



CREATING  
A CIRCULAR  
**FUTURE**

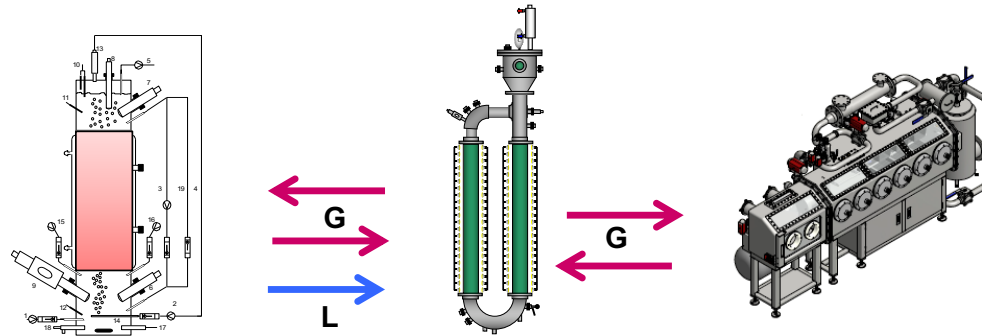
## Continuous operation of C3, C4a and C5 Compartments in the MELiSSA Pilot Plant interconnected in gas and liquid phases

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# INTEGRATION WP6: C3 + C4a connection in liquid phase and C3 + C4a + C5 in gas phase



**Integration  
WP1**

**C4a + C5 gas**

**Integration  
WP3**

**C3 + C4a liquid**

**Integration  
WP4**

**C4a + C5 gas**

**C3 + C4a liquid**

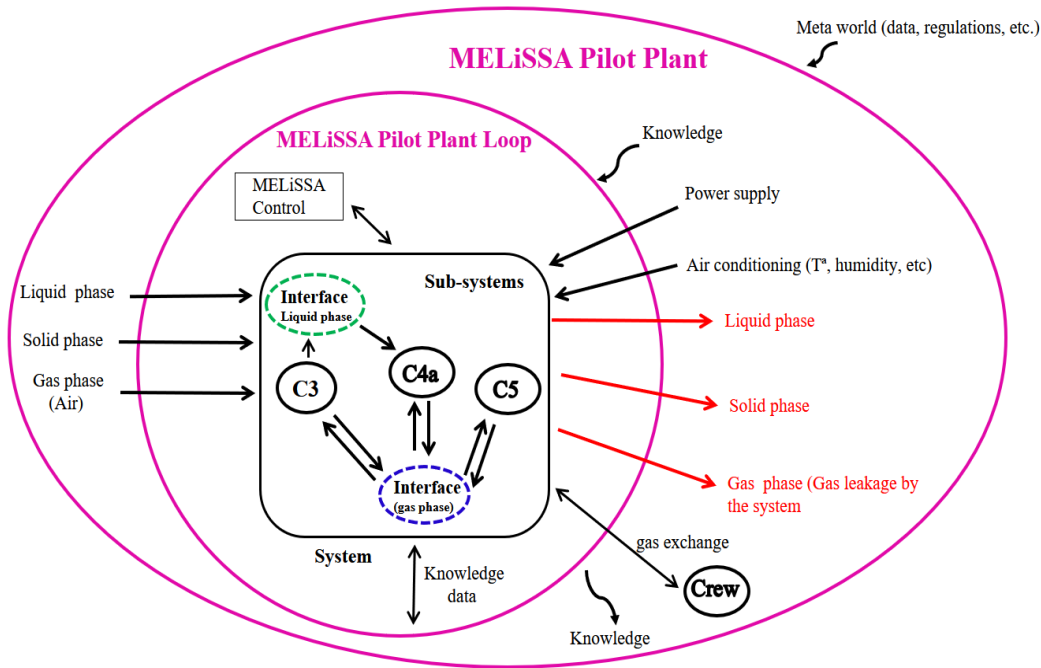
**Integration  
WP6**

**C3 + C4a + C5 gas**

**C3 + C4a liquid**

Objective: integration of the liquid and gas phase of nitrifying packed-bed bioreactor (C3), air-lift photobioreactor (C4a) and rats isolator as a mock crew (C5)

# WP6: WP6 Context Diagram



## Entering into the system:

- Solid phase: Food, bedding, rats, C3 and C4a inocula.
- Liquid phase: Drinking water and medium components (C3 and C4a nutrients).
- Gas phase: Air interaction (Air entering for leak compensation to the system).

## Exiting the System:

- Solid phase: C3&C4a biomass, rats faeces, bedding and food residues.
- Liquid phase: Urine, medium, condensates.
- Gas phase: Gas leaked from the system.

## WP6: Main requirements



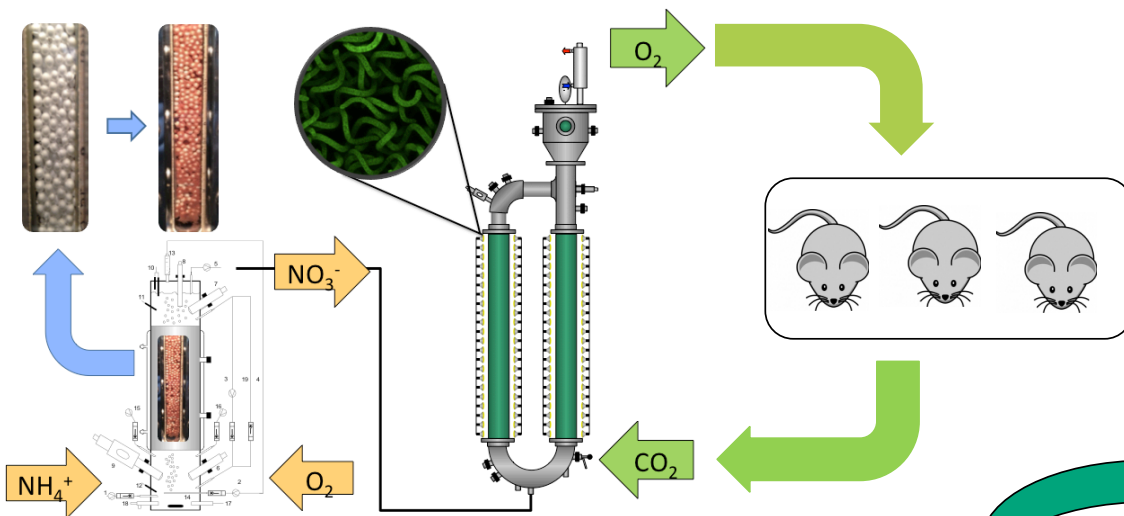
- The system shall address the dynamic **oxygen demand for 3 rats** while addressing the oxygen demand of C3 and achieving highest gas loop closure.
- The system shall **prevent chemical/microbial contamination** in the liquid/gas phase which could be toxic for C3, C4a process and the crew.
- The system shall **not leak more than 20%** of the initial gas volume in 4 weeks.
- Gain knowledge on **closure regenerative life support system**: C3, C4a, C5.
- Demonstration/validation of control **model robustness**.
- Validate **long term continuous operation** of processes, technology items.

