

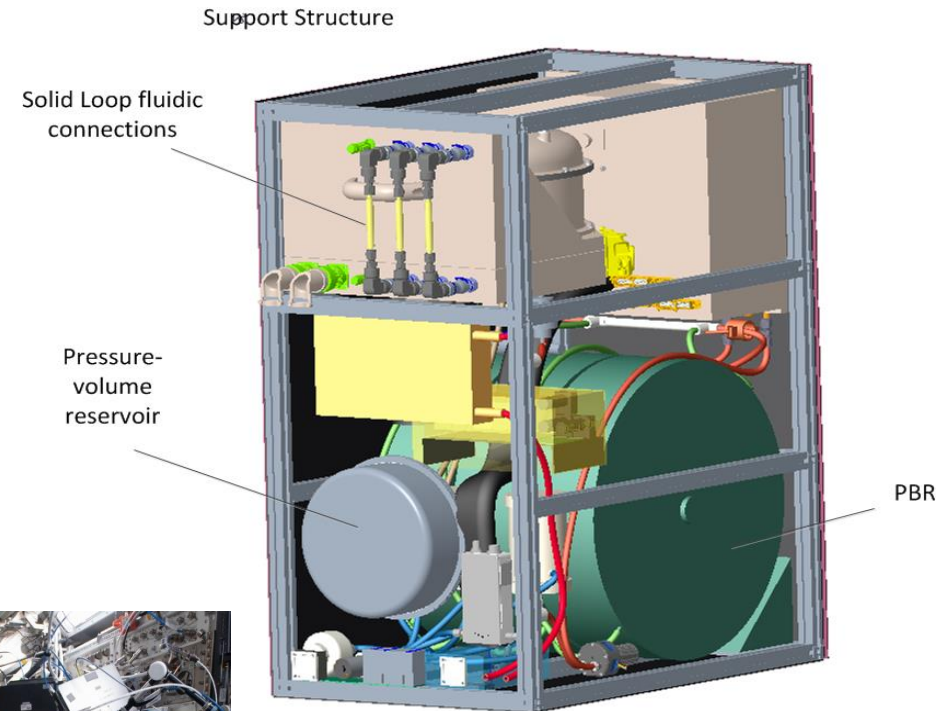
**Design & operation of a bread board model  
of spirulina photobioreactor  
equipped with a harvesting system  
to support ISS On-Board Demonstrator development**

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- Biorat-1 On-board Demonstrator
- Bread Board Model
  - Previous design
  - New functionalities
  - Life test results
- Conclusions & Way Forward

# BIORAT-1 OBD Objectives

- BRT1 On-board demonstrator (OBD)
- Objectives:
  - Demonstrate operation of Photoautotrophic Bacteria Compartment (IV4) in microgravity
    - Demonstrate recycling of CO<sub>2</sub> directly from ISS cabin into O<sub>2</sub> for crew by the mean of a photobioreactor & spirulina (*Limnospira indica* PCC 8005)
    - Demonstrate **optimal process control**:
      - Precise regulation O<sub>2</sub> production on demand
      - Validation of model predictive strategy
      - Generate **edible biomass**
  - (operate in axenic conditions, avoiding contamination)
  - Demonstrate **long term operation and controllability** (90 days demonstration sequence)
- Accommodation
  - ISS, European Drawer Rack 2 (EDR2)



BRT1 OBD Concept design



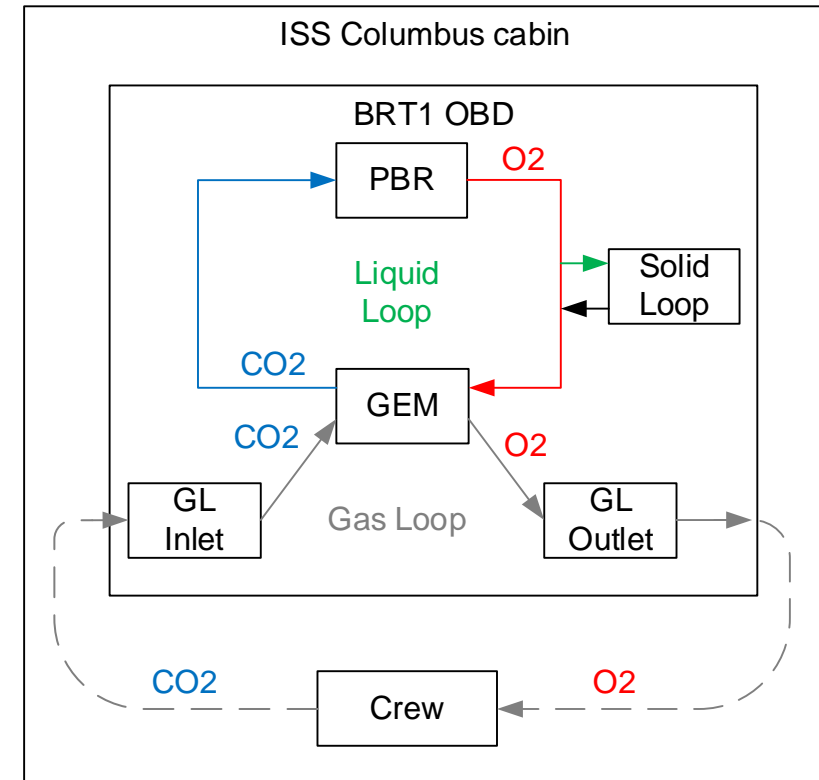
Installation of EDR2, source ESA

# BIORAT-1 OBD

## Operation Principle

- Limnospira indica PCC 8005 oxygen production stoichiometry
 
$$\boxed{HCO_3^-} + 0,70 H_2O + \boxed{0,17 NO_3^-} + 0,007 SO_4^{2-} + 0,006 HPO_4^{2-} + 0,197 H^+ \xrightarrow{\langle r_x \rangle} \boxed{CH_{1,58}O_{0,46}N_{0,17}S_{0,007}P_{0,006}} + \boxed{1,39 O_2} + OH^-$$
- BRT-1 OBD key components & functions
  - Liquid Loop (LL): transfer of chemical species in liquid
  - Photobioreactor (PBR): O<sub>2</sub> & biomass production
  - Gas Exchange Membrane (GEM): O<sub>2</sub> & CO<sub>2</sub> transfer between gas & liquid phase
  - Gas Loop (GL): Transfer of O<sub>2</sub> to the LL & CO<sub>2</sub> to the cabin
  - Solid Loop (SL):
    - Harvesting: Biomass concentration control,
    - Feeding: Nitrate supply in Zarrouk medium
- **Life tests with Bread Board Models (BBM) to validate OBD design**

