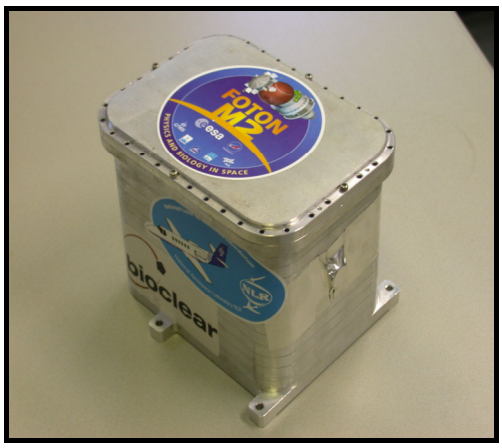
Nationaal Lucht- en Ruimtevaartlaboratorium
National Aerospace Laboratory NLR

Executive summary

Indications for reduced bacterial growth rates in micro-gravity

Design & results of the BIOFILTER experiment flown on FOTON M2



BIOFILTER experiment box as flown on FOTON M2

Problem area
For application of biological air filters in manned spacecraft, research is done on bacterial growth under micro-gravity conditions. For the BIOFILTER experiment, flown in 2005 on FOTON M2, eight turbidity sensors to measure the growth rate of the bacteria *Xanthobacter Autrophicus GJ10* were used. Also thermal management provisions were implemented to control the internal temperature.

Description of work
The design and performance of the BIOFILTER equipment as well and results of the biological ground reference experiments performed in 2006 are discussed. High performance thermal (vacuum) insulation (λ=0.7mW/mK) and Phase Change Material were implemented, keeping the BIOFILTER internal temperature below 16°C during the 4 days integration period between transport and launch. After launch, in micro-gravity, the growth of XA GJ10 was successfully triggered by a temperature increase using an internal heater to 26 °C.

Results and conclusions
Although the operation of the sensor electronics was not fully satisfying, the bacterial growth was measured with the sensors, revealing growth rates between 0.046–0.077 hr^{-1} in micro-g, i.e. approximately 1.5-2.5 times slower than routinely measured on earth under optimal laboratory

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Author(s)
R.C. van Benthem
W. de Grave
J. Krooneman
H Hammenga-Dorenbos

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conditions. For the ground-reference experiments the equipment box, containing the eight sensors, was placed on a Random Positioning Machine (RPM) performing random rotations at 0.5^{o}/min (settling compensation) and 90^{o}/min (micro-g simulation) while the environment was controlled, accurately repeating the BIOFILTER internal temperature profile. Despite the rotation speed differences, growth rates of $0.115\ hr^{-1}$ were confirmed by both the ground reference experiments. Biological and physical interpretation of the results is ongoing.

Applicability
Biological airfilter systems.

Nationaal Lucht- en Ruimtevaartlaboratorium, National Aerospace Laboratory NLR

Anthony Fokkerweg 2, 1059 CM Amsterdam,
P.O. Box 90502, 1006 BM Amsterdam, The Netherlands
Telephone +31 20 511 31 13, Fax +31 20 511 32 10, Web site: www.nlr.nl

Nationaal Lucht- en Ruimtevaartlaboratorium
National Aerospace Laboratory NLR

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R.C. van Benthem, W. de Grave, J. Krooneman[1] and H. Hammenga-Dorenbos[1]

[1] BIOCLEAR

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INDICATIONS FOR REDUCED BACTERIAL GROWTH RATES IN MICRO-GRAVITY

DESIGN & RESULTS OF THE BIOFILTER EXPERIMENT FLOWN ON FOTON M2

Roel van Benthem[1]
National Aerospace Laboratory NLR

Janneke Krooneman[2]
BIOCLEAR BV

Wubbo de Grave
National Aerospace Laboratory NLR

Hilma Hammenga-Dorenbos
BIOCLEAR BV

ABSTRACT

For application of biological air filters in manned spacecraft, research is done on bacterial growth under micro-gravity conditions. For the BIOFILTER experiment, flown in 2005 on FOTON M2, eight turbidity sensors to measure the growth rate of the bacteria *Xanthobacter Autrophicus GJ10* were used. Also thermal management provisions were implemented to control the internal temperature. The design and performance of the BIOFILTER equipment as well and results of the biological ground reference experiments performed in 2006 are discussed. High performance thermal (vacuum) insulation (λ=0.7mW/mK) and Phase Change Material were implemented, keeping the BIOFILTER internal temperature below 16°C during the 4 days integration period between transport and launch. After launch, in micro-gravity, the growth of XA GJ10 was successfully triggered by a temperature increase using an internal heater to 26°C. Although the operation of the sensor electronics was not fully satisfying, the bacterial growth was measured with the sensors, revealing growth rates between 0.046–0.077hr^{-1} in micro-g, i.e. approximately 1.5-2.5 times slower than routinely measured on earth under optimal laboratory conditions. For the ground-reference experiments the equipment box, containing the eight sensors, was placed on a Random Positioning Machine (RPM) performing random rotations at 0.5°/min (settling compensation) and 90°/min (micro-g simulation) while the environment was controlled, accurately repeating the BIOFILTER internal temperature profile. Despite the rotation speed differences, growth rates of 0.115hr^{-1} were confirmed by both the ground reference experiments. Biological and physical interpretation of the results is ongoing.

NOMENCLATURE

ESA-MAP	Molecular tools for monitoring and control of pathogenic bacteria in advanced life support systems
ISS	International Space Station
XA GJ10	*Xanthobacter Autrophicus* GJ10
NLR	National Aerospace Laboratory NLR

[1] P.O. Box 153, 8300 AD Emmeloord, The Netherlands, T: +31527248231, F: +31527248210, E: benthem@nlr.nl

[2] P.O. box 2262, 9704 CG Groningen, The Netherlands, T:+3150 5718455, F: +3150 5717920, E: krooneman@bioclear.nl

1 INTRODUCTION

For long duration manned missions such as the International Space Station ISS biotechnological recycling and cleaning processes are promising tools in order to achieve sustainability. For optimal process control of such biotechnological applications knowledge need to be available on the effects space conditions may have on the activity of the microbes used. In addition, by using biotechnological systems in closed environments such as the ISS, hygienic aspects are of significant concern as well. Up till now still little is known on the behavior of these bacteria under space conditions inside a spacecraft. Past research gave indications that:

- microbes tend to grow faster and produce more biomass
- are able to form biofilms and are able to attach to a large variety of surfaces
- deterioration of materials happens due to microbial activities
- locally high concentration of pathogenic microbes may develop and as such cause bio-hazardous situations.

In 2002 an opportunity came along with ESA's FOTON flight M1 offering a 16 days spaceflight for 39 scientific experiments. This gave us the opportunity to study the effects of microgravity on microbial growth-kinetics, and to study the capacity of microbes to adhere to materials that are often used in space. One of the conditions for a space-experiment onboard this FOTON flight was that the experiments needed to take care for their own resources. For the biological experiment a standard experiment box (16.4x11.2x14.5cm, Figure 1) bolted inside the FOTON capsule became available to BIOCLEAR BV a company based in Groningen, The Netherlands working in the field of environmental and sustainable biotechnology. Since BIOCLAER required technical support in the field of thermal control and space qualification for this box, The National Aerospace Laboratory, NLR, The Netherlands, became involved in the BIOFILTER project. For NLR the project was a unique opportunity to design and build an experiment from the start-on and to space qualify the in-house developed biomass sensor.

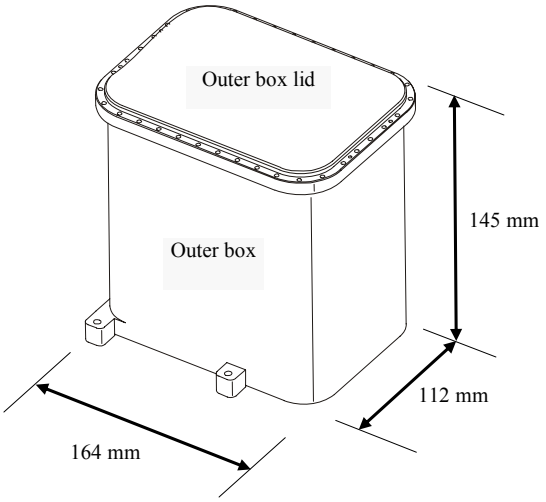


Figure 1: Standard experiment box as available for experiments on FOTON

The BIOFILTER project was a BIOCLEAR initiative in cooperation with international partners: NLR, the University of Groningen, The Netherlands, the University of Milan, Italy and Vermicon GmbH, and incorporated two biological experiments:

I Biofilm formation and molecular tools under µg
II Microbial growth kinetics under µg

Roughly half of the box was available for experiment I focusing on biofilm-formation in space and microbial detections (ESA-MAP project [*Molecular tools for monitoring and control of pathogenic bacteria in advanced life support systems*] and the other half for experiment II, studying microbial growth kinetics, using NLR's sensor equipment. For the ESA-MAP project ninety samples of representative materials as commonly used in manned spacecraft such as polypropylene, stainless steal, aluminum and Kapton® foil were placed in hermetically sealed bags together with mixtures of bacterial cultures. These bags were incorporated in the experiment box. Biofilm formation and material deterioration were investigated after flight.

