Feasability study of food sources (proteins) and biopolymers for astronauts

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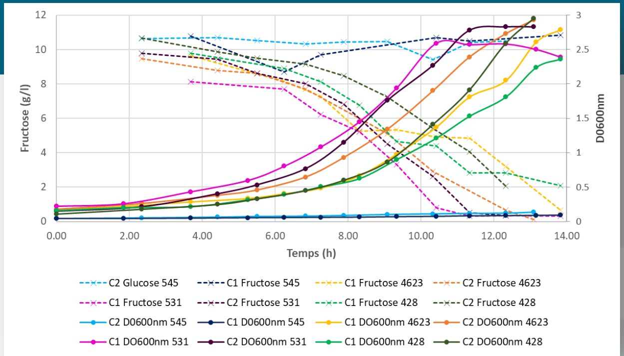
AstroPOU project aims

- **Demonstrate** the recycling potential of organic (urine and faeces like) wastes using heterotrophic fermentation of *Cupriavidus necator*
- **Produce** single cell proteins and bio-based plastics
- **Evaluate** the biomass and products quality for food and material applications
- **Optimize** the process in terms of reactor monitoring and inlet composition

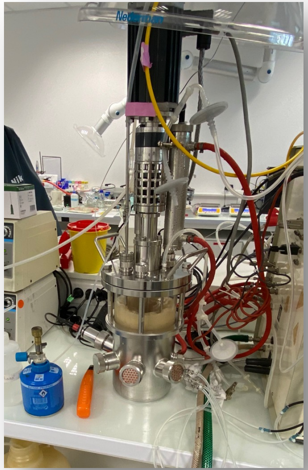
Experimental strategy



1st – Strain screening on urea consumption ability and protein content

→ *Cupriavidus necator* CECT 4623

2nd – Continuous fermentations on synthetic medium mimicking anaerobic digestate from organic wastes:



→ Modulation of biomass composition at different growth rates (µ)

→ Comparison with reference experiments on glucose

→ Investigation of the inlet stream composition variability

Cupriavidus necator

Heterotrophic metabolism

- Organic compounds = source of carbon and O_2 = source of energy
- Other elements: water, N, P, Na, K etc…
- Production type monitored:

Cupriavidus necator

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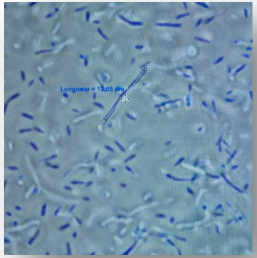
- Production type monitored:

PHA : Polyhydroxyalcanoate

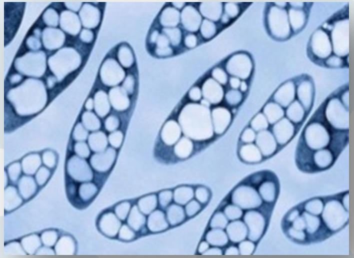
PHB : Polyhydroxybutyrate

PHV : Polyhydroxyvalerate



C/N < 10

C/N > 20



Carbon limitation and nitrogen excess
→ **Protein accumulation**

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→ **PHA accumulation**

Cupriavidus necator

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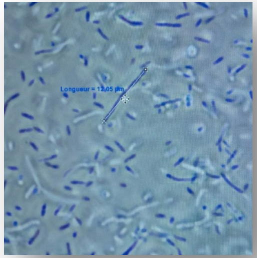

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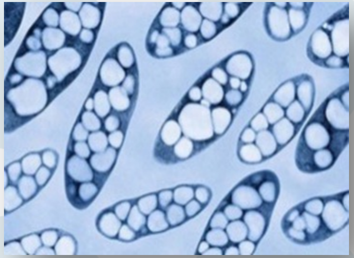


