Model analysis of an *Arthrospira* photobioreactor running on ISS

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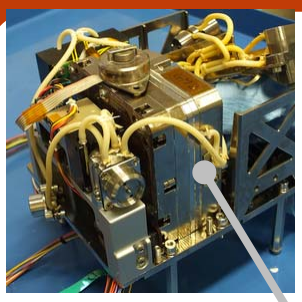
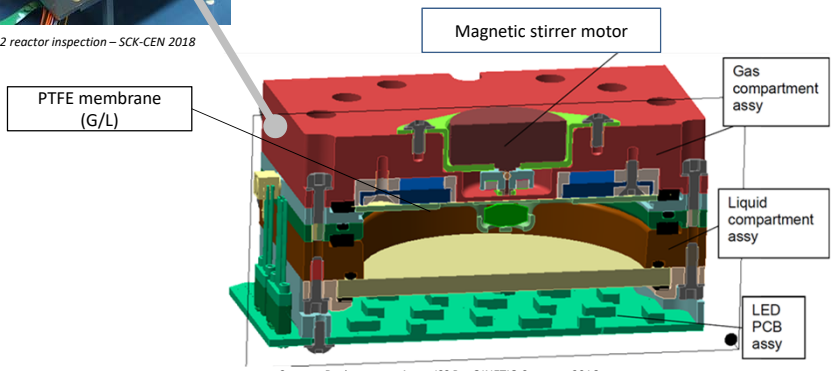


Source : GM2 reactor inspection – SCK-CEN 2018

ArtemiSS :

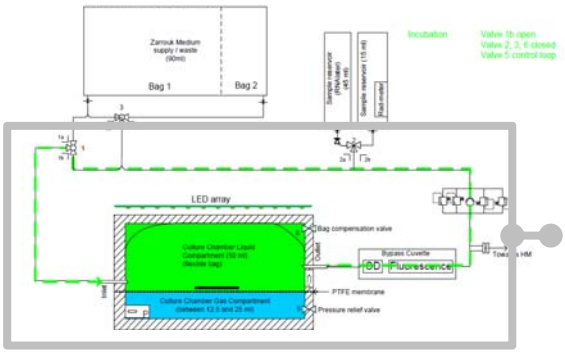
- on-line assessing of growth of *A. platensis* on ISS by gas pressure measurement

- Photobioreactor → analysis using a classical approach for bioprocesses : modelling and simulation



Source : Design report ArtemISS B – QINETIQ Space nv 2016

Photobioreactor diagram (batch incubation operation)

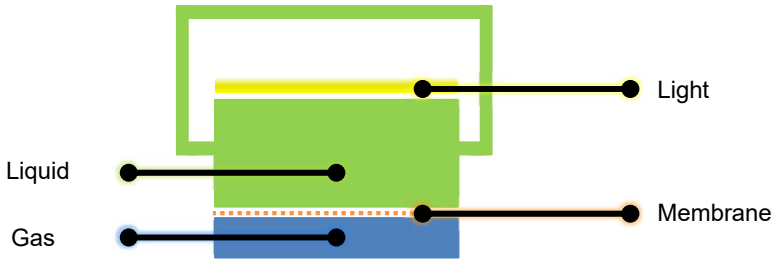


Model of the photobioreactor

Objectives :

- Use of a predictive model built from the knowledge of previous works (MASK, MELiSSA photobioreactor, lab scale photobioreactors)
- Analysis of results
- Comparison Ground / ISS (? Differences)

ArtemISS photobioreactor model principles



Liquid : Model of a batch culture (light dependent)
Several consecutive batches:
Resetting initial conditions of a batch after a volume dilution with Zarrouk medium

Gas : Model of a pressure increase up to a threshold → venting with air
Periodic manual venting with air (discrete events)

Membrane : Model of Liquid ←→ Gas transfer (O_2/ CO_2/ N_2 / Water)