For evaluation and interpretation of the experiment results, understanding of the BIOFILTER equipment (Figure 2) and its operation is necessary. Therefore, the paper presents detailed information about the thermal design and the operation of the sensors. The paper starts with an historical overview of the FOTON M1 & M2 flights, the BIOFILTER design and presents the requirements and hardware implementations. Thereafter the BIOFILTER flight experiments as well as the results of the ground reference experiments are discussed. Biological and physical interpretation of the results is ongoing.

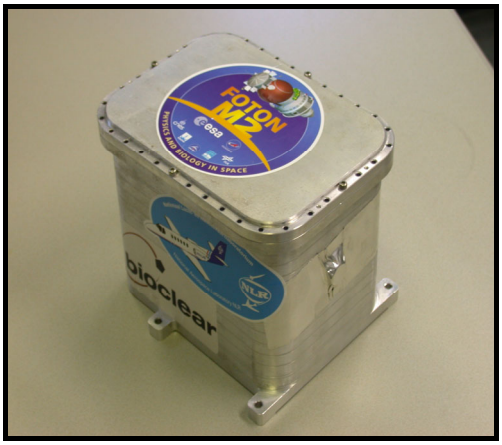


Figure 2: BIOFILTER experiment box as flown on FOTON M2

2 FOTON M1 & M2 FLIGHTS

The first flight of BIOFILTER on FOTON M1 in 2002 was unsuccessful: on October 15th the FOTON rocket exploded shortly after launch from Plesetsk, Cosmodrome. A few weeks later the heavily damaged BIOFILTER box was found back (Figure 3). Fortunately the temperature and sensor data could be retrieved from the memory and were analyzed until the explosion giving valuable information about the performance of the design until the explosion.

Figure 3: Heavily damaged BIOFILTER box after the accident with FOTON M1

Using spare parts from FOTON M1 the BIOFILTER experiment was prepared for a second flight. This time, on May 31st 2005 FOTON-M2 was successfully launched at 12:00 from Baikonur, Kazakhstan. The flight lasted 15.8 days. On June 16th 2005 the capsule landed safely 170km south of Kostanay, Kazakhstan, 13:37 local time (Figure 4). After the M2 flight when the BIOFILTER box was safely returned to The Netherlands, the BIOFILTER box was opened, its contents examined and analyzed and the results were evaluated.

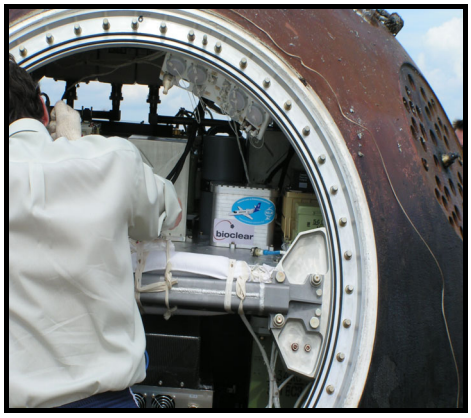


Figure 4: Recovery of BIOFILTER on FOTON-M2 in Kazagstan, June 16th 2005

In 2006-2007 ground reference experiments were done using the BIOFILTER flight hardware. The results of both the FOTON M2 flight and the ground reference experiments are discussed in section 6. In section 3, the requirements, the design and hardware implementations of both the thermal subsystem and sensor equipment are thoroughly discussed. The reliability and trustworthy performance of the equipment is critical for the biological interpretation of the results.

3 BIOFILTER DESIGN

BIOFILTER equipment was modified and improved by NLR involved and Inner an Outer box and an Equipment box, containing a set of eight biomass sensors, the maximum which could be accommodated inside the available space. See Figure 5. The Inner and Outer box were implemented for thermal insulation purposes.

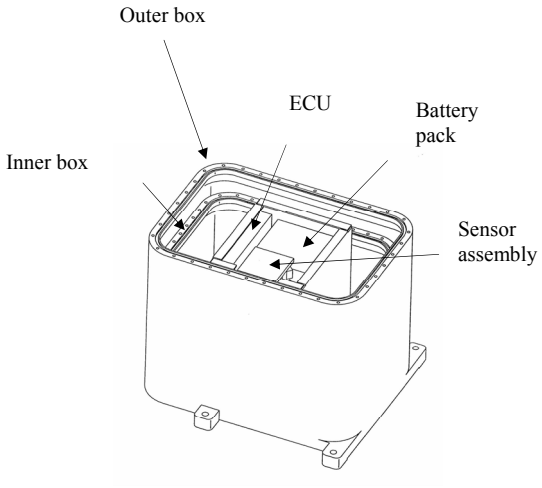


Figure 5: BIOFILTER equipment designed by NLR

The Inner box holds an ambient pressure and contains the experiment samples (exp I) and the Equipment box containing temperature sensors, batteries[3], electronics and a stack of eight samples, with medium[4] and bacteria (*Xanthobacter Autrophicus, XA GJ10)*, for measurement of bacterial growth rates. The on-line measurements were performed with an optical turbidity sensor selected and improved by NLR for this application. After launch, the internal temperature was raised to 26^{o}C, thereby initiating bacterial growth resulting in increasing turbidity. During growth measurements were performed every five minutes and stored in memory. After landing read-out and analysis were done.

The design of the BIOFILTER experiment box has been based on a standard bio container type 3 (Figure 1) that was made available by ESA for the FOTON flights. NLRs involvement to the design of the BIOFILTER box was split into two contributions:

- **Thermal Subsystem**
- **Sensor Equipment**

The requirement and design implementation for both contributions are discussed in the next paragraphs. Since no external resources were available when the experiment box was mounted on the FOTON rocket, the BIOFILTER experiment is fully autonomous. This required the application of an on-board battery. To prevent the on-set of bacterial growth during the transport and integration on earth the equipment was kept cool. To start the growth, once in orbit, the temperature was raised to 26^{o}C.

3.1 Thermal Subsystem Design

The objective of the thermal design of the BIOFILTER was to achieve adequate *internal* temperatures required for a controlled growth of the bacteria (*XA GJ10*), this to ensure that the bacteria only start growing under micro-gravity conditions and not during the preflight phases. For the thermal design the *external* temperature conditions anticipated during the subsequent phases of the FOTON flight, indicated with respect to the Launch (L), and the bacteria behavior were considered. (Figure 6)

[3] The battery pack consists of 9 in parallel-connected Ultralife® batteries, 6 V each, type U6VF-K-T-2

[4] Clear fluid with minerals and nutrients added

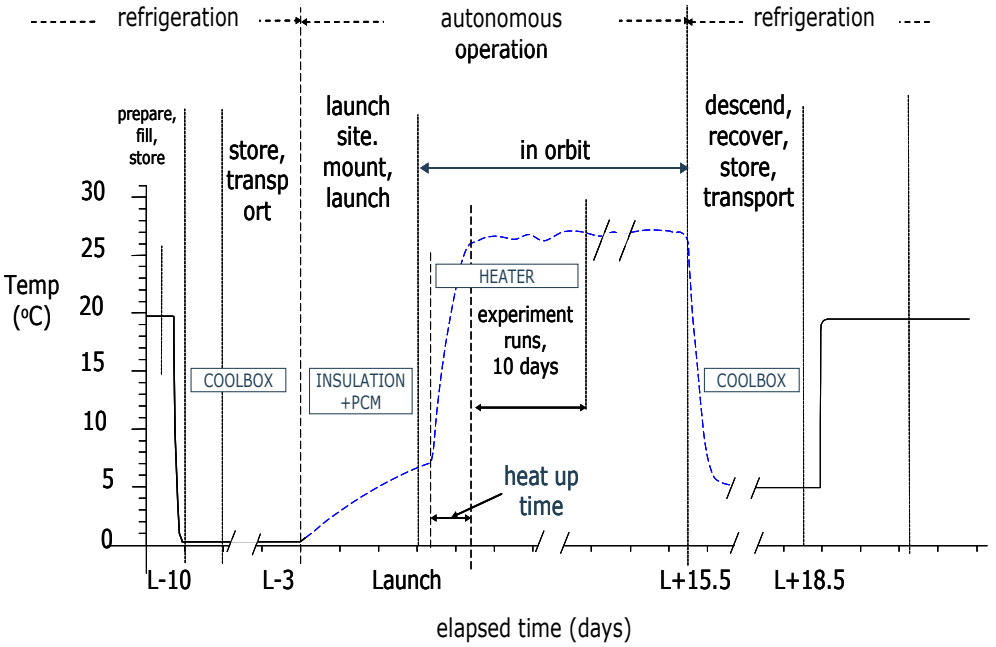


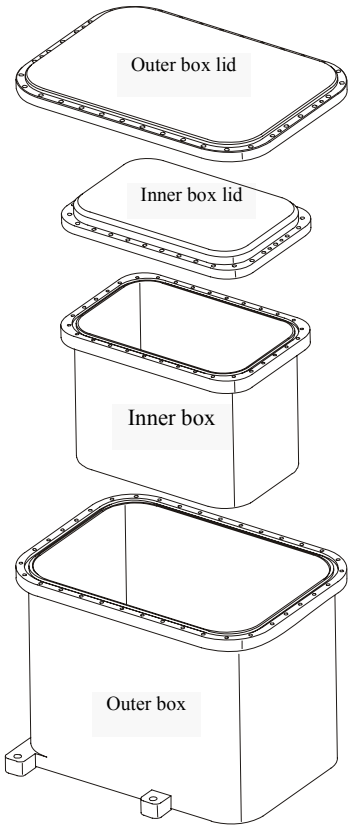
Figure 6: BIOFILTER design temperatures

The activity of the bacteria at low temperatures (1-2 degrees Celsius) is very low, sufficient for maintenance energy and to stay viable, but too low to develop growth. Therefore the temperature was kept just above 0°C during the transport and integration phase. After launch the temperature was raised to approximately 26°C, allowing the bacteria to enter their exponential growth phase. An overview of the temperature requirements and the implemented solutions is given in Table 1 and discussed below.