Carbon limitation and nitrogen excess
→ **Protein accumulation**

Good single cell protein quality

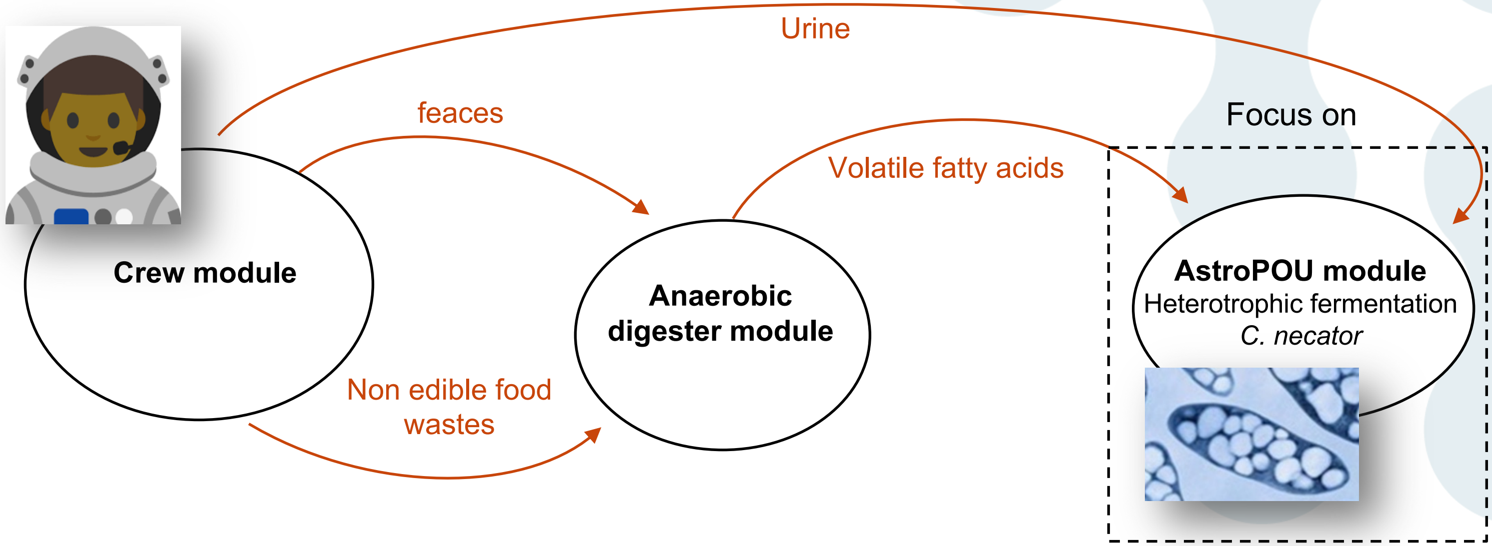
- High protein content (>50%)
- Optimal amino-acid composition
- Nucleic acid content below 2%

Carbon excess and nitrogen limitation
→ **PHA accumulation**

Good biobased plastics quality

- High content
- PHV/PHB ratio around 20%

Carbon and nitrogen feed composition



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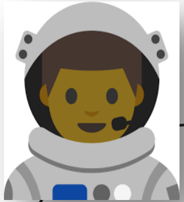
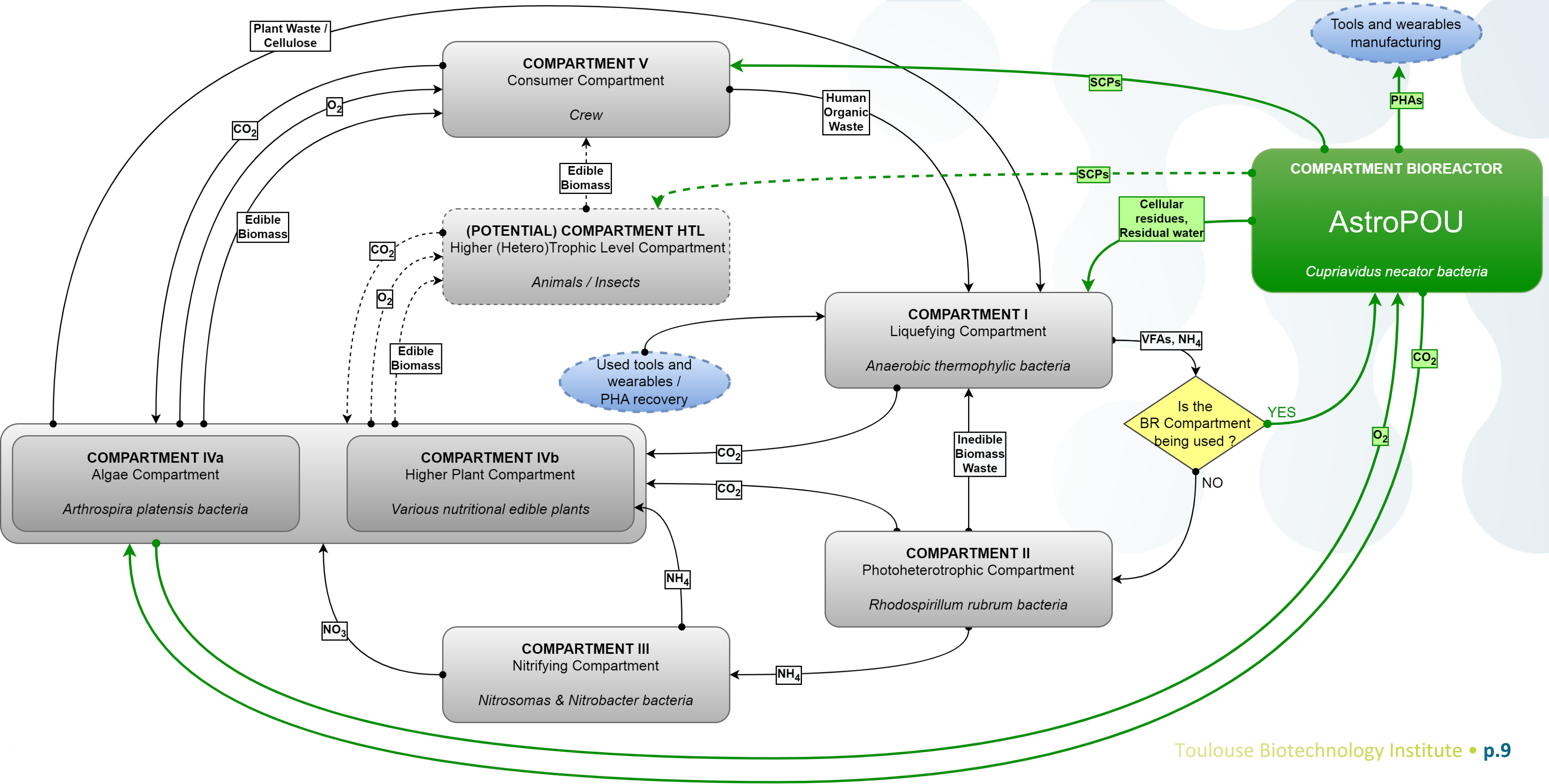