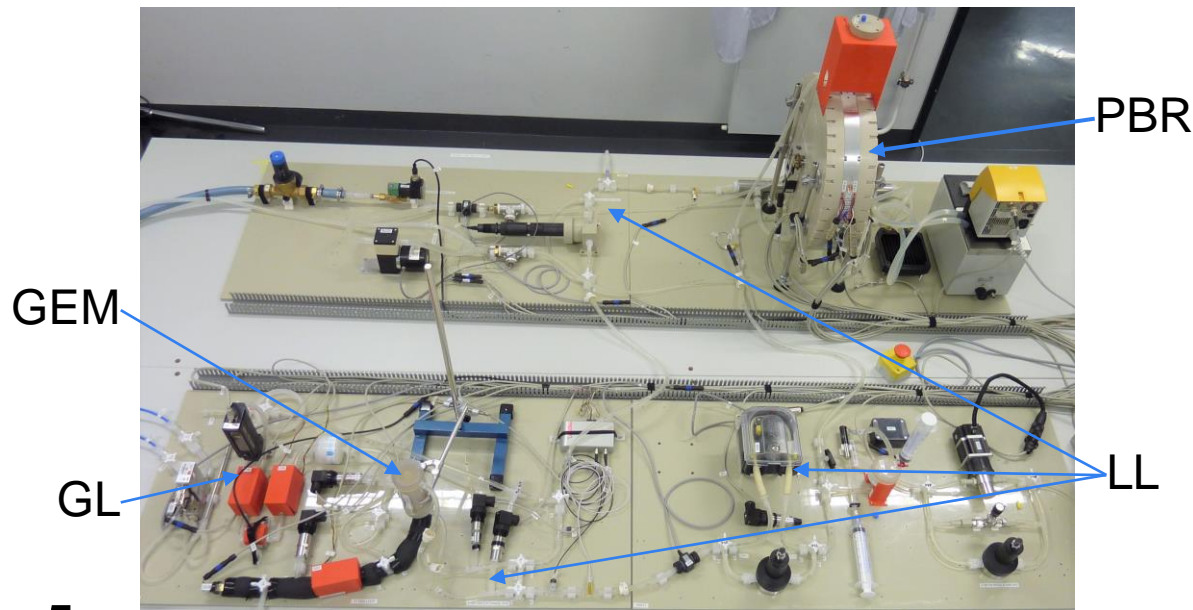
Ref. Biorat, MELiSSA demonstration breadboard, Final Presentation 2000



# Biorat-1 BBM

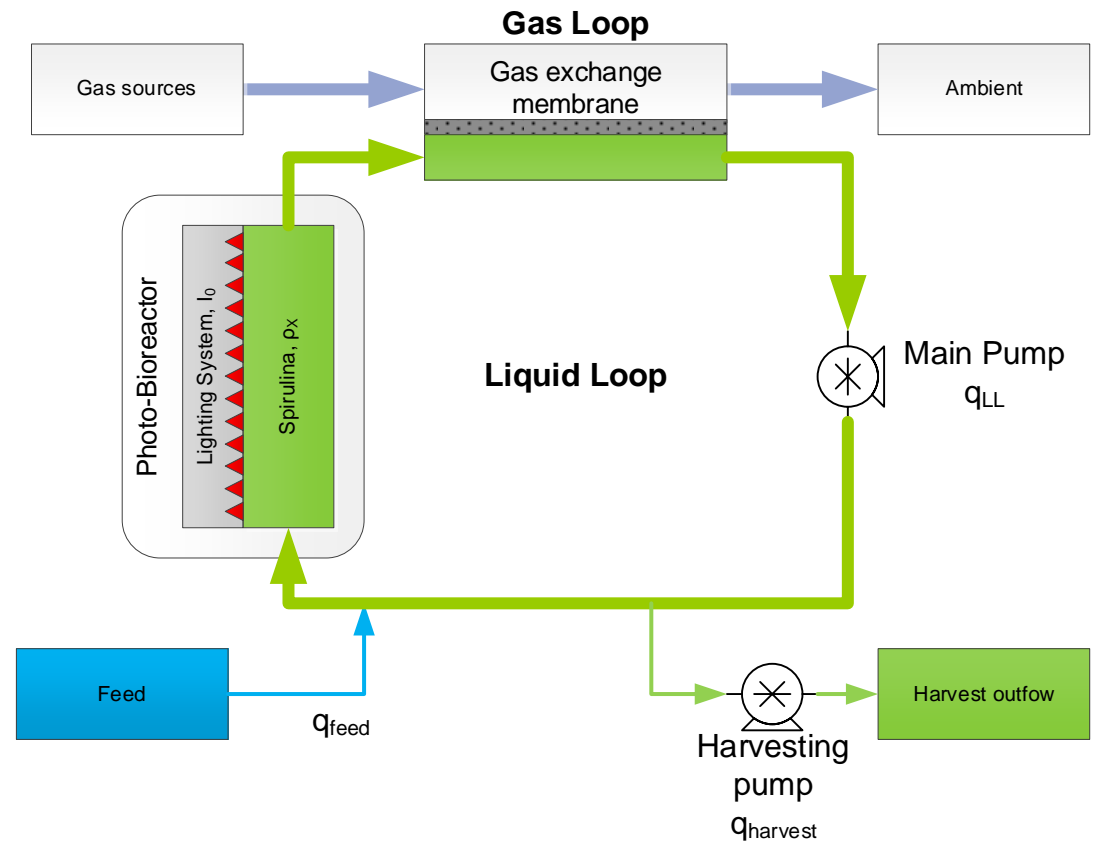
## Previous BBM design

- 2019 BBM Life test, performed to validate Photobioreactor & Liquid Loop
  - PBR 2.6L, O<sub>2</sub> production 6.8mmol/hr
  - Chemostat operation (no solid loop)
  - ➔ high feed consumption
  - No process control
  - ➔ Light intensity, harvesting flow rate set manually



