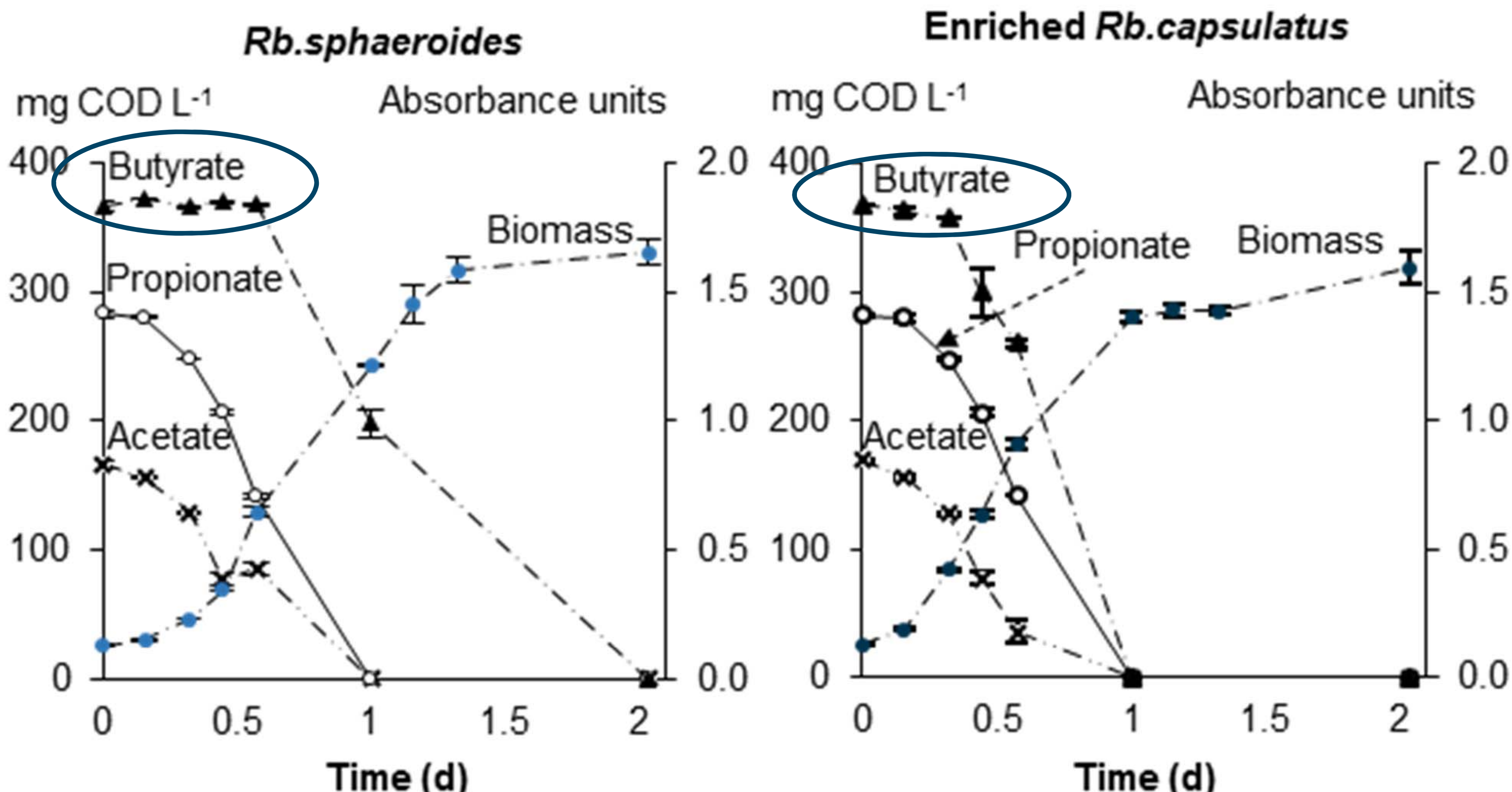


Growth rates => Select species based growth rate vs. carbon source for **MELiSSA**