Phase	Required Internal Temperature	External Temperature	Duration Days	Purpose	Implementation
Transport to Russia	±2°C	±2°C	6	Keep bacteria in lag phase	Coolbox set at 2°C
Integration	2 < T < 10°C	±20°C	4.5	Keep bacteria in lag phase bridging 4.5 days in an ambient environment without coiling	1cm vacuum cavity with MLI and getter between inner- and outer box + thermal buffer (PCM)
Experiment/flight	±26°C	20-28°C	6 (only needed during growth)	Trigger bacteria growth in µ-gravity. Raise & control internal temperature to 26°C 24hr after launch	Temperature controlled heater
Transport back to The Netherlands	±2°C	±2°C	4	Keep bacteria cool for conservation	Removal of vacuum insulation by opening lid after landing Coolbox set at 2°C

Table 1: Summary of thermal requirements and design implementation

During transport a coolbox temperature was used to control the external temperature around 2°C, ensuring that the *internal* temperature was low enough to prevent growth. The most delicate part of the thermal design was bridging the 4.5 days integration period with the BIOFILTER box when mounted on the FOTON rocket, without cooling available. As a design target a maximum temperature increase to +10°C was allowed in 4.5 days before launch.



To achieve this in an ambient environment, a very good thermal vacuum insulation in combination with Phase Change Material (PCM[5]) was applied. The PCM absorbs the remaining incoming heat during its melting, effectively delaying the box internal temperature increase. It was estimated that a heat leak, less then about 0.7kJ was allowed to raise the temperature from 0°C to 10°C during the 4.5 days integration period resulting in a very low thermal conductivity coefficient through the walls of the experiment box of $\lambda \approx 0.1$ mWK^{-1}m^{-1} was needed. The very good thermal insulation has been achieved by implementation of an air thigh inner box wrapped in Multi Layer Insulation (MLI) placed in the outer-box. (**Figure 7** and **Figure** 8) hold into position with 12 plexi-glass fixtures with a minimal cross-section. The 1 cm cavity was evacuated to a pressure of $P \leq 10^{-4}$ mBar by placing the outer box -without lid- together with the inner box –containing the experiments- inside a vacuum chamber.

After out-gassing a couple of days, the outer box was closed by placing the lid on it, using a mechanical construction inside the vacuum chamber. When the chamber was re-pressurized to ambient conditions, the outer box closes hermetically by the pressure difference, thus maintaining the internal vacuum. In order to keep the pressure low after closing the box an internal getter is applied to absorb all out-gassing materials. To secure the lid, bolts were additionally applied.

Figure 7: BIOFILTER inter box and outer box

For FOTON M1 155 grams of N-tetradecane was selected as PCM (melting point at 5.9°C, Cp=2.1kJ/kg, h_{melt}=230 kJ/kg) stored in plastic bags and placed in some spare rooms the inner box. From the FOTON M1 data retrieved it appeared that the N-tetradecane worked to short, thus a larger thermal buffer was required. Also n-tetradecane was leaking out of the bags. To increase the storage capacity and also to improve implementation for FOTON M2, 200 grams of deuterium-oxide (melting point at +3.9°C, Cp = 2.9 kJ/kg, h_{melt} = 318 kJ/kg was selected as PCM. Unfortunately, by the very good insulation of the box the internal temperature never dropped below 3.9°C, the freezing point of deuterium-oxide, during the transport phase for FOTON M2. For details about the thermal design of the BIOFILTER box one is referred to [3].

[5] PCM => M1: 155 grams of N-tetradecane, M2: 200 grams of deuterium oxide ('heavy water')

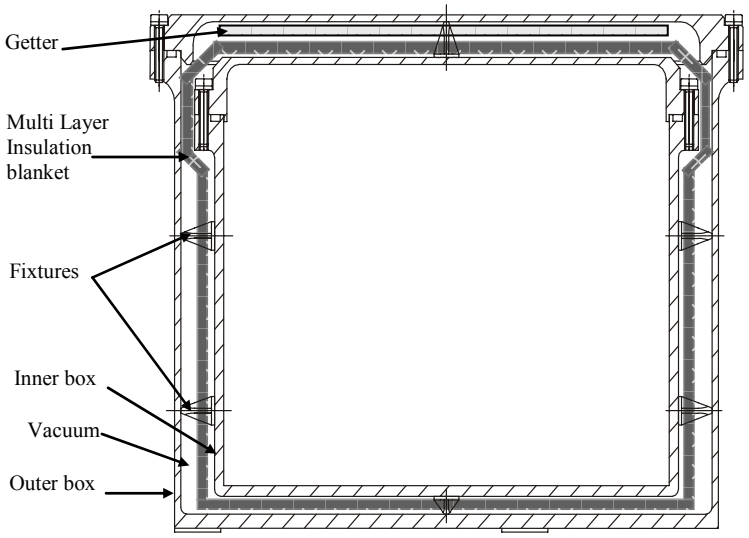


Figure 8: BIOFILTER experiment box cross-section

To trigger the growth -once in orbit- the temperature was raised to 26°C, 1 day after launch using an internal heater. The heater was set to switch on automatically 24 hours after launch to cope with an eventual launch delay of 1 day. For a comparison with laboratory conditions a stable temperature of 26°C had to be achieved during the flight. The on-board temperature inside the capsule was estimated to be around 25°C during the flight, so it was anticipated that internal heating power (battery) would be hardly used by the small temperature difference. Nevertheless, after six days, when the anticipated growth of the bacteria was completed, the heater was switched off, to spare the batteries for sensor operation and readout.

After re-entry and landing 16 days after launch, the BIOFILTER box was dismounted from the capsule and transported back to the Netherlands inside a cool box, mainly for conservation purposes. Directly after landing, the outer box lid was partially opened, letting some air in, thus disrupting the thermal insulation and ensuring better cooling of the interior.

3.2 Biofilter sensor design

Accurate determination of bacterial growth curves during spaceflight requires a sensor that is inherently stable, capable of continuous measurements and low power consumption. Work on the biomass sensor started in NLRs laboratory in 1999 [1], [2] with a trade between two types of optical sensors, both in principle suitable for on-line measurement of bacterial growth. The first method was based on the measurement of light transmission, using a submerged prism, whereas the second method was a turbidity sensor measuring both the transmitted and scattered light. Although the turbidity sensor needed a few improvements to enhance its sensitivity in the low range it had been selected for its attractive features. The sensitivity of the turbidity sensor was significantly improved by implanting logarithmic amplification and using diaphragms to narrow the beams, making it suitable for the application, e.g. on-line measurement XA GJ10 growth. The improved turbidity sensor was selected for:

- on-line measurement of growth
- inherent stability by self compensation
- insensitive for foiling
- non invasive, sample volume inside sensor
- high accuracy (after improvements)

- low power consumption

A more detailed discussion about the selection process and the improvements can be found in section 4.

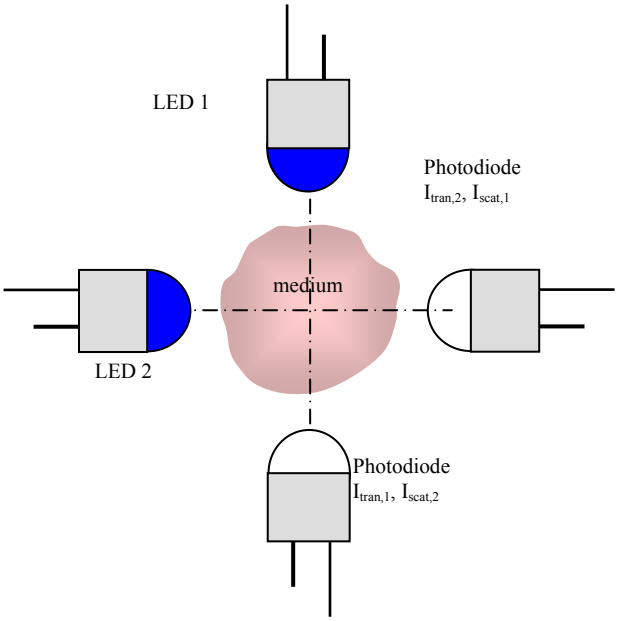


Figure 9: Turbidity sensor with dual Ratio concept as applied in BIOFILTER

The implemented sensor for BIOFILTER is based on dual turbidity measurement, using two LED and two photodiode arranged in a cross around a medium (Figure 9). It combines two scatter and transmission measurements. The ratio eliminates LED emission and detector sensitivity fluctuations. Optical properties and geometry of the particles (e.g. bacteria) which produce the turbidity, require appropriate calibration. A blue LED is selected (λ=450nm), the same wavelength as used in the laboratory to measure the Optical Density (OD) with a photo-spectrometer.
The geometrical mean value of the alternately obtained ratio values as induced by each light source and detected in real-time. Accordingly, R is calculated on-line:

$$R = \sqrt{\left(I_{scat,1}/I_{tran,1}\right)\times\left(I_{scat,2}/I_{tran,2}\right)} \quad (1)$$

with:

R	dual ratio signal.
Iscat,1	scattered intensity signal, induced by LED 1
Itran,1	transmitted intensity signal, induced by LED 1
Iscat,2	scattered intensity signal induced by LED 2
Itran,2	transmitted intensity signal, induced by LED 2

The ratio eliminates LED fluctuations and the geometric mean calculation eliminates detector circuit instability and the influence of fouling of surfaces in the optical paths. The detected light energy depends on the wavelength spectrum, the illuminated medium volume, the properties of the suspended particles (optical properties, characteristic dimensions and shape) and the suspended particle density (=number of bacteria).
For BIOFILTER eight sensors were accommodated in a stack (Figure 10): seven were used for the measurement of bacteria growth and one reference. Each sensor has a 2mm high circular Sample Unit containing the medium with bacteria, in close contact, via Accurel sheets with two

air chambers. Each sensor has two LEDs and two photodiodes arranged in a cross. One sensor unit is used as a reference using an epoxy with aluminum particles added to achieve a fixed turbidity . In section 4 details about the sensor design are discussed.