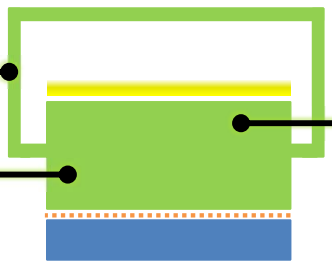
Light : Model of light transfer in culture chamber

ArtemISS photobioreactor model principles

Liquid volume
(~60mL)

VLL: Liq. Loop
~ 10mL
Non illuminated

VCC : Cult.Chamber
~50mL
Illuminated



Physical Liq. variables
V_{CC} and Vbubbles
V_{CC} illuminated fraction
pH
Tot. liq. pressure

Compounds Liq. Variables
P_{CO2} (dissolved CO_2)
P_{O2} (dissolved O_2)
P_{N2} (dissolved N_2)
X (biomass)
HCO_3^- ; CO_3^{2-}
NO_3^-
H^+ ; OH^-

Reaction model (*A. platensis*) :
Mass balanced stoichiometric equation = f(light)
Growth rate: Rx = f (light , T , X, Monod limiting : HCO_3^- , NO_3^-)
Compound : Rate = f (Rx , stoichio. Yields)

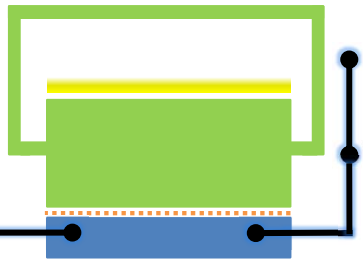
pH model :
Electroneutrality = cst
Ionic equilibrium = f(T, pH, activities)

Hydrodynamics & mass balance model:
liquid <u>perfectly mixed</u>
Mass Balance = reactions + G/L transfer

ArtemISS photobioreactor model principles

Gas volume
(~24mL)

Pressure
0.97→1.25 bar



Physical gas variables
Total Pressure

Compounds gas Variables
P_{CO2}
P_{O2}
P_{N2}
P_{water}

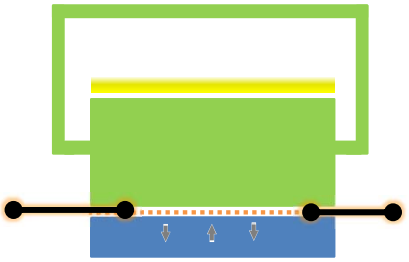
Venting :
→ Ambient air pressure
→ Ambient air composition

	Ground	ISS *
O2	21%	22%
CO2	0.04%	0.4% (~3 mmHg)

** Not always venting with habitat atmosphere composition can vary in time (and in ISS modules)*

Pressure model :
P_i balance = G/L transfer
+ Discrete events :
- overpressure release (+venting)
- "daily" venting operation

ArtemISS photobioreactor model principles



Membrane
PTFE hydrophobic and porous (0.2 µm) membrane
O_2 , N_2, CO_2

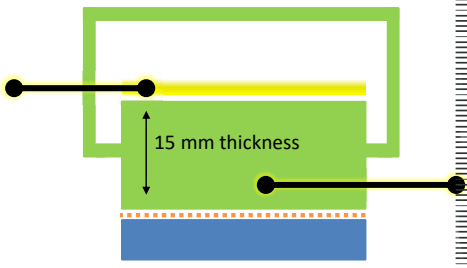
Parameters
Area (A) : 2.6 10^{-3} m^2
Permeability (k) * :
O_2 : 2 10^{-5} $mol.Pa^{-1}.m^{-2}.h^{-1}$
**Fitted from previous experiments*

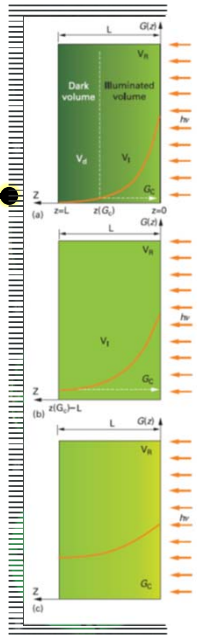
G/L equilibrium constant :
Henry constant (H) = f(temp.)