- VFA mix **boosts** the growth rate for all strains/communities
- **Max µ** for *Rba.capsulatus* enrichment community and *Rba. sphaeroides* (VFA mix)

Full usage of COD: ~ 1 g COD to biomass/g COD removed **in line with literature**

Batch growth: Preferential carbon uptake for *Rhodobacter*



- **Lag of butyrate** for both enrichment community and *Rhodobacter sphaeroides*
- Do not overdose with synthetic medium
- Same observation for ***Rsp.rubrum*** experiments of De Meur (UMons, 2017)

Study objectives

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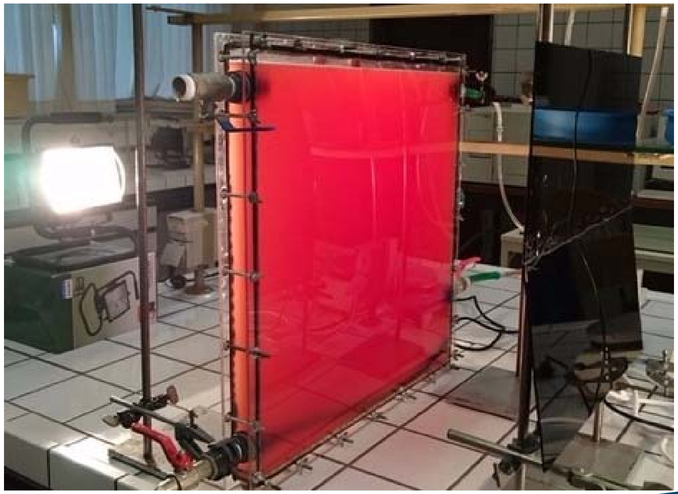
Part 2: Continuous growth

- **Optimize operational conditions of a photobioreactor to maximize protein production**

Part 3: Terrestrial valorization of PNSB



Continuous growth: Operation of a photobioreactor

Goal

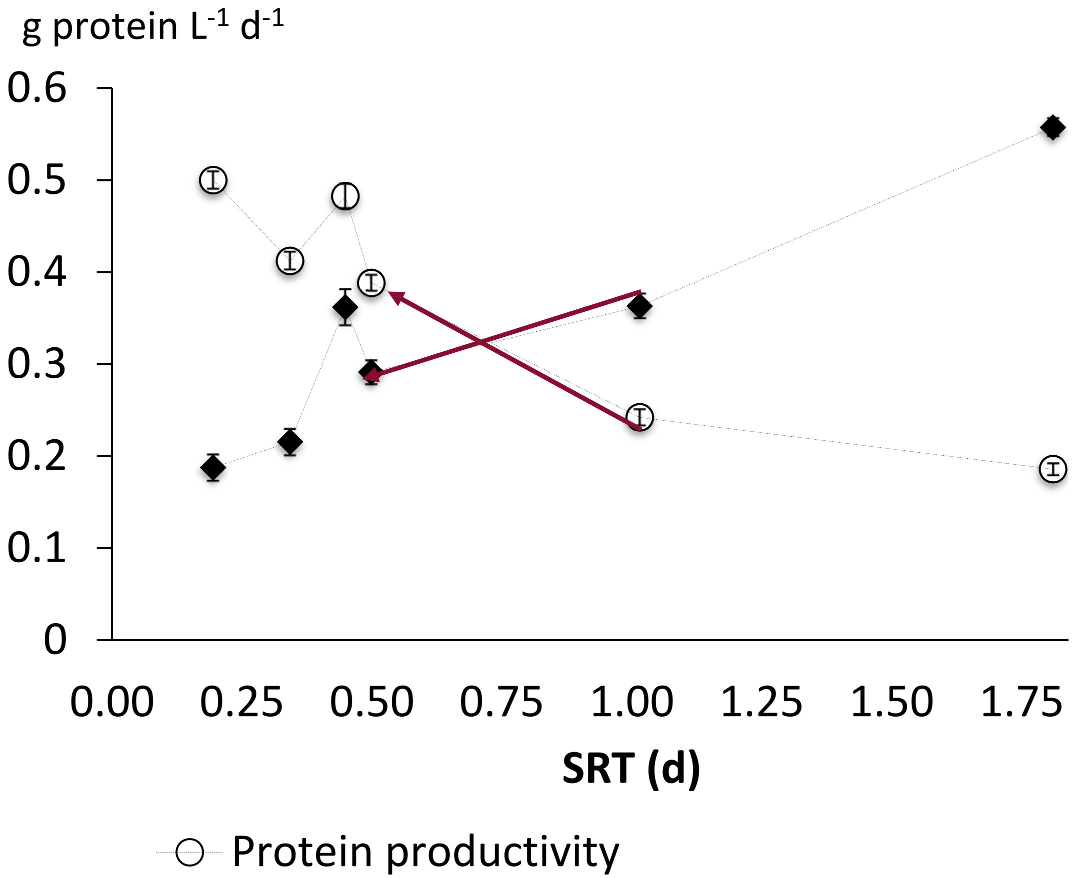
- Optimize operational conditions of a photobioreactor to maximize protein production
- Operate reactor at 6 different SRT 2 d, 1 d, 0.5 d (2 times) , 0.38 d and 0.25 d