- ❑ C4a should produce the **oxygen needed** in C5 and C3 compartments (rats+nitrification), with different demand dynamics:
  - O<sub>2</sub> demand in C5 depending on **day/ night cycles** of the rats
  - Several **O<sub>2</sub> concentration set-points** in C5 will be tested (19-21%)
  - O<sub>2</sub> demand in C3 depending on N load (range 710-1500 ppm/day to be tested)
  
- ❑ C3 should produce the **nitrate needed in C4a** to produce oxygen for C3 and C5 (3 rats)
  - Min. load approx. of **710 mg N/L·d** ( $Y_{N/O} = 0.06$  g N/g O<sub>2</sub> in C4a, experimental data from MPP)
  
- ❑ Prepare **future integration steps** in the MPP:
  - Test N loads corresponding to the **potential urine introduction** into C3 compartment (higher oxygen demand in C3 and potential C limitation in C4a (C input required))
  - Consider potential inputs/outputs to future connection of the **higher plants chamber** gas loop.

# WP6 integration; C3&C4a connection by liquid phase and C3&C4a&C5 by gas phase



## Oxygen demand in C3 and C5 (previous tests):



PREVIOUS TEST RESULTS						
WP3 test results C3 compartment Oxygen needs			WP1 test results C5 comp. oxygen needs (g O <sub>2</sub> /h) (average)		WP3 test results C4 Compartment Oxygen production (high light intensity)	
N- Load (mg N/L d)	L O <sub>2</sub> /d	g O <sub>2</sub> /h	1 rat	3 rats	Inlet flow (L/d)	Oxygen production (g O <sub>2</sub> /h)
438	10.2	0.56	0.59	1.77	20	2.44
876	20.9	1.14			30	2.76

**Average O2 needs: around 2,8 g/h (C3+C4a)**

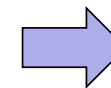
## C4a Characterization (New illumination system)



### Max. Oxygen demand in C3 and C5 (WP6):

O2 Max. needs for Integration WP6 Test				
N-NH <sub>4</sub> <sup>+</sup> Load (ppm/d)	C3 O <sub>2</sub> needs (g/h)	Max C5 O <sub>2</sub> needs (g/rat/h)	Max C5 O <sub>2</sub> needs for 3 rats (g/h)	Total O <sub>2</sub> WP6 req (g/h)
710	0.91	0.73	2.19	3.10

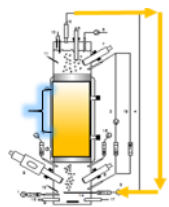
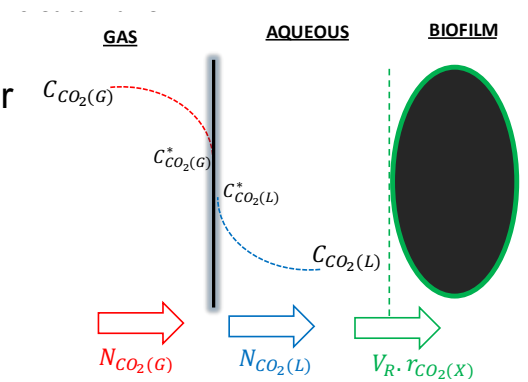
Condition	Q <sub>L</sub> (L/d)	W/m <sup>2</sup>	CDW (g/l)	O <sub>2</sub> prod (g/h)
1	20	364	2.25	3.26
2	30	364	1.74	3.80
3	40	364	1.27	3.94
4b	50	250	0.96	3.53
4c		300	0.98	3.61



- C4a production 3.30-4 gO<sub>2</sub>/h
- All conditions can be tested in WP6 mode 1

# WP6 Oxygen needs for C3 Compartment

- Depending on N load according to stoichiometry  $\text{NH}_4^+ \rightarrow \text{NO}_3^-$
- Keeping **constant  $K_La$**  as far as hydrodynamic conditions are maintained in the bioreactor
- Depending on gas-liquid transfer rate  $\rightarrow$   **$\text{O}_2$  partial pressure** in gas phase



### INPUTS

C3 Volume (L)	7
C3 Temperature (°C)	30
C3 Pressure (mbarg)	80
$C^*_{\text{DOL}}$ (AIR, 30°C) (g/L)	0.00757
Weight $kLa$ TOP Section (%)	85

MODE *	C3 Feed Load (ppm/d)	C3 Actual Load (ppm/d) Conv.:93.3%	Total Air flowrate		$kLa$ TOP		$kLa$ LOWER		(% mol/mol) $\text{O}_2$ C3 Gas Phase	TOTAL	MATERIAL BALANCE
			(L/min)	(L/h)	(1/h)	(1/s)	(1/h)	(1/s)		TOTAL $\text{N}_{\text{O}_2}$	
										TOTAL $\text{O}_2$ trans. flow gas to liquid (g $\text{O}_2$ /h)	$\text{O}_2$ consumption by Bacteria (g $\text{O}_2$ /h)
MODE 1	251	235	3.0	180	31.995	0.0089	52.785	0.0147	19.0	0.3359	0.3140
MODE 1	486	453	3.0	180	31.995	0.0089	52.785	0.0147	22.0	0.6140	0.6062
MODE 2	1286	1199	3.0	180	31.995	0.0089	52.785	0.0147	33.4	1.7250	1.6046
MODE 2	1714	1599	3.0	180	31.995	0.0089	52.785	0.0147	40.4	2.3954	2.1380