G/L mass transfer rate model :
$R_{G/L} = k \cdot A \cdot (P_{liq} - P_{gaz})$

ArtemISS photobioreactor model principles

Light (white LEDs)
light flux (q_0)
Off
~7.6 $W.m^{-2}$
~9.2 $W.m^{-2}$





Light diffusion model :

L (illuminated thickness) and Vcc illuminated = f(q_0 , X)

<J> = f(L, q_0 , X) ← radiant light energy absorbed

<Rx> = f (<J> , T , X, Monod limiting : HCO_3^- , NO_3^-)

ArtemISS photobioreactor model principles

Model equation (example for O_2)

$$\frac{dP_{O2.liq}}{dt} = \left(-k.A.\left(P_{o2.liq} - P_{O2.Gas}\right) + Y_{O2}.< R_x >\right).\frac{H}{V_{Liq}}$$

Stoich. Equation — *G/L equilibria* — *G/L transfer* — *f (<J> , T , X, Monod limiting : HCO3- , NO3-)* — *Light diffusion model* — *Ionic equilibria pH model*

$$\frac{dP_{O2.Gas}}{dt} = \left(k.A.\left(P_{o2.liq} - P_{O2.Gas}\right)\right)\frac{R.T}{V_{Gas}}$$

Model computing

Matlab : ODE/DAE + overpressure event detection “ode15s” solver used

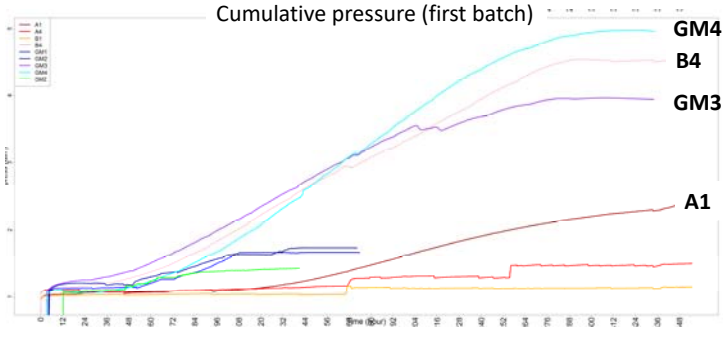
Model(s) references & validation

- Light diffusion model (J-F. Cornet , MELiSSA + lab scale photobioreactors)
- pH model : MASK experiments (G. Cogne, 2003, pH range 9,5-10)
- G/L membrane transfer model (G. Cogne, 2003, MASK photobioreactor)
- *A. platensis* growth & mass balance model : MELiSSA (since 1992 .. up to 2017)

→ Predictive model : no parameters fitted from ArtemISS experiments

ArtemISS results

Cumulative pressure (first batch)