$$SRT(d) = \frac{Volume\ reactor\ (m^3)}{Flow\ rate\ (\frac{m^3}{d})}$$

Hypotheses

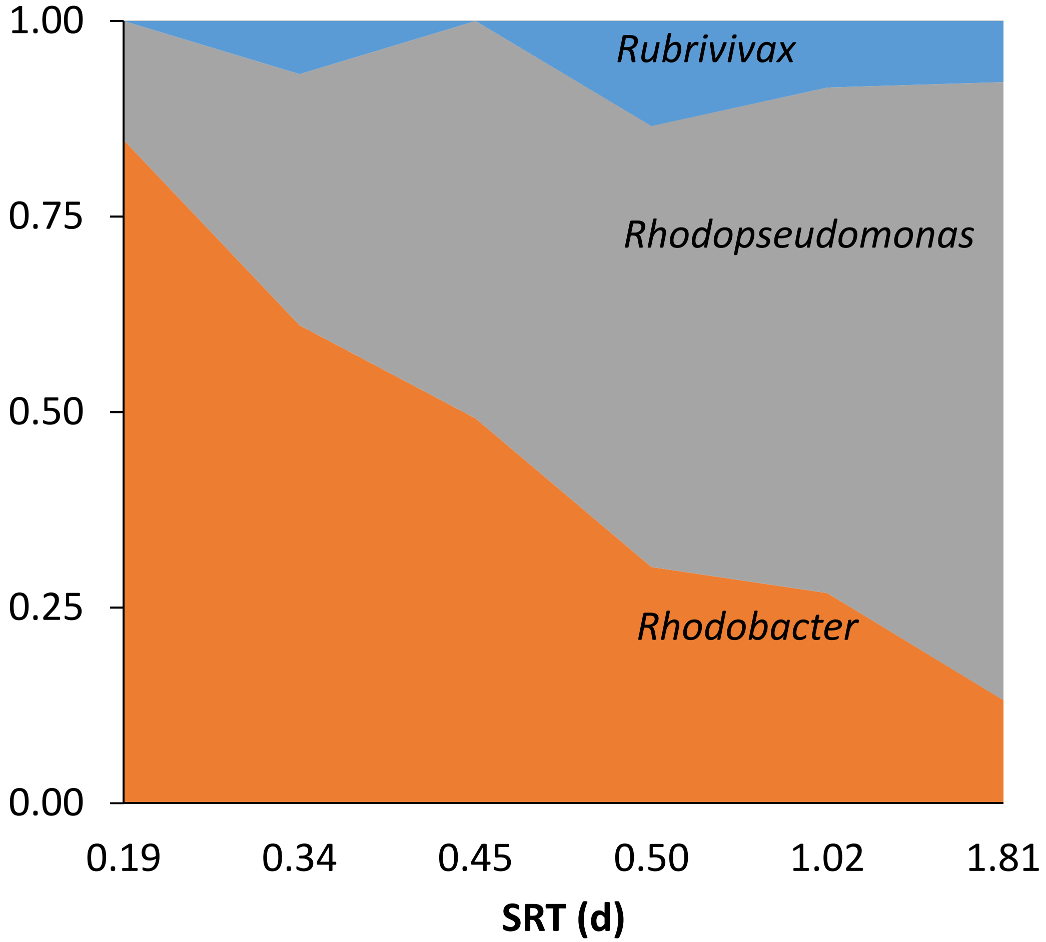
- Shorter SRT
  - Will increase the protein productivity
  - Result in a more dominant PNSB community

