TECHNICAL REPORT

TRUSTER®

TESTING & VALIDATION

November 26th, 2025

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1 INTRODUCTION

This technical report describes the analytical determinations and experimental procedures carried out by ARCHA Srl for the **testing and validation** of the *Truster* machinery. The device is designed so that waste is introduced from above into a dedicated loading compartment. Once the sealed door is closed, the material is shredded and conveyed into a trolley positioned inside the sterilization chamber below. Both the loading compartment and the sterilization chamber are subsequently subjected to a sterilization cycle based on moist heat.

The experimental trials were conducted using simulated test materials, with the objective of verifying the capability of the machine to simultaneously shred and sterilize the input material, thereby producing a final waste stream of homogeneous particle size and characterized by a substantial reduction of the microbial load.

All tests were structured to demonstrate that the system operates with high levels of efficiency and effectiveness, meeting market requirements, and that its use does not introduce risks to worker health and safety (H&S) or to the surrounding environment.

The validation is required to certify that:

- the system is actually a sterilizing apparatus,
- there is a repeatability of the sterilization cycle in time,
- chemical and microbiological pollution don't occur in the surrounding environment,
- the machine is totally reliable and safe during working cycle
- the system does not create any risk to workers for H&S aspects.

The initial objectives are achieved thanks to:

- the sterility of the final product
- the absence of environmental pollution.

Task 1 involves the verification of the sterilization process to ensure complete inactivation of the microbial load. The tests are performed both directly on materials obtained from the sterilization cycle (waste and leachates) and by using biological indicators placed in several positions within the waste inside the machinery. In parallel, environmental monitoring is carried out to evaluate the overall safety of the equipment, with particular reference to the absence of emissions that could pose a risk of biological exposure for workers and end-users.

Task 2 focuses on assessing the environmental compatibility of the system with respect to Health and Safety (H&S) aspects, specifically by monitoring chemical micropollutants and potential odorous impacts, as well as demonstrating the non-polluting nature of the developed technology. These tests are conducted in compliance with the main European standards.

A detailed description of **Tasks 1 and Task 2** is provided in the following sections.

Task 1
MICROBIOLOGICAL TESTS TO ASSESS THE EFFICIENCY OF STERILIZATION

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2 VALIDATION OF STERILIZATION CYCLES

2.1 Test material

For the validation of the sterilization cycles, vials containing the commercial biological indicator *Geobacillus stearothermophilus* (ATCC 7953) were employed, due to their established suitability for verifying steam sterilization processes applied to both solid and liquid matrices.

Each vial contains a microbial population exceeding 10^6 Colony-Forming Units (CFU). For liquid matrices, the microorganisms are suspended in a nutrient solution, whereas for solid matrices they are immobilized on a small inert carrier disk. In both cases, the nutrient medium is enclosed within a sealed glass ampoule located inside the vial. At the end of the sterilization test, the glass ampoule is mechanically broken, thereby allowing contact between any surviving spores and the culture medium. The occurrence of microbial growth is then used as the endpoint criterion to evaluate sterilization efficacy (Figure 1).

Figure 1. Commercial vials containing spores in liquid form (left) or enclosed in a carrier (right). At the bottom of the vial on the right, the paper disc containing the spores is clearly visible.

The vials are incubated at 58°C for 24-48 hours so that any survived microorganisms could proliferate inducing a colour change of the culture medium.

This type of test is “qualitative” but extremely sensitive, since the survival of a single microbial spore leads to its proliferation and, consequently, to a colour change in the culture medium and failure of the validation cycle.

2.2 Validation tests for different loading amounts

An initial series of validation tests on the *Truster* autoclave was performed to evaluate its performance under three different waste load conditions (low, medium, and high).

The input material was designed to simulate realistic operating conditions, including solid components (e.g., latex and nitrile gloves, cloth, non-woven fabric, medical gauze), organic material (e.g., meat, bones, blood), and additional items representative of typical waste streams processed by the system (e.g., syringes, needles, paper, cardboard). Examples of the simulant materials are shown in Figure 2.

Following the shredding phase, commercial vials containing the biological indicator were inserted into the system for both liquid and solid matrices. The vials were placed in three different positions within the shredded material. The sterilization cycle was then performed for 20 minutes at 140°C.

Figure 2. Pictures of input materials used as simulant for the validation of truster system.

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The vials containing the biological indicator (for both wet and solid matrices) were placed inside the collection trolley of shredded waste in 9 different positions, so that during the cycle they were located at the four upper and lower corners as well as at the core of the shredded material.

This arrangement allows for a mapping of the sterilization cycle's effectiveness throughout the entire mass of shredded waste. To facilitate their spatial placement and retrieval, the vials were placed inside small steel sieves (Figure 3).

Figure 3. Sieves containing the vials (above) and their positioning inside the container for collecting shredded waste.

The material was subsequently subjected to a sterilization cycle with a treatment time of 20 minutes at 140°C.

At the end of the cycle, the vials were retrieved and transported to the laboratory for incubation and observation in order to assess the effectiveness of the sterilization cycle. In Figure 4, the shredded and sterilized waste at the end of a full-load cycle can be observed. Part of the material was removed to allow the recovery of the vials located at the bottom of the trolley (lower left of the photo).

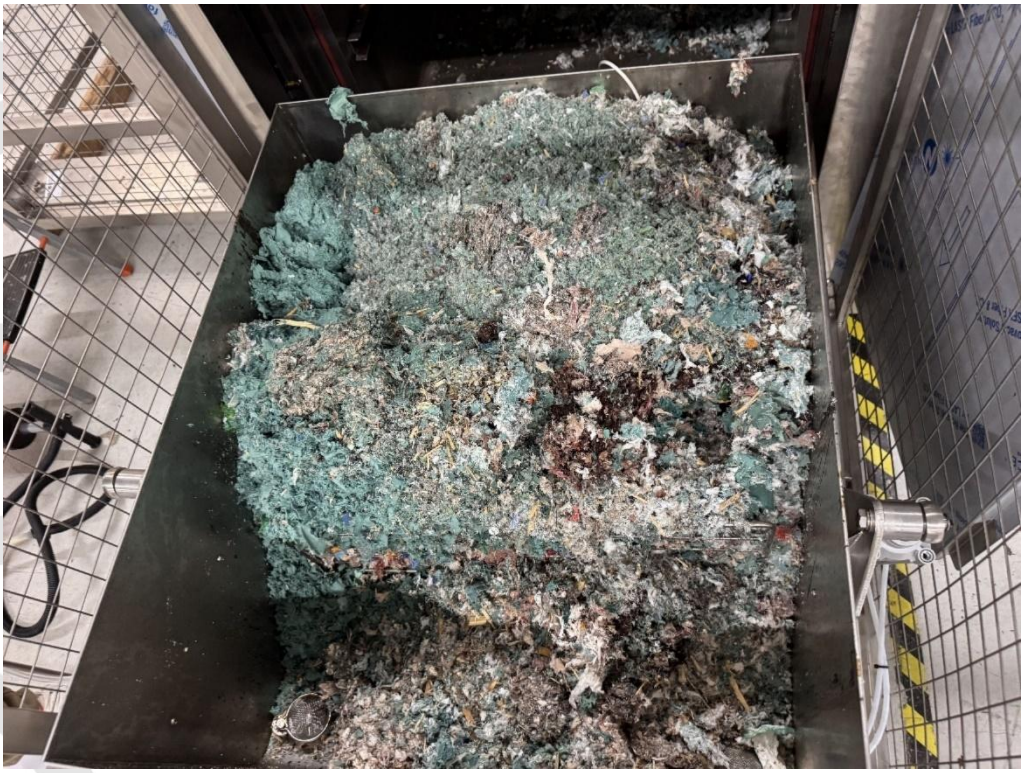

Figure 4. Shredded and sterilized waste at the end of a full load cycle.

The tests demonstrated the absence of microorganisms surviving thermal treatment in all vials placed at various positions within the load, confirming the effectiveness of the sterilization cycle, regardless of the location within the shredded waste. As expected, the three different trials conducted with different waste loads showed equivalent effectiveness in achieving inactivation of spores present in both types of vials.

2.3 Validation tests for liquid waste

An additional validation test of the Truster device was performed to evaluate the sterilization efficacy on liquid waste subjected to thermal treatment at 121°C for 15 minutes. For this purpose, the collection trolley of shredded waste was filled with water, to which 1 liter of a broth culture containing 10^8 CFU/ml of spores from four different lactic bacteria species was added as a simulant (Figure 5).

Figure 5. Addition of the lactic bacteria solution through the shredding chamber.

In this case, the vials containing the biological indicator (intended exclusively for liquid matrices) were placed inside the collection trolley of shredded waste in three different positions, so that during the cycle they were located in an upper corner, a lower corner, and at the core of the liquid mass to be sterilized. At the end of the cycle, the vials were retrieved and transferred to the laboratory, where they were incubated and subsequently examined to evaluate the effectiveness of the sterilization process.

The tests showed the absence of viable microorganisms surviving the thermal treatment in all vials placed at different positions within the load, thus confirming the effectiveness of the sterilization cycle regardless of their location within the liquid waste.

2.4 Determination of the effectiveness according to official ISO 11138-1 method

2.4.1 ISO 11138-1 standard

According to ISO 11138-1, the mean sterilization time (UHSK) related to the inactivation of *Geobacillus stearothermophilus* spores can be calculated by introducing commercial vials containing the test microorganism into the autoclave and performing sterilization cycles of increasing duration at fixed and regular intervals (e.g., 2, 4, 6, 8, n minutes), in order to assess microbial survival within the vials.

The method used, known as the **Limited Holcomb-Spearman-Karber Procedure (LHSKP)**, is described in the standard and requires that the number of test samples remains constant across all exposure times.

The statistical calculation is carried out on at least five exposure times, whose results must include:

- one set in which all samples show growth (i.e., survival after sterilization)
- two sets of samples in which partial survival is observed
- two sets of samples from consecutive exposure times in which all samples show no growth.

Each sample set should consist of at least 20 vials: the carrier has the nominal charge of $2.5x10^8$ spores in each vial.

The average time of sterilization, reported to the type of device employed and to the microbial concentration tested, is calculated as follows:

$$U_{HSK} = U_k - d/2 - d/n * \sum r_i$$

where:

U_{HSK} average time of sterilization

U_k first exposure level with no growth in replicates

d time interval in minutes between two exposure levels (in this case