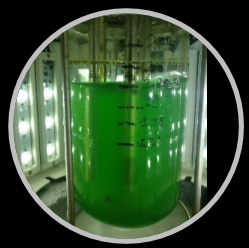
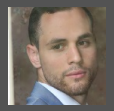


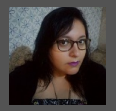
Ecological Engineering of Purple and Green Photoorganoheterotrophic Mixed Cultures for Water Resource Factories

David Weissbrodt
Assistant Professor

Marta Cerruti
PhD candidate



Abbas Alloul
Postdoctoral researcher



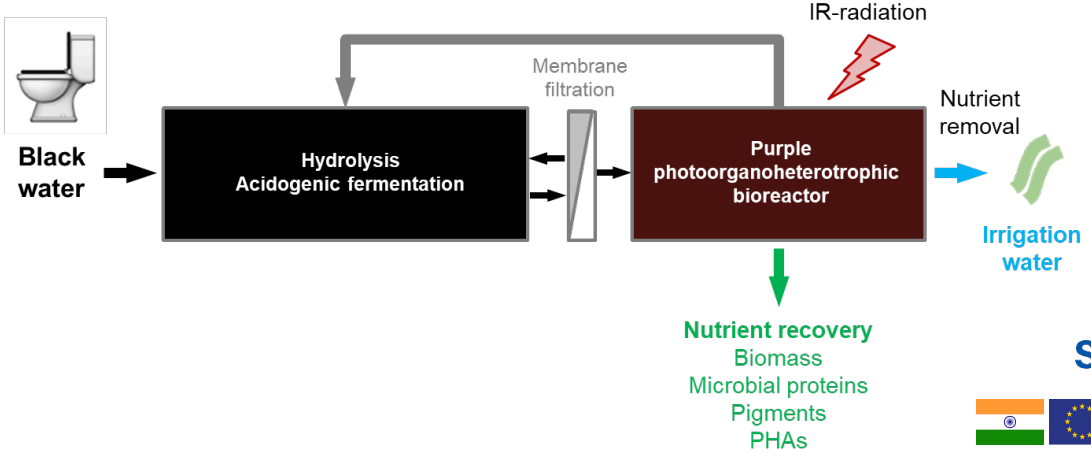
Maria Paula Giulianetti de Almeida
PhD candidate

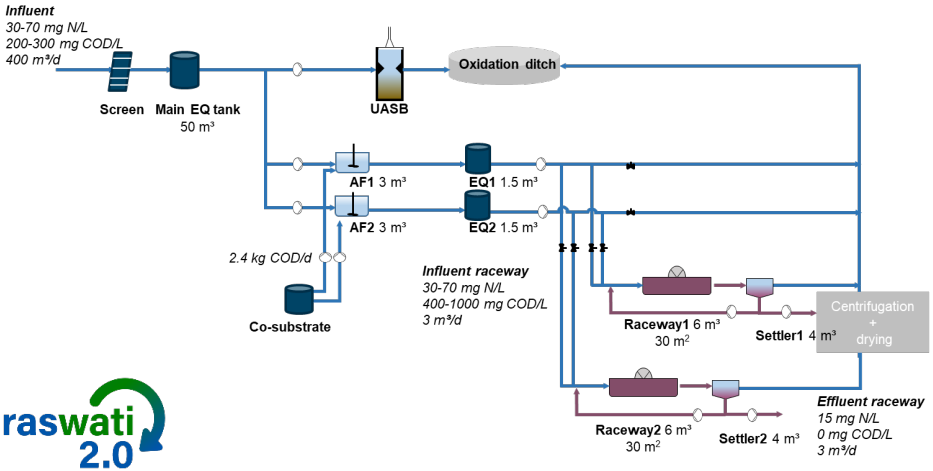
Delft University of Technology | Department of Biotechnology | Environmental Biotechnology Section
Weissbrodt Group for Environmental Life Science Engineering

URL www.tudelft.nl/davidweissbrodt
✉ d.g.weissbrodt@tudelft.nl
@WeissbrodtLab

Application context

Coupling acidogenic fermentation and photoorganoheterotrophy for nutrient capture and water recycling

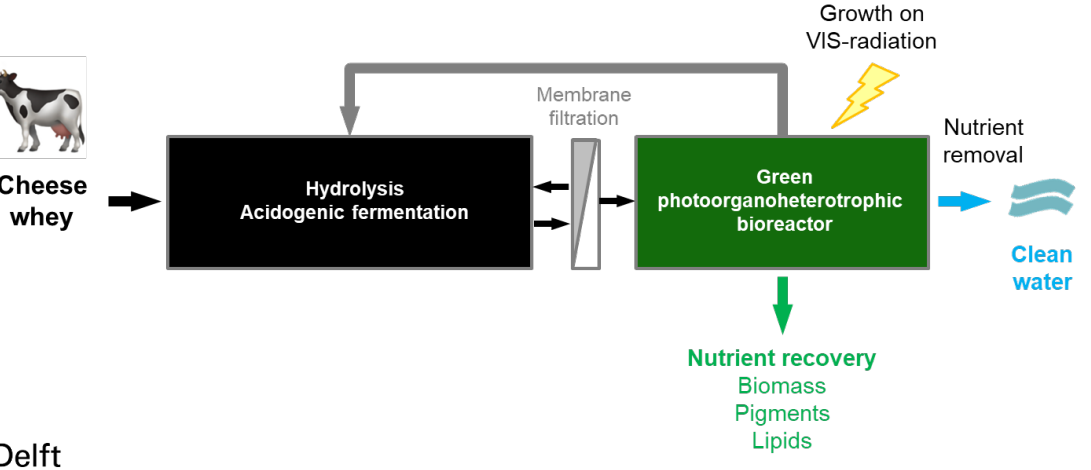




Alloul (2020)

Saraswati 2.0

Horizon 2020
European Union Funding
for Research & Innovation



CAPES
Ministério da Educação
BRASIL

D.G.Weissbrodt@TUDelft.nl | ESA MELiSSA 2020

TU Delft

Microbial diversity

Anoxygenic vs. Oxygenic phototrophy

a

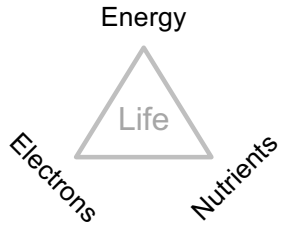
Mahoney Lake Ecological Reserve, USA

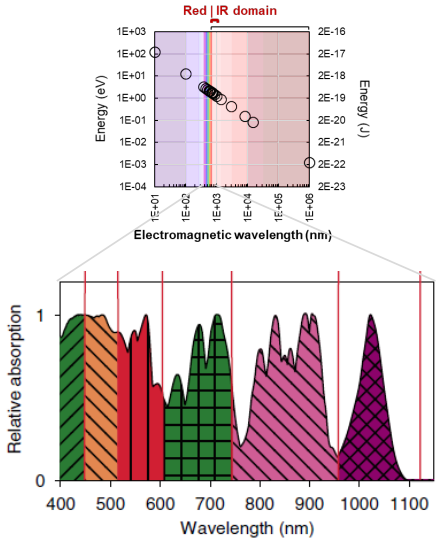
https://preservedlight.com/p48822125/h873D6A8B#h873d6a8b

Lake Erie, USA

https://earth.esa.int/web/earth-watching/environmental-hazards/content/-/article/algal-blooms-in-lake-erie-north-america-