Continuous growth: Effect of sludge retention time on protein productivity and protein concentration



- Protein **productivity** increased with shorter SRT (no clock form observed), yet **trade of between** harvesting => **economic** analysis required

Continuous growth: Change in microbial community during reactor operation



- ***Rhodopseudomonas*** dominant at long SRT
- ***Rhodobacter*** at short SRT

Study objectives

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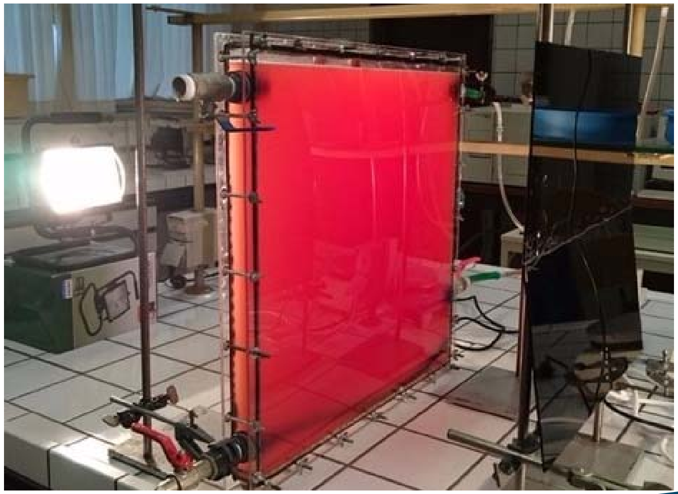
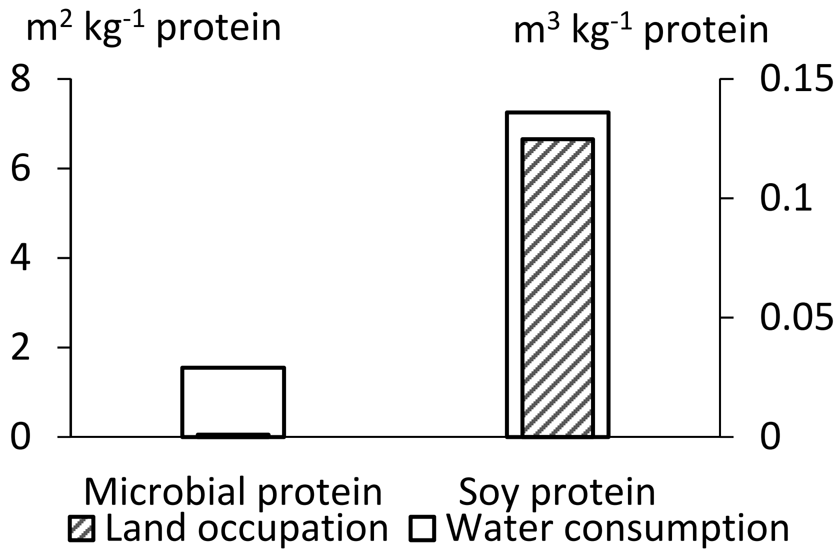
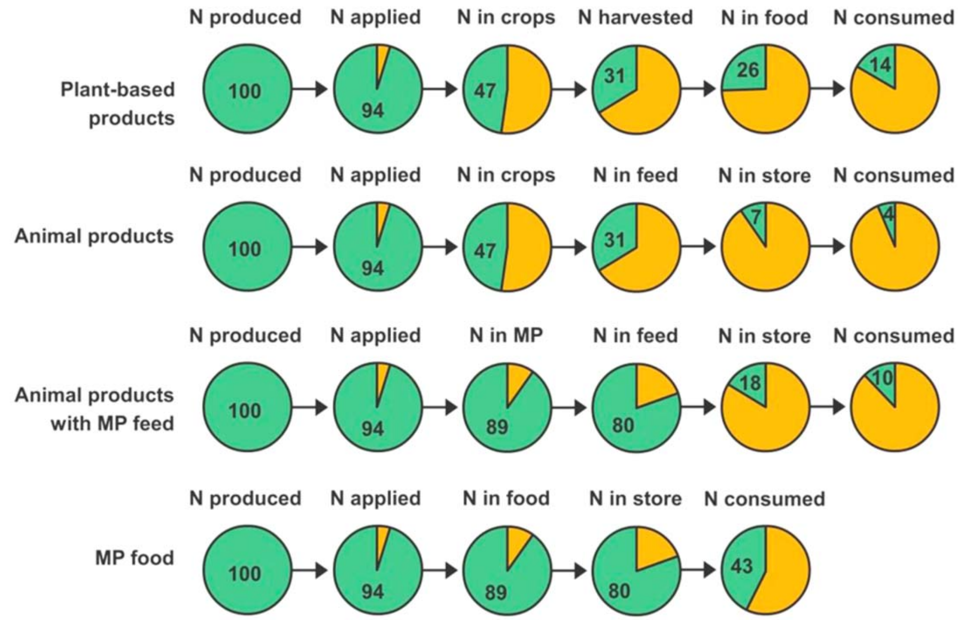