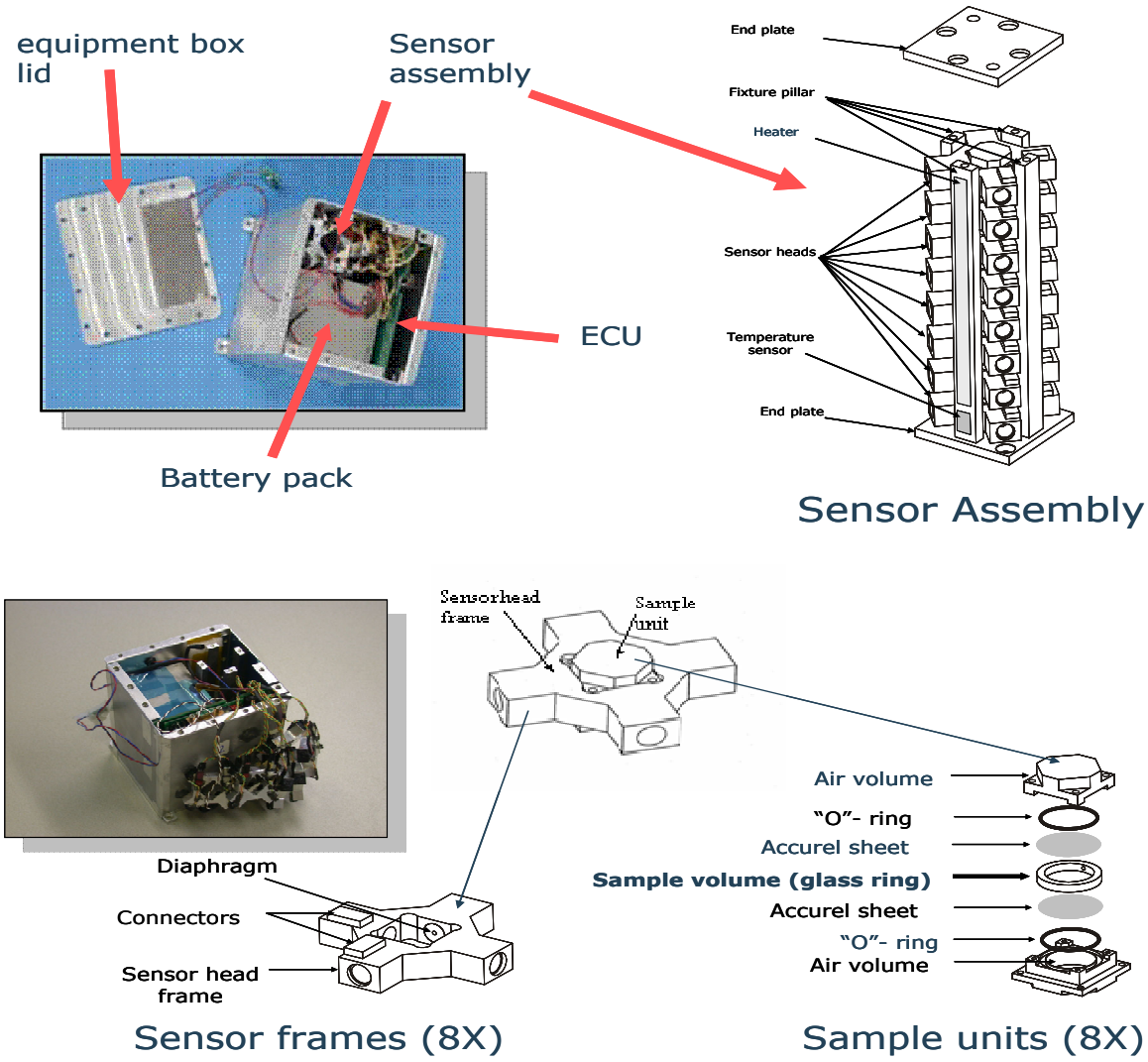


Figure 10: BIOFILTER sensor stack

4 TURBIDITY SENSOR OPERATION

The turbidity sensor investigated was originally designed for high turbidities and is now-a-days routinely used for feedback and control by the measurement of water pollution in household dishwashers and washing machines [6]. Disadvantage of the investigated turbidity sensor was its poor sensitivity for low turbidity's in the range required for the measurement of bacterial growth. However, attracted by its very good stability, which allows for a one time calibration only, investigations were started by NLR to improve the sensor sensitivity in the lower range.

It appeared that the problem was related to the dynamic range of the amplifiers, since they need to operate in both the high intensity transmission as well as the low intensity scatter modes simultaneously. For the envisaged application the scattered light appears to be 6 to 9 orders of magnitude weaker that the transmitted light, it is difficult to find an appropriate amplifier. Such

an enormous difference in dynamic range is normally not achieved with a single proportional amplifier. The solution was to use logarithmic amplification instead, that has a very large dynamic range up to 12 orders of magnitude, both very sensitive for weak scattering signals and less sensitive for high signal levels. Interesting side effect of using logarithmic amplifiers is that the taking the ratio (multiplication) now become a simple subtraction. Since logarithmic amplification is temperature sensitive (see next paragraph), absolute temperature compensation is required. The accuracy of the sensor was further improved by careful alignment and using diaphragms for both the LED as detectors to narrow down the light beams produced by the LED and the detector field of views to limit cross over. These improvements were implemented in a prototype design that was successfully tested to measure a bacterial growth (Figure 11). The maximum growth rate under laboratory conditions, $\mu=0.16hr^{-1}$ was found at $30^{o}C$ is calculated from the doubling time t_d=5.25hr with $\mu=\ln(2)/t_d$.

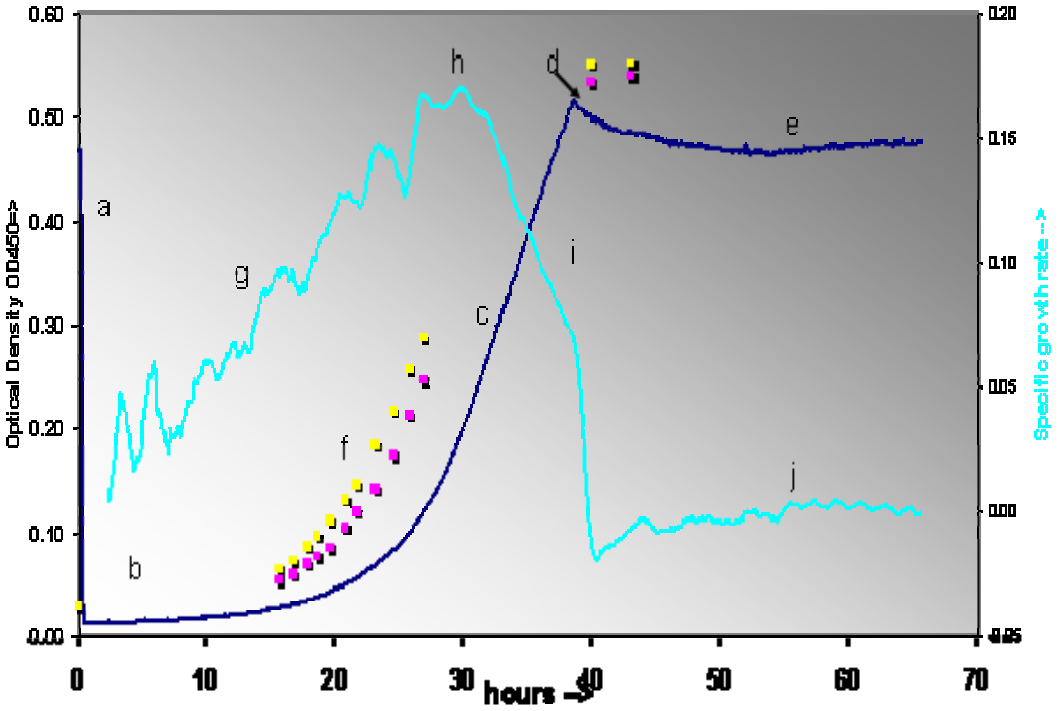


Figure 11: Exponential growth curve (dark blue) and derived specific growth rate (light blue) of Xanthobacter Autotrophicus GJ10 *obtained with the improved turbidity sensor*

a) switch-on, b) lag phase, c) exponential growth, d) substrate completion, e) stationary phase, f) reference cultures, g) growth acceleration, h) maximum specific growth at $30^{o}C$, $\mu=0.16hr^{-1}$, i) growth deceleration, j) stabilization

4.1 Logarithmic amplification

The light intensity level differences between the transmitted and scattered light for this application is typically in the range of 1 to 10^{-3} to 10^{-6} respectively, normally requiring a very large detection range and the use of at least a 16 bits A/D converter. Disadvantages are that a large "part" of the A/D converter is never used and an equal sensitivity for both weak and strong signals. When using logarithmic instead of linear amplification, the detection range is roughly reduced 1 to 10^{-1}. In this case logarithmic amplification is achieved by connecting a photodiode to the emitter of a transistor using its logarithmic amplification property. See Figure 12 for the circuitry.

