Biocontamination Integrated Control of Wet Systems for Space Exploration (BIOWYSE)

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Control of microbiological contamination within spacecraft is of huge importance for long-duration manned space missions: such systems must guarantee crew well-being, health, and subsistence. The development of materials and methods to prevent, monitor, and mitigate environmental microbial contamination and its harmful effects are thus required. Considering the application of such systems to spacecraft, possible solutions must be safe, automated, lightweight, reliable, efficient, and require minimal energy, consumables, maintenance, etc. The "Biocontamination Integrated Control of Wet Systems for Space Exploration" (BIOWYSE) project focuses on the development and demonstration of a compact, integrated, and automated solution (hardware & software) to biocontamination control. The BIOWYSE system is designed to prevent, monitor, and mitigate the risk of microbiological contamination in water systems and humid surfaces onboard International Space Station (ISS) and in future human space exploration missions. Automation and synergy of these processes lead to reduction in crew time, decreased energy requirements, procedure simplification, and additional safety measures. Prevention and real-time monitoring, together with an appropriate control system, can reduce the decontamination effort requirement and radically improve efficiency.The BIOWYSE system and its subsystems and modules are described in this paper.

Nomenclature

CM = Control Module
DM = Decontamination module
HAS = Humid Area Sampler
MM = Monitoring Module
PM = Prevention module
SM = Service Module

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I. Introduction

AS demonstrated in several years of ground studies and actual space operations, humid areas and wet systems are the most prone to biocontamination. Space exploration requires the development of more reliable, rapid, significant and safe methods for preventing, monitoring and controlling biocontamination within human confined environments[1]. These methods have to be automated, simple, lightweight and with minimal consumables. Both space and terrestrial monitoring and prevention/mitigation methods are currently working separately, rather than in synergy. The BIOWYSE project foresees development and demonstration of *an integrated biocontamination control system for water and humid areas, for space exploration habitat management within infrastructures, experimental payload, cargo and crew transportation elements*. BIOWYSE stems from the results of actual flight experiments, such as Viable ISS, the biocontamination control requirements of experts working groups, and state of the art prevention, monitoring and mitigation technologies.

A key requirement in identifying control measures to prevent water contamination is that their performance should be monitored. Monitoring frequencies are normally planned by terrestrial water utilities in order to ensure that corrective actions can be introduced in a timely fashion to avoid development of hazardous situations. Control measures are considered effective if monitoring results comply with certain limits. If not, appropriate actions need to be taken immediately to bring the measure back under control. The critical time lag between sample analysis and, when necessary, corrective intervention, represents a major challenge for the achievement of optimal water quality standards and conceals the risk of a contamination spread. The general principle is that frequent performance of quick (or even better real-time) field tests is preferable to infrequent and expensive laboratory-based testing.

For all of these reasons a real-time online monitoring strategy would be highly desirable to test drinking water at the point of use with extreme rapidity and independently from the possibility to culture the microorganisms.Several biomonitoring methods are available for the assessment of drinking water quality. For example, Flow Cytometry and DNA amplification-based methods (e.g., LAMP, PCR, q-PCR) can provide accurate quantification and detection of a wide range of different microorganisms, including viruses, prokaryotes, protists and human pathogens. Next-Generation Sequencing analysis can additionally provide a detailed description on the microbial species eventually resistant to the mitigation and prevention treatments. These approaches are now starting to become available as required for “sample-to-answer” methodology (i.e WetLab-2 experiment[2]) .

BIOWYSE combines real-time (thus culture-independent) microorganism monitoring with almost instantaneous UV-based disinfection (which also means no harmful by-products), thus providing an innovative tool with a wide application potential in a large number of situations. By adopting a clear modular and concurrent approach, the BIOWYSE consortium has designed, built and tested innovative prevention, monitoring and mitigation modules, which are integrated in a compact system.

II. Technical and Scientific goals

The BIOWYSE project main technical goal is demonstrating the capability of realizing a system which is able to manage the potable water according to the expectations of storage, bacterial growth prevention, quality monitoring, decontamination and delivery. Non-specific decontamination technology coupled with a non-specific monitoring technique allows optimizing time and energy consumption, ensuring a non-selective log reduction (system requirement of a microbial load reduction of at least 99,99%) and avoiding deep investigation on microbial species.