Sustainability advantages of microbial protein (MP): **Water, space and fertilizer** efficiency





5X less water 128X less arable land

2.5-11X more nutrient-efficient than animal based products

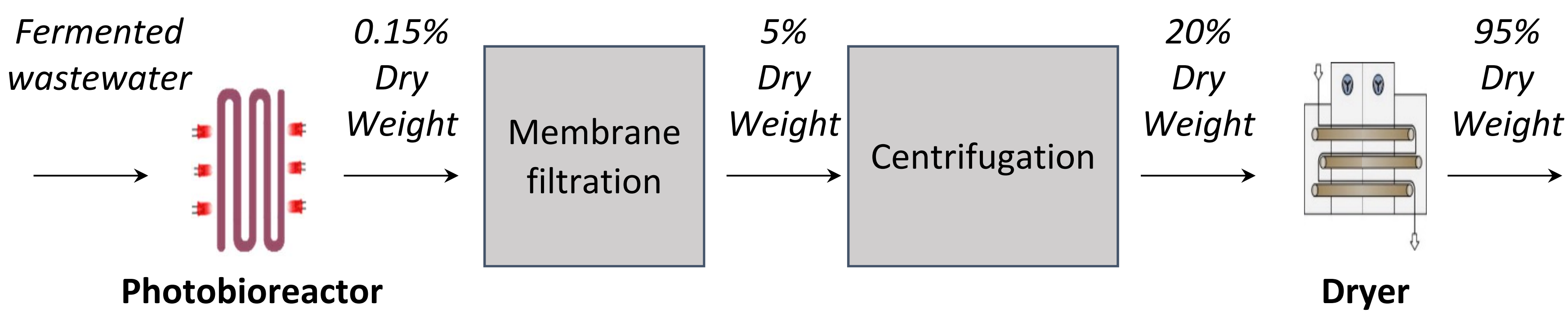
(based on Matassa et al., 2016)