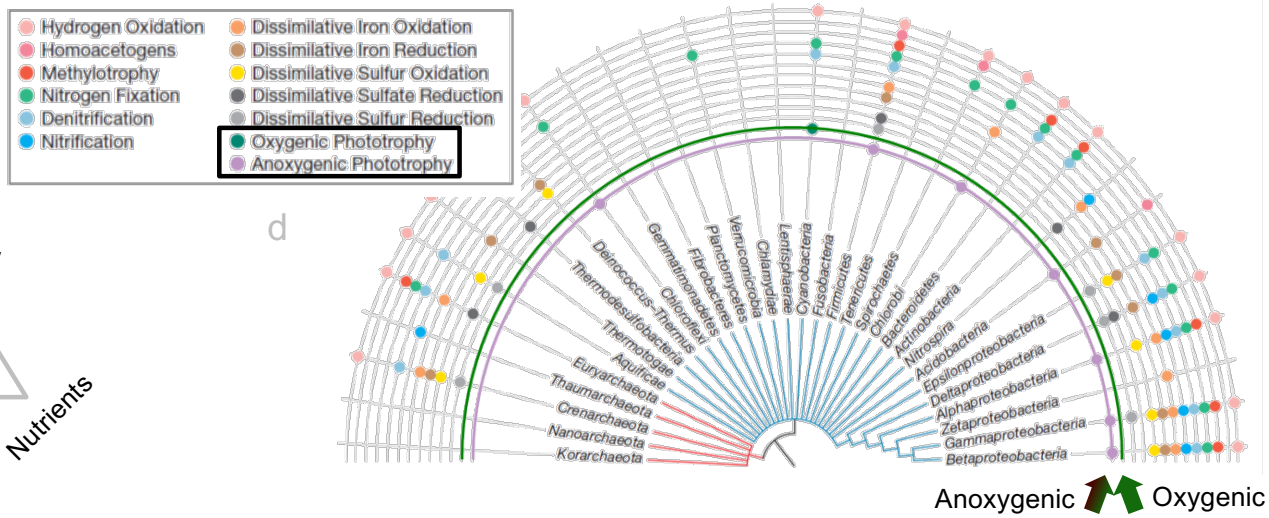
b





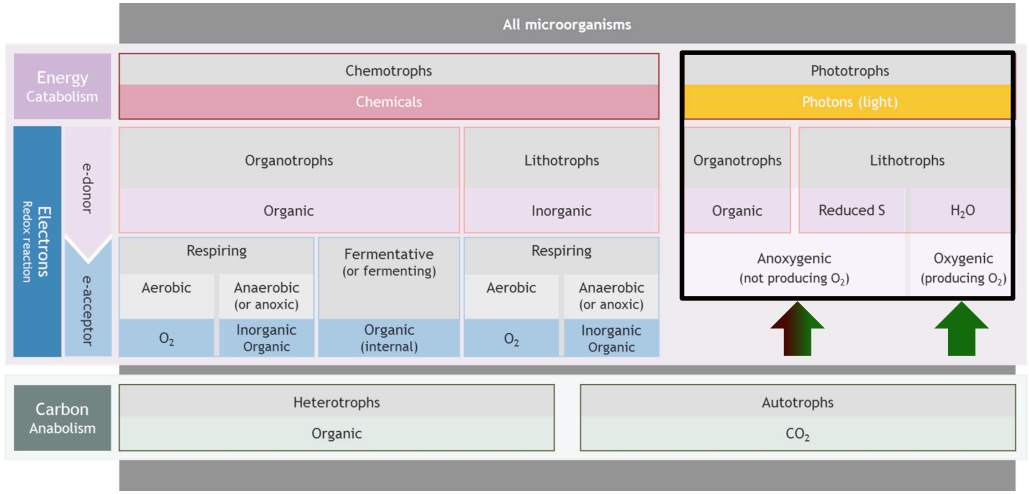
Stomp *et al.* ISME J. 2007, 1: 271-82

d



Madigan *et al.* (2018) Brock Biology of Microorganisms, 15th ed., Pearson.

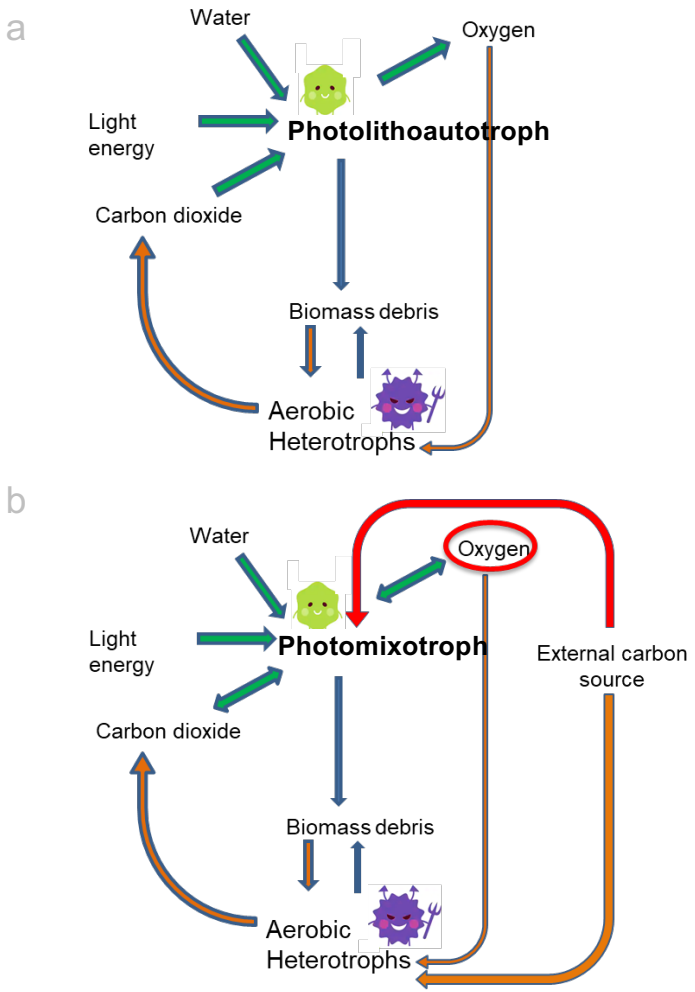
c



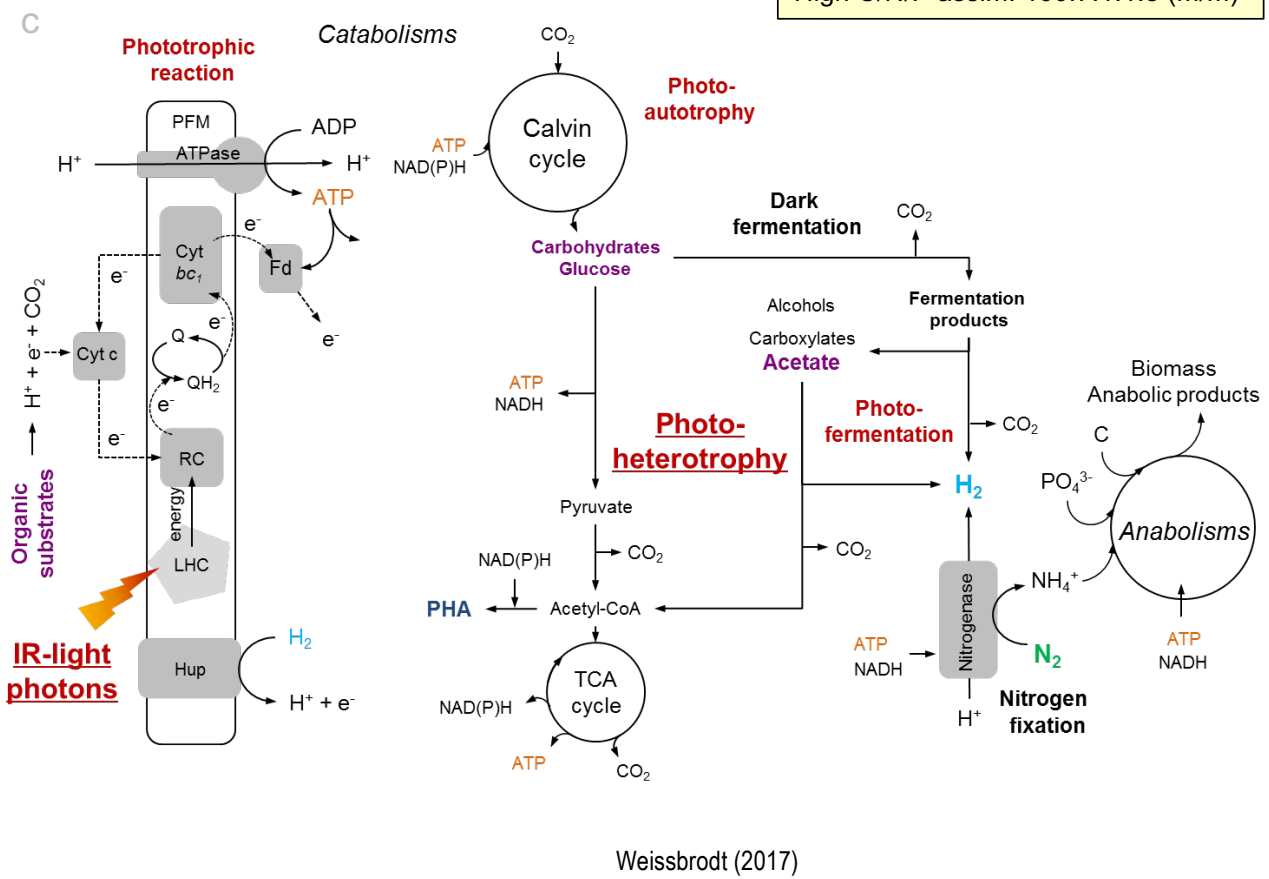
Weissbrodt *et al.* (2020) Chap. 2: Basic Microbiology & Metabolism. Biological Wastewater Treatment, 2nd edition, IWA Publishing.