2019 BRT1 BBM

5



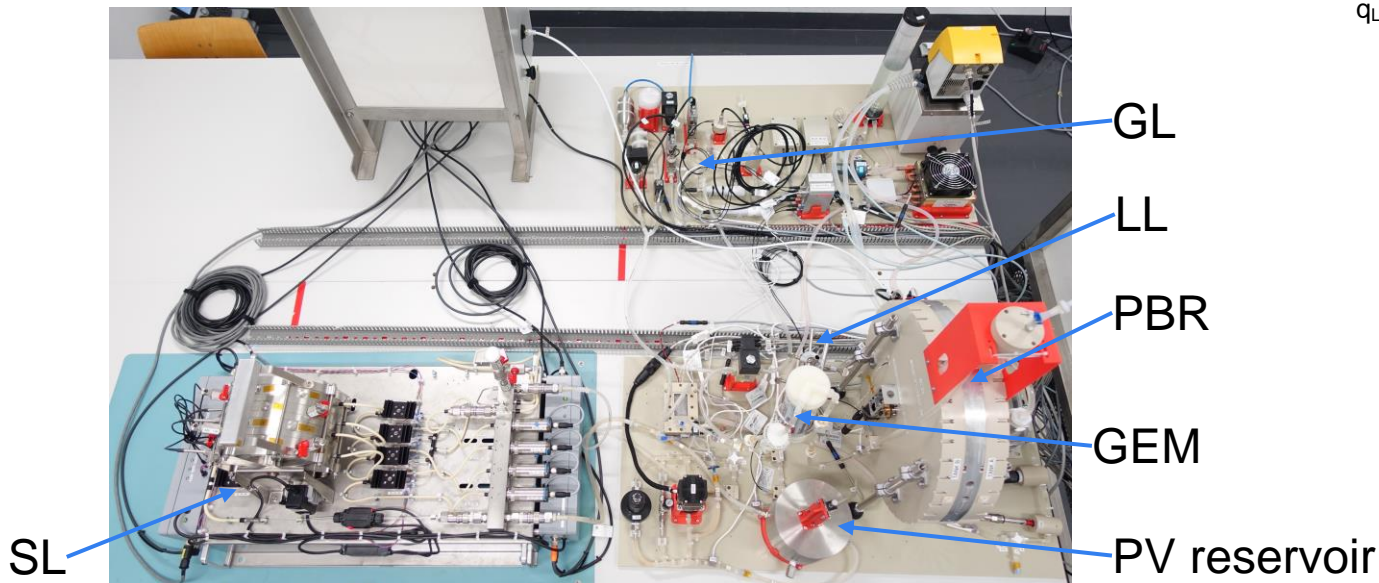
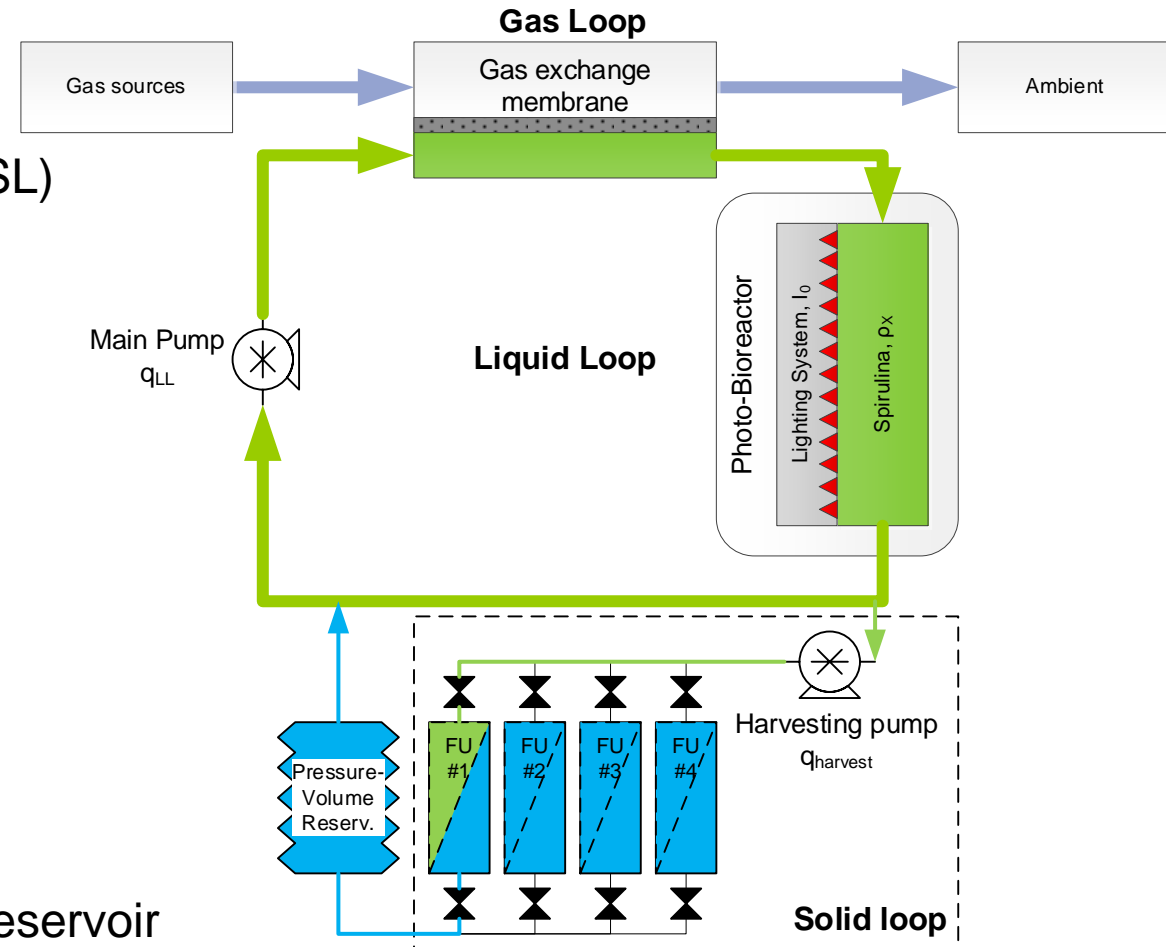
2019 BRT1 BBM P&ID

# Biorat-1 BBM

## 2021 Life test objective

Perform sequence representative to OBD demonstration sequence

- Maintenance operation every 30 days
  - Medium consumption reduction with Solid Loop (SL)
- Autonomous operation
  - Process control to be implemented