(Pikaar et al., 2017)

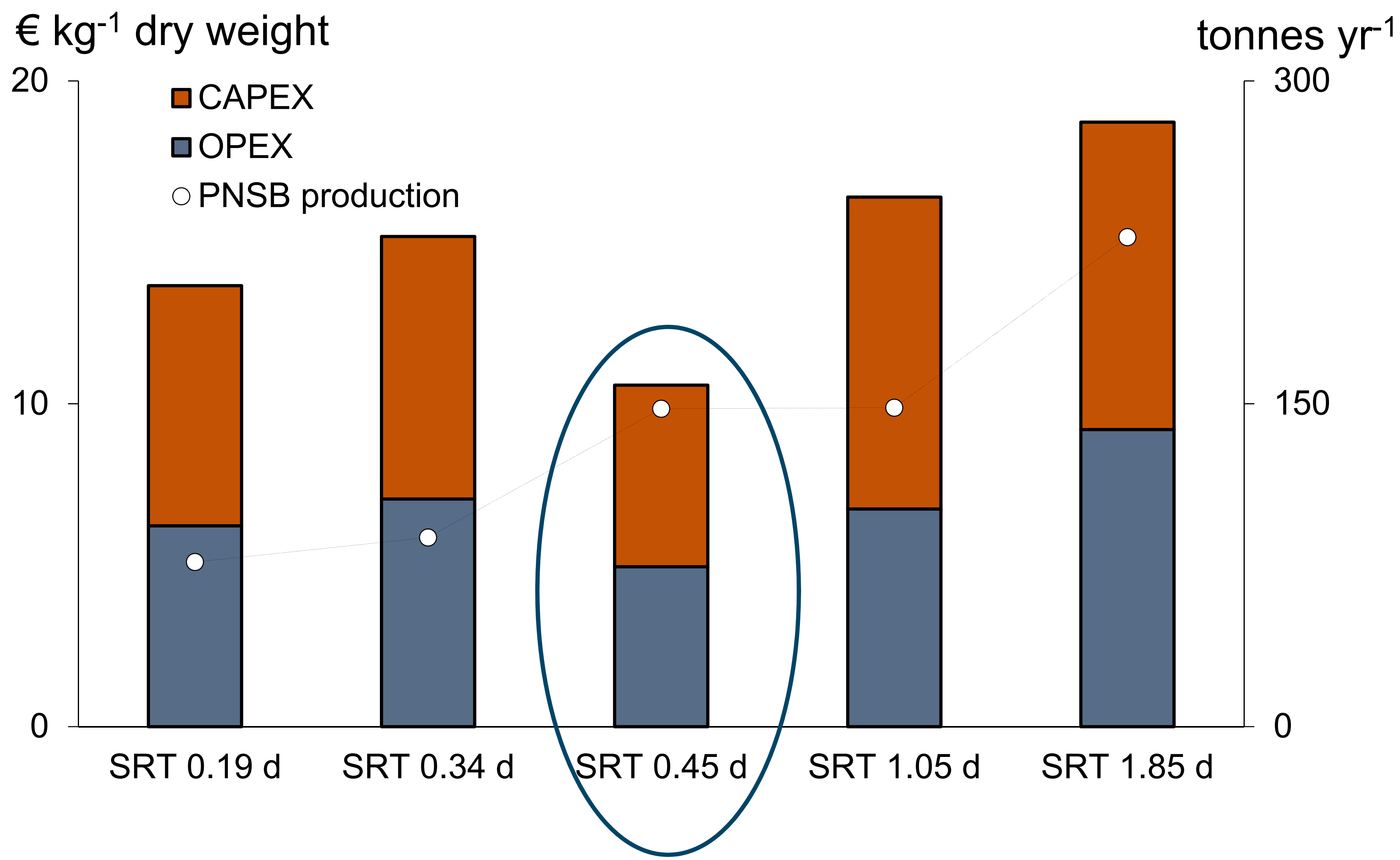
Terrestrial valorization: Economic assessment for PNSB