Mixotrophy and metabolic versatility

Growth under ever-changing environmental conditions



Mondini (2019) TU Delft MSc thesis



Weissbrodt (2017)

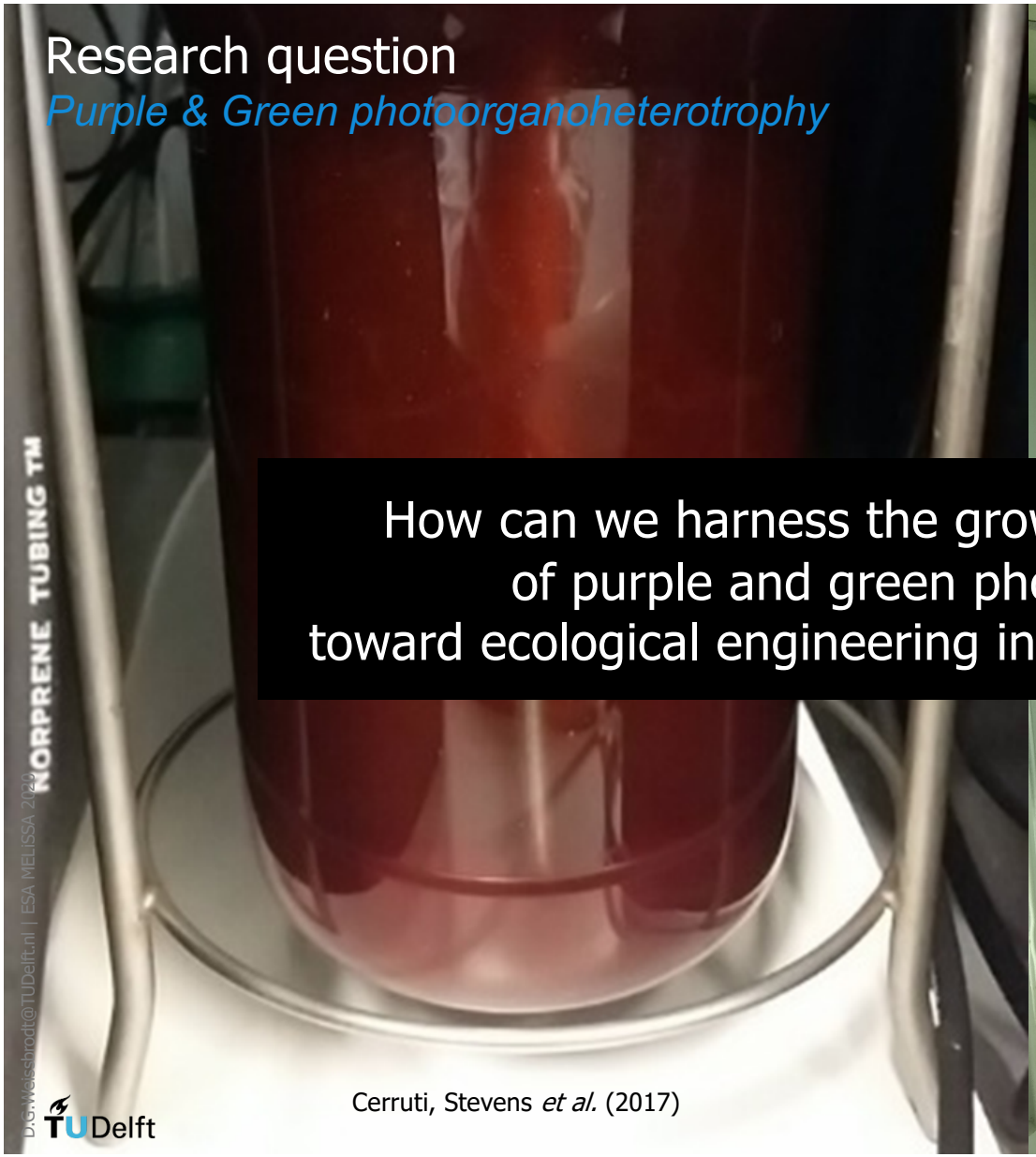
Growth

$C_1H_{1.8}O_{0.38}N_{0.18}$
High γ_{PNSB} = 4.5 mol e^- C-mol^{-1} X
High $Y_{X/S}$ ~ 1 g COD_x g^{-1} COD_S
High C/N/P assim. 100:7.1:1.8 (m/m)

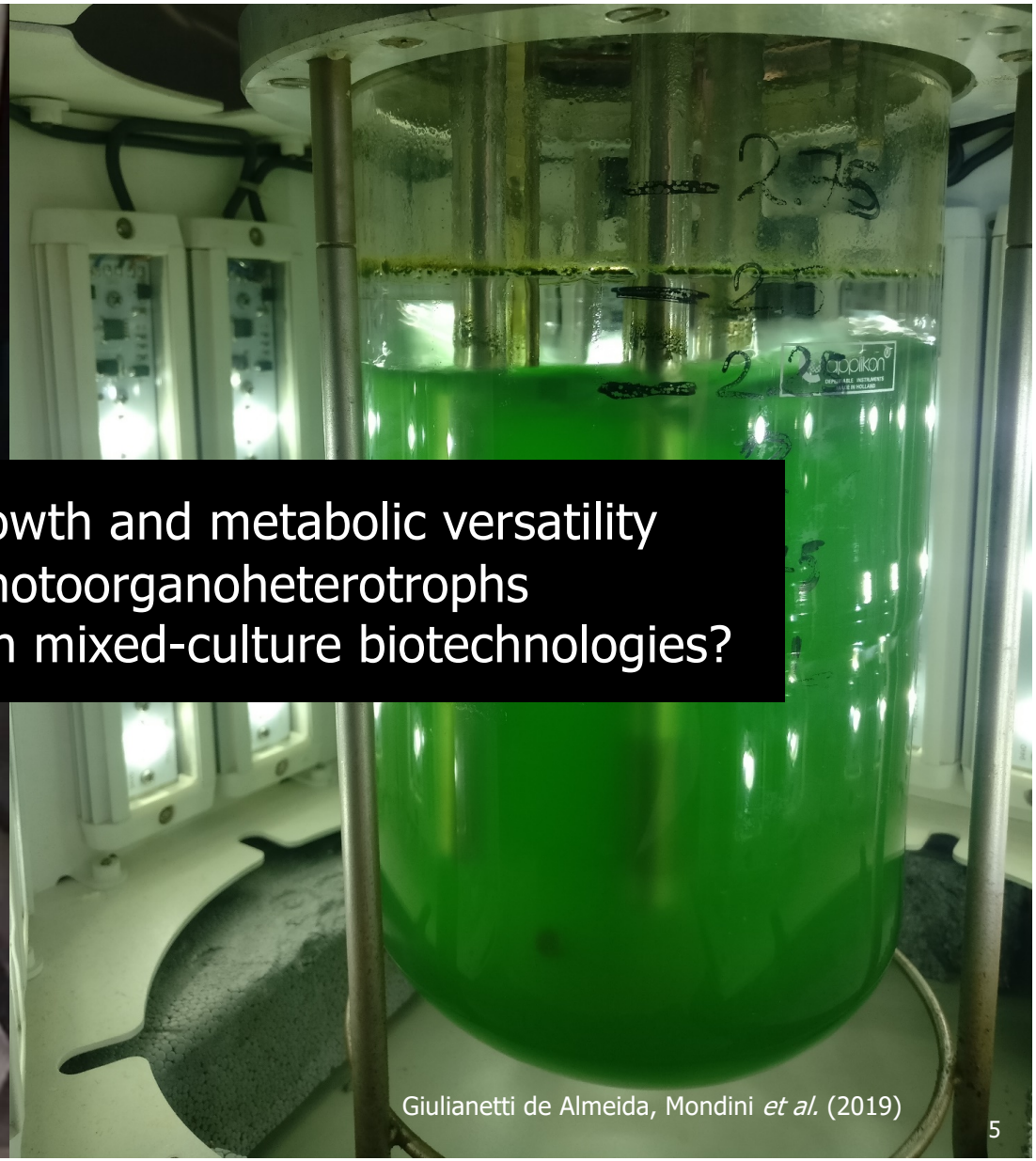
Research question

Purple & Green photoorganoheterotrophy

How can we harness the growth and metabolic versatility
of purple and green photoorganoheterotrophs
toward ecological engineering in mixed-culture biotechnologies?