VFA (Cmol.L^{-1})	Cruvellier, 2018	Poughon et al, 2013	AstroPOU for proteins	AstroPOU for bioplastcs
Acetate	0.026	0.100	0.100	0.177
Propionate	0.011	0.015	0.044	0.078
Butyrate	0.007	0.101	0.018	0.033
Iso butyrate	0.005		0.027	0.049
Iso valerate	0.007	0.018	0.030	0.053
Valerate	0.007		0.027	0.048
Total	0.062	0.233	0.246	0.437

AstroPOU & MELiSSA complementarity

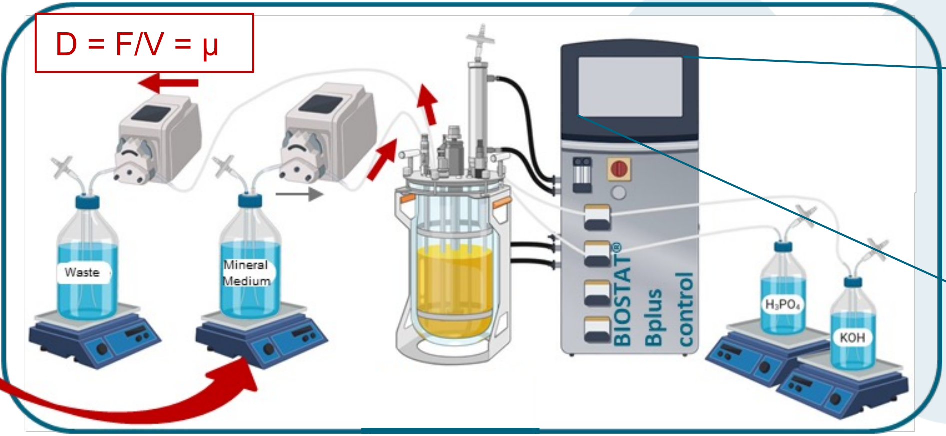
From E. Perrin, “Assessing the integration of a bioreactor producing SCPs and PHAs from organic waste into global environment systems”, 2022 MELiSSA conference



Experimental setup

Limiting substrates

Glucose, VFA mix, VFA, Urea



- V = 2L
- pH = 7
- T = 30°C
- pO_2 = 30%
- Online gas analysis

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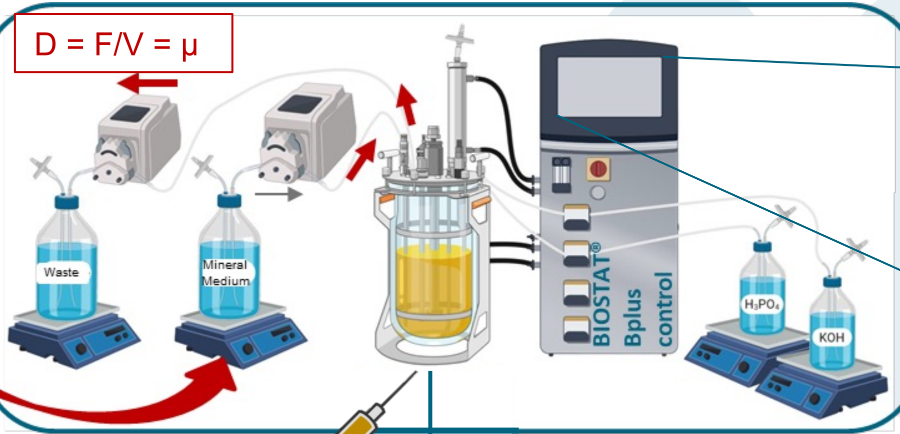

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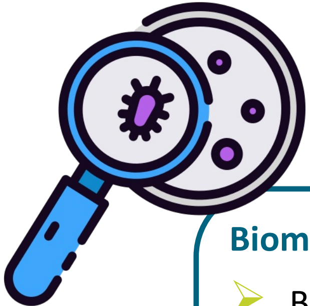

Biomass analysis

- Biomass quantity
- Proteins
- PHB and PHV
- Nucleic acids

Supernatant analysis

- Substrates consumption (carbon and nitrogen)
- Product synthesis (Ethanol or organic acids)