Assumptions

- Fermented brewery wastewater
- Tubular photobioreactor intermittent (1sec on/ 1sec off) with infrared LED 810 nm
- Variables: SRT and final dry weight concentration reactor (results experiments)

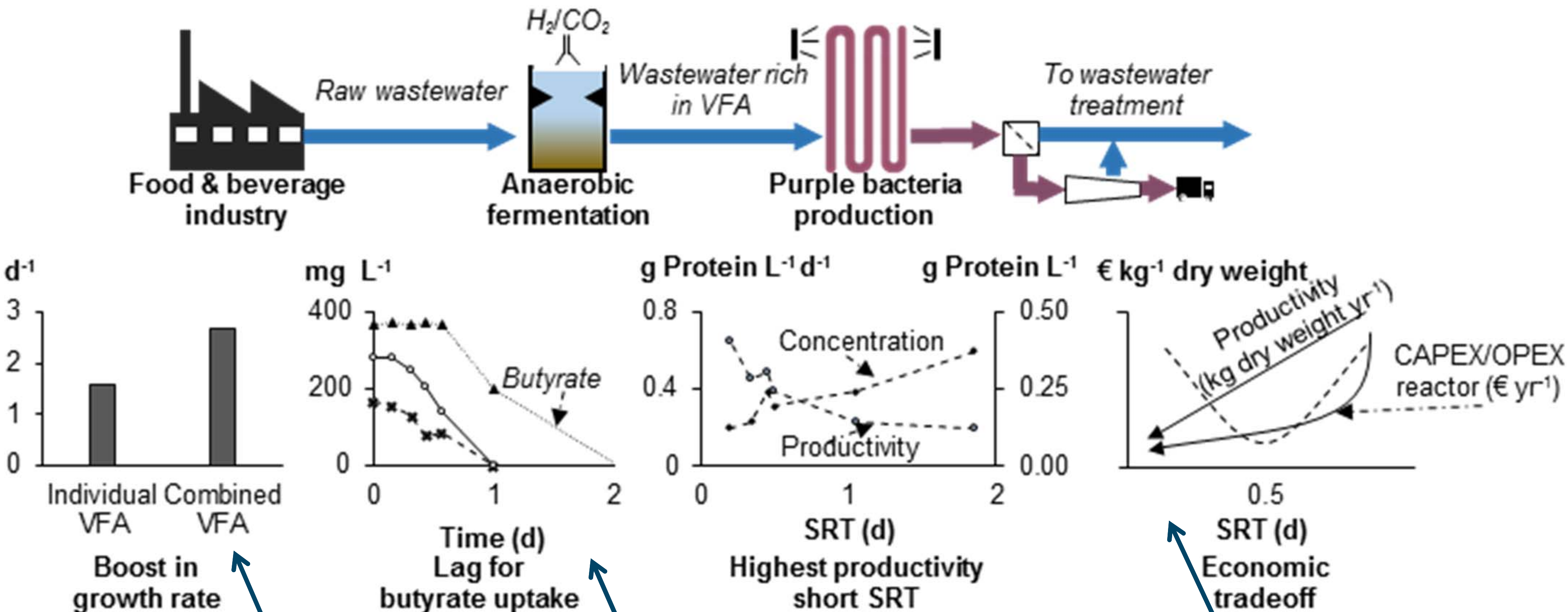


Tradeoff between production cost and biomass production



SRT 0.50 day best option for protein production => **10 euro/kg dry weight**

New opportunities for purple protein production on fermented waste(water)



Economically optimal conditions: SRT of 0.50 days
=> **10 euro kg^{-1} dry weight**

Propionate is the **preferential** C source

More similar to realistic **fermentate**
Fastest conversions obtained with ***Rhodobacter** spp.*, not ***Rhodospirillum rubrum***