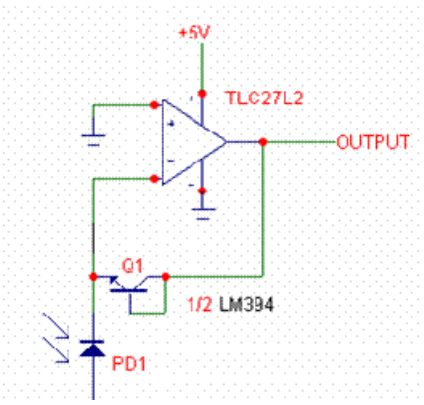


Figure 12: Logarithmic amplification circuitry

Advantage of this approach is that the full detection range can be covered with a 12 bit A/D converter only and that the sensitivity for the weak (scatter) signals is improved. Disadvantage is that a logarithmic amplifier is slightly temperature sensitive since the output (2) is determent by the base-emitter voltage of a transistor typically:

$$V_s = kT/q \ln (I_s/I_d) + V_o \quad (2)$$

With kT/q usually called the thermal voltage V_T. The offset voltage Vo is applied to adjust the output voltage V_s within the range of the 12 bits A/D converter.

With:

V_s	=	forward voltage [V]
V_T	=	thermal voltage [V]
V_o	=	offset voltage [V]
I_s	=	current generated by the light [A]
I_d	=	dark current [A]
T	=	absolute temperature [K]
q	=	electron charge [C]
k	=	Boltzmann constant [J/K]

Due to logarithmic calculation laws, calculation of the ratio now becomes a subtraction of scatter signal and the transmission signal. The dual ratio calculation ln R* is temperature sensitive when using logarithmically amplified signals:

$$\ln R^*(T) = \ln s1 - \ln t2 + \ln s2 - \ln t1 \quad (3)$$

with:

ln s1 = V_{s1} scatter measured with LED1 on
ln s2 = V_{s2} scatter measured with LED2 on
ln t1 = V_{t1} transmission measured with LED1 on
ln t2 = V_{t2} transmission measured with LED2 on

The procedure can be automatically performed in the electronics or later during evaluation of the raw data. Note that since scatter signal usually has the lowest numerical value and the

transmission signal the highest, ln R* becomes a negative number in most cases. The result is that the dual ratio signal ln R* is slightly temperature sensitive. By measuring the absolute temperature T of the electronics, the temperature dependence of ln R(T)* is compensated in the software using a reference measurement at 293K:

$$\ln R^* |Tref = Tref/T * \ln R^*(T) \quad (4)$$

Under the assumption that at the reference temperature Tref= 293K and ln R*|293K = ln R*(293K). The validity of this procedure has been verified by the NLR with the electronics box placed in a temperature-controlled chamber in the range of 5^o to 35^o. The temperature sensitivity in found to be the range of 0.1 units/K for the sensor filled with water. Around 293K the temperature sensitivity of the logarithmic amplification is fully compensated using procedure (4).

4.2 Sensor calibration

In the operational range it has been discussed [2] that the sensor signal ln R* (3) can be approximated using a polynomial function with coefficients b_i, to be determent by calibration, with [c] is the concentration of cells.

$$\ln R^* = b_0 + b_1*[c] + b_2*[c]^2 + b_3*[c]^3 + \quad (5)$$

From spectral characterization tests on samples it is found that when ln R* measured at a fixed wavelength (LED), in most cases it is a function of the cell concentration. Using the inverse relation the cell concentration can be derived from the sensor signal R=ln R* using:

$$[c] = c_0 + c_1 * R + c_2 * R^2 + c_3 * R^3 + c_4 * R^4 \quad (6)$$

The coefficient c_0 to c_4 are determent by calibration and are used for evaluation of the raw data sensor data or programmed in the software.

Note that due to a malfunction in the electronics the scatter signals were corrupted for FOTON M2 and the ground reference tests. The dual ratio and the above calibration procedure could not be employed during evaluation. An alternative method, however less accurate, using the transmitted signals only is discussed in the section 5.2.1.

5 BIOFILTER FLIGHT EXPERIMENT RESULTS

The discussion about the results of the BIOFILTER flight experiment in this paper is focused on two subjects:

- **Thermal results**
- **Sensor results**

5.1 Thermal results

Both the internal and external temperatures were monitored during the FOTON missions using external loggers and internal sensors. The major events occurring during the **FOTON M1** and **M2** mission are indicated with a label or a number.

5.1.1 FOTON M1 temperatures

For FOTON M1 the recorded temperatures stop by the explosion of the rocket, shortly after launch. See Figure 13. Note that during transport the internal temperature was too low, around 0^oC. This could be related to the low set-point of the coolbox at 0^oC. Possibly some of the bacteria might be frozen during transport. To prevent this for FOTON M2 the coolbox

temperature was set at 2°C during transport. During integration, the internal temperature increased to 18°C prior to launch. It also can be seen that the PCM worked around 6°C nicely leveling off the temperature increase for about 1 day. Unfortunately, for FOTON M1 the external temperature logger was not found back. From the internal temperature increase during integration the thermal insulation value is estimated λ=0.69mW/mK [3] Since the thermal insulation for M1 and M2 was comparable, most likely the environmental temperature at the launch pad for M1 was slightly higher than for M2 resulting in a higher pre-launch temperature.

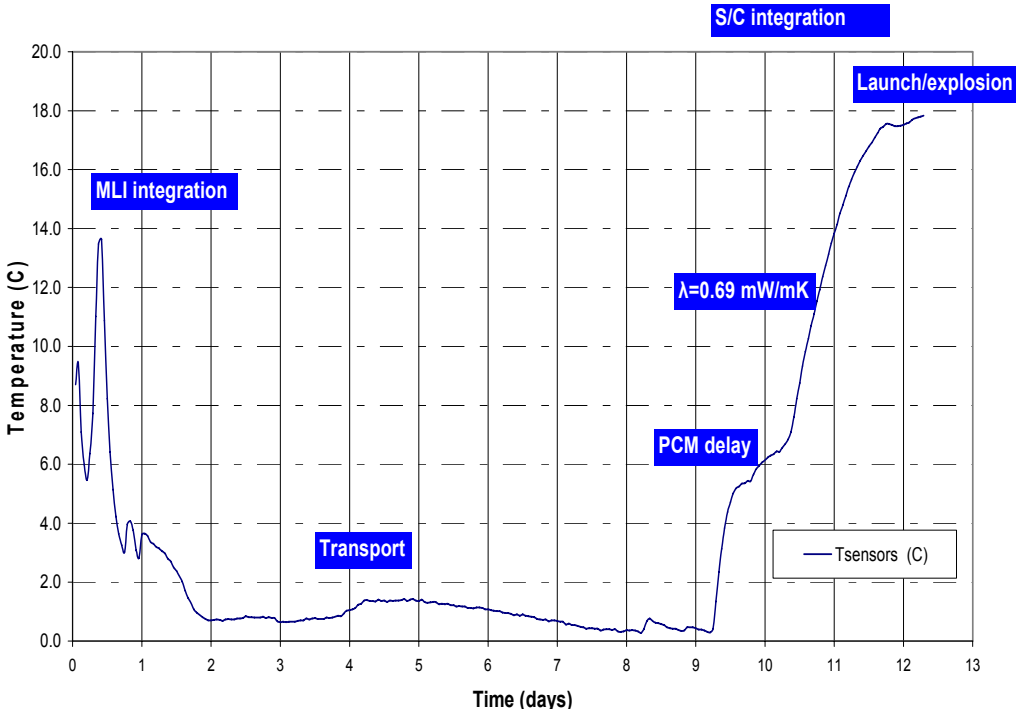


Figure 13: BIOFILTER temperatures measured until the explosion shortly after launch of FOTON M1 (recovered data)

5.1.2 Result summary thermal subsystem for FOTON M1 (recovered data)

- The internal temperature during transport was around 0°C by the low set point of -1/0°C the coolbox. There was a concern that the bacteria might have been frozen.
- The PCM N-tetradecane was activated before/during the transport phase and stabilized the temperature around 6°C for about 1 day during payload integration. From the internal temperature increased from 0°C to +18°C in 4.5 days before launch, a thermal insulation of λ=0.69mW/mK was achieved.