Sampling

At least four independent samples during the steady-state (after 5 residence times)

Experimental strategy sum up

- **Continuous cultures on glucose and VFA mix for protein production at several dilution rates**

Production type	**Proteins**					
Limiting substrate / **Carbon source**	Glucose			VFA mix		
D (h^{-1})	0.06	0.14	0.23	0.06	0.12	0.25

Experimental strategy sum up

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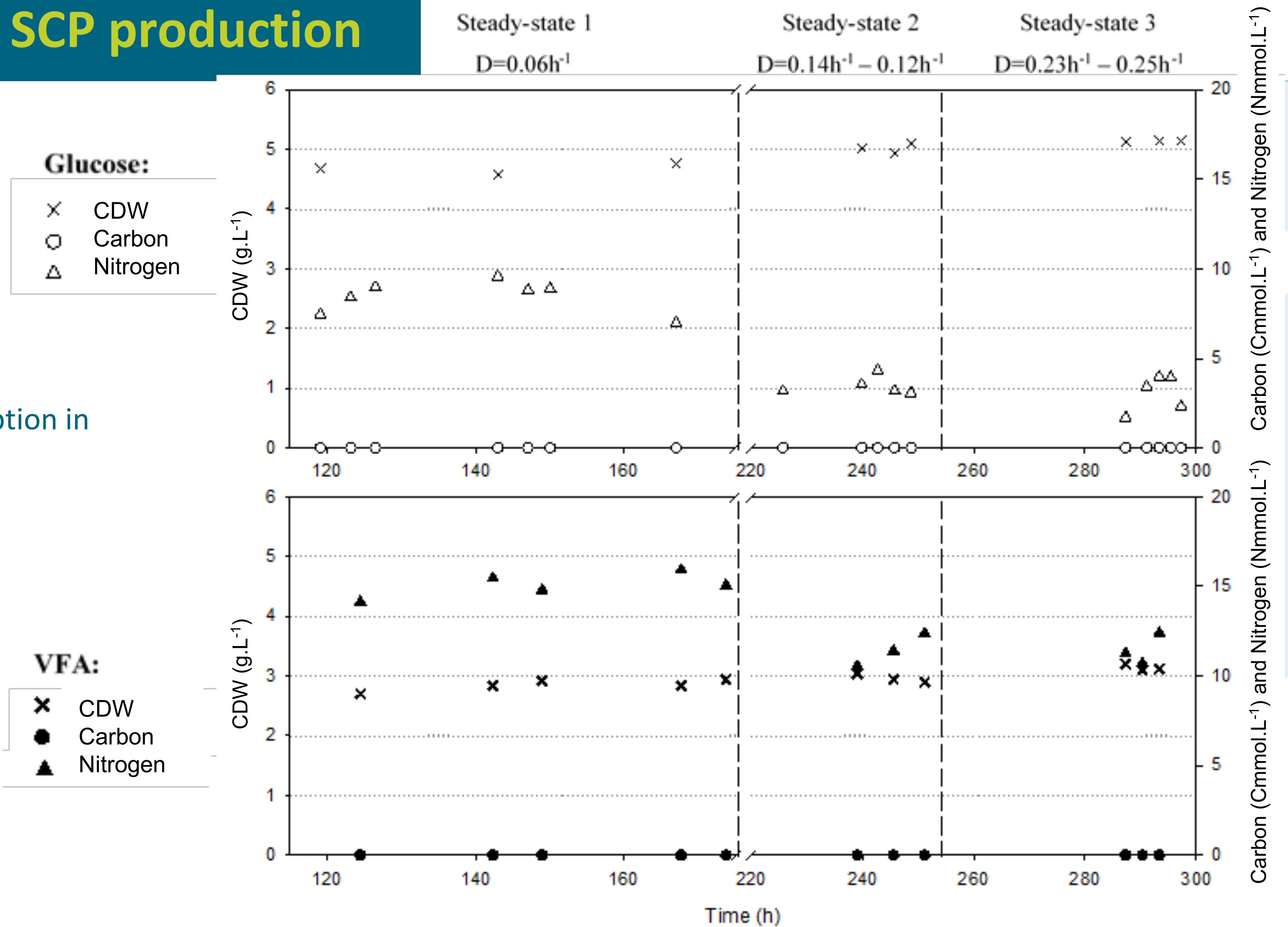
- Continuous cultures on glucose and VFA mix for bioplastic production at several dilution rates

Production type	Bioplastics	
Limiting substrate	Urea	
Carbon source	Glucose	VFA mix
D (h^{-1})	0.11	0.06*