More specifically, the projects *goals* can be summarized as:

1. Real-time detection of water microbial load with high accuracy without the burden of false negative results intrinsic to traditional culture-dependent methods.
2. Increase the analytical sensitivity to detect viable-not-cultivable microorganisms largely disregarded by traditional culture-dependent methods.
3. Identify threshold limits of the selected monitoring parameter corresponding to safe drinking water
4. Define ranges of physical, chemical, and microbiological quality assuring an optimal System analytical performance.
5. Increase sensitivity compared to other established real-time monitoring technologies such as analyzers for dissolved oxygen, nitrate, UV-VIS spectra, ammonia, pH, temperature, conductivity, redox potential and turbidity.

6. Avoid dangerous disinfection by-products in the resulting drinking water.

III. Outline of the BIOWYSE terrestrial system (breadboard)

The integrated BIOWYSE breadboard, is the main hardware output of the BIOWYSE project. The breadboard is designed with the aim to test the system principles behind the concept and used off the self components as much as possible. The breadboard is planned to be tested in the laboratory and in the field.

The breadboard is composed of the following modules:

1. **Prevention Module (PM)**: a coated tank for inlet water buffering. It will prevent microbial load in-crease during temporary storage. In future space systems the same surface treatment could be applied also on surfaces.
2. **Monitoring Module (MM)**: the analysis system applicable to both water and surface samples, based on bioluminescence. It receives the inlet “stabilized” water and performs bacterial total count;
3. **Decontamination Module (DM):** a UVC-LED unit followed by a regenerable Compact Filter Unit (CFU) filter, applied to water loops in the breadboard, applicable also on surfaces in a future system. It is actuated by the control module when analysis results are increasing or exceeding the imposed thresholds levels for microbial contamination. Once turned on, it allows microorganisms deactivation (by UV light) and trapping (by fine filtration);
4. **Control Module (CM)**: power, command and data handling unit for the monitoring and decontamination modules. It receives and elaborates analysis data via dedicated control laws, regulating the decontamination modules operation (power, duration, on/off cycles) depending on the biocon-tamination monitoring results (above / below thresholds). It provides caution, warning and emergency alarms.
5. **The Service Module** (**SM)** which provides structural support and accommodation volume for the other Modules, the Cooling Subsystem, and the water distribution network.