5.1.3 FOTON M2 results

As can be seen in Figure 14 with the temperature results for FOTON M2, the PCM was not activated prior to integration. During transportation the box internal temperature (due to the 2°C set point of the coolbox) became not below the solidification point of the deuterium-oxide at +3.9°C. Since in this cases the PCM was not activated the internal temperature increased to +15°C (instead of the anticipated design value of +10°C) before launch. Despite the somewhat higher pre-launch temperatures the bacteria remained in lag phase as confirmed by the sensors) prior to flight. From the internal temperature increase during integration, the external temperature and the inner box mass the thermal insulation value was estimated in the range of 0.7mW/mK. Since the BIOFILTER box was thermally insulated and under control by an

internal heater, the experiment was not influenced by the lower than expected on-board temperature

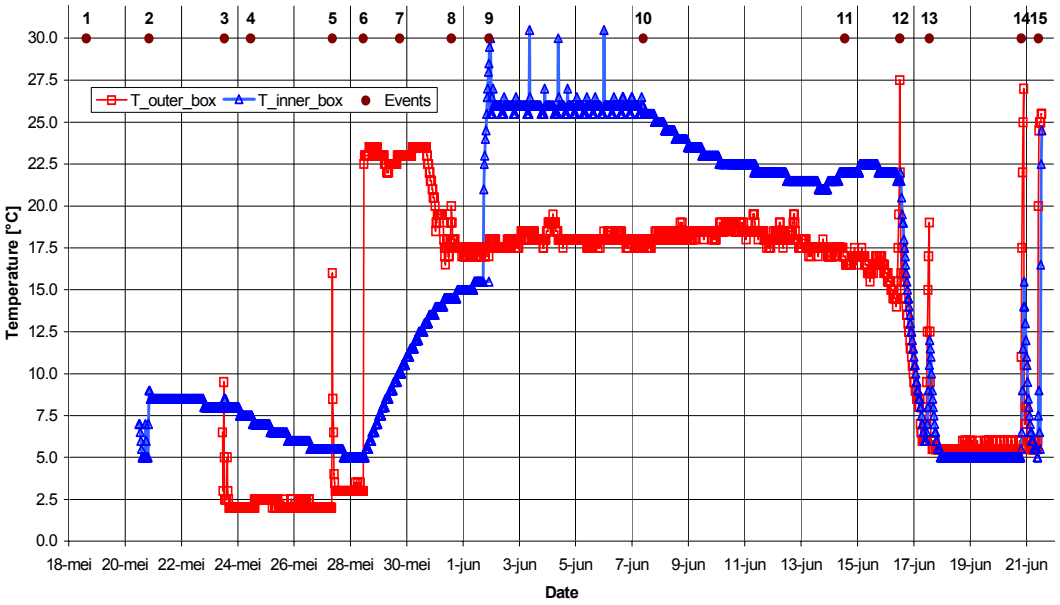


Figure 14: BIOFILTER external and internal temperatures as measured during FOTON M2.

FOTON M2 events:
1. Start sensor measurements. 2. Box placed in vacuum chamber 3. External box closed and placed into VEBA # 1027 4. Box delivered to ESTEC 5. Incoming inspection after transport 6. Biofilter placed on FOTON in ambient integration hall 7. Internal temperature increases to above 10°C 8. Launch 9. Programmed heater switch-on 10. Programmed heater switch-off 11. End of flight/re-entry 12. Vacuum insulation disrupted (air in) and box placed into VEBA # 1027 13. Box placed into VEBA #1025 14. Temporary malfunction of VEBA #1025 15. Box opened and inspected in laboratory

One day after launch (event 9) the internal temperature was automatically increased by a heater to +26°C to initiate bacterial growth. The heater was switched off automatically (event 10), after six days time window for the bacteria growth) to spare the batteries. After re-entry the vacuum insulation was removed from the outer-box, by letting air in, before placement in the coolbox for transportation back home. Because the coolbox #1027 was not working properly BIOFILTER box was placed in a spare coolbox #1025.

5.1.4 Result summary thermal subsystem for FOTON M2

- For FOTON M2 the internal temperature remained above the melting point of deuterium-oxide at +3.9°C during the transport phase caused by the set point of the coolbox at +2/3°C and was therefore not capable delaying the temperature increase during integration.
- In the integration hall at +22.5°C, the internal temperature increased from +5.2°C to +15.6°C in about 100 hours indicating a thermal insulation for FOTON M2 to be λ=0.7mW/mK, comparable as found for FOTON M1.
- Most likely the ambient temperature on the launch day was a few degrees lower than for FOTON M1 resulting in a 2 degrees lower pre launch temperature.

5.2 Sensor results

As concluded form the thermal data the actual achieved FOTON M2 temperature profile was close enough to the designed values to ensure bacterial growth in µg. However, due to significant problems with the electronics[6], the scatter measurements appeared corrupted and could therefore not be used for the dual ratio or for calibration. A work around was found using transmission data; each sensor gave two readouts, by the two perpendicular directions of the light beams. The accuracy of this approach is discussed below.

5.2.1 Sensor operation & calibration

Since the dual ratio method, introduced for full compensation for both the LEDs as well the photo-detector instabilities, could not be applied, an alternative solution was needed when using the transmission data only. LEDs are known to give repeatable and stable outputs for several hundred hours of nominal operation [4]. Since for BIOFILTER the LEDs operate for 10 seconds per measurements, (1x per hour during transport and 20x per hour during heater switch on) they operate for less than an hour during the total experiment. Therefore, LED intensity output changes by aging can be fully neglected. Also the sensitivity of the photodiodes hardly changes over time [5]. Compensation of temperature influences in the LED outputs, photo detectors sensitivity and amplifiers is performed by dividing the detector outputs by the reference sample signal with a fixed turbidity and epoxy with aluminum particles included. Since all LEDs and photodiodes for the eight sensors, are selected from the same batch and are distributed closely together and run at about the same temperature, it is reasonable to assume that they respond the same to temperature changes. The conclusion is that, although less precise, the accuracy of the transmission approach is guarantied. Note that intermediate offsets can not be compensated for because the calibration is only performed at the start and end of the experiment.
The transmission data was used for the determination of the Optical Density (OD) which is proportional to the bacteria dry-weight. The OD is defined as the logarithmic ratio between the transmission T_1 and the reference transmission T_o measured with a clear medium, and is proportional [k] to the bacteria dry weight.

$$\text{Bacteria dry-weight [gr/cc]} = k * OD \tag{7}$$

with:

T_1 = transmitted light through sample
T_0 = transmitted light through reference (clear) sample
OD= optical density (= - log T_1/T_0)
k = calibration factor [dry-weight/OD]

For the calibration of the transmission data towards OD values it was considered that at the start of the experiment the reference transmission T_o is measured since we than have a clear medium (with only very few cells added) and the OD is zero by definition. Also it was considered that the lowest transmission T_1, e.g. the highest bacteria density, was measured at the end, after sample vortexing, during the post flight inspection. The bacteria culture was extracted from the samples and the OD was measured using a calibrated photo spectrometer. These two fixed OD measurement points have been used to determine the in-between sensor outputs related to OD values. Note that if in this case offsets occur during the 16 days flight, they are not fully compensated when using the above method.

[6] This is caused by application of a multiplexer in the electronics, requiring a redesign to fix this. Current leakages in the range of nA appeared to be in the same order of magnitude as the scatter induced photo-currents. It was proved that replacing the multiplexer with dedicated amplifiers fully overcomes this problem.

5.2.2 Conclusion for the sensor subsystem

- Scatter data/dual ratio and calibration could not be used by a fault in the electronics.
- Although less accurate the transmission measurements could be used for evaluation since:
  - LEDs output and photo-detectors sensitivity are comparable and inherently stable.
  - Temperature influences are compensated using a reference sample with a fixed turbidity.
  - OD calibration performed at the start (e.g. clear medium, OD=0) and end (e.g. fully grown bacteria, uniform distribution after vortexing, OD=0.45)
  - Sensitive for intermediate offsets

5.2.3 Post flight inspection

During post flight inspection when the samples were opened bacterial growth was seen in all 7 sensors. The Optical Density (OD) of the fluid inside the sensors was diluted and measured in the laboratory with a photo spectrometer at 450 nm showing the expected level of completed bacterial growth (OD=0.47±0.04), same as found for the reference cultures (OD=0.48±0.02).

5.2.4 Sensor flight results

The data transmission data from the sensors have been processed according to the discussed procedure in section 6.2. The result for FOTON M2 is visible in
Figure 15 taking the averaged of both directions for all sensors, accept for the corrupted sensor 1 and 2 (reference). Soon after launch (t=330hr) an array of growth curves with growth rates of 0.046–0.077hr^{-1} up to an OD= 0.26 during flight is visible.