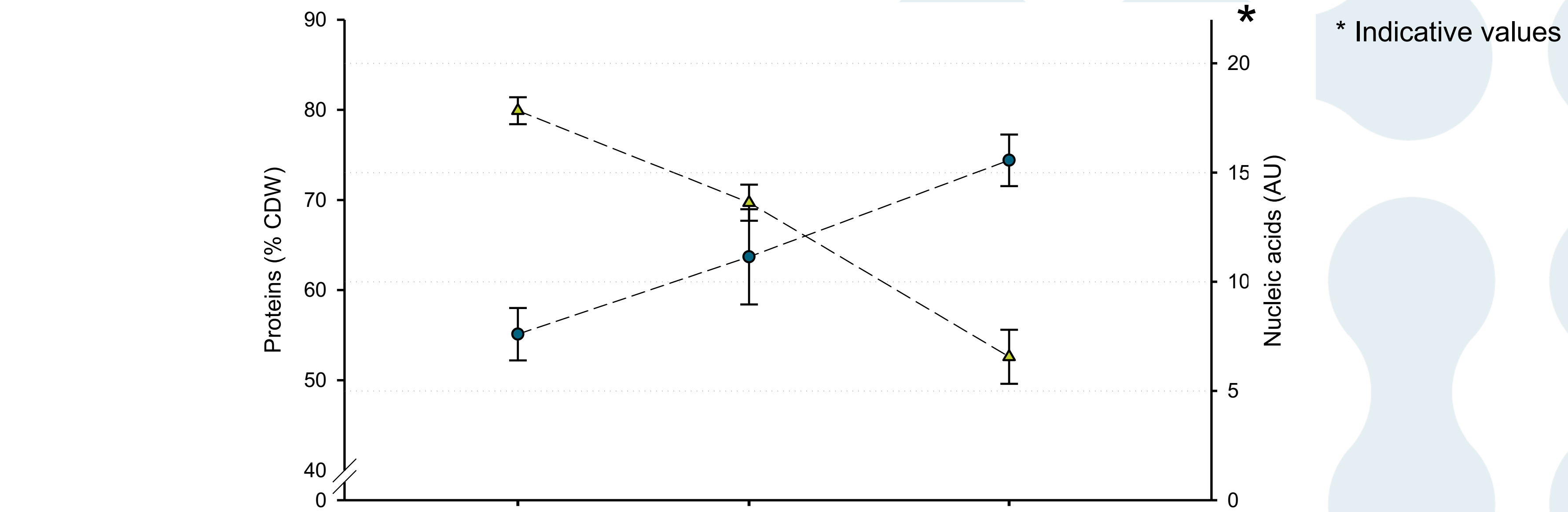
- Aragao, Glaucia Maria Falcao de. 1996. « Production de poly-bêta-hydroxyalcanoates par Alcaligenes eutrophus : caractérisation cinétique et contribution à l'optimisation de la mise en oeuvre des cultures ». PhD Thesis, INSA de Toulouse.
- Estelle Grousseau. 2012. « Potentialité de production de PHA chez C. necator sur substrats de type AGV : études cinétiques et metaboliques ». PhD Thesis, INSA de Toulouse.

Kinetics for SCP production



- ✓ Total carbon consumption in any case

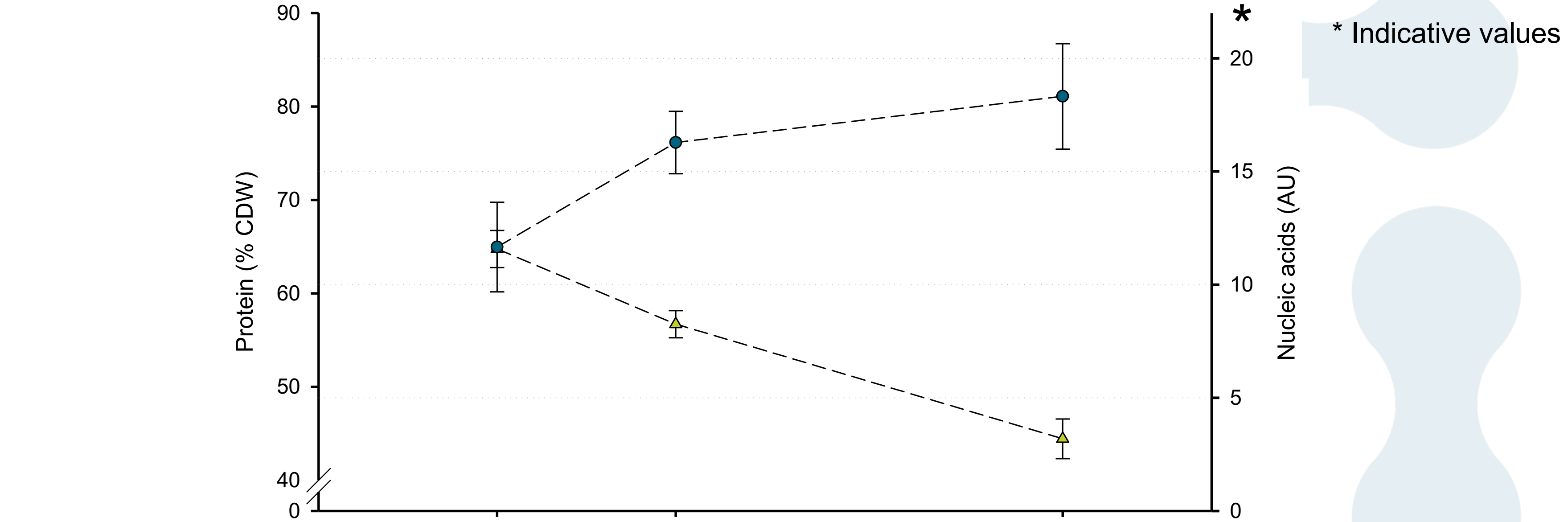
Biomass characterization on glucose



0.06	0.14	0.23	μ (h^{-1})
96 ± 8	100 ± 7	98 ± 7	**Carbon balances (%)**
91 ± 13	95 ± 12	88 ± 8	**Nitrogen balances (%)**