Cerruti, Stevens *et al.* (2017)



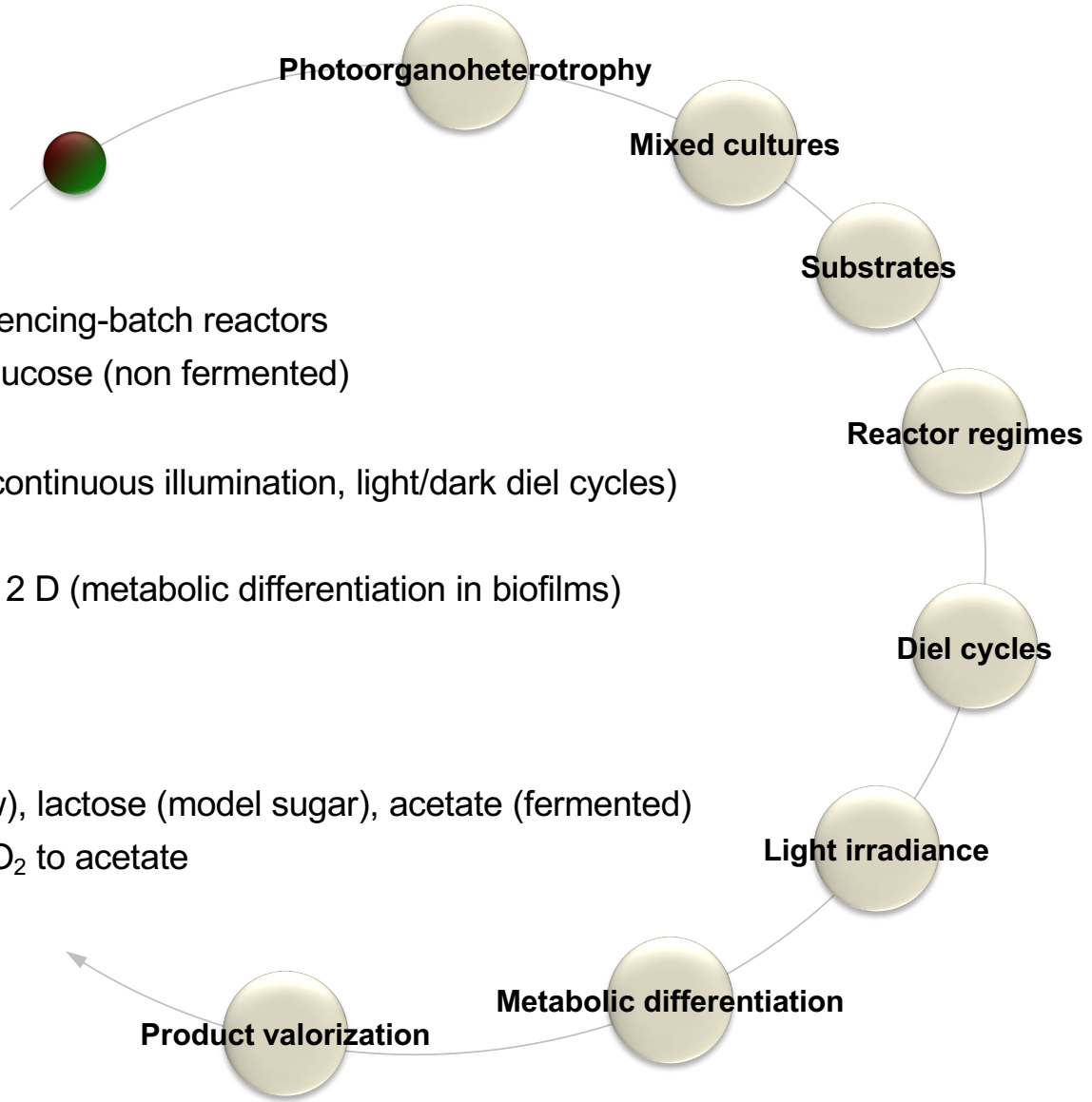
Giulianetti de Almeida, Mondini *et al.* (2019)

Investigations

Wet-lab and dry-lab approaches

- **Purple photoorganoheterotrophs**
  - Mixed cultures vs. pure cultures
  - Enrichment cultures and bioaggregation in sequencing-batch reactors
  - Substrates: volatile fatty acids (fermented) vs. glucose (non fermented)
  - Reactor regimes: batch vs. continuous-flow
  - Light irradiance (350 → 0 W m^{-2}) and patterns (continuous illumination, light/dark diel cycles)
  - Pigments extraction and analysis
  - Mathematical models: 1 D (metabolic switches), 2 D (metabolic differentiation in biofilms)

- **Green photoorganoheterotrophs**
  - Mixed cultures
  - Substrates testing in batches: cheese whey (raw), lactose (model sugar), acetate (fermented)
  - Enrichment culture in chemostat: switch from CO_2 to acetate
  - Metabolic model of triacylglyceride production



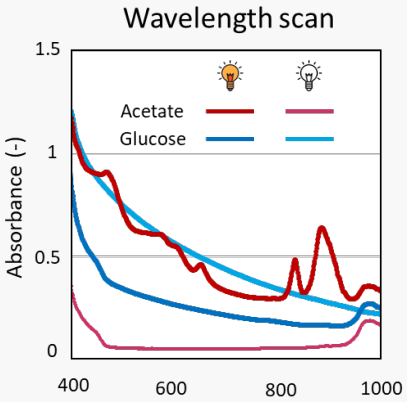
Open mixed cultures: substrates and reactor regimes

Cerruti, Crosset-Perrotin, *et al. IWA AD & MEWE 2019*

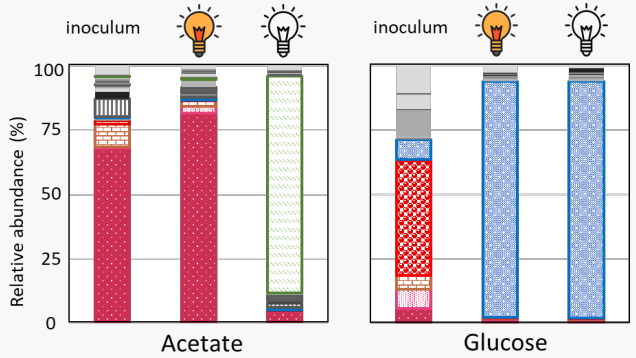
Purple and green photoorganoheterotrophs are best selected with pre-fermented organics

a

Batches



Amplicon sequencing



Rhodopseudomonas
Rhodobacter
Blastochloris
Thiobaca
Enterobacter
Acinetobacter
Bifidobacterium
Others