(\*) MODE 1: LOW N LOAD MODE ; MODE 2: OXYGEN CONCENTRATION

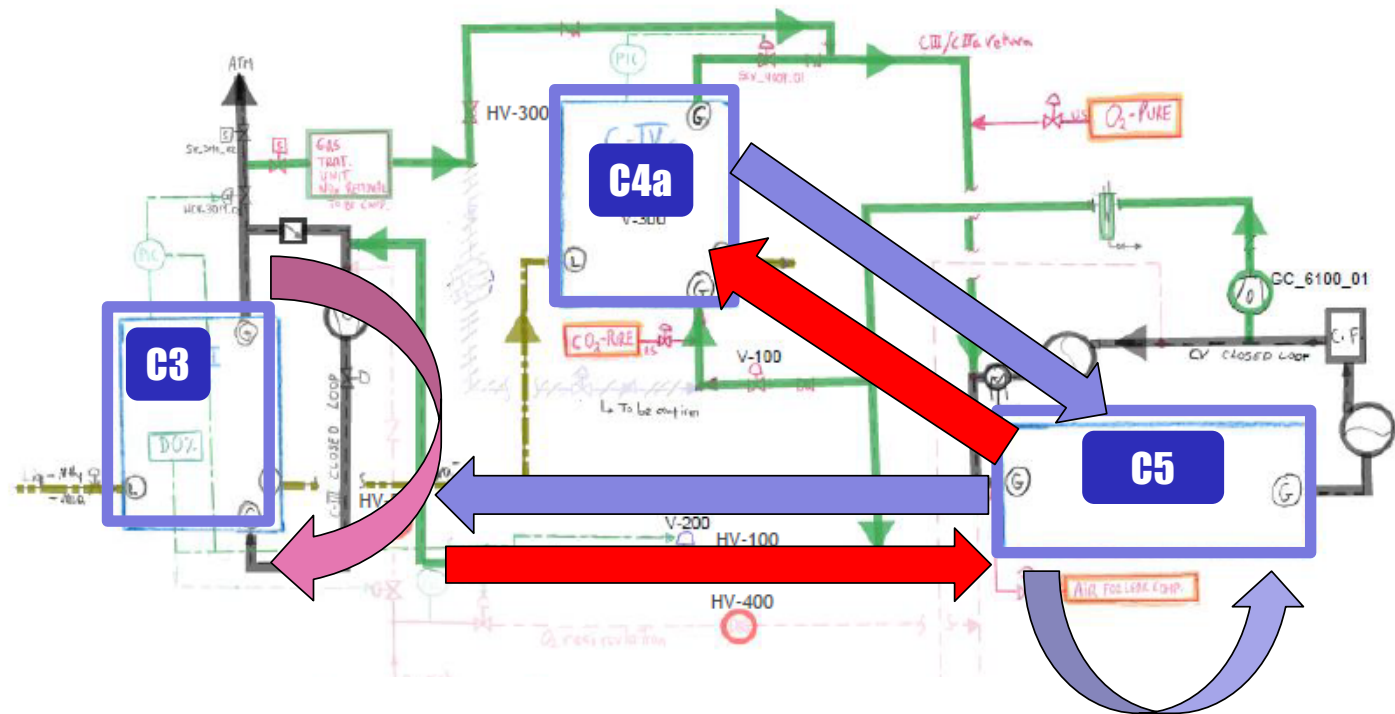


# WP6: PFD FOR NOMINAL OPERATION MODE 1



## LOW AMMONIUM LOAD in the system

- ❑ C4a outlet is sent to C5 compartment.
- ❑ C5 outlet is divided to C4a and C3 compartments.
- ❑ C3 outlet is sent to C5 compartment.
- ❑ C3 closed gas loop is maintained (pulses addition when required).

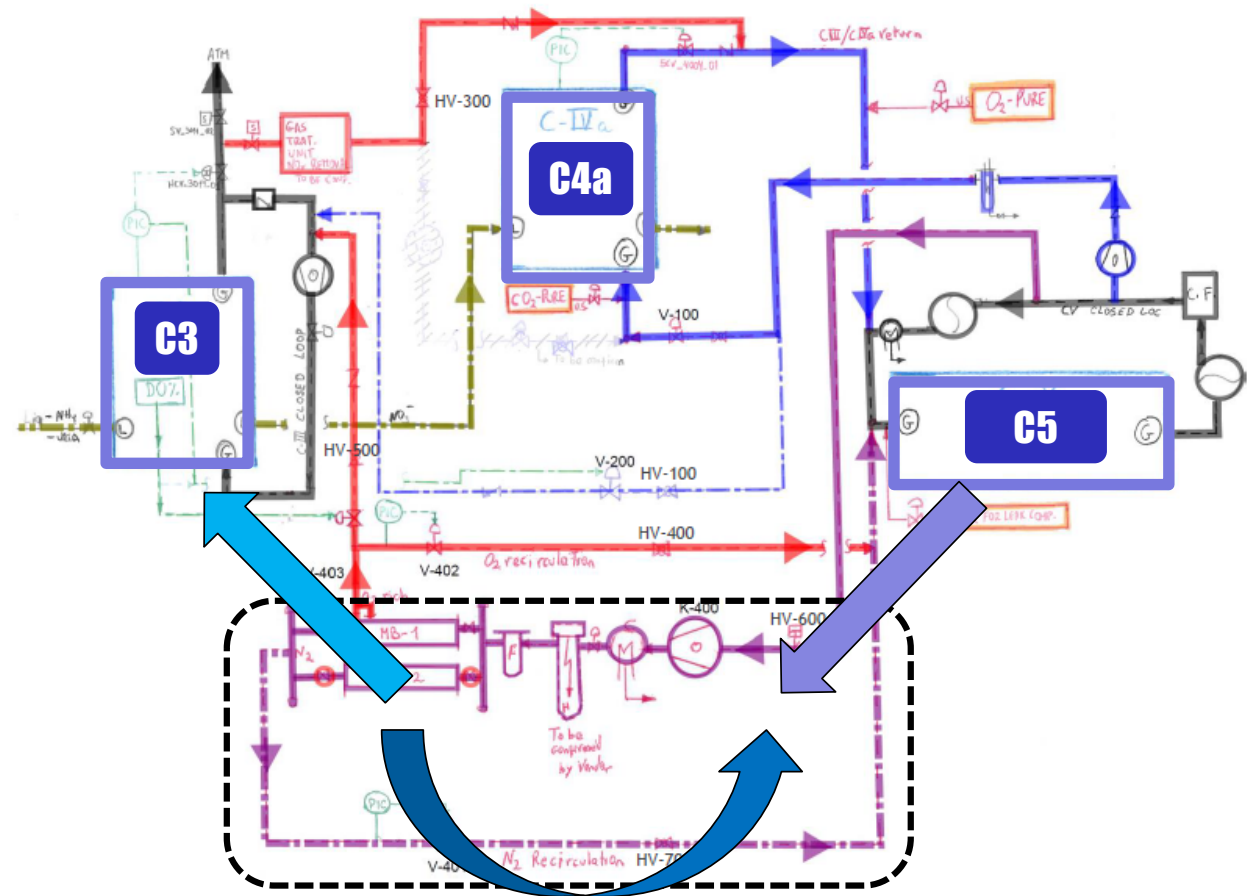


## WP6: PFD FOR NOMINAL OPERATION MODE 2



### HIGH AMMONIUM LOAD in the system

- ❑ Part of C5 outlet is sent to C4a compartment
- ❑ Part of C5 outlet is sent to an oxygen enrichment membrane module to fulfil C3 oxygen requirements.




# Oxygen enrichment unit (OEU) design



Evaluated technologies for Oxygen concentration:



- Cryogenic air separation units (ASUs)
- Pressure swing adsorption (PSA)
- Membranes separators 

## Critical points in the OEU design:

- High operating pressure (**6-8 bar**) compatible with very low P in C5 (**2 mbar**)
- Regulation of **retentate Flow, temperature and Pressure**
- Avoiding presence of **condensates and NH<sub>3</sub>** → cooler+automatic drainage; coalescing filter; C filter in C5
- Dedicated **O<sub>2</sub> analyser** at the permeate side