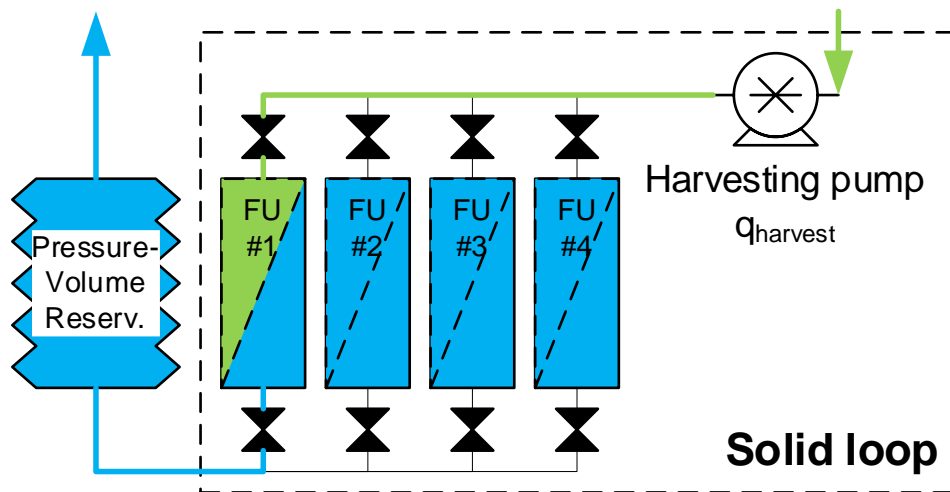


2021 BRT1 BBM

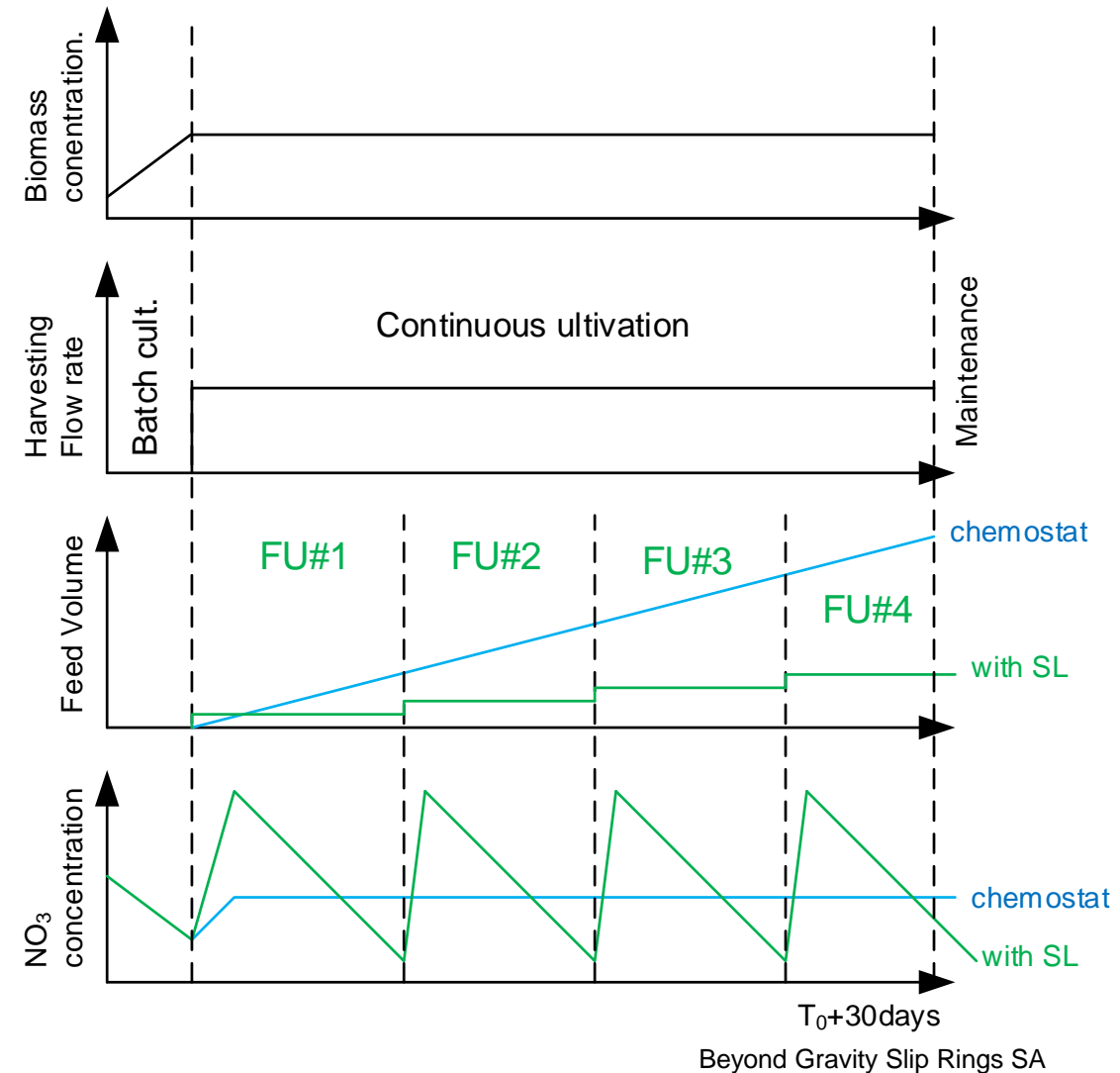
# Biorat-1 BBM

## Solid loop operation

- LL operation at constant biomass concentration (1.5g/L)
- **Batch cultivation:** biomass increases, no harvesting
- **Continuous cultivation:** Harvesting flow rate enabled
  - Filter Unit (FU) #1 activation
  - **Harvesting:** Biomass accumulated in the FU
  - **Feeding:** NO<sub>3</sub> in Zarrouk medium released into LL
  - Sequential activation of the other FUs in function of NO<sub>3</sub> concentration
- **Maintenance operation:** replacement of the FUs



beyond gravity



# Biorat-1 BBM

## Solid loop operation

Pros & cons of discrete/batch feeding:

+ Less volume medium needed from typically 1.8L/day to 0.11L/day by

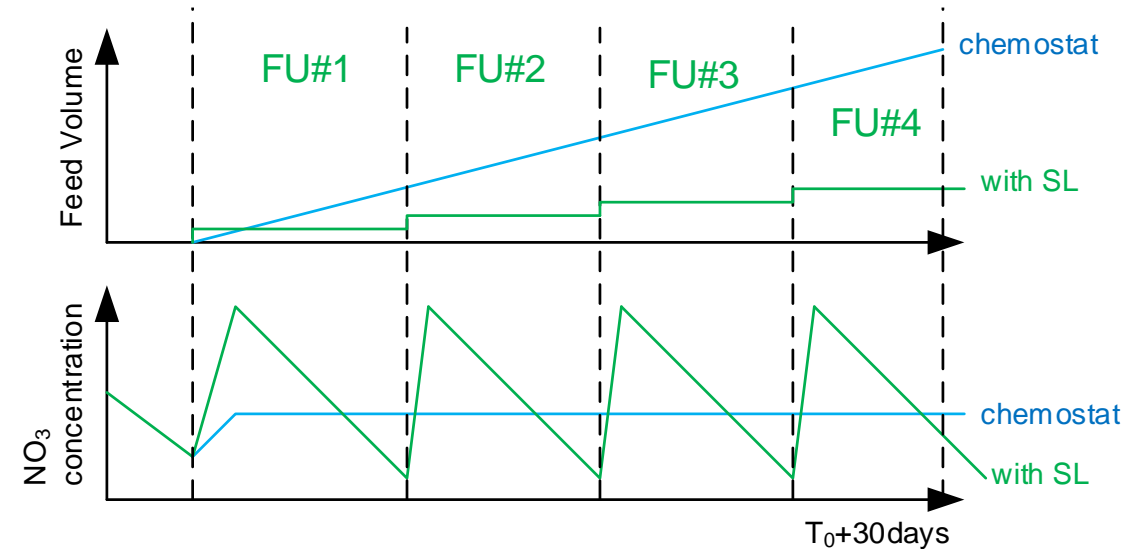
- decoupling between harvesting flow rate & feeding
- concentrated Zarrouk up to (7.3x reference concentration) stored in FUs

+ Constant volume operation, fresh medium replaced by the harvested biomass in the FUs

+ No dewatering needed for the harvested biomass

-Variation of  $\text{NO}_3$  concentration in LL

- Switching of FUs & maintenance operation must be performed at low  $\text{NO}_3$  concentration to prevent overshoot
- $\text{NO}_3$  concentration variable needed for process control



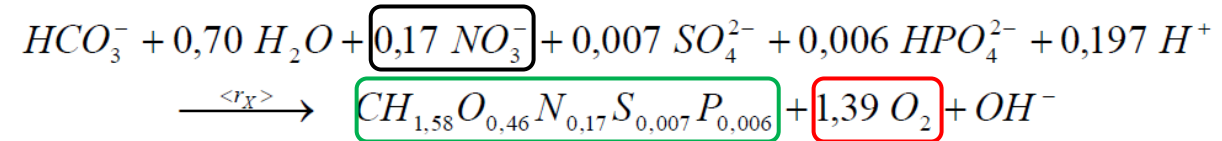


# Biorat-1 BBM

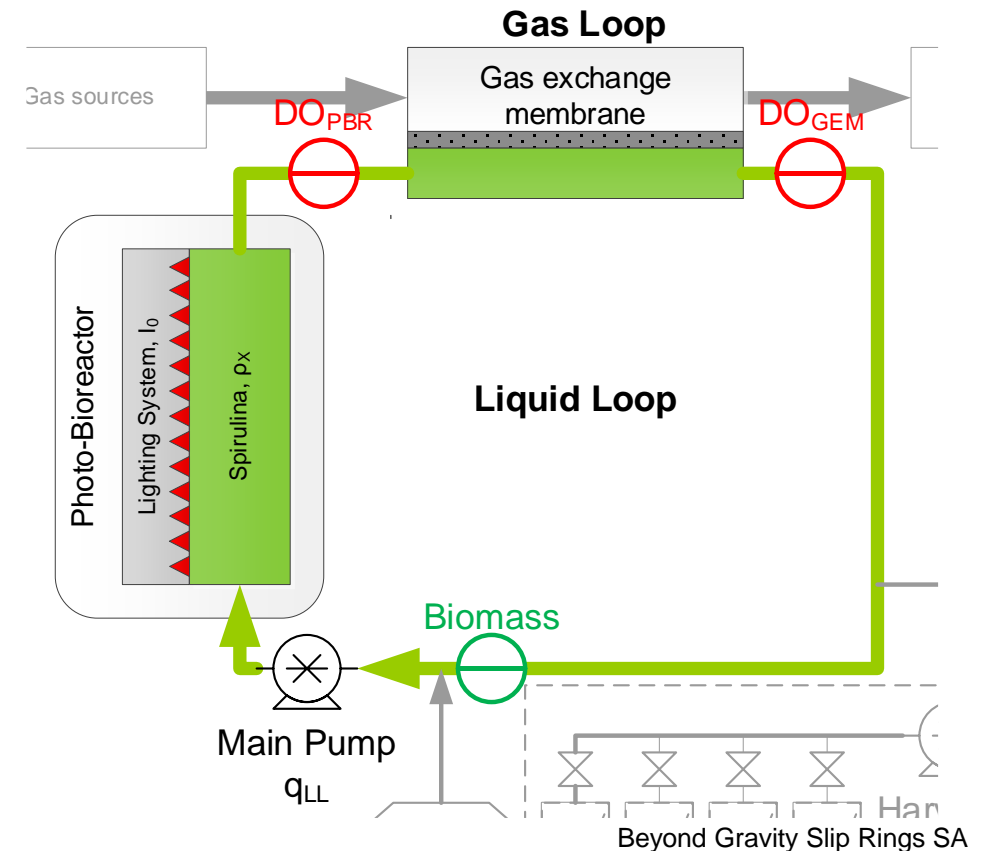
## Key process variables measurement

beyond gravity

- Existing
  - **Oxygen** production measurement
    - Measurement: dissolved O2 mass balance before and after the GEM
    - Hardware: Pyroscience, Firesting O2 & OXROB3
- Upgrade
  - **Biomass**
    - Measurement: optical density sensor
      - Hardware: Hamilton, Dencytee
    - Alternative: software estimator based on O2 production measurement
  - **Nitrate**
    - No online sensor suitable
    - Software estimator based on O2 production measurement
    - NO3 Calibration function to correct NO3 estimator and O2 measurement.