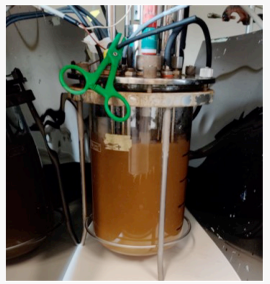
b

Chemostat

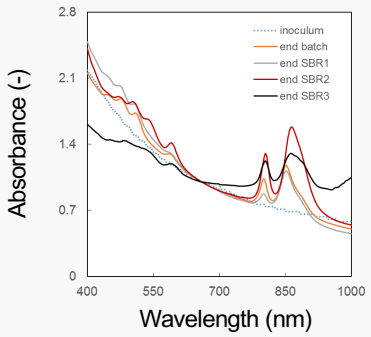
16h / 8h

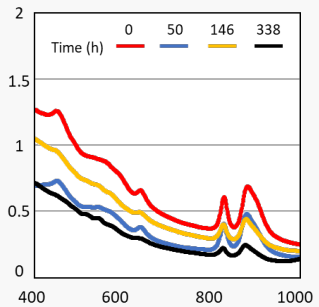
Acetate

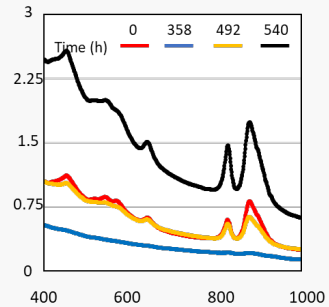
Glucose

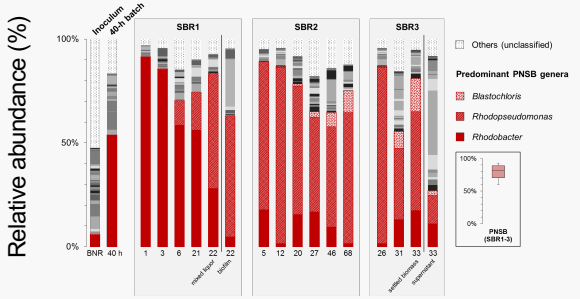


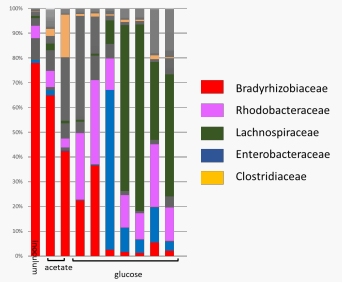
Glucose



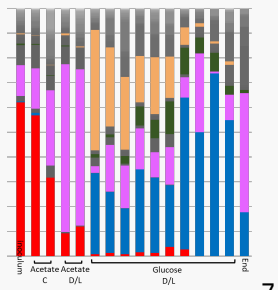








Bradyrhizobiaceae
Rhodobacteraceae
Lachnospiraceae
Enterobacteraceae
Clostridiaceae



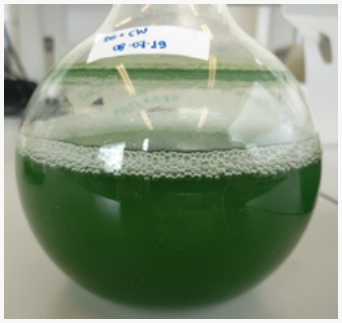
Open mixed cultures: substrates and reactor regimes

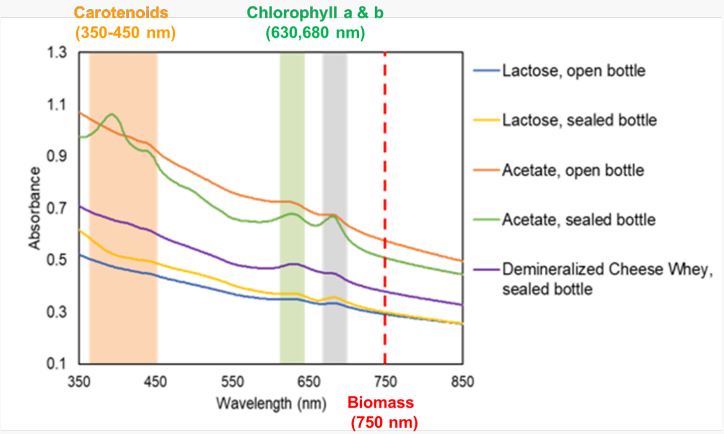
Giulianetti de Almeida, Mondini, *et al. ACS Fall Meeting 2020*

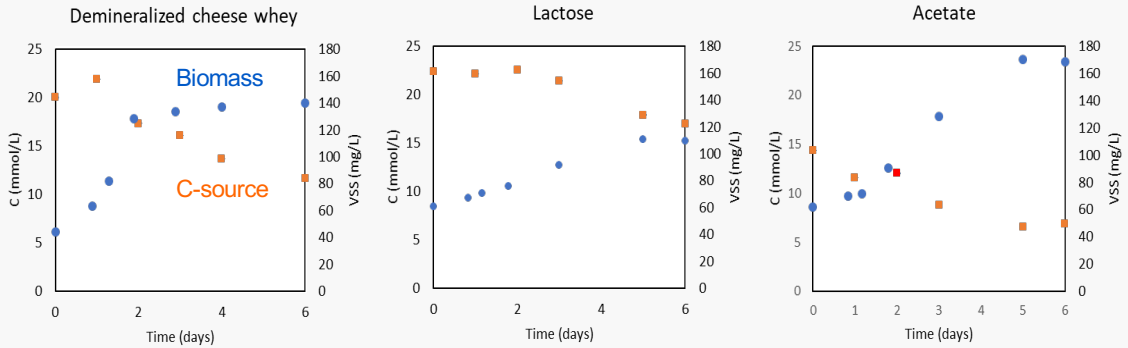
Purple and green photoorganoheterotrophs are best selected with pre-fermented organics

a

Batches







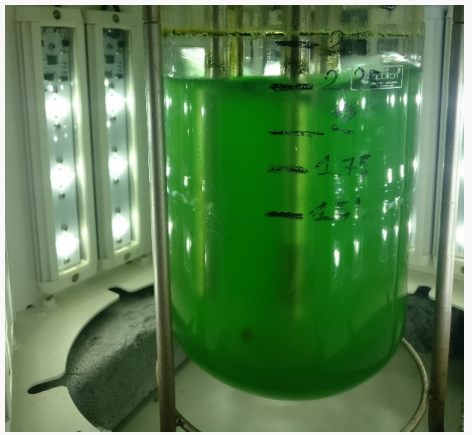
ΔC: 100 mg VSS L $^{-1}$
μ_{max} : 0.56 d^{-1}
$Y_{X/S}$: 0.82 mg VSS mg C $^{-1}$

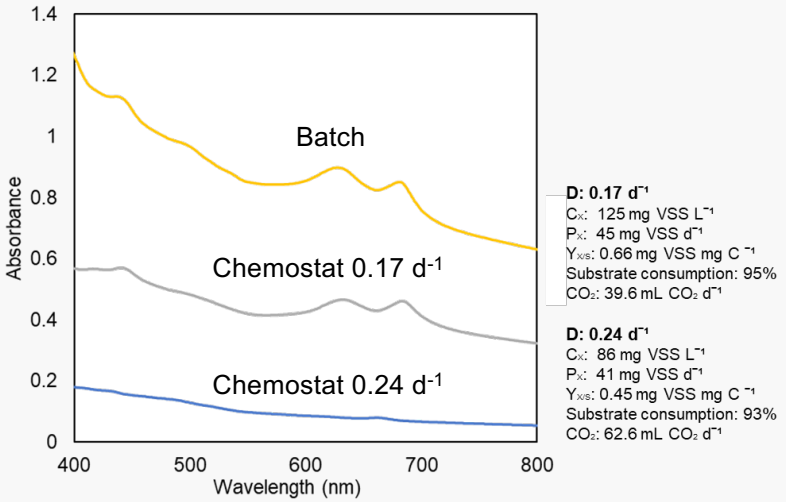
ΔC: 80 mg VSS L $^{-1}$
μ_{max} : 0.14 d^{-1}
$Y_{X/S}$: 0.65 mg VSS mg C $^{-1}$

ΔC: 110 mg VSS L $^{-1}$
μ_{max} : 0.25 d^{-1}
$Y_{X/S}$: 1.15 mg VSS mg C $^{-1}$

b

Acetate-fed chemostat





D: 0.17 d^{-1}
C_X: 125 mg VSS L^{-1}
P_X: 45 mg VSS d^{-1}
$Y_{X/S}$: 0.66 mg VSS mg C $^{-1}$
Substrate consumption: 95%
CO_2: 39.6 mL CO_2 d^{-1}