Case

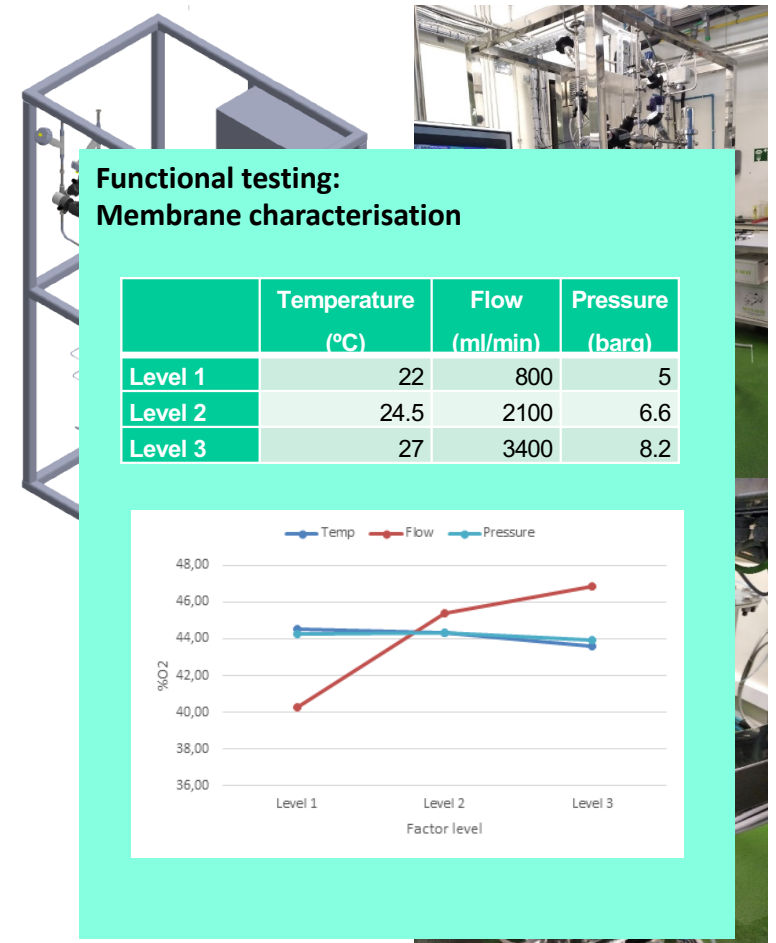
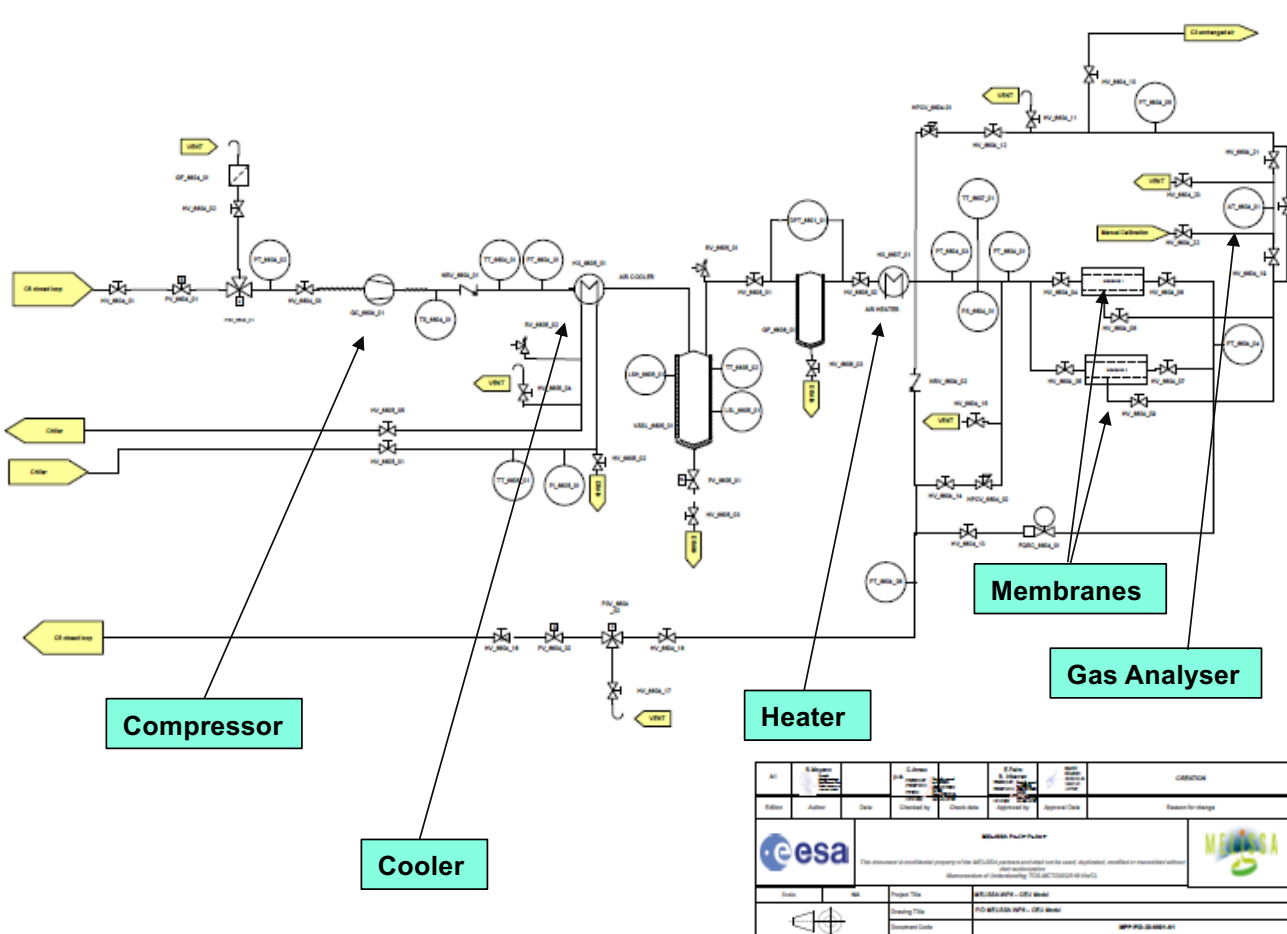


Hollow Fibers

End Caps

PA1010-P3 membrane (AIR PRODUCTS)