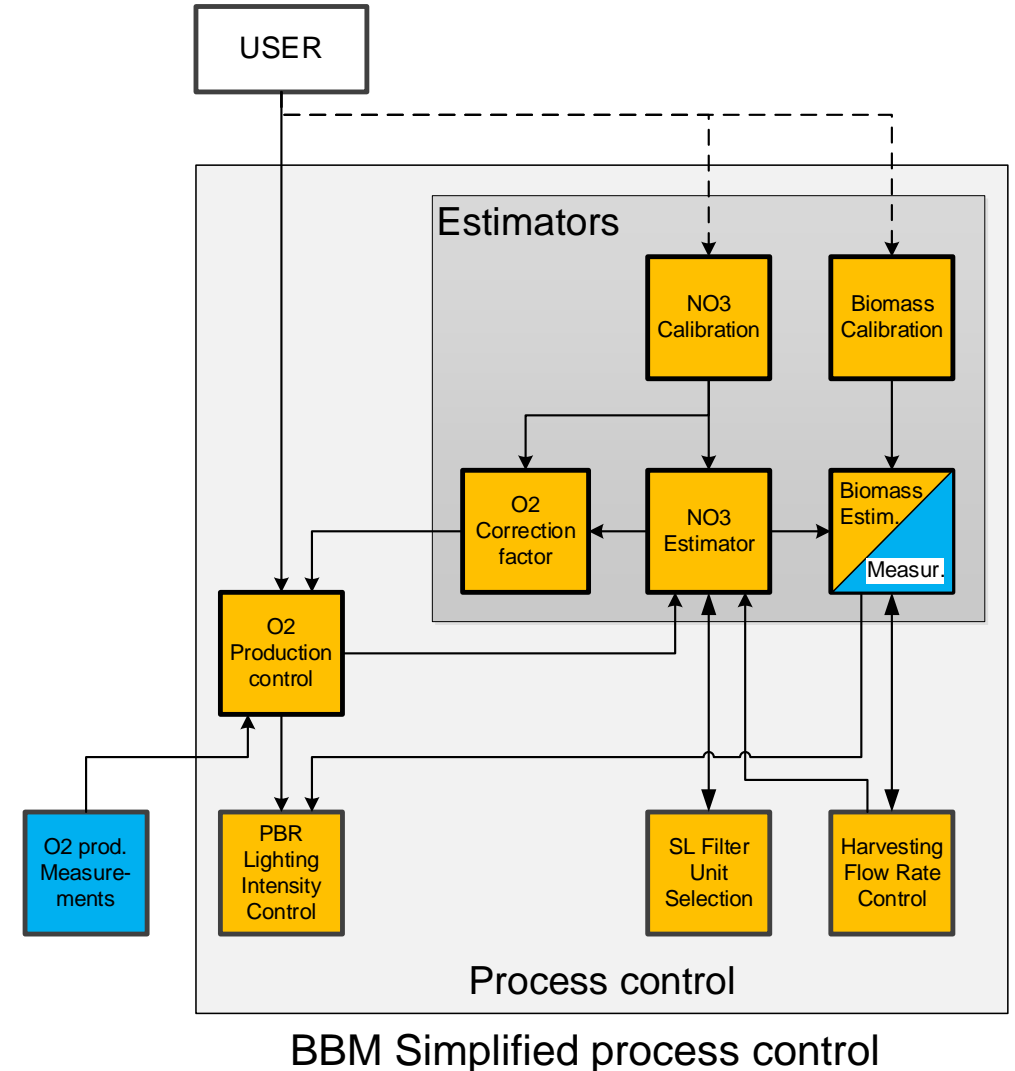


Ref. Biorat, MELiSSA demonstration breadboard, Final Presentation 2000



# Biorat-1 BBM Process Control Software

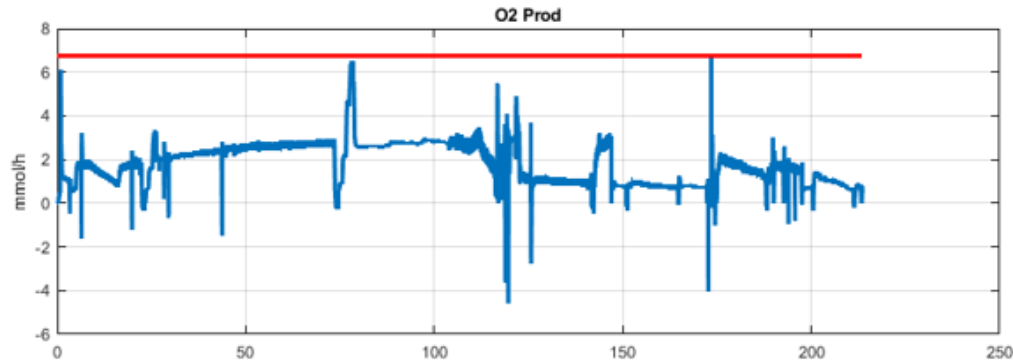
- Existing Low level control loops:
  - LL flow rate & pressure, PBR Light intensity control, LL temperature control, Feed flow rate, GL flow rate, etc...
- Update:
  - Process control:
    - Estimators for NO3 & Biomass
    - Control of O2 production, SL Filter Unit selection and SL Harvesting flow rate
  - System Inputs:
    - O2 production measurement
    - LL NO3 concentration estimation
    - LL Biomass concentration estimation or measurement
    - Active FU's
  - User inputs:
    - O2 set point
    - When needed: NO3 or Biomass calibration value



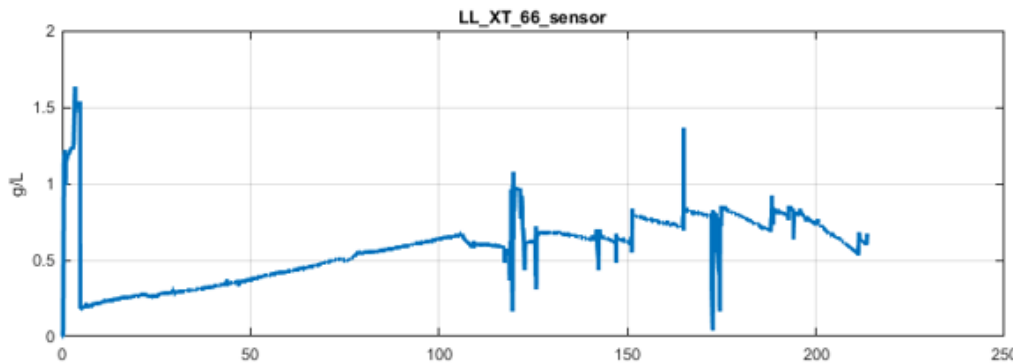
# BRT1 BBM Life test Process Control Results

- Process control up to 5 days (99hr)
  - GEM Clogging Issue @99hr
- ➔ Process control validated for batch cultivation mode

O2 production



Biomass conc.