- ✓ The protein content decreases when the dilution rate rises
- ✓ The nucleic acids content increases with the dilution rate

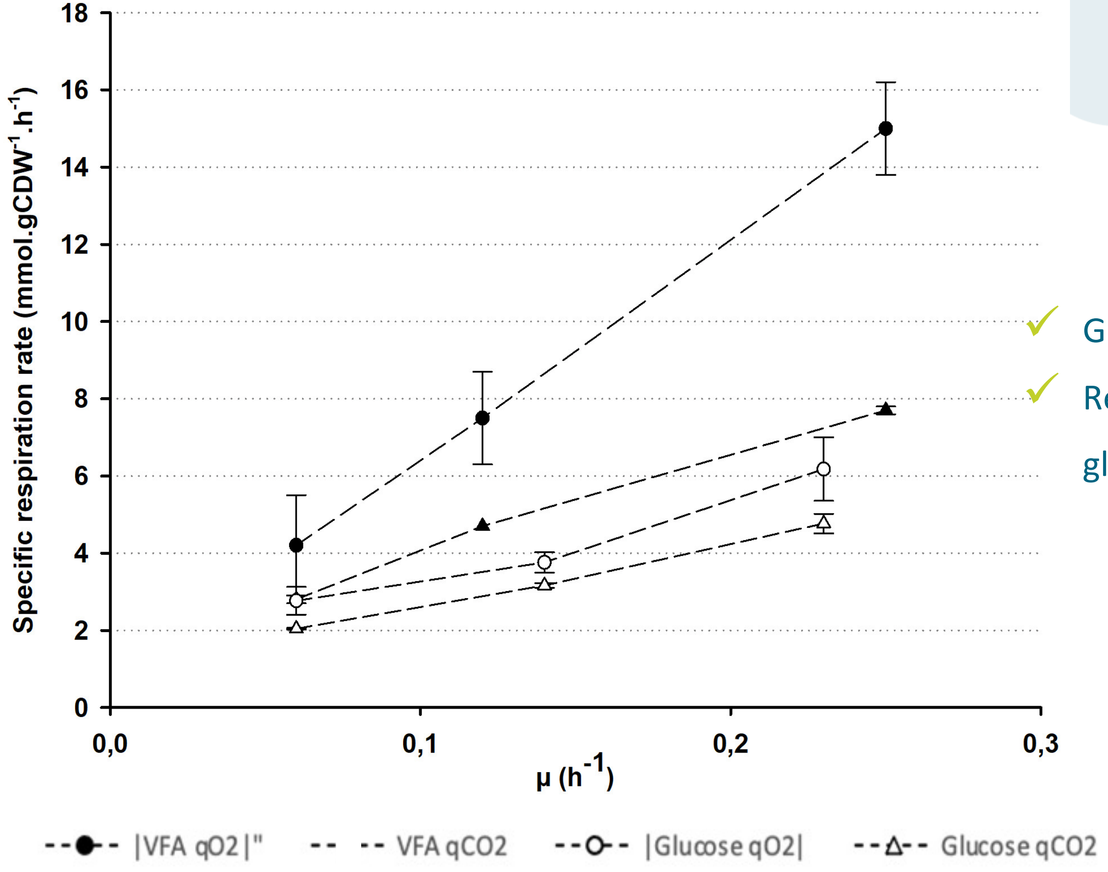
Biomass characterization on VFA mix



0.06	**0.14**	**0.23**	**μ (h^{-1})**
114 ± 6	103 ± 4	92 ± 2	**Carbon balances (%)**
98 ± 9	108 ± 2	106 ± 5	**Nitrogen balances (%)**

- ✓ The same evolutions according to the dilution rate are observed
- ✓ There are less proteins on VFA mix than on glucose

Respiration rates comparison



- ✓ Glucose or VFA conditions show the same behavior
- ✓ Respiration rates on VFA are higher than those on glucose