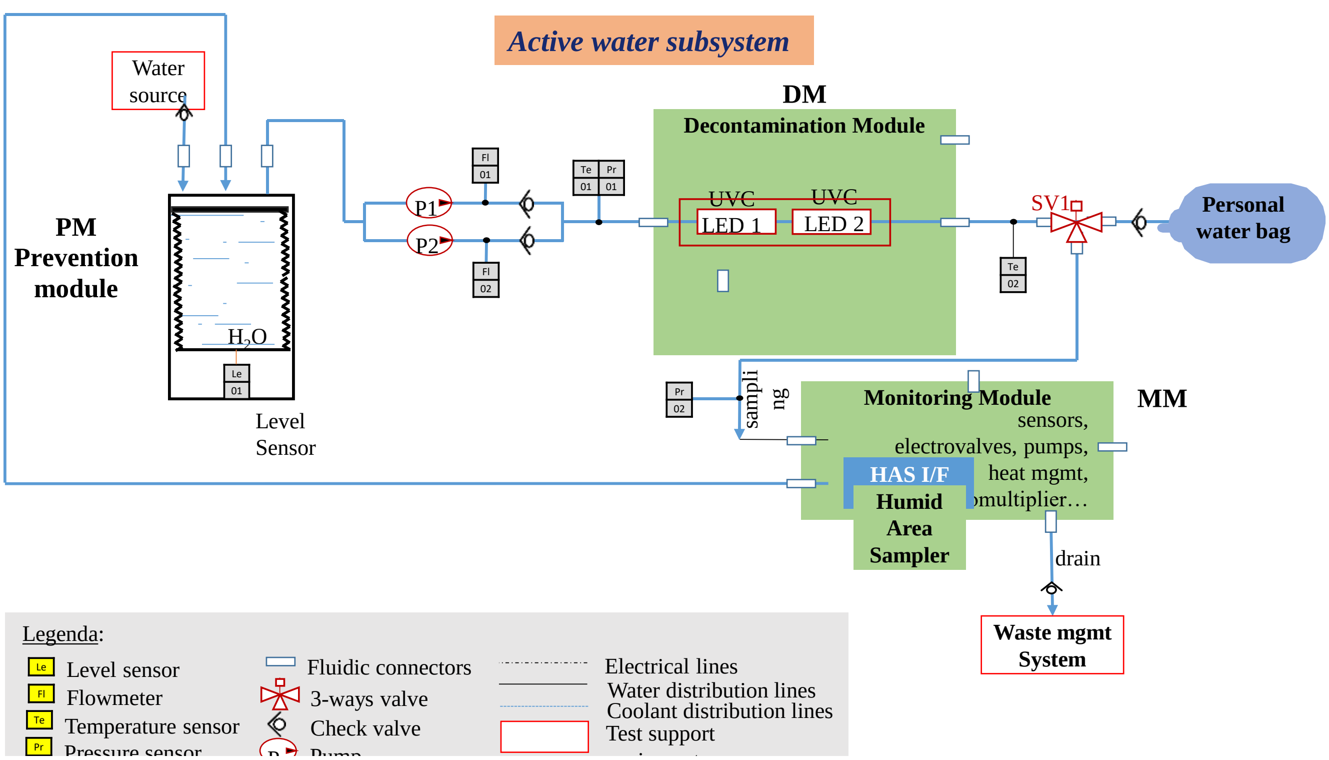

Figure 1: BIOWYSE active water sybsystem PM, MM and DM are parts of the active water subsystem. This has the function to storage, decontaminate, monitor and distribute the water coming from an external water source. Decontaminated water is deliverd to the personal water bag. The MM has an intergace with a Humid Area Sampler, which is used forsurface sample analysis.

The BIOWYSE modules thus form a set of three main interacting Subsystems designed with the aim to storage, decontaminate, monitor and deliver the water to an external user.

1. The active water Subsystem (Figure 1), whose functions are to store, decontaminate, monitor, and distribute the water coming from an external water source. The Prevention Module, the Monitoring Module and the Decontamination Module are parts of this subsystem.
2. The Data Management and Subsystem Interface, whose function is to receive, collect, store, elaborate and transmit the data, to monitor and control the overall System performance, and to provide an interactive interface with the external user. The Control Module is part of this subsystem.
3. The cooling Subsystem, whose function is to remove the heat from the subcomponents in order to assure their correct operation.
4. In addition to the automatic and in-line water sampling provided by the Active Water Subsystem, BIOWYSE includes the Humid Area Sampler (HAS) which interfaces the MM for surface samples analysis.

The system is a very compact assembly, designed to be installed into EDR2 drawer (Figure 2). The allowable envelope dimensions are: 460 X 525 X 720 [mm] . The breadboard structural box dimensions are: 400 X 505 X 720 [mm], designed for the correct installation of the system into the drawer. The goals of the structure box are to provide easy and stable installation of modules, manual accessibility of all components, visual accessibility in order to detect off-nominal behavior, and minimize mass (for transportation on the field). For easier re-configurability, transport the breadboard has an external structural frame. Thus, the breadboard used in the field will have structural supports leading to the following dimensions: 500 x 600 x 850 [mm].

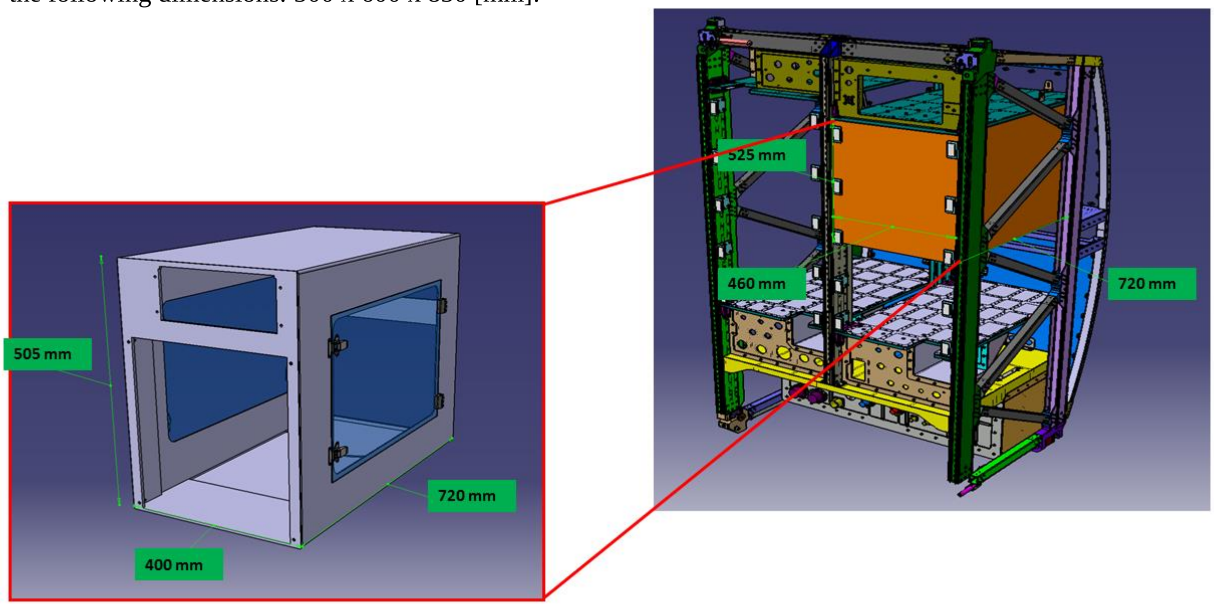

Figure 2: BIOWYSE envelope and installation in EDR2 drawer

During operations, the User will interface the BIOWYSE front side and the PC tool, see Figure 3 and Figure 4. The CM front panel provides the electrical interfaces, while the rest of the SM front panel provides hydraulic and mechanical interfaces for maintenance and operations.

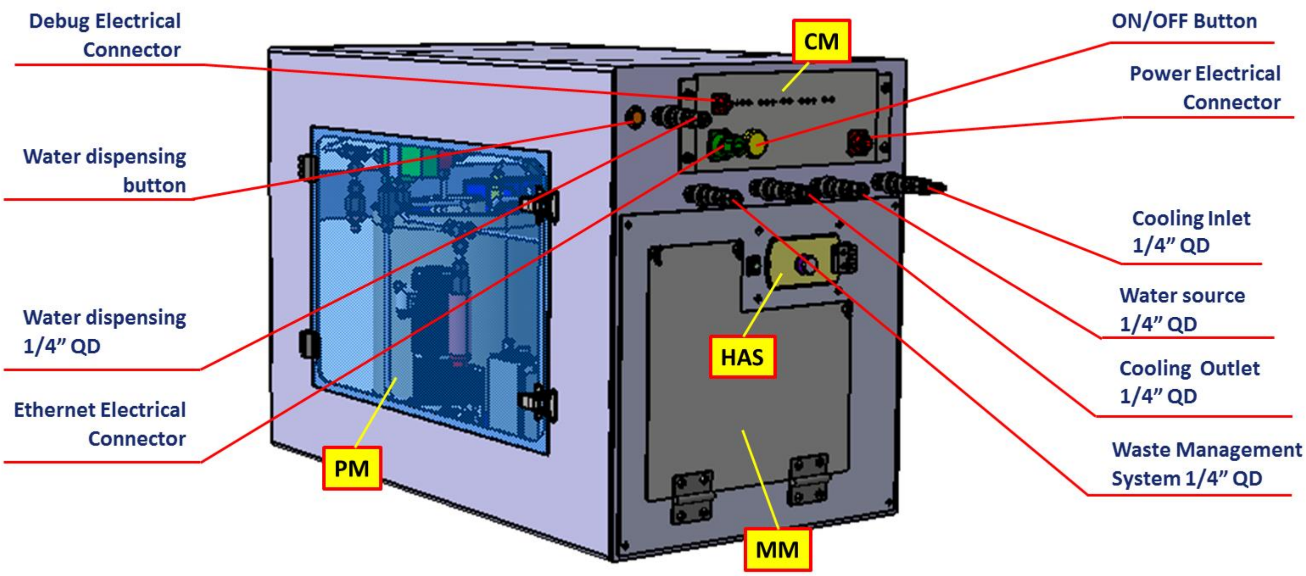

Figure 3: BIOWYSE external interfaces

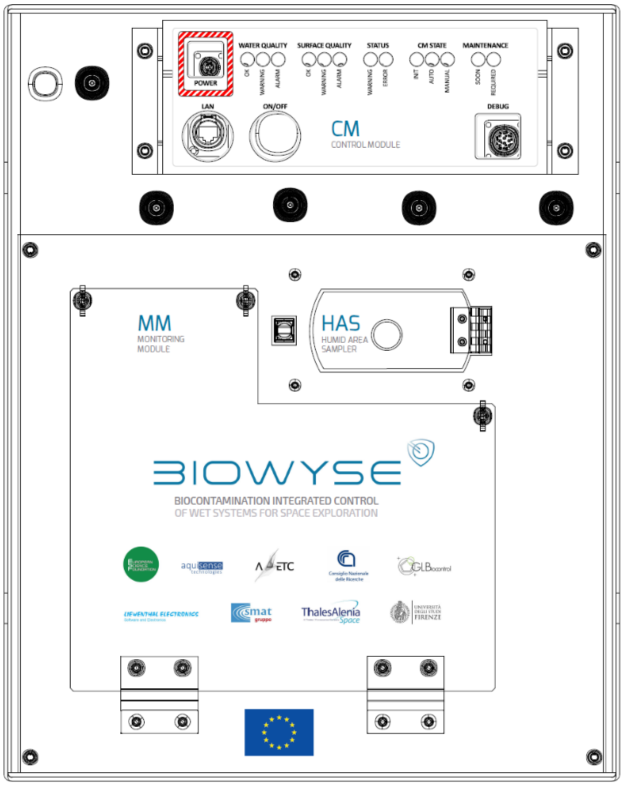

Figure 4: BIOWYSE front panel appearance

The BIOWYSE breadboard configuration and transportation for field testing is depicted in Figure 5. As ground support equipment there are four main elements:

- Mechanical, off-the-shelf trolley for transportation of BIOWYSE and support equipment
- Electrical, customized AC/DC power supply
- Fluidic, for start-up (priming) and final operations
- Thermal, COTS compact chiller (to simulate EDR-2 liquid cooling system)

The laptop has been provided together with the CM.

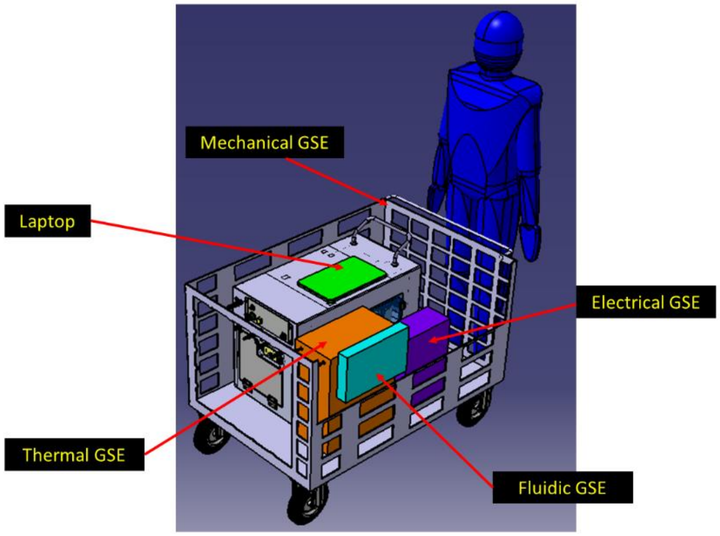

Figure 5: BIOWYSE support equipment

Mass and power budget requirements were 56 kg and 267 watt – total estimations at the Critical Design Review had respectively a margin of 6 kg for mass and 100 W for power.

IV. Microbiological control in BIOWYSE

The following sections will sketch out the three modules in BIOWYSE that deal directly with microbiological control, namely the PM, MM, and DM.

A. Prevention Module - PM

Microbial control measures for space habitats are currently active techniques such as HEPA filter for removal of airborne microorganisms from the atmosphere, biocide agents for guarantee the required potable water microbial quality and regular on-board analyses and cleaning procedures for water, air and surfaces. Compared with the application of countermeasures that attenuate an on-going or already occurred contamination event, preventive measures on the raw materials are strategic to make them inhospitable for microorganisms, thus blocking biological growth. This approach does not require neither chemicals addition nor further removal or on-board energy consumption. Moreover, the same effective prevention method can be employed for both external and internal surfaces of Life Support Systems and habitats. The BIOWYSE project used the results of the VIABLE-ISS experiment and made good use of these up-to-date available ISS data for selecting the most promising prevention method to be applied on the internal surfaces of the water buffer tank.

The Prevention Module is based on a biocide surface treatment of the buffer tank. Compared with the application of countermeasures that attenuate an on-going or already occurred contamination event, preventive measures block biological growth, counteracting proliferation. Moreover, the same effective prevention method can be exploited also for habitats external and internal surfaces. It is designed to receive water from chemical treatment systems, prevent microbial load increase during temporary stowage, and store water prior to processing for bio contamination removal. Microbial load control is given by the internal addition of silver[4], acting as a bacteriostatic and antimicrobial agent.

B. Monitoring Module - MM

The Monitoring Module is based on luminescence, instead of culture-based monitoring systems, therefore it allows reducing consumables and timely results. The analysis is automated, from reagents dosing, to internal calibration, reaction mechanism, radiation detection, response and cleaning, going beyond limitation due to archival samples return. Its main function is to monitor the biomass level in the water circuit and on humid surfaces. The Monitoring Module will be based on the bioluminescence phenomenon using the ATPmetry technique. This analysis system is rapid, compact, automated, not using toxic or flammable chemicals, applicable to multiple sources and with low requirements for energy, consumables and maintenance. Water sampled will be analysed using the quantitative

ATPmetry (quantitative evaluation of microbial biomass with adenosine tri-phosphate, or ATP, which is the major source of energy for cellular reactions) technique developed by GL Biocontrol, one of the BIOWYSE project partners.

Nowadays, a classical technique used for microbiological risk management in terrestrial water networks is bacterial growth on plate count agar. However, this technique is time consuming and requires important man power resources. Furthermore, it is limited to cultivable microorganisms. For many years, ATPmetry has been a good proxy for microorganisms' detection because it has the advantage of providing real-time results and allows the evaluation of total flora (cultivable and non-cultivable). This technology is already used on earth for the microbiological control of industrial, sanitary or ultrapure water networks but only manually.

The MM, plugged after the DM, will analyse water coming from the tank. Water sampled will be reinjected into the tank while reagents will be sent to the waste. Furthermore, the MM will be plugged to the water cooling loop system. The purpose of the MM is to avoid results (and thus intervention) delay due to storage and transport periods from sampling to analysis; avoiding delays from response and counteraction (possible risks for crew health); reducing the necessity of decontamination from "constant" to "if needed" intervention; and reducing crew time and complexity for water and surface microbial control.

The results, expressed in pg ATP/ml, will alert the crew in case of high value. For this purpose, four thresholds were defined dering the project regarding the contamination level of the water: drinkable (safe), drinkable (warning), drinkable (alarm) and not drinkable.

Tests were performed in collaboration with SMAT and CNR-IRSA to support or better define, if necessary, the threshold values chosen . A linear correlation was obtained between ATP measurement and total cell density (flow cytometry, epifluorescence microscopy count, qPCR) and HPC. These results are being prepared for publication.

C. Decontamination Module - DM

The Decontamination Module is based on UVC LED technology. The technology has the advantages of reaching full intensity within nanoseconds, excluding chemicals and minimizing energy consumption. Moreover, from the perspective of reducing consumables and crew time cost, the combination of a UV and a filtration system is not recommended. In brief, even considering filters which are regenerable by thermal or vacuum procedures, significant crew-time demanding steps still would count in the integration to a complete system (i.e.draining of the process water loop, removal of the unit, system inactivity for 1 hour for maintenance process (1/year)).

Further, considering the 95% regeneration efficiency, the filter performance would drop to about 80% after the fourth regeneration cycle (i.e. four years), and so it may reasonably be assumed that across a 10-year lifetime at least three filters would be required to account for routine degradation. Additionally, the inclusion of a filter implies additional components such as a pressure sensor, gas trap, gas pump, and sampling points. This would increase system complexity and potential sites for microbial fouling—concern has been raised particularly on the additional risk of biofilm formation within gas traps in process water loops[3]. By introducing a filter to trap inactivated bacteria after the UV unit, the microbes are stored in a location downstream of the point of disinfection. Following UV treatment microbes may undergo a process known as 'dark repair'[4], where the damage introduced by the UV may be recovered over a period of hours or days. Thus, by introducing a post-UV filtration unit the 'dark repair' processes are facilitated, and a potential recontamination risk introduced.

The design of a simple system is the ultimate goal when considering robustness, reliability, and maintainability; thus, the necessary additions brought by the filtration are unwelcome in the design. However, a final fine filtration step is foreseen at the point-of-use, after the final water outlet, on the personal bag to be filled with water. This to ensure that unexpected contamination from the last branch of the water circuit do not affect outlet water quality.

The DM of BIOWYSE will supply UV radiation (at ~275nm UV-C) to delivery water passing through the BIOWYSE system, such that the irradiation produces a disinfection effect within that water. The DM will utilise UV LEDs, arranged about a reaction flow cell such that a sufficient dose is delivered for dissinefection. Aquesence UK design and manufactured the UV LED reactor. The solution has been selected based on prior experience of producing UV treatment reactors capable of litres per minute (LPM) flow rates. Selection of the Aquisense PearlAqua™ model E as the base allows for the inclusion of a dual-lamp system, as a redundancy mechanism (the disinfection can take place with one lamp only), without compromising the structural integrity of the reactor body.

From a safety standpoint, UV-inactivated microbes do not pose a health risk by definition. UV-C disinfection predominantly causes direct DNA damage, inhibiting the ability of an organism to reproduce. The harm that pathogens may cause to humans is generally based on their ability to reproduce in large numbers. Removing this ability from the microbes therefore renders them safe for consumption, and there is no need for removal once inactivated. Best-practice places the UV unit as close to the point of delivery as feasible, such that the time/latency between treatment and usage is minimized. The most UV-sensitive organisms are protozoan pathogens, followed by vegetative bacteria such as E. coli and Salmonella. Viruses tend to be the least sensitive, together with bacterial spores[5].

V. Toward the BIOWYSE flight system

BIOWYSE has been conceived to be installable into EDR2 drawer. Breadboard and Flight system have same/similar requirements for: Functions and performances, Operations, RAMS and Design (e.g.: Dimensions, Power, Cooling). Thus, the breadboard will represent a possible Flight DEMO for form, fit and function. Materials are chosen according to space standards as long as compliant with schedule and cost budgets. Processes and technologies are selected to offer new solutions based on future exploration needs – in particular, for minimizing consumables and increasing autonomous operations.

The development logic goal is validating the integrated System for functional and performance requirements. The verification campaign is planned by TAS Italia (as System Engineer) and reviewed by CNR-IRSA (as Scientific Coordinator) for end of 2018. The integrated breadboard is to be verified in laboratory and tested on the field, to demonstrate efficiency and reliability on-board spacecraft and in remote terrestrial areas.

Space/terrestrial platforms for water/surface monitoring tests will be performed on: EDEN ISS items, Cargo Transportation Bags or Cygnus-PCM (by TAS Italia). Field tests in water kiosk (“Punto Acqua”) and in cave (“Grotta del vento”) will be led by SMAT.

Verification test techniques definition, and different analysis approaches application (culture-based, portable flow cytometry, molecular methods) for characterization of microbial community and identification of pathogens in the water will be performed by CNR-IRSA.

The tests on the breadboard will lead to fine-tuning of the BIOWYSE integrated System and consolidation of the key elements for future flight demonstrations and utilisation, as well as terrestrial applications.

The TRL logic is depicted in Figure 6.

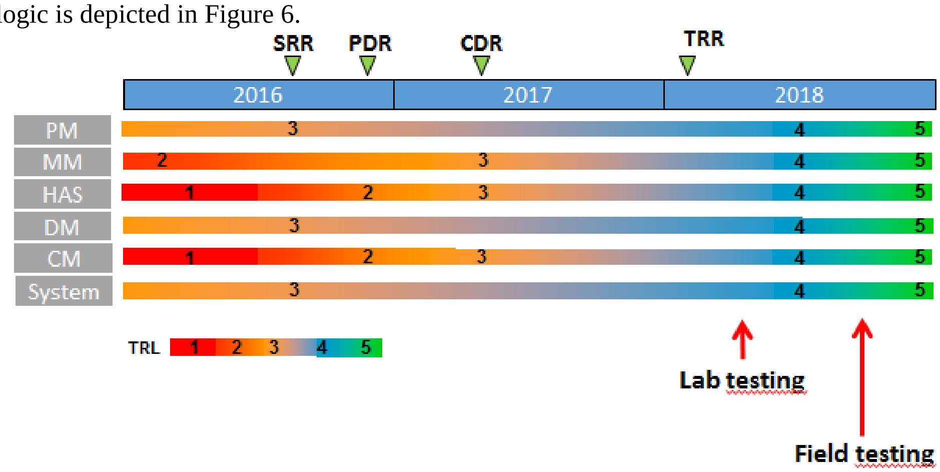

Figure 6: TRL logic

The BIOWYSE model philosophy can be summarised as follows:

- BIOWYSE breadboard:
  - ✓ includes CAM/COTS items – representative in form, fit and function for in-flight utilization with respect to electrical, mechanical and thermal interfaces
  - ✓ drawer rack accommodation can be used also as training model for future flight models
  - ✓ field tests to confirm reliability and performance of automatic procedures and integrity of mechanical parts after transportation and in different environmental conditions
  - ✓ allows updating and improving the System and its parts for commercialization and on-orbit demonstration and future utilisation

- BIOWYSE Flight DEMO:
  - ✓ design started since project beginning, looking at EDR-2 as hosting facility on ISS

- ✓ design will be progressively refined and optimized, based on all relevant aspects encountered during breadboard development and verification
- ✓ on-board tests will be aimed at validating PM, MM, operating procedures and CM reliability

The BIOWYSE test logic can be summarised as follows:

- Laboratory testing
  - ✓ will apply to single Modules and then to the Integrated Breadboard
  - ✓ Modules will be individually validated to ensure required functionality, performances and verify operational procedures
  - ✓ Once integrated, some tests will be repeated to guarantee System well-functioning
  - ✓ Test Sequence at System and SM level include verification of Functional, Operational, RAMS, Design and Interface requirements

- Field testing
  - ✓ EDEN ISS items, Cargo Transportation Bags or Cygnus-PCM
  - ✓ water kiosk (“Punto Acqua”) and in cave (“Grotta del vento”)

- On-orbit testing aimed at validating at least:
  - ✓ Prevention Module and Monitoring Module in microgravity conditions and with the ISS microbial population
  - ✓ operating procedures with strict safety regulations and minimal available crew time
  - ✓ Control Module reliability by on-ground cross validation tests

VI. Conclusions

Many teams work on prevention, monitoring and decontamination fields. As far as we know, no team is dealing with an “integrated” system with the advantages of BIOWYSE. BIOWYSE has full potential for exploitation within several manned space programs. The integrated system developed in BIOWYSE includes the unique opportunity to combine biostatic/biocide action with real-time (thus culture-independent) microorganism monitoring and almost instantaneous UV-based disinfection (which also means no harmful by-products), thus providing an innovative tool with a wide application potential in a large number of situations. The possibility to prevent microbiological growth and to take immediate action through the decontamination module upon check vs thresholds by the monitoring module represents a significant step forward compared to existing real-time and on-line monitoring devices. Maximal optimization of the system compactness and automation and control will be beneficial for ISS and future Exploration crew members.

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