MicroNOD
www.micronod.be

Microbial nutrients on demand:
Waste -> 3 Types of microbes -> Fertilizer

Purple non-sulfur bacteria;
Rhodobacter sphaeroides

Consortium of aerobic bacteria

Microalgae; Spirulina

- Resources: COD, N, P in **waste and side streams**
- Recovery: Grow **three types of micro-organisms**
- Reuse: Dried microbial biomass is **as effective as commercial organic fertilizer** at the level of:
  - Mineralization
  - Fertilization: parsley, ryegrass, tomato, surfinia

-> Promising as **sustainable next-generation fertilizers** (NGF)

Sustainable Energy, Air & Water Technology University of Antwerp
GHENT UNIVERSITY
Avecom
AgrAqua
GREENYARD

Sakarika, M., Spanoghe, J., Wambacq, E., Muys, M., Alloul, A., Spiller, M., Gomes de Sousa, G., Derycke, V., Stragier, L., Verstraete, H., Fauconnier, K., Grunert, O., Haesaert, G. & Vlaeminck, S.E.

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(UAntwerpen)

Prof. Ilse Smets
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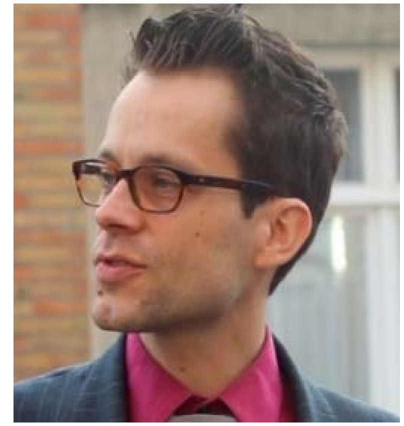
Kenneth Simoens
(KU Leuven)



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Acknowledgments

i-CLEANTECH VLAANDEREN enabling the future

KU LEUVEN

Sustainable Energy, Air & Water Technology University of Antwerp

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Last scientific presentation: tomorrow **14h25** in Sala **Marconi**

Variability in nutritional value and safety of *Arthrospira* and *Chlorella* biomass necessitates smart production of microalgae for human spaceflight

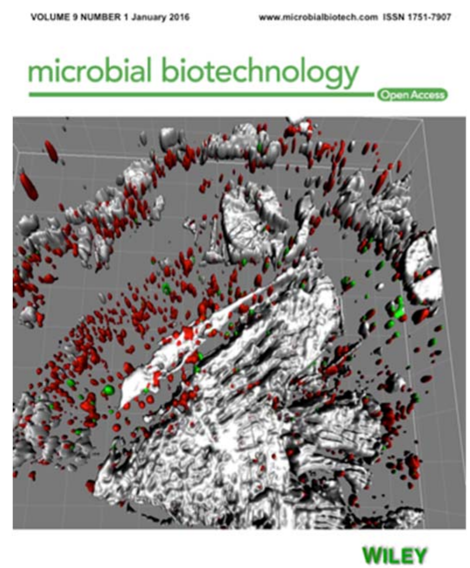
Siegfried E. Vlaeminck, Sui Yixing, Pieter Vermeir & Maarten Muys

society for applied microbiology (sfam)

microbial biotechnology

WILEY-BLACKWELL

- **Open access** journal (°2008)
- Editors: Kenneth N. Timmis, Juan Luis Ramos, Willem de Vos, Siegfried E. Vlaeminck, Auxiliadora Prieto
- 2016 ranking:
    - Impact factor: **3.5**
    - **Top 30%** in Microbiology (36/124) and Biotechnology & Applied Microbiology (40/158)



Environmental biotechnology - Green chemistry - Food, beverages and supplements - Bioenergy - Agriculture - Bioremediation - Biopolymers, biomaterials - Technology development - Process engineering

– AND MUCH MORE

Looking forward to receive your best work!