# Oxygen Enrichment Unit (OEU) design and installation



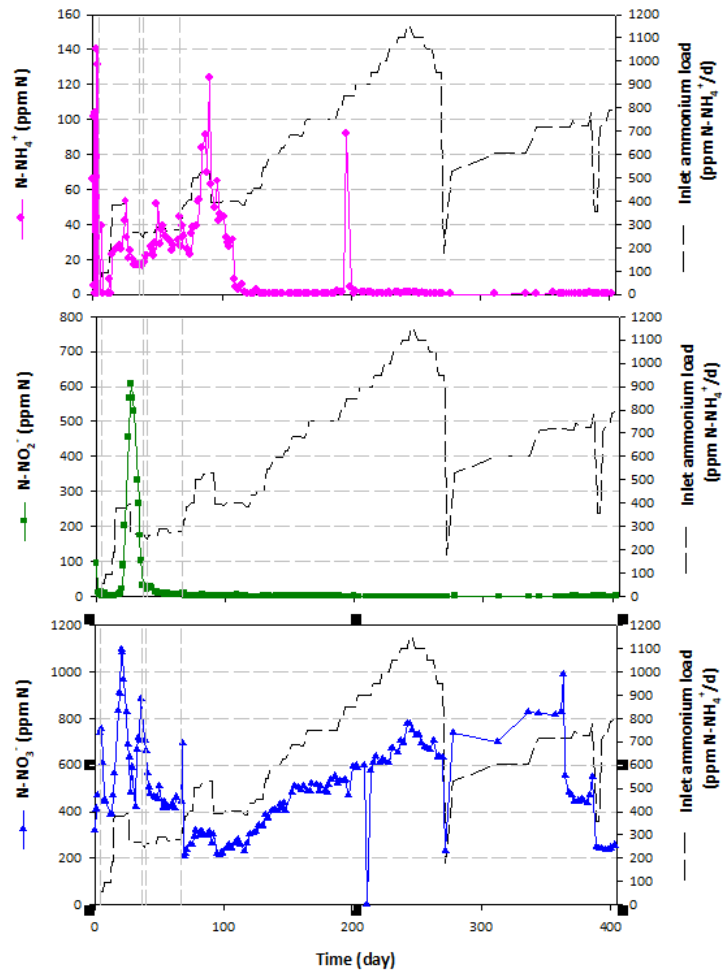
## WP6 MODE 1 CONDITIONS



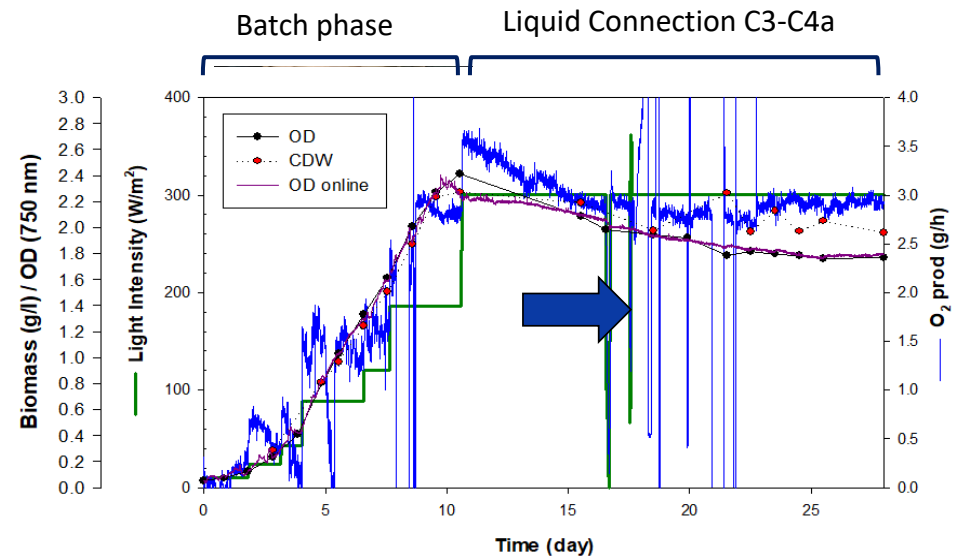
C3 compartment					WP6 OXYGEN NEEDS	
Inlet ammonium load in C3 (mg N-NH <sub>4</sub> /L/d)	N elimination in C3 (%)	O <sub>2</sub> consumption. (gO <sub>2</sub> /h)	DO set-point	Inlet gas concentration (%)	Max. O <sub>2</sub> consumption per rat (C5; night period)	Total O <sub>2</sub> consumption (C3+C5) <u>3 rats</u>
<b>710</b>	99%	0.90	50	21.29	0.73	3.10
			45	19.18		
			30	17.10		

- INLET FLOW FIXED at 20-30-40-50 L/day (3 HRT at steady state)**
- AMMONIUM LOAD fixed at 710 mg N/L/d**
- OXYGEN SET-POINT in C3 CONTROLLED at 45%**
- SEQUENTIAL CHANGES of OXYGEN SET-POINT in C5 (21-19-20-21%)**
- NUMBER OF RATS 3.**

# C3 and C4a start-up, stand-alone and liquid connection phases)



- C4a Initial batch phase with target OD of 2.5
- **Liquid Connection to C3 at 20 L/d**
- **Stable O<sub>2</sub> production level**
- **Nitrate conc. kept above 30-50 ppm (no N limitation)**
- **TIC kept above 200 ppm (no C limitation)**