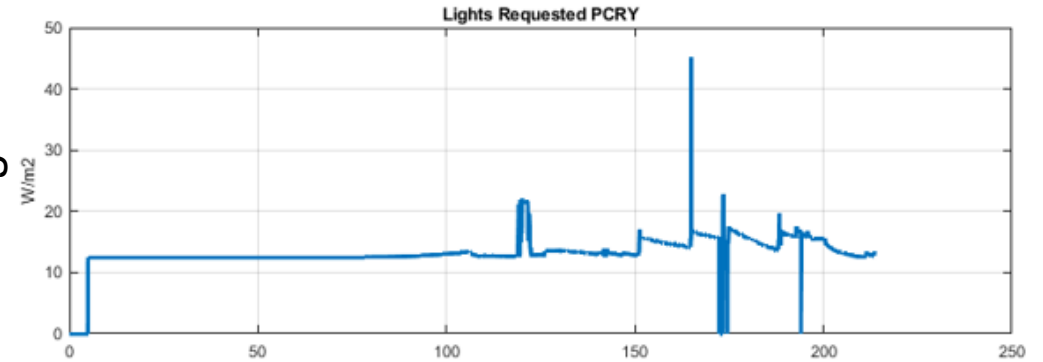


Process Control

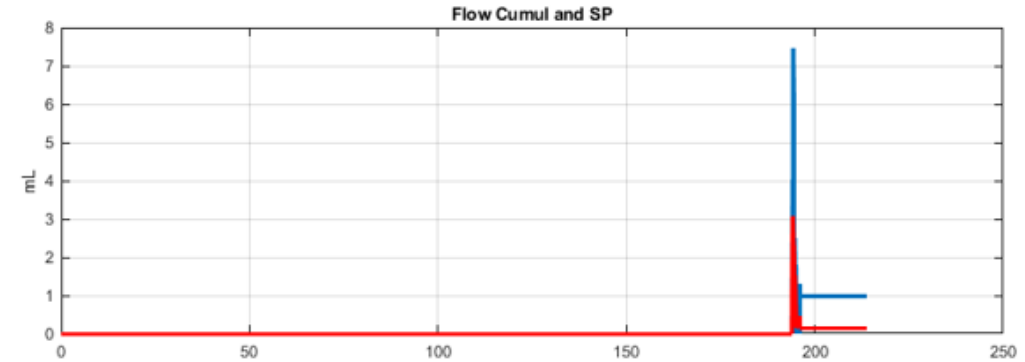


beyond gravity

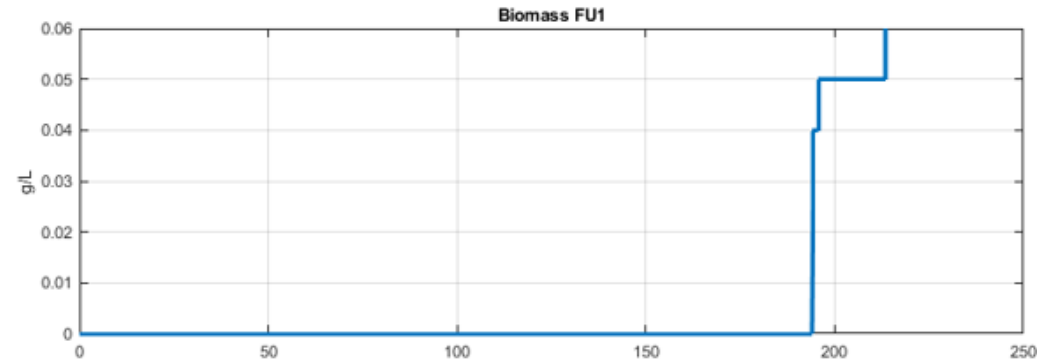
PBR Light int.



Harvesting SP



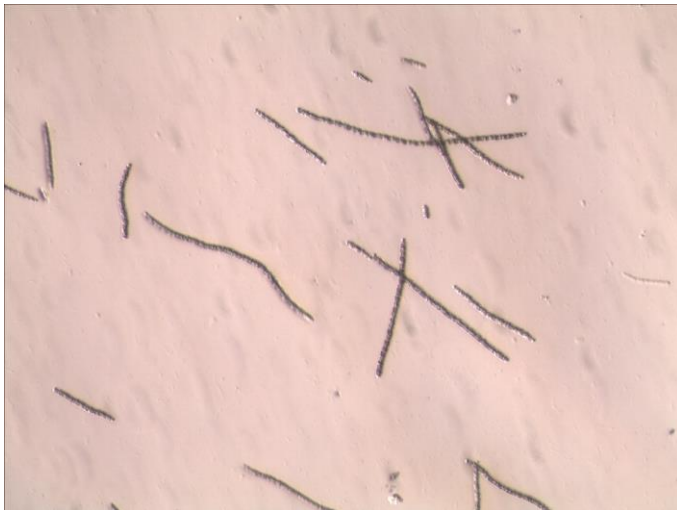
Filter Unit Load.



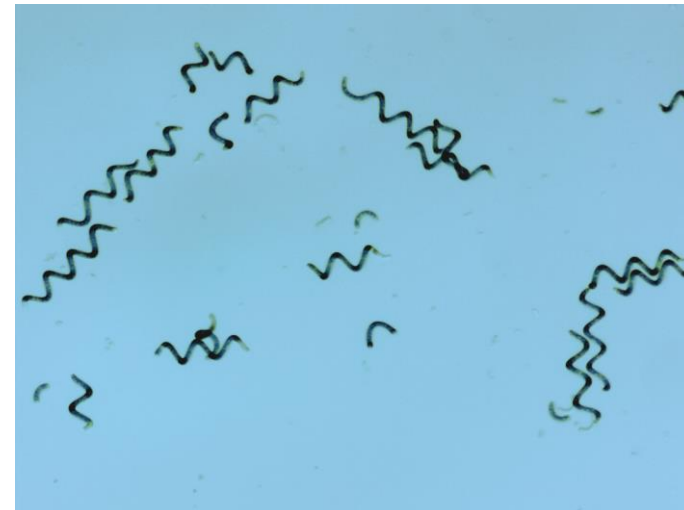
# BRT1 BBM Life test

## Spirulina morphology issue

- GEM clogging anomaly
  - 2017 & 2019 life tests: *Limnospira indica* PCC 8005, with **straight morphology** → Design & GEM validated
  - 2021 Life test: *Limnospira indica* PCC 8005 with **helix morphology**
    - GEM clogging anomaly at 0.65g/L
    - Hollow fiber diameter (200  $\mu\text{m}$ ) not compatible with helix shape (dia. 18-20  $\mu\text{m}$  or trichomes clustering)
- Consequence: Life test sequence forced to be stopped during batch mode
- Lessons learned: Increase of GEM hollow fibers diameter in next BBM iteration