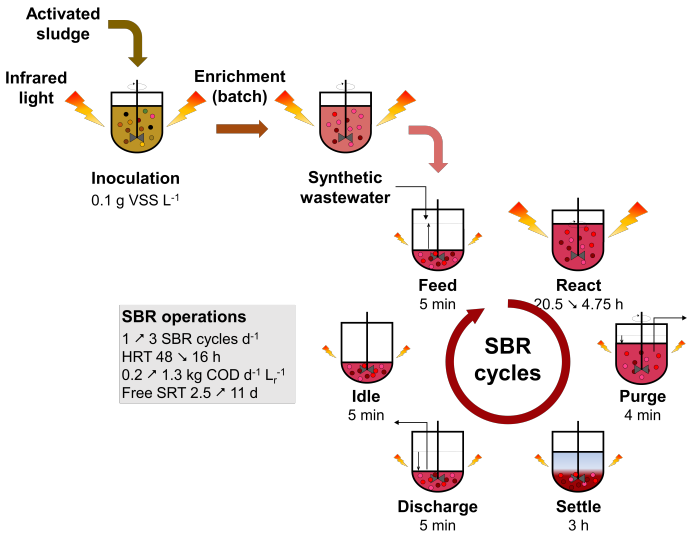
D: 0.24 d^{-1}
C_X: 86 mg VSS L^{-1}
P_X: 41 mg VSS d^{-1}
$Y_{X/S}$: 0.45 mg VSS mg C $^{-1}$
Substrate consumption: 93%
CO_2: 62.6 mL CO_2 d^{-1}

Bioaggregation and nutrient removal

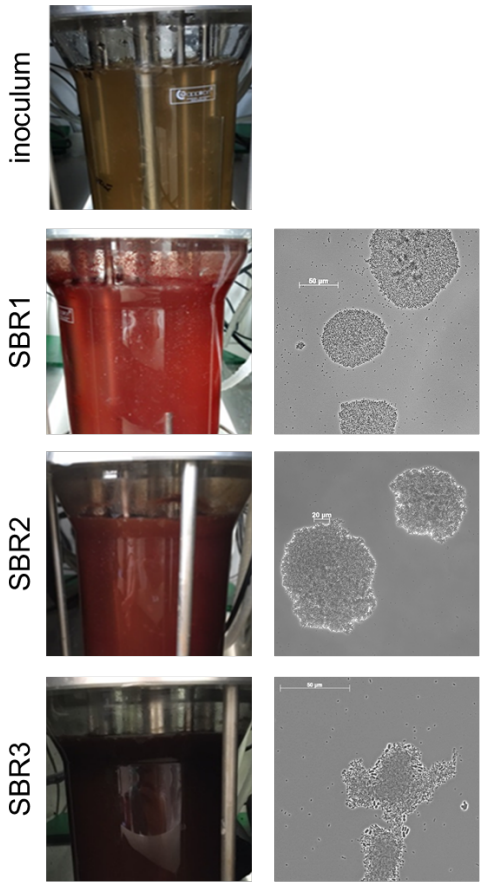
Cerruti *et al. bioRxiv* 2020.01.08.899062

Photoorganoheterotrophs can efficiently aggregate, facilitating nutrient removal and solid/liquid separation

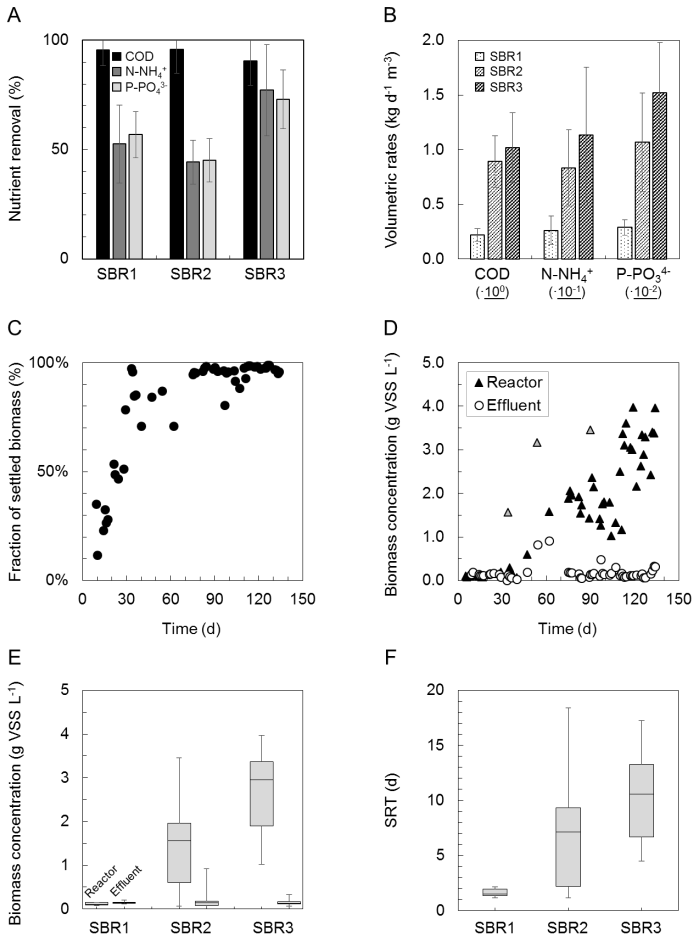
a



b



c

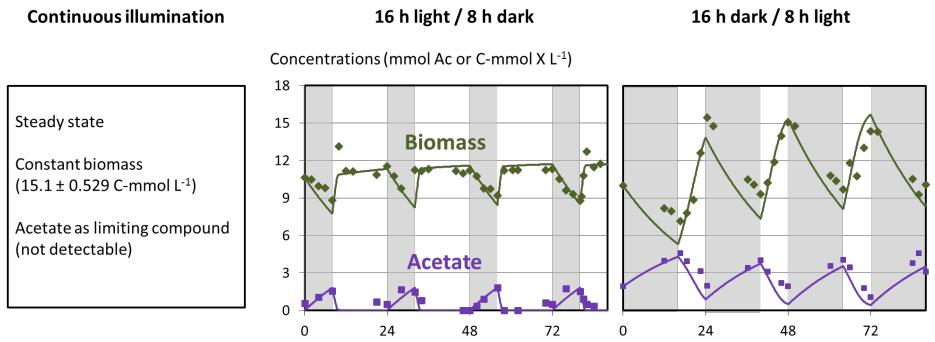


Light / dark diel cycles

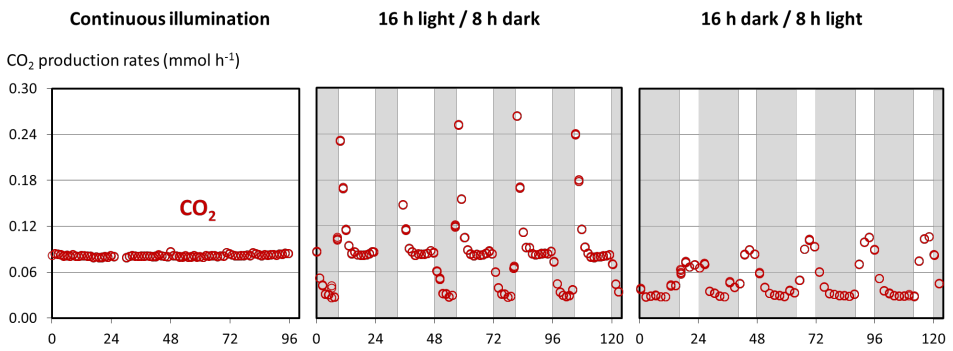
PHAs or H_2 act as carbon and/or electron sinks depending on diel regimes