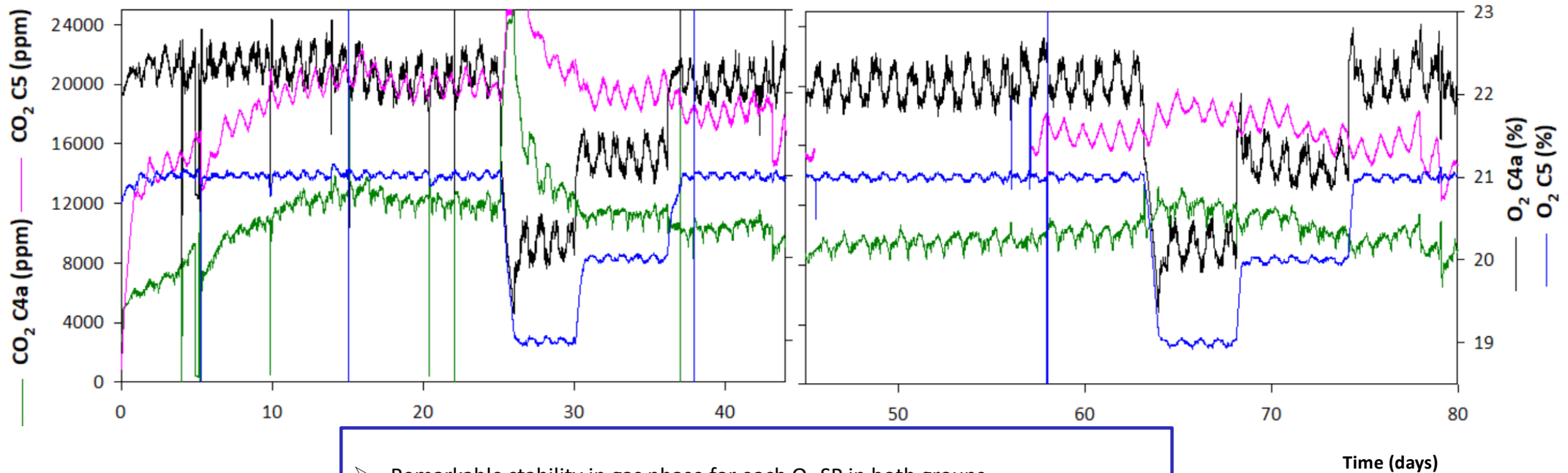


# C4a-C5 O<sub>2</sub> and CO<sub>2</sub> evolution



1st group; 20 L/d

2nd group; 30 L/d



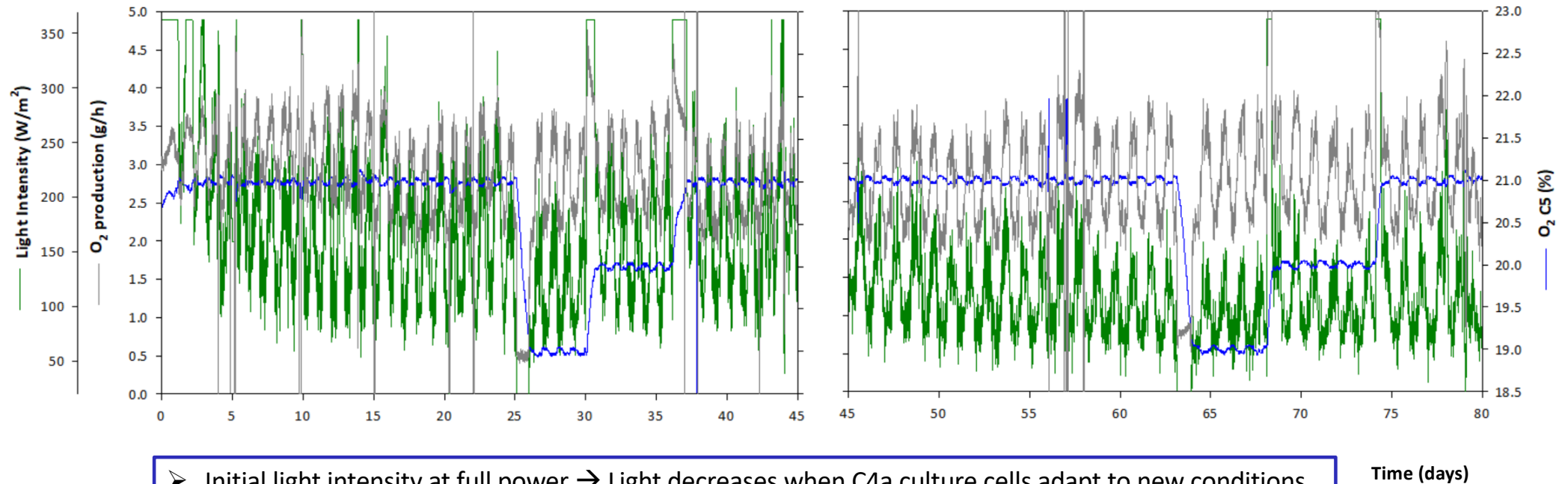
- Remarkable stability in gas phase for each O<sub>2</sub> SP in both groups
- CO<sub>2</sub> maintained ≤2% most of condition, except during a few transient phases

# C4a-C5 O<sub>2</sub> production and Light evolution



1st group; 20 L/d

2nd group; 30 L/d



- Initial light intensity at full power → Light decreases when C4a culture cells adapt to new conditions
- Lower light intensity is needed in 2nd group (higher dilution rate) for the same O<sub>2</sub> production

Time (days)

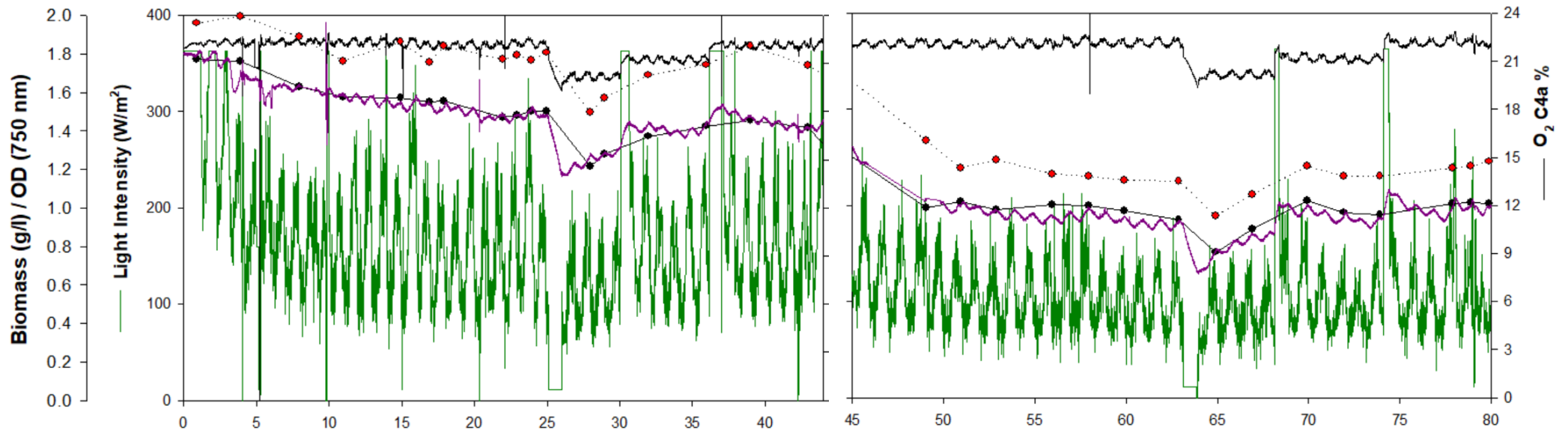


# C4a Biomass evolution



1st group; 20 L/d

2nd group; 30 L/d



● OD  
● CDW  
— OD online

- Stable biomass levels at 21% O<sub>2</sub> SP
- Day and night cycles are perfectly reflected in online biomass monitoring
- Same behaviour of second group, at a lower biomass level

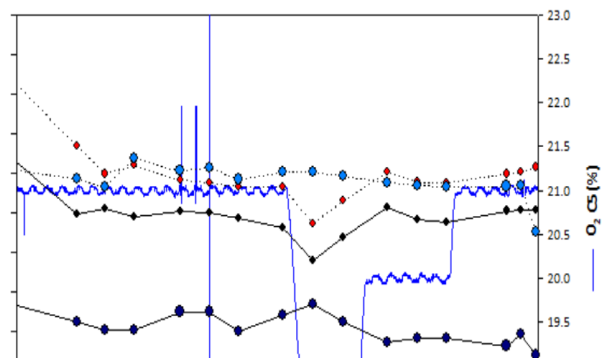
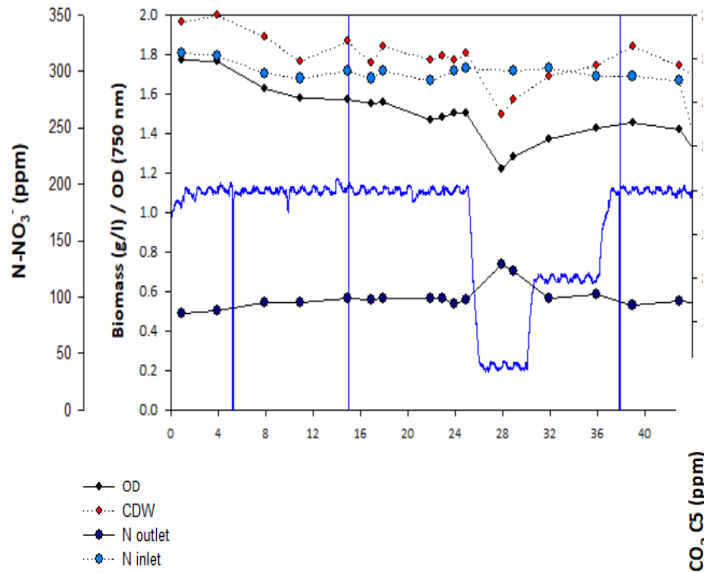
Time (days)