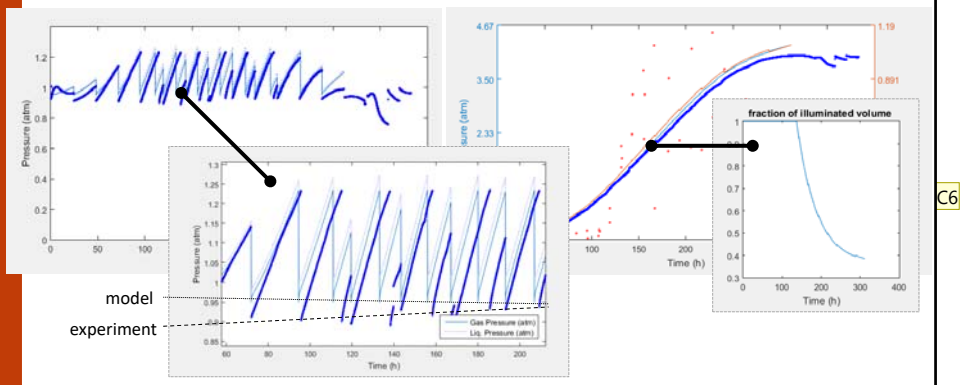


ArtemISS experiments analysed using the predicted model

→ Ground reactors
- GM3
- GM4

→ ISS reactors
- A1
- B4

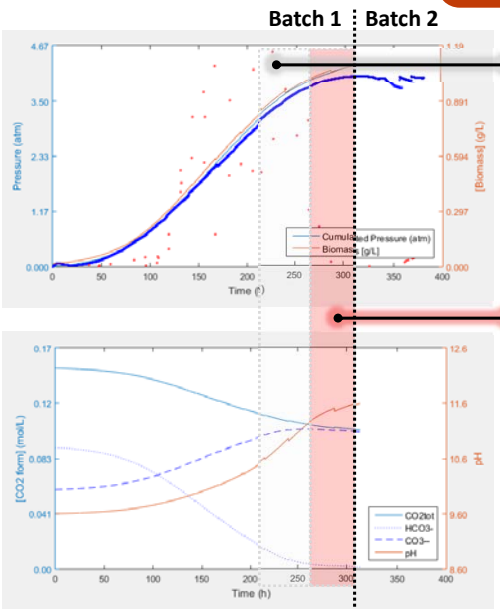
GM4 Ground experiment vs Model



C6

- Very **good fitting of the predictive model with the GM4** – batch 1experiment (pressure)
- At 150h decrease of the illuminated volume → light limitation →linear growth
  (model slightly higher , probably small difference of the light flux value)
- Differences in overpressure sequences are due to a different pressure baseline after venting between model and experiment (not constant in experiment).

GM4 Ground experiment vs Model



Limitation :

high pH →
1) Lower quantity of HCO_3^-
   (← Monod limitation in model)
2) Direct pH effect: biomass lysis

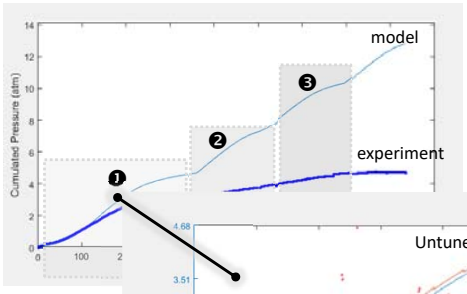
Biomass death at high pH:

- At 275h predicted pH=11.39
- Biological model not developed for pH higher than 10.5
- **No recovery of biomass activity** after dilution for 2nd batch

Slide 11

C6 je vois pas ce que représente la petite figure à droite
Céline, 04/05/2018

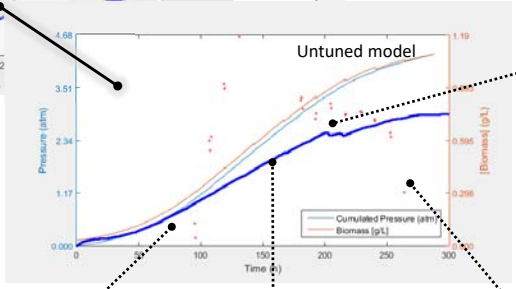
GM3 Ground experiment vs Model



- Results are different of the GM4
→ will require to tune the predictive model

- Batches **2** and **3** : slow gas production rate
(identified after reactor examination as a stirring issue)

Unknown incident
(Probably stirring)



First 100h hours :
exponential growth

Slower (linear)
increase
of the pressure

At ~260h : death
of biomass (as GM4)

GM3 Ground experiment vs Model

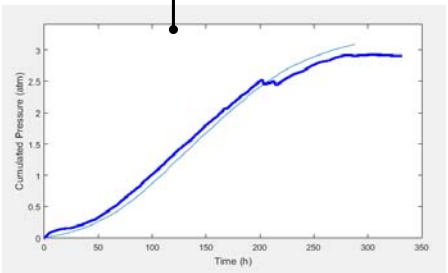
Slower increase of the pressure may be due to:

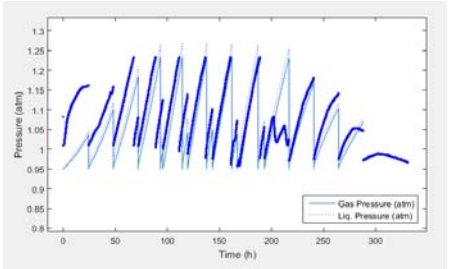
1) a lower light flux ←checked – not fitting

2) a mixing issue ←cannot be tested – required new model (CFD approach)

3) a gas bubble in Vcc contributing to the total gas volume of the reactor

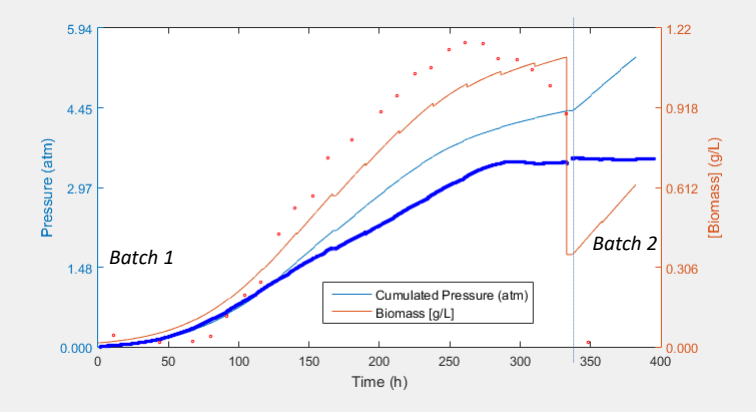
6 mL (= 25% of the volume of gas compartment)





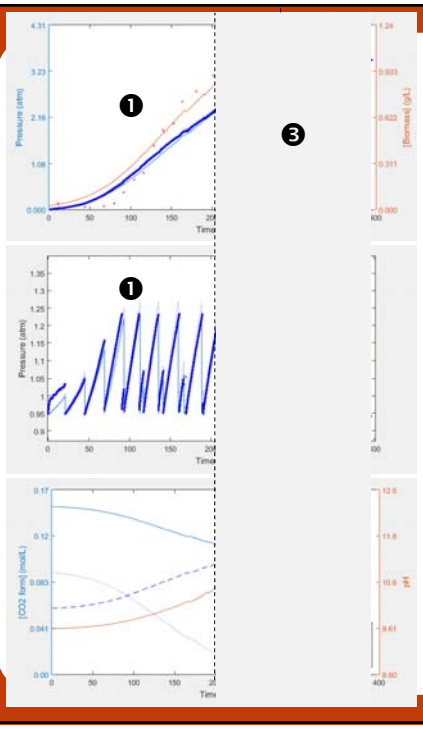
Cumulative pressure and pressure sequence perfectly fitted
Biomass collapse at a predicted pH = 11.4

B4 ISS experiment vs Model



- Exactly the same trend as the ground reactor GM3 (untuned model cannot perfectly fit the experiment)
- Biomass decrease after 275h
- No recovery of the growth after the batch

Same trend as for GM3 → testing the same assumption for tuning the model : Bubble of 6mL contributing to the gas phase



B4 ISS experiment vs Model

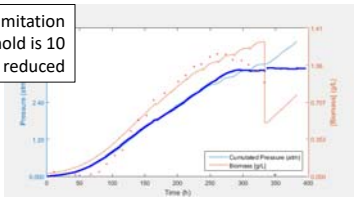
❶ **Model perfectly fit experiment while there is no substrate limitation**

❷ **Limitation is not observed**

- **Biological** : threshold for HCO_3^- limitation appears to be lower in microgravity