Cerruti *et al. bioRxiv* 2020.08.19.258533

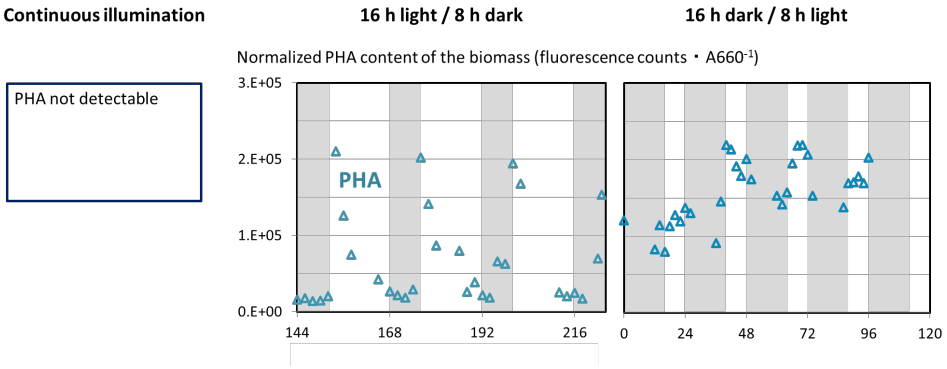
a – Acetate and biomass



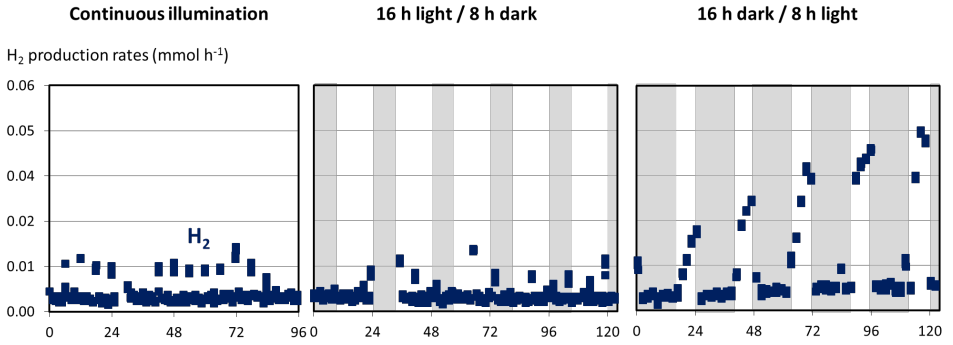
b – Carbon dioxide (CO_2)



c – Poly-β-hydroxyalkanoates (PHAs)



d – Dihydrogen (H_2)

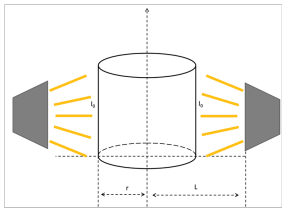


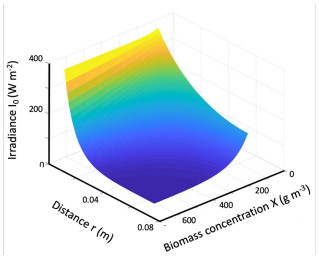
Light irradiance

Light intensity impacts PNSB physiology and biomass settling

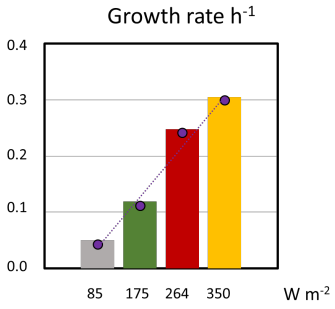
Cerruti, Kim, *et al. IWA AD 2019*

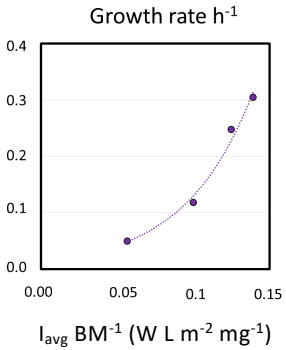
a – Biomass concentration and light penetration

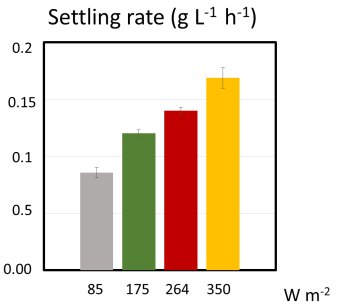




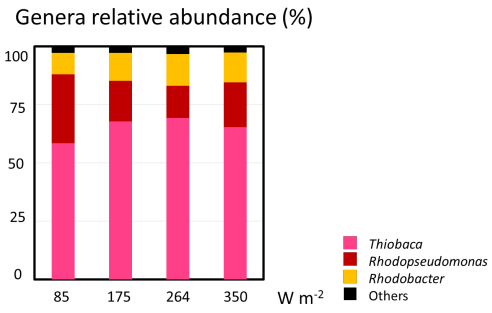
b – Irradiance impacts on biomass growth



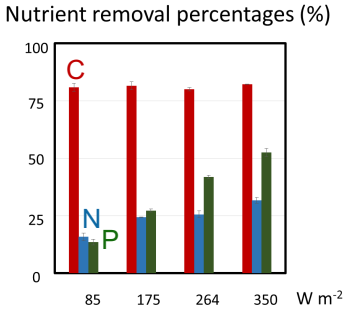


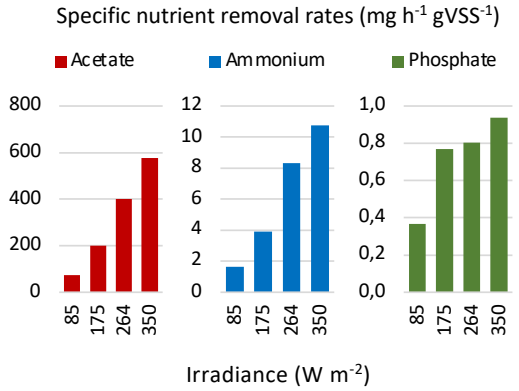


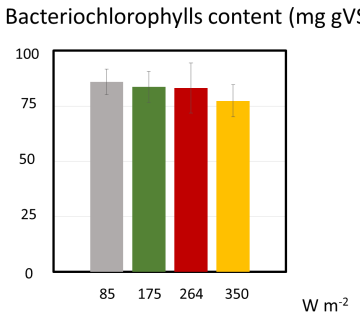
c – Community composition



d – Irradiance impacts on nutrient removal and pigment content







(over the tested irradiance range from 350 to 85 W m^{-2}; to be continued)

D.G.Weissbrodt@TUDelft.nl | ESA MELiSSA 2020

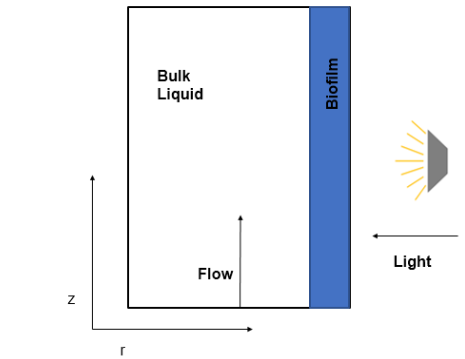
Metabolic differentiation

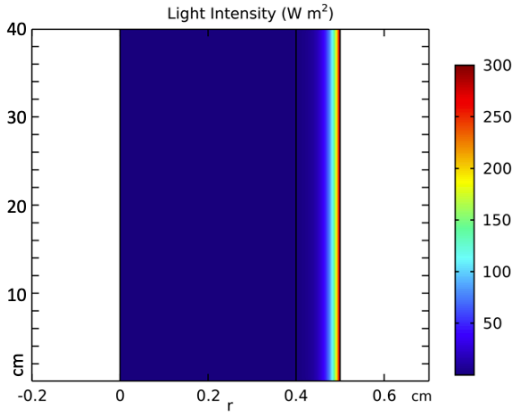
Chasna (2020) TU Delft MSc thesis

Single strains can exhibit different metabolic states along gradients in biofilms

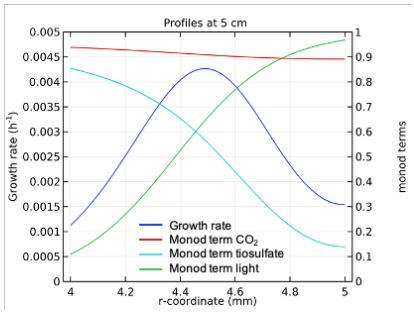
- 2-D model of single-specie biofilm of *Rhodopseudomonas palustris*
- e-donor mixture: acetate (photoorganoheterotrophy) + thiosulfate (photolithoautotrophy)