# C4a Nitrogen and Carbon content



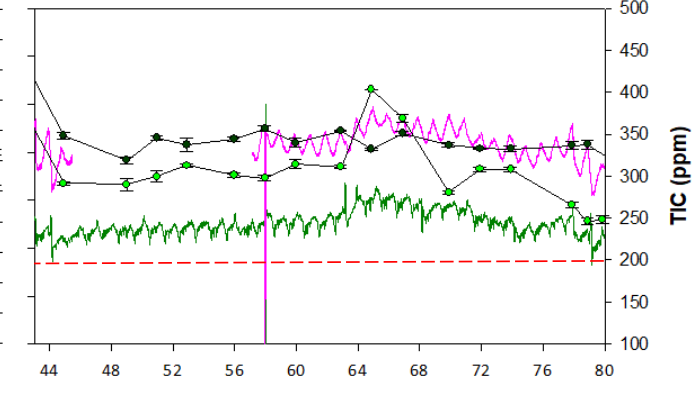
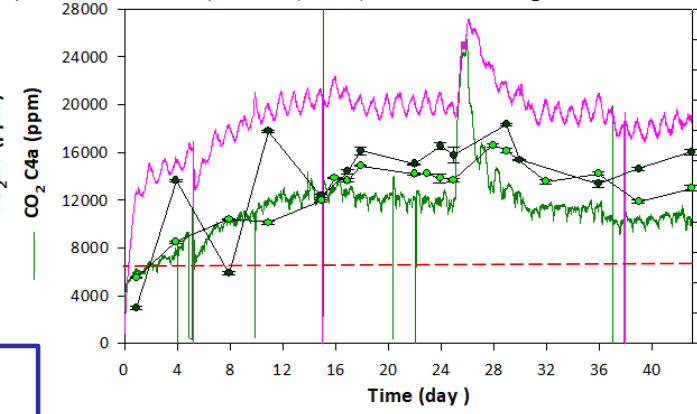
1st group; 20 L/d

2nd group; 30 L/d



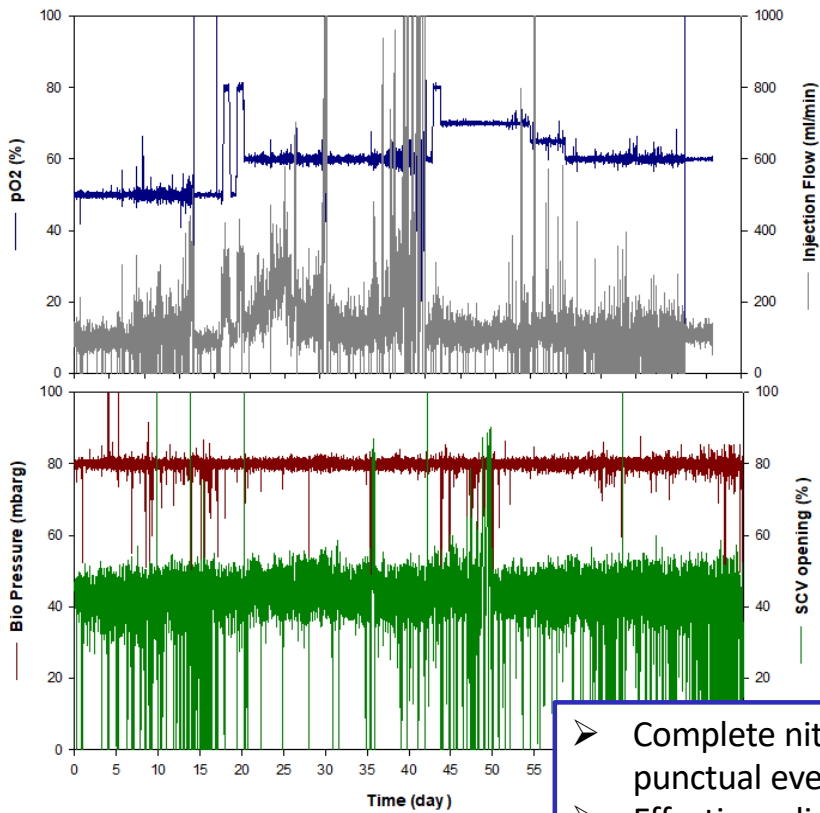
➤ Residual N in liquid phase >50 ppm avoiding potential N limitation

➤ Residual C in liquid phase >200 ppm avoiding potential C limitation

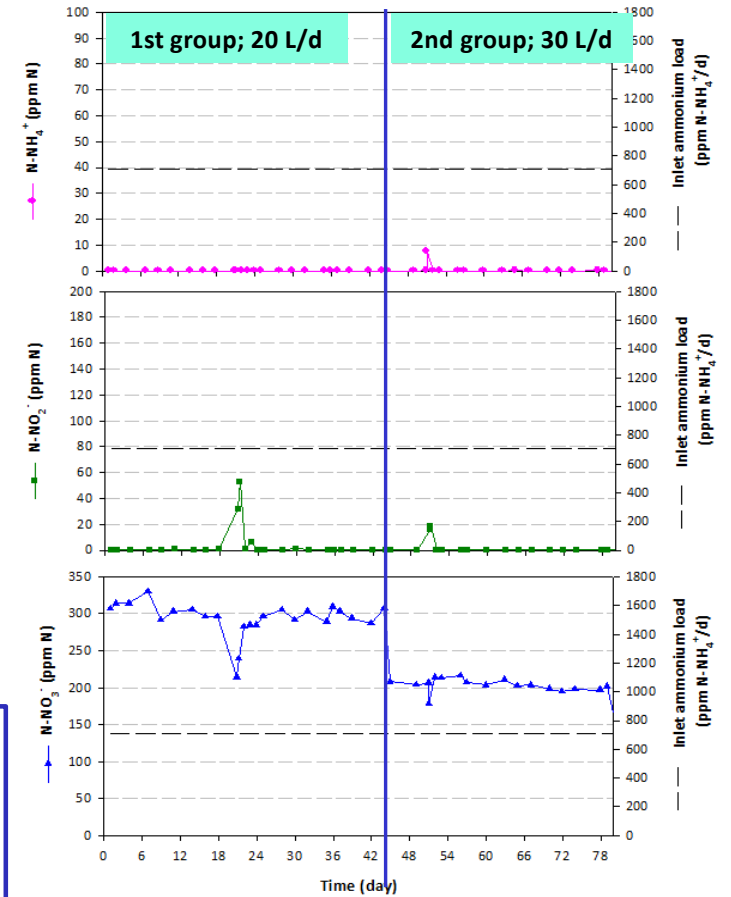


—●— TIC Inlet  
—●— TIC Outlet

# C3 gas and liquid phases



- Complete nitrification during the test except punctual events
- Effective adjustment of D.O. level (50-70%)
- Correct regulation of C3 head pressure