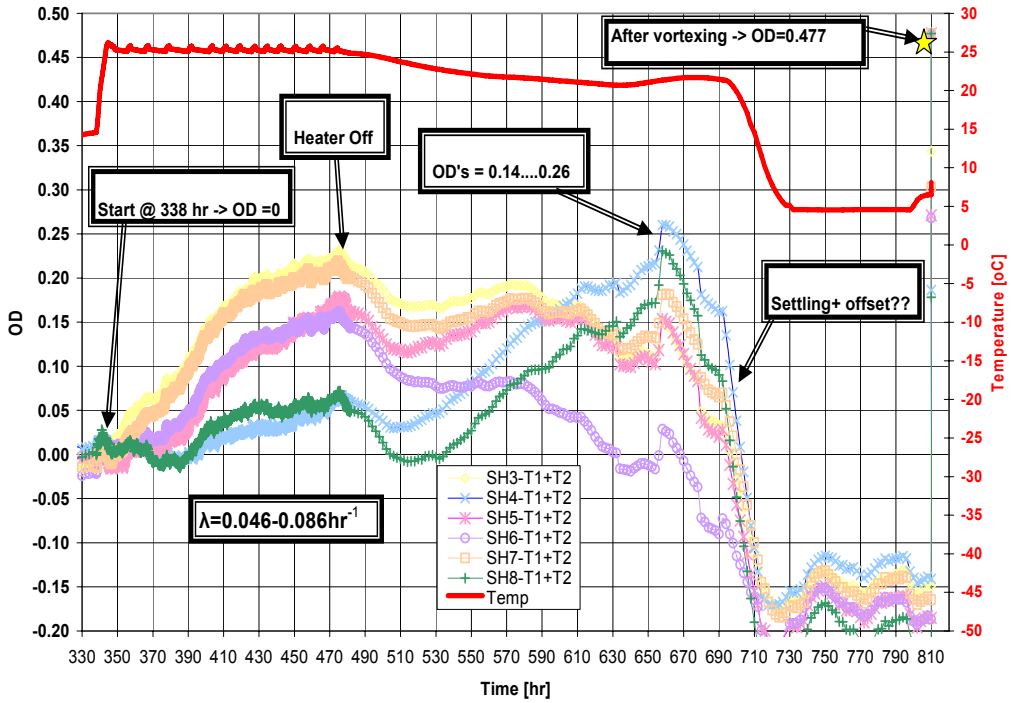


Figure 15: FOTON M2 sensor results. OD measurement was derived from the averaged transmission data.

As can be seen in **Figure** 15 the bacteria growth started in all sensors some time after the switch on of the heater. Directly after switch-off of the heater after 6 days, all curves drop a little, indicating a temporarily growth stop. Two measurements from sensor 4 and 8 show an exceptional long lag-time up to 150 hours, both however only measured in one direction, indicating a non-uniform growth and/or distribution of bacteria inside a sensor. In fact a couple of unknown influences that vary in time during the growth determine the results making biological interpretation more difficult. Since in μg the fluid in the sensor is most likely not mixed, the measurement signal is incidentally influenced during/ after growth by:

- the number of bacteria in the light path
- distribution of the bacteria in the sensor
- temperature
- offsets

For OD calibration at the end, all the sensors were vortexed in the laboratory during the post flight inspection, to ensure an uniform distribution of bacterial cells and the highest possible Optical Density for the fully grown cultures. As confirmed by the raw data the *transmission* value's (maximum OD) after vortexing were the lowest found. Note that during/after re-entry and landing, a combined influence of settling of the bacteria under the influence of gravity and an offset indicated by a negative OD value around -0.15 is visible. However an OD=0 would be normally expected after settling. From previous laboratory experiments performed with this bacteria it is know that settling of the cells occurs at a velocity of about 1.2 mm/day in static cultures. Since the sensor light beams are ca 1mm across, the bacteria settle within less then 20 hours in the present of gravity. The offset is possibly related heavy loads during re-entry and affects the calibration of the OD levels during the flight, however will not affect the conclusion of a slower growth. Also interesting is the fact that a small peak is visible before launch in the data that was related to the erection of the rocket from a horizontal into a vertical position on the launch pad prior to launch.

5.2.5 Summary of the sensor flight results

- post flight inspection revealed bacterial growth in all sensors
- averaged OD=0.477 (comparable with the references cultures) found after vortexing in all sensors
- array of growth curves (μ=0.046–0.077hr^{-1}) after heater switch-on
- indication of non-uniform distribution of bacteria in sensor, possibly by poor fluid mixing in μg
- indication of slower growth rate's
- settling after landing under influence of gravity
- possible offsets occurred during re-entry indicated by negative OD values after settling.
- biological interpretation difficult

To increase confidence in the equipment and the calibration methods applied and biological interpretation of the flight results, two ground reference experiments that are discussed in the next chapter have been performed, using the BIOFILTER hardware and replying flight conditions.

6 BIOFILTER GROUND REFERENCE EXPERIMENTS

Objective for the ground reference experiments was to increase confidence in the operation of the sensor technology, in which case the differences in growth (rates) between the flight and ground experiments can be determined. It is anticipated and confirmed by other flight experiments that gravity has an effect on the growth of bacteria. The ground reference experiment was carried out using BIOFILTER hardware, procedures and internal temperatures as applied for the FOTON M2 mission. Any differences found might expected to be related to the influence of (or absence of) gravity.

6.1 Ground reference set-up

Two ground reference experiments using BIOFILTER flight hardware (e.g. the equipment box including the eight sensors) mounted on a Random Positioning Machine (manufactured and supplied by Dutch Space) The temperature environment is controlled using a coolbox (with heater) -simulating the transport phase- and a climate chamber -replaying the FOTON-M2 internal flight temperature profile. The rotation speed of the RPM is the major difference between the ground experiments. A fast random rotation is applied to simulate μ-g conditions and a slow rotation speed is applied to prevent settling of the bacteria to the bottom of the sample cells when exposed to 1g conditions. Two ground reference experiments[7], both lasting one month, were carried out:

0g ground experiment
BIOFILTER equipment box mounted on a *fast* random rotating RPM (90^o/min) with the intention to simulate μg conditions.

1g ground experiment
BIOFILTER equipment box mounted on *slow* random rotating RPM (0.5^o/min) with the intention to prevent bacteria settling under normal gravity (1g) conditions.

For the 0g experiment a rotation speed of 90^o/min was selected because this was the highest possible rotation speed. Centrifugal forces 4cm away from the axis of rotation, roughly were the outer sensors are located were smaller than 0.01g. For the 1g experiment a 0.5^o/min ($=180^o$/6hr) rotation was selected to compensate for bacteria settling effectively turning the 2mm cells up-side-down four-times-a-day.

6.2 Temperature conditions

The environmental conditions for both 0g and 1g experiments were correctly carried out, simulating the internal BIOFILTER temperature within $\pm 1^oC$ throughout the significant periods.

6.3 0g experiment results

Some time after start-up of the 0g experiment (with the RPM rotation at 90^o/min) it appeared that the bacteria culture used was not growing as expected. Both the parallel references cultures showed poor growth to an OD of 0.109±0.015 whereas an OD between 0.4-0.5 is expected. The poor growth was confirmed by the low the averaged OD as extracted from all the sensors, after completion of the 0g experiment.

[7] As a result of the ground reference experiment it appeared that the names 0g and 1g were not fully correctly chosen with respect to the simulation of the conditions during flight. For convenience we keep them throughout the paper.

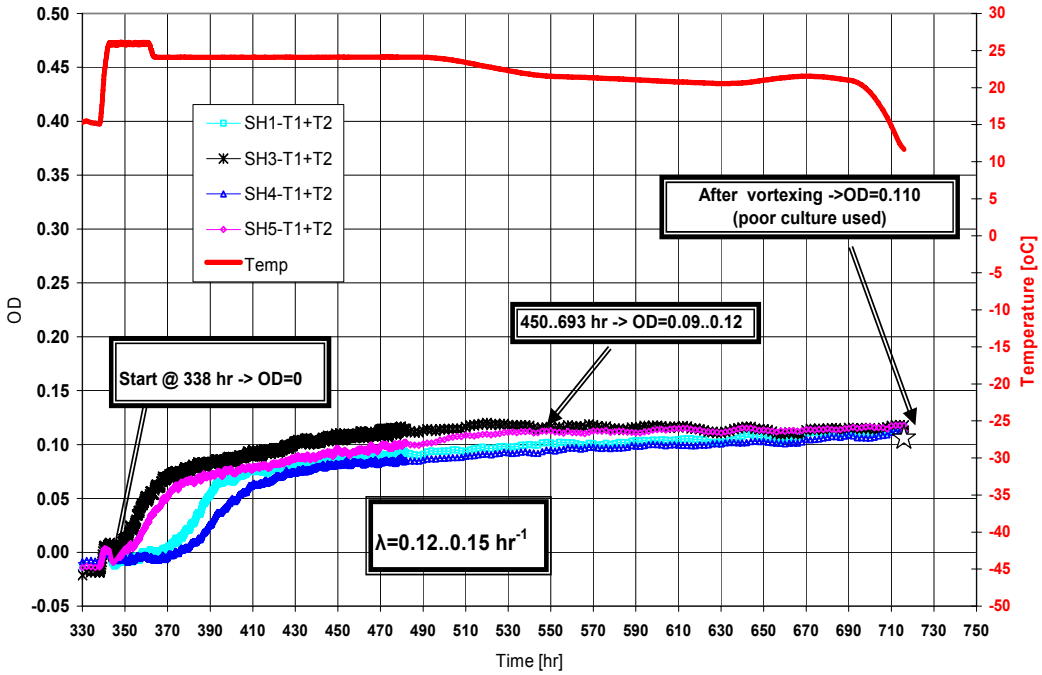


Figure 16: 0g experiment (RPM at 90°/min). End OD is too low due to a poor growing bacteria culture.