Kinetics for bioplastic production

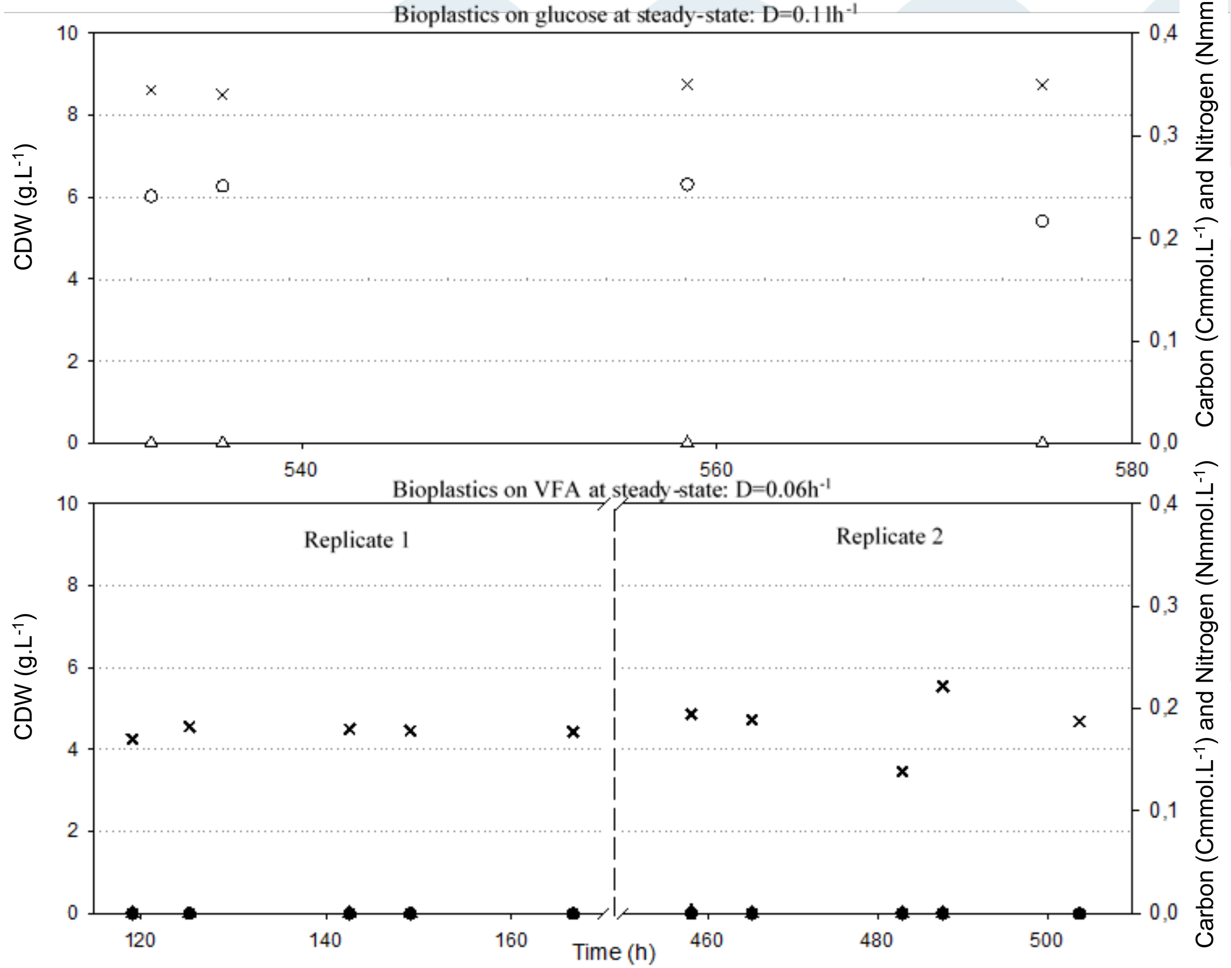
Glucose :

× CDW
○ Carbon
△ Nitrogen

- ✓ On glucose : total nitrogen consumption
- ✓ On VFA : total nitrogen and carbon consumption

VFA :

✖ CDW
● Carbon
▲ Nitrogen



Biomass characterization on glucose

On glucose

D (h^{-1})	CDW ($g.L^{-1}$)	Proteins (%CDW)	PHB (%CDW)	PHV (%CDW)	PHV/PHB	Carbon balances (%)	Nitrogen balances (%)
0.11	8.7 ± 0.1	31.0 ± 1,3	55.5 ± 5.7	*	1	88 ± 5	102 ± 4

* Detected but not quantifiable

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On VFA mix

D (h^{-1})	CDW ($g.L^{-1}$)	Proteins (%CDW)	PHB (%CDW)	PHV (%CDW)	PHV/PHB	Carbon balances (%)	Nitrogen balances (%)
0.06	4.4 ± 0.1	60.4 ± 1.4	20.1 ± 1.0	4.2 ± 0.2	0.21	107 ± 1	108 ± 2
	4.7 ± 0.7	53.3 ± 0.8	25.5 ± 0.7	6.0 ± 0.2	0.24	104 ± 2	103 ± 11

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✓ Bioplastics with around 80% PHB and 20% PHV have the best mechanical properties for material applications

Conclusion

Proof of concept

- ✓ The total transformation of organic wastes such as VFA and urea into proteins or bioplastics has been demonstrated

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Protein production

- ✓ High protein contents (almost 70% of the dry biomass) was reached
- ✓ We demonstrated that low dilution rate allowed whatever the C source:
    - To increase the protein content
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Bioplastic production