# Production calculations



Flow	O <sub>2</sub> SP	Biomass (g/l)	O <sub>2</sub> prod (g/h)	LI (W/m <sup>2</sup> )	C3 need stoich. (g/h)	O <sub>2</sub> rats (g/h)	CO <sub>2</sub> prod (g/h)	RQ (rCO <sub>2</sub> /rO <sub>2</sub> )	N-NO <sub>3</sub> in (ppm)	N-NO <sub>3</sub> - out (ppm)	Y <sub>N/X</sub> (g/g)	
20 L/d	21%	1.79	2.9	156.8	0.93	2.0	2.64	0.95	298	97	0.11	
	21 - 19%	-	-	11		2.0	-	-	-	-	-	-
	19%	1.51	2.8	118		1.9	2.76	1	300	126	0.12	
	19 - 20%	-	4.1	364		1.9	-	-	-	-	-	-
	20%	1.72	2.9	146		2.0	2.65	0.95	299	100	0.12	
	20 - 21%	-	3.8	364		2.2	-	-	-	-	-	-
	21%	1.79	2.7	155		1.8	2.53	1	294	94	0.11	
30 L/d	21%	1.14	2.8	97		1.9	2.11	0.82	210	80	0.11	
	21 - 19%	-	-	11		1.9	-	-	-	-	-	-
	19%	1.00	2.8	88		1.9	2.12	0.81	209	79	0.13	
	19 - 20%	-	5.1	364		1.8	-	-	-	-	-	-
	20%	1.17	2.8	102		2.0	2.12	0.78	199	64	0.12	
	20 - 21%	-	4.9	364		1.9	-	-	-	-	-	-
	21%	1.20	2.9	96		1.9	2.05	0.79	198	68	0.11	

Theoretical need (710 N-ppm/d)

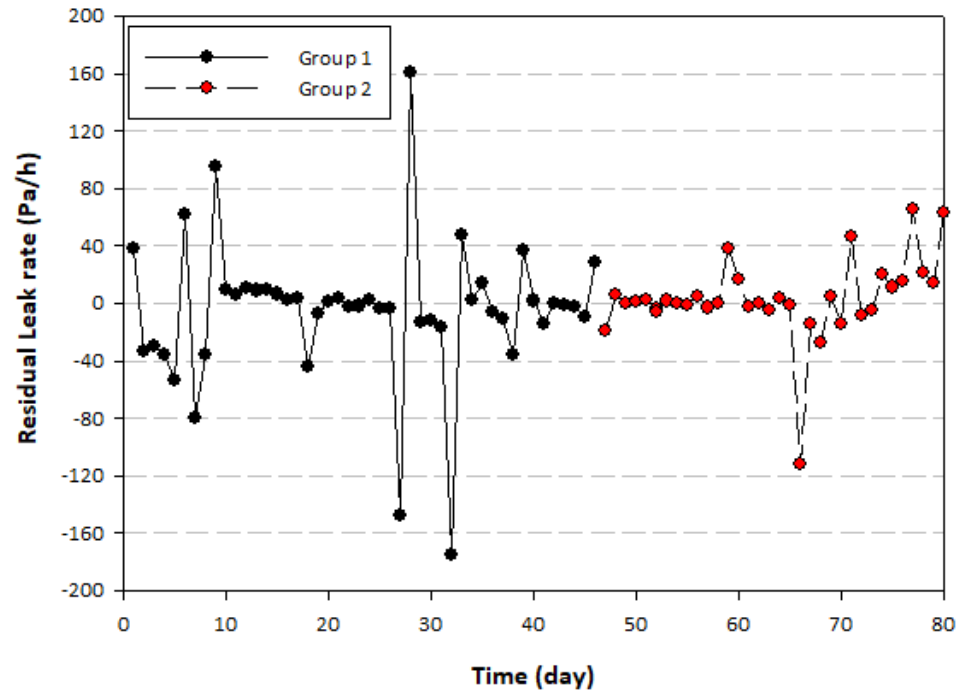
Expected: 0.08-0.10

# Mode 1 – next phases schedule



Condition #	HRT C3/C4a (h)	Inlet N-NH4+ conc. (ppm)	Inlet N-NH4+ load	Flow (L/d)	TIC inlet (ppm)	C5 O <sub>2</sub> SP	pO <sub>2</sub> C3	Estimated duration (days)	Flexibility (days)	Rats	Status
1						21%		25		New group	Completed
2						19%		1+4			Completed
3	8.3/99.6	245		20	445	20%		2+4	4		Completed
4						21%		2+2			Completed
5						21%		17+2		New group	Completed
6	6.55 / 66	165		30	355	19%		1+4	2		Completed
7						20%		1+4			Completed
8						21%		1+3			Completed
9			710			21%	45%	12+4		New group	Ongoing
10	4.14 / 49.8	122		40	327	19%		1+4	5		
11						20%		1+4			
12						21%		1+3			
13						21%		10+4		New group	18/11/2020
14	3.13 / 39.8	98		50	300	19%		1+4	0		
15						20%		1+4			
16						21%		1+3			16/12/2020

# System tightness



Group	Injected air (L)	Air flow (L/h)	Residual leak (Pa/h)	Total leak (Pa/h)
1	362	0.34	-0.7	-22.3
2	181	0.22	+3.5	-11.3

Leak specification: <30 Pa/h

## WP6 MODE 2 CONDITIONS



### Oxygen Enrichment Unit (OEU): O<sub>2</sub> enrichment available 40-50%

C3 compartment					OXYGEN CONSUMERS		
Inlet flow (L/d)	Inlet ammonium load in C3 (mg N-NH <sub>4</sub> /L d)	O <sub>2</sub> consump. (gO <sub>2</sub> /h)	DO set-point	Inlet gas concentration (%)	Max .O <sub>2</sub> consumption per rat (C5; night period)	Total O <sub>2</sub> consumption (C3+C5)	Total O <sub>2</sub> consumption (C3+C5) considering 3 rats
<b>40</b>	900	1.2	80	29.57	0.73	1.93	3.35
	1200	1.6	80	34.14		2.33	3.74
	1500	1.9	80	37.2		2.63	4.12

- INLET FLOW FIXED at 40 L/day (3 RT at steady state)
- AMMONIUM LOAD from 710 mg N/L d to 1500 mg N/L d
- OXYGEN SET-POINT in C3 CONTROLLED at 80%
- SEQUENTIAL CHANGES of OXYGEN SET-POINT in C5 (21-19-20-21 %)
- NUMBER OF RATS 3.

Preparing urine introduction in C3: expected N load of 2174 mg N/L day

Preparing HPC future integration: potentially pure O<sub>2</sub> injection

## Integration WP6 (in progress) main conclusions

- ❑ WP6 hardware design and validation completed
- ❑ Test conditions defined
- ❑ Testing phase (Mode1) progressing successfully, achieving continuous operation during 3,5 months of three compartments in gas and liquid phases, demonstrating the robustness of the system
- ❑ Mode 2 conditions defined; still to be modulated based on knowledge gained during Mode1
- ❑ Paving the integration of relevant future integration steps: introduction of urine and connection with next compartments (HPC) in the MELISSA loop



# MELISSA



MICRO-ECOLOGICAL  
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## THANK YOU.

**Enrique Peiro**

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