- **Physical** : P_{CO2} of gas used for venting higher on ISS (~0.3% -0.4%) when it is 0.04% on Earth. Higher P_{CO2} in liquid → passive diffusion of CO_2 in cells while active transport for HCO_3^- → bypass of the limitation for high pH value

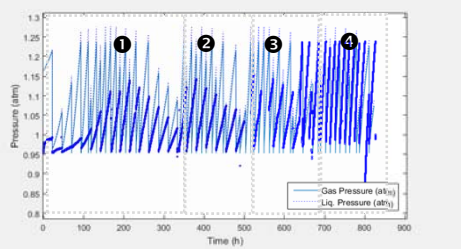
Results if limitation treshold is 10 times reduced

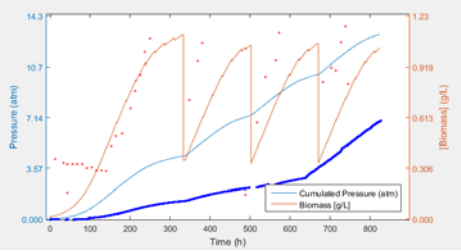


❸ **Biomass death (no activity + lysis)**

At predicted pH = 11.38

A1 ISS experiment vs Model





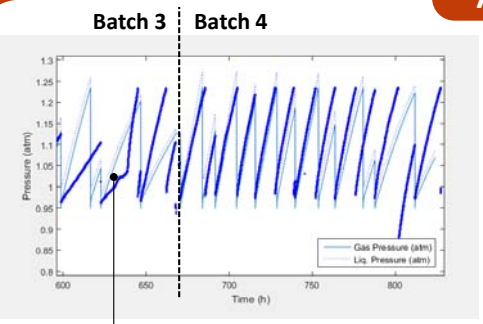
A1 : ISS exp. with 4 batches:

- Low gas production rate for batch 1, 2 and 3
  → Not fitted by model
- Recover a « normal » behavior for batch 4
  → fitted by model
- No biomass death :
  ! lower growth → lower pH

Mixing (stirrer) problem is suspected (same as GM3 batches 2 and 3) :

Cumulative pressure slope : A1 batch1 : ~700 $Pa.h^{-1}$ A1 batch2 : ~580 $Pa.h^{-1}$
GM3 batch 2 ; batch3 : 460 $Pa.h^{-1}$

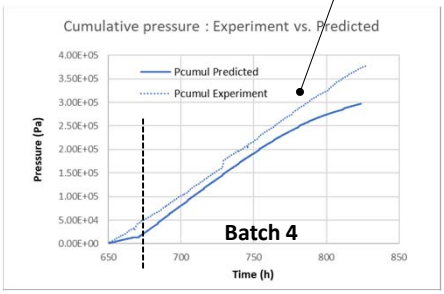
A1 ISS experiment vs Model



Bach 4 : fitted by predictive model (without assumption of gas bubbles)

Same trend as GM4 for the end of the batch : limitation is not observed

Magnetic stirrer working again ?



Predictive Model :

Pressure increase measurement is an excellent method for on-line assessing of growth rates. It is **challenging in terms of modeling** both on ground and in space. Predictive **model fits experimental values on ground and on ISS**, even if the few available repetitions obtained in experiments limits the ability to verify the model

Two main experimental issues for model application :

- pH (predicted by model) and biomass death
- Mixing (stirrer failure), or presence of gas bubble

Ground vs. ISS :

- No differences in low pH (<10.5) conditions → low or limited effect of µgravity
- When HCO_3^- limitation (low HCO_3^- concentration at high pH value) the behavior is different in ground and ISS condition.

Perspectives

Flow model :

Reactor operated without mixing (A1) → model for not perfectly mixed reactor

Growth model :

Need to be developed for high pH (>11) ,

…. or experiments must avoid high pH (reducing batch duration of at least 2 days)

Experiment :

Low HCO_3^- + high pH + high PCO_2 in ground condition must be tested to check if HCO_3^- growth limitation is reduced

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Thank you for your attention

Questions ?

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