- ✓ Bioplastic content of 30% was reached with a good PHV - PHB composition
- ✓ Both total consumptions of urea and VFA was shown

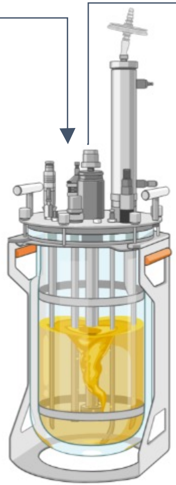
AstroPOU module for protein production, D = $0.06 h^{-1}$

Inlet (consumptions)

O_2
$rO2 = -12\ mmol.L^{-1}.h^{-1}$

Nitrogen (urea)
$rN = -1.6\ Nmmol.L^{-1}.h^{-1}$

Carbon (AGV mix)
$rC = -13\ Cmmol.L^{-1}.h^{-1}$



Outlet (productions)

CO_2
$rCO2 = 7.9\ Cmmol.L^{-1}.h^{-1}$

Biomass
$Qx = 0.17 gDM.L^{-1}.h^{-1}$

72% of proteins
$Qp = 0.12 gProteins.L^{-1}.h^{-1}$

Inlet concentrations

Nitrogen ($Nmol.L^{-1}$)	Carbon ($Cmol.L^{-1}$)
0.041	0.212

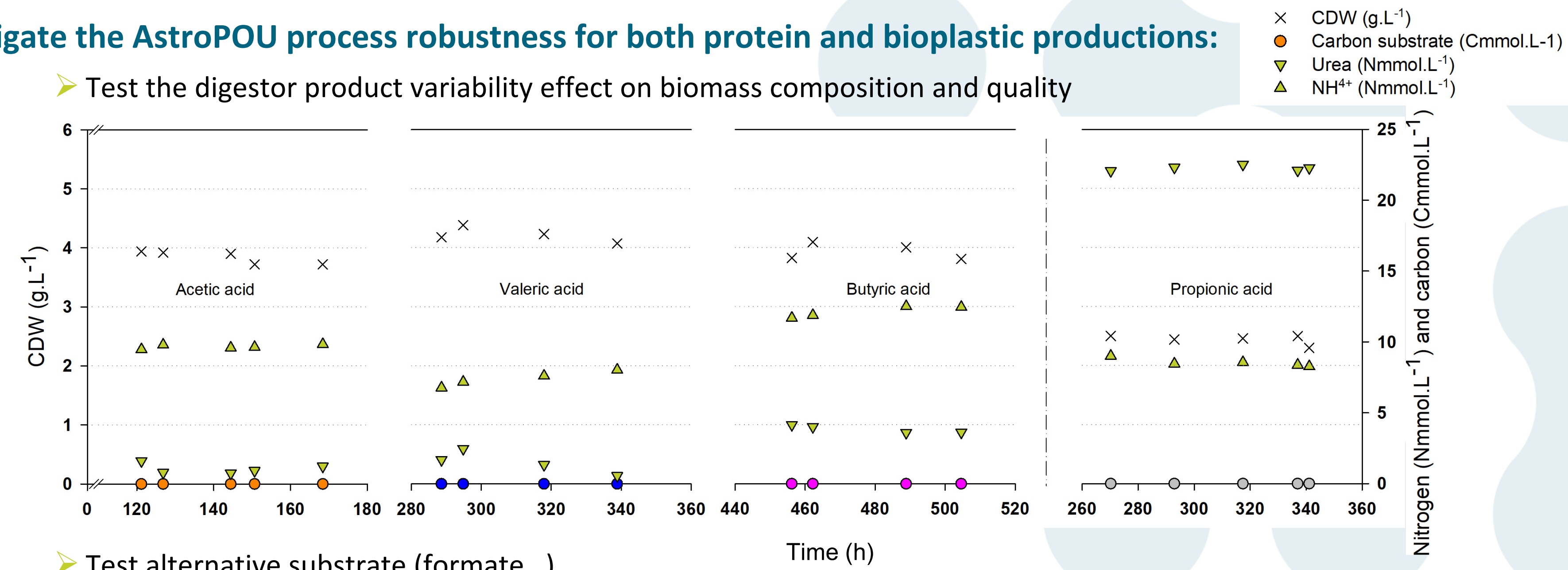
Outlet concentrations

Nitrogen ($Nmol.L^{-1}$)	Carbon ($Cmol.L^{-1}$)	Biomass ($g. L^{-1}$)
0.015	0	2.82

Perspectives

Investigate the AstroPOU process robustness for both protein and bioplastic productions:

- Test the digestor product variability effect on biomass composition and quality



- Test alternative substrate (formate…)
- Define an optimal VFA mix composition to maximize the protein and bioplastic amounts and quality

Gradually increase the complexity of our system:

- Test synthetic urine effect on biomass composition and quality
- Implement a medium recycling loop

Thank you !

Acknowledgments

Spaceship FR

All the FAME team

www.biorender.com