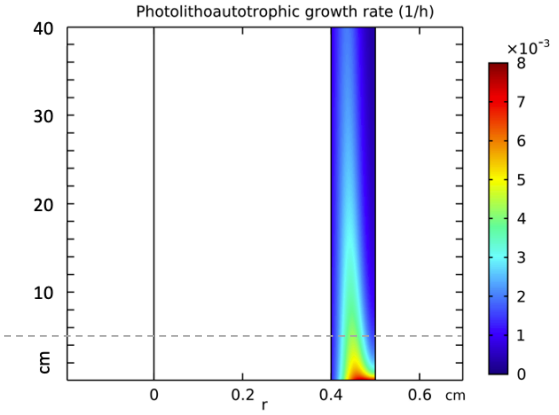
a

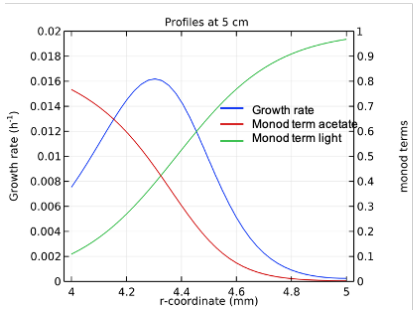


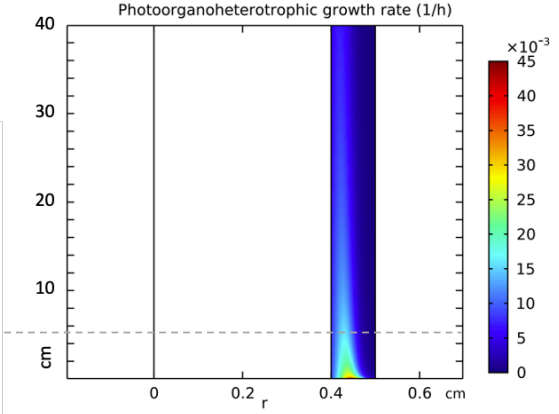


b







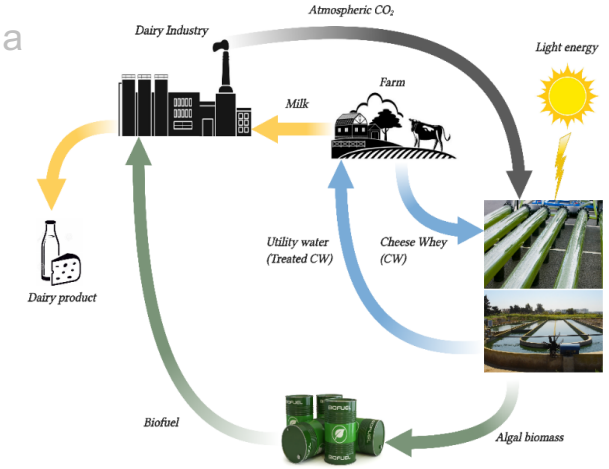


Product valorization

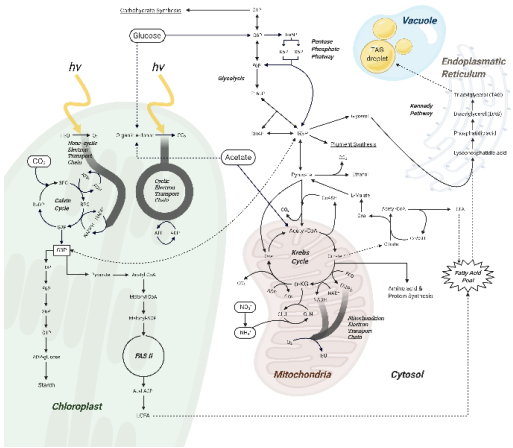
Khayat (2020) TU Delft MSc thesis

Tryacylglyceride production with green photomixotrophs in mixed culture

a

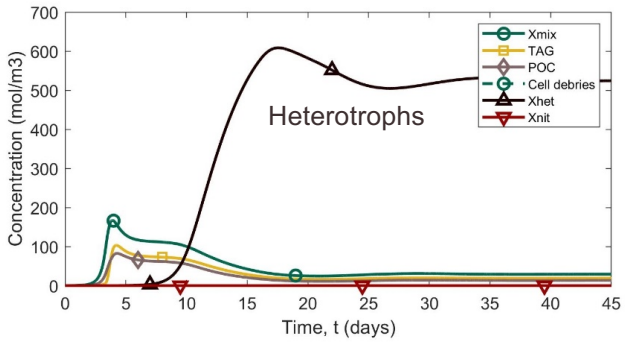


b

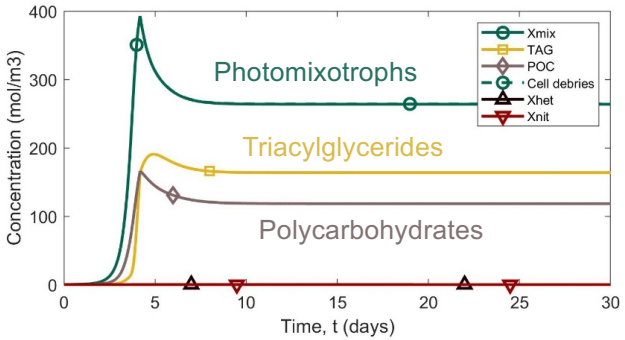


c

Raw cheese whey
Chemostat (continuous light)

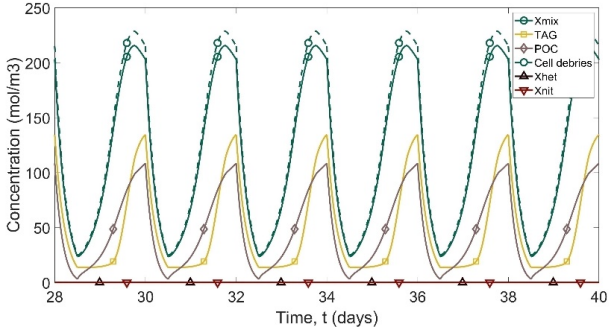


Modified cheese whey (C/N, C/P)
Chemostat (continuous light)

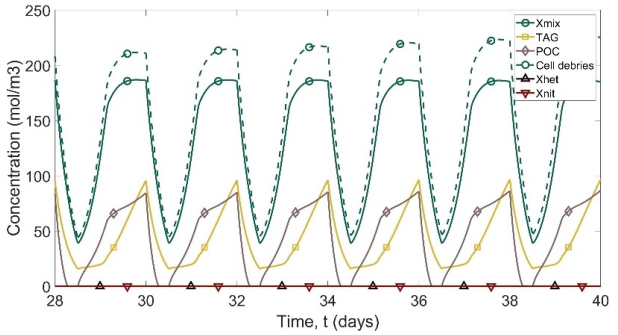


d

Raw cheese whey
Cyclic chemostat (light-feast / dark-famine)



Prefermented cheese whey
Cyclic chemostat (light-feast / dark-famine)



Take home

Purple to green photobiotechnology for carbon/nutrient capture and water recycling

1. Photo(organohetero)trophs exhibit high microbial diversity and metabolic versatility.
2. Photomixotrophs are not monofunctional and display high metabolic power for resource recovery.
3. Pure-culture and mixed-culture investigations are essential for (eco)physiological understanding.
4. Microbial lineages exhibit different metabolic states along gradients (biofilms).
   To be considered in process management.
5. Engineering of photo(organohetero)trophic biosystems rely on managing light (irradiance, diel cycles), substrates (with(out) pre-fermentation), electrons allocations, and reactor regimes among other keys.

TU Delft
Department of Biotechnology

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Phototroph team: going purple and green

Thank you !

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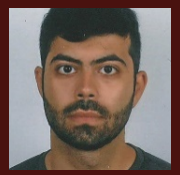

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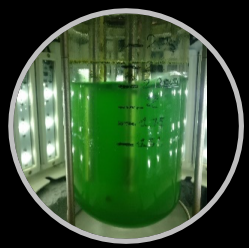

Ecological Engineering of Purple and Green Photoorganoheterotrophic Mixed Cultures for Water Resource Factories

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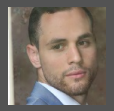
Assistant Professor

Marta Cerruti
PhD candidate



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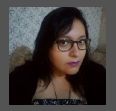

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