Although the growth was poor and the sensor signals were noisy it was possible to evaluate the results. In Figure 16 the results of the 0g experiment are plotted after OD calibration. Sensor 1, 3 ,4 and 5 are selected giving the best results. Interesting is that the final-OD measured after vortexing showed no difference with the end-value obtained during the 0g-experiment. This indicates that the fast rotation of the RPM ensured uniform distribution of the bacteria during the experiment. Doubling times were found between 4.5-6 hours e.g. 'normal' growth rates at 26°C, resembling growth rates in the range of between 0.12-0.15hr^{-1}. The growth spreading is most likely related to the poor & noisy signal (low OD) and a 1°C lower environmental temperature for the some of the delayed growing cultures (sensor 1 & 4). Note that no offsets occurred.

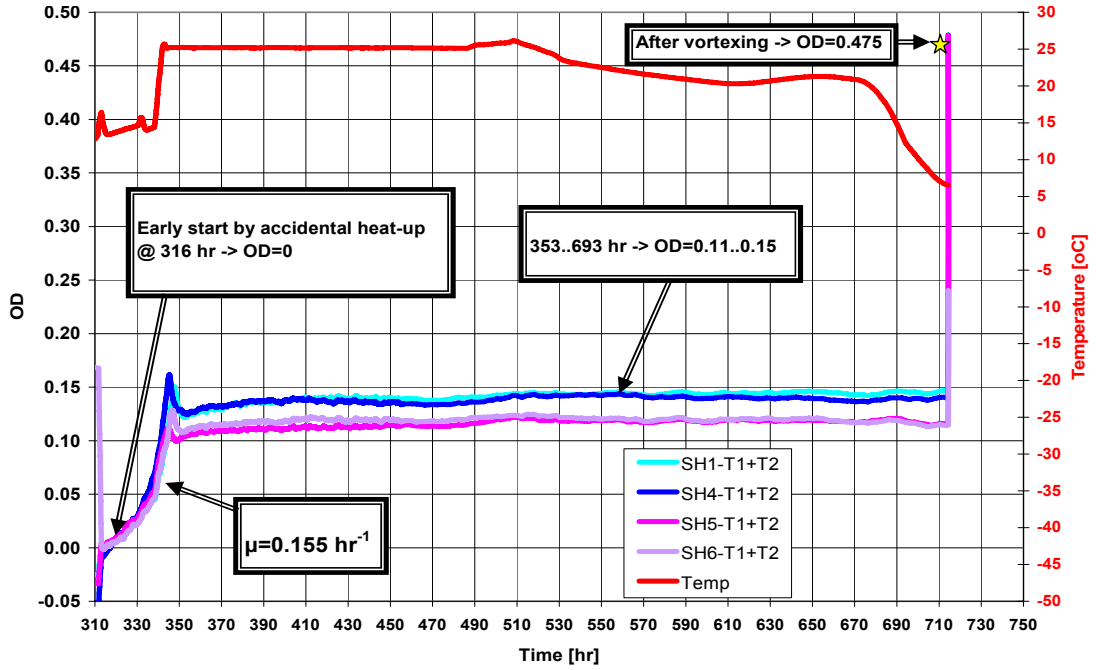


Figure 17: 1g experiment (RPM at 0.5°/min). End OD is lower due to poor mixing

6.4 1g experiment results

For the 1g experiment the reference cultures showed a final OD of 0.39±0.02. The averaged OD measured after the 1g-experiment (vortexed, extracted and diluted from the samples) revealed a slightly higher value OD=0.475±0.06. This value is used for the calibration of the sensors. From Figure 17 the averaged OD during the experiment was found between 0.11-0.15, significantly lower than measured after fortexing, indicating that due to the slow rotation of the RPM the bacteria partially settled outside the range of the optical beams. After temperature stabilization, a doubling time of ca 6 hr is measured, indicating a normal growth rate of μ=0.12hr^{-1}. Note that no offsets occurred.

6.5 Ground reference experiments results summary

Below the results of both the ground experiments are summarize and compared with the BIOFILTER results.

Experiment	Conditions RPM rotation 24-25°C	Doubling time [hr]	Growth rates [hr^{-1}]	'End' ODs (flight)	Final OD (after vortexing)	Reference culture OD
0g experiment	Ω=90°/min	4.5-6	0.115-0.154	0.11±0.01	0.11±0.01	0.109±0.015[1]
1g experiment	Ω=0.5°/min	6	0.115	0.12±0.02[2]	0.475±0.06	0.39±0.02
FOTON-M2	μ-gravity	9-15	0.046-0.077	0.05-0.45	0.477±0.04	0.48±0.02

Table 2: Results summary BIOFILTER and ground reference experiments

[1] *Poor growing culture used, however no influence on growth rate*
[2] *The lower end-OD is most likely related to poor mixing due to the slow rotation of the RPM.*

To give an overview of the results in a graphical form in Figure 18, where the laboratory, BIOFILTER and ground reference results are normalized to an OD=1 and schematically plotted.

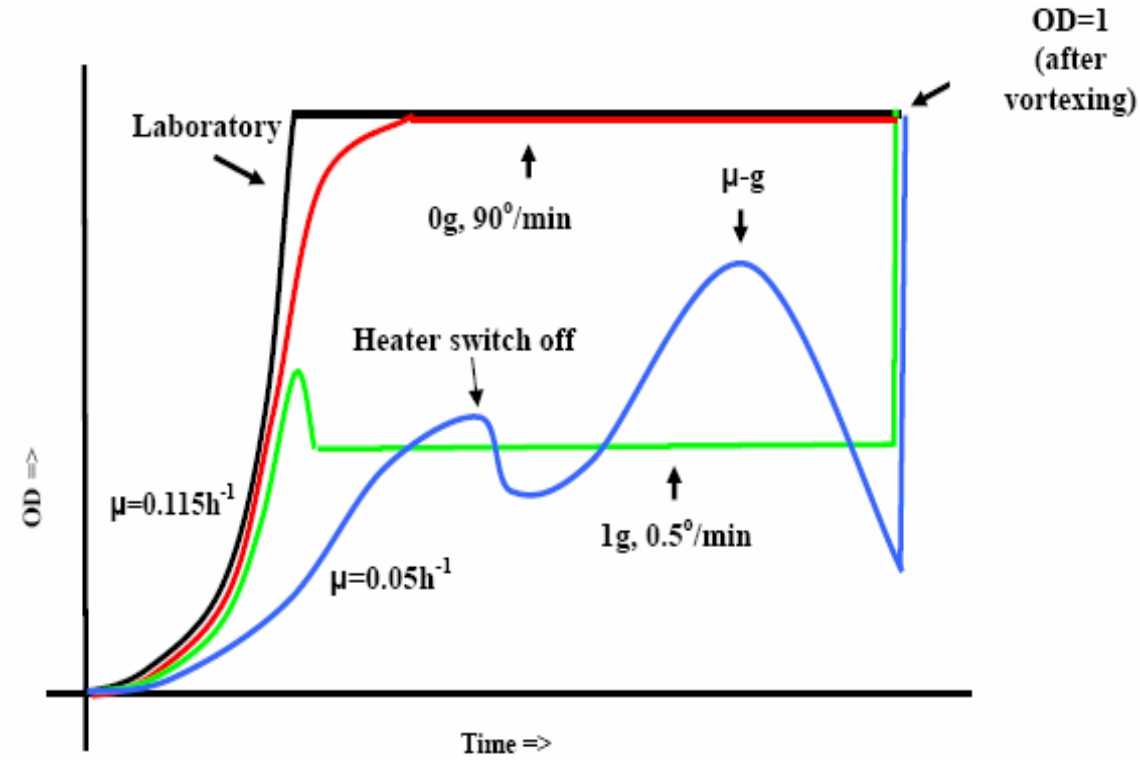


Figure 18: Normalized picture of the BIOFILTER flight and ground reference sensor test results.

Biological interpretation should take into account sensor limitations and physical influences such as poor mixing and non-uniform bacterial distribution in μg and rotational speeds of the RPM.

7 CONCLUSIONS

The BIOFILTER experiment on board FOTON offered an excellent platform for BIOCLEAR to study the effects of microgravity on microbial growth kinetics. The BIOFILTER project offered NLR a unique opportunity to design, build and space qualify both the thermal and in-house developed sensor subsystem. To increase confidence in the hardware and flight experiment results the BIOFILTER design was thoroughly discussed in the paper. The thermal design of the box performed fully as expected, despite a minor problem with the PCM. The bacterial growth, trigged by a heater, started nicely in orbit. The thermal design might be useful for further projects requiring autonomous control of the temperature. The performance of the sensor subsystem was degraded by malfunction of the electronics. A work-around was found, revealing an array of growth curves and slower growth rates in flight. Biological interpretation is compromised by unknown phenomena such as biofilm formation, heterogenic cell distribution, sensor instabilities, poor mixing and non-uniform distribution of the bacteria in μg. Sensor offsets, most likely related to re-entry, has only an influence on the OD level and not on the measured (slower) growth rate. To increase confidence in the equipment and the flight results, two ground reference experiments were performed, using BIOFILTER hardware. Both ground experiments show a nice growth curve at least indicating a correct functioning of the equipment and methods applied. The 1g experiment (slow rotation RPM) proved that mixing is necessary to see the full bacterial growth. The 0g experiment (fast rotation RPM) shows precisely the same growth rate as found under laboratory conditions on earth. Does this mean that the growth rate is not influenced by (simulated) μ-gravity or that the RPM does not fully simulates μg

conditions? Perhaps bacterial growth measurements under these conditions in a fluid are not ideal? These questions will be addressed when results from more flight experiments on bacterial growth kinetics become available in the future.

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[6] As an example see http://www.siemens.com search for WM14S794GB