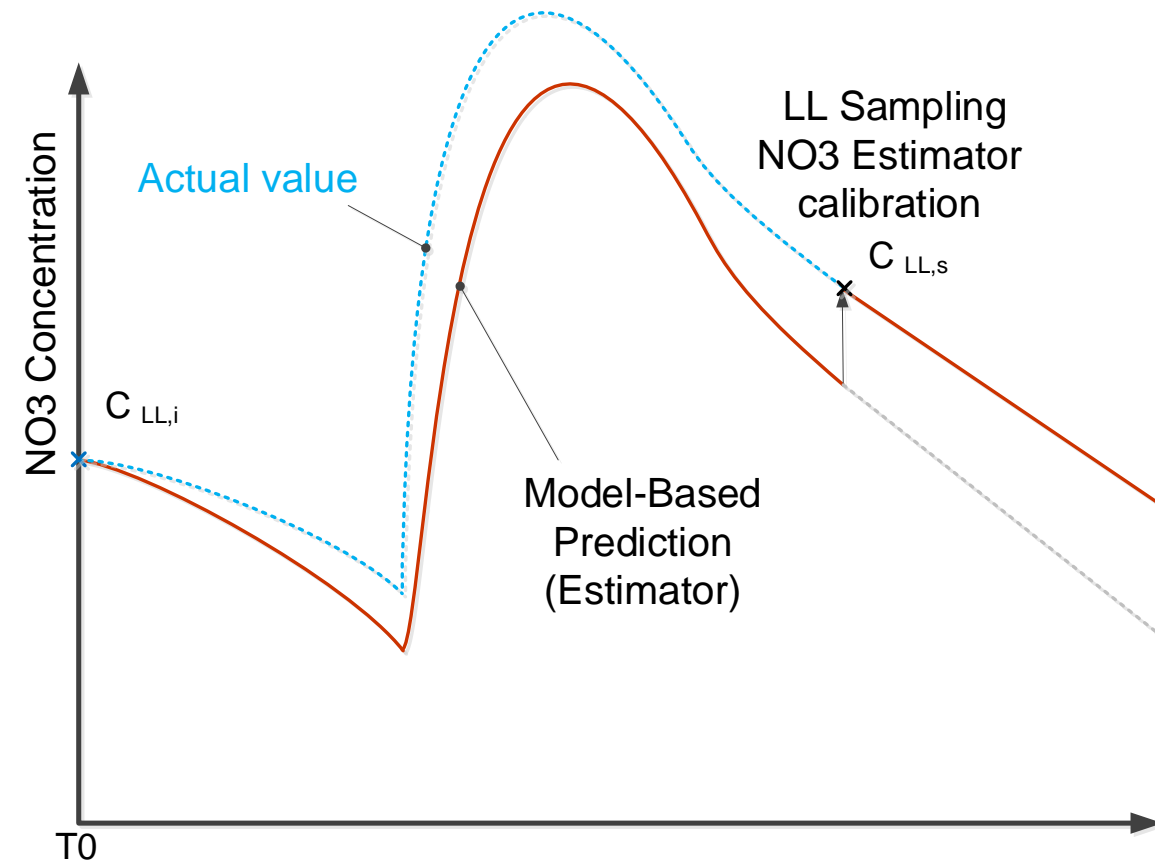
Straight morphology spirulina



Helix morphology spirulina

# BRT1 BBM Life test Nitrate Estimator Calibration

- Need of estimator calibration:
  - Low uncertainty: Stoichiometric coefficient
  - High uncertainty:  $O_2$  production measurement
    - $q_{LL}$  measured LL flow rate
    - $H$  Henry coefficient of liquid
    - $p_{DO\_GEM}$ ,  $P_{DO\_PBR}$  measured dissolved oxygen
- Solution:  $NO_3$  estimator calibration:
  - Input: Measured  $NO_3$  value on a sample
  - Output:
    - Calculation of a  $O_2$  production correction factor
    - Update of  $NO_3$  estimator value
- NOTE:  $NO_3$  titration not possible on-board of ISS

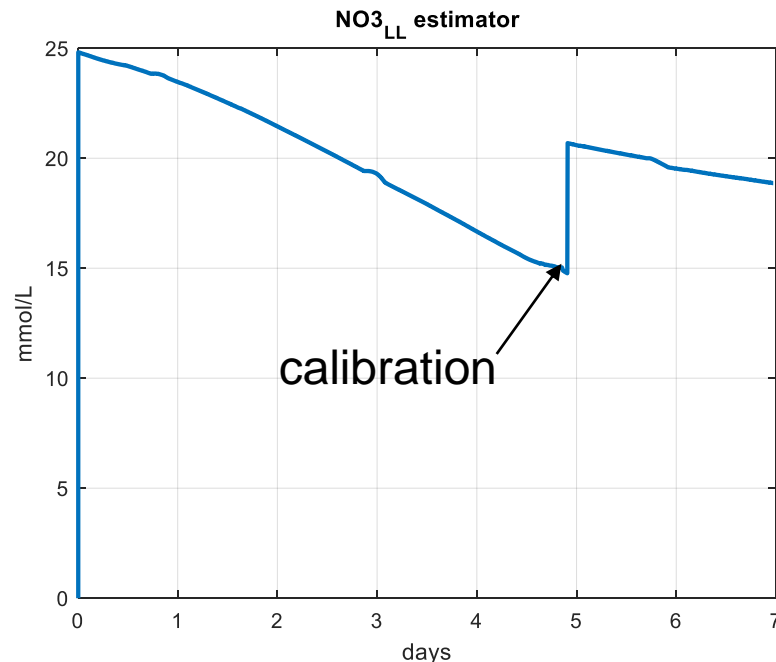


# BRT1 BBM Life test

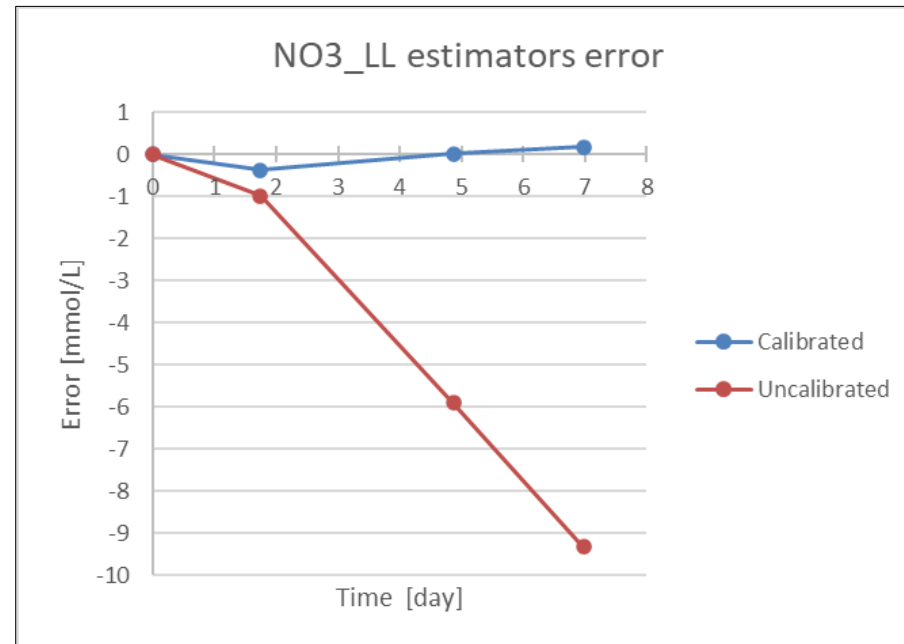
## Nitrate Estimator Correlation

- Sampling time: @day#0, #2, #5, #7
- Calibration time @day #5
- O<sub>2</sub> prod correction factor: 0.41 (Identified by the process control)
- After calibration: NO<sub>3</sub> estimator consumption better than **20% error**

→ NO<sub>3</sub> estimator & calibration validated



NO3 estimator logging

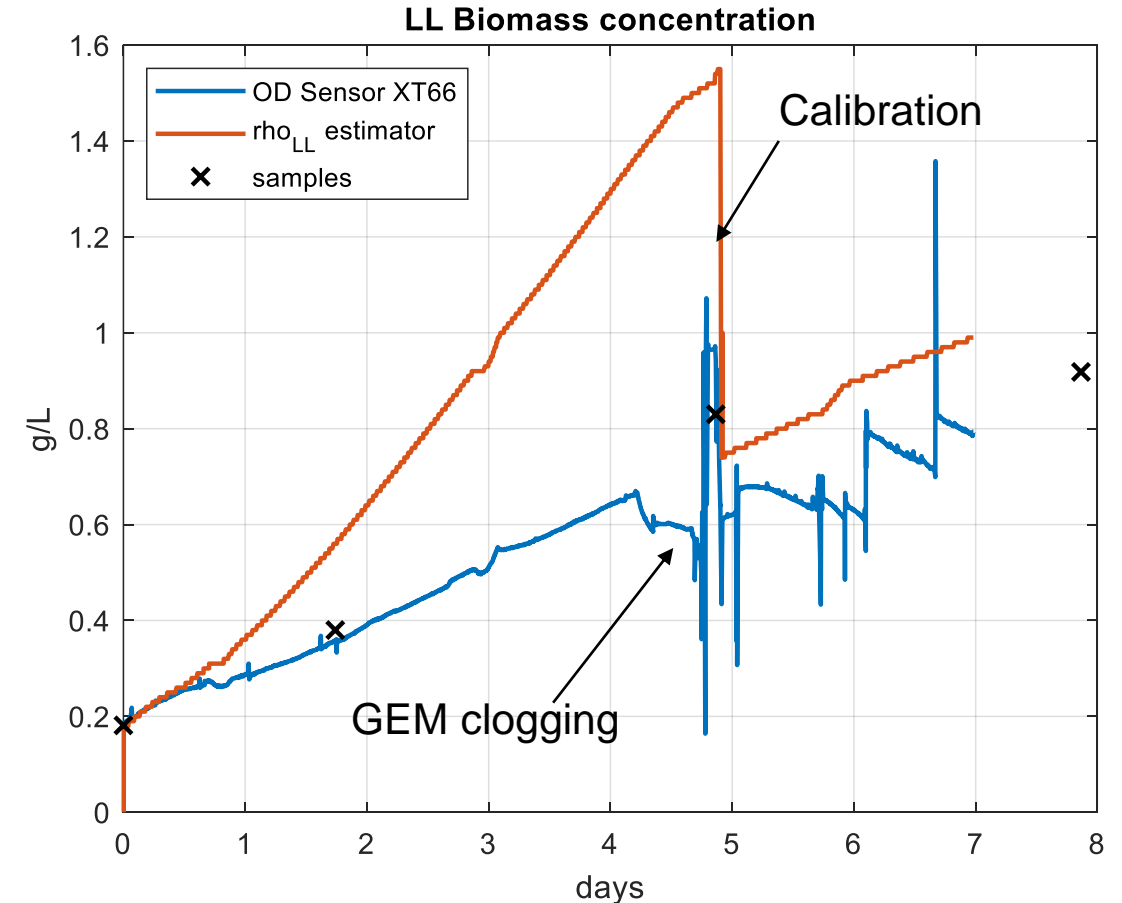


Post-processed errors with samples value

# BRT1 BBM Life test

## Biomass measurements comparison

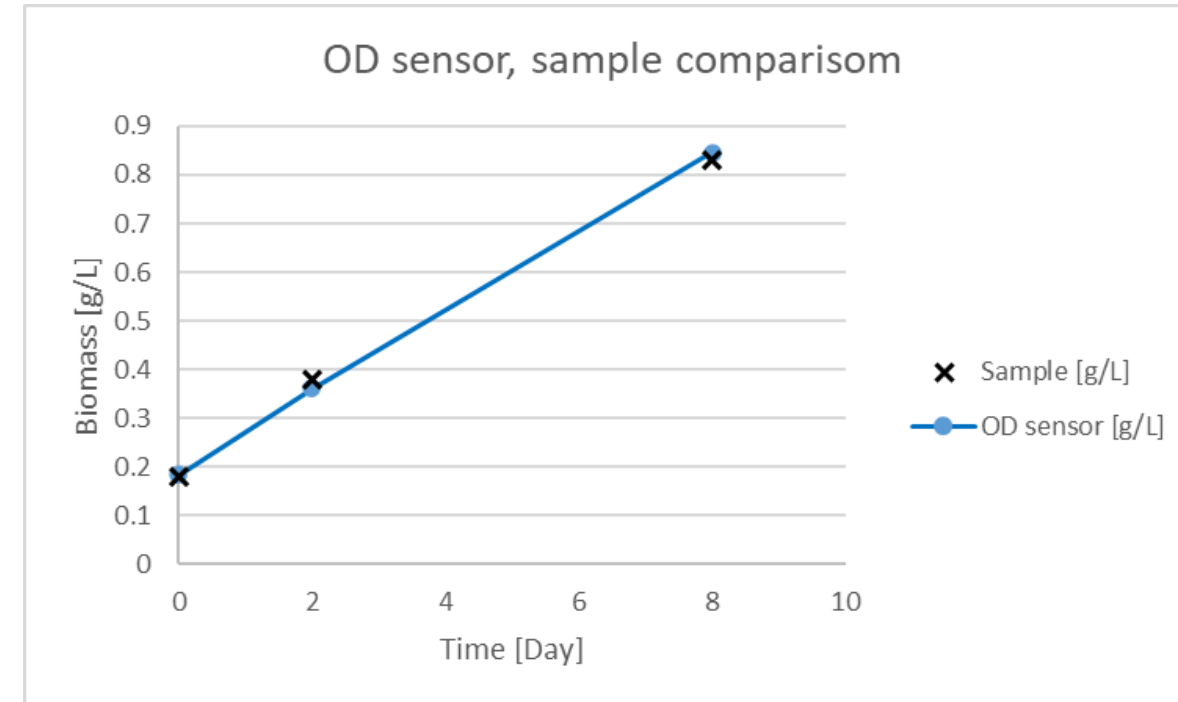
- Sampling performed in BBM activities
    - Samples @Day #0, #2, #5, #8
  - Comparison of LL concentration:
    - Biomass estimator (model-based)
    - On-line Optical Density sensor (Hamilton Dencytee)
  - Results:
    - Biomass estimator
      - divergence with actual value in case on anomaly
      - need calibration like  $\text{NO}_3$  estimator
    - Optical density measurement
      - + actual biomass concentration measurement
- ➔ **OD sensor better than biomass estimator**



# BRT1 BBM Life test

## Optical Density-Biomass Correlation

- Optical density 2-points calibration with day#5 sample
- Errors after calibration (over a 0.2g/L to 0.8g/L range):
  - Maximum absolute error: 0.02[g/L]
  - Maximum relative error: 5%
- OD sensor validated
- Correlation factor determination prior experiment:
  - Live calculation based on absorbance value prior & after inoculation
  - Recommended procedure: correlation with a laboratory spectrophotometer on the inoculum & scaling. **Higher accuracy**





## Conclusion

- Results validated over batch mode
  - Process control operation
  - NO<sub>3</sub> Estimator & Calibration function
  - Biomass OD sensor validated
- Lessons learned
  - GEM Design compatible with helix morphology spirulina needed.

## Way Forward

- To be validated in next life test
  - Process control operation in continuous mode & during maintenance operations
  - SL operation in continuous mode
    - Harvesting
    - Feeding
- Upgrade for next BBM & OBD design
  - Robust NO<sub>3</sub> management compatible with ISS operation (without titration)
  - Axenic design

# Acknowledgements

IN COOPERATION WITH



Life test performance  
Spirulina expertise



Control System Electronic & Software  
Solid loop



Control System Software  
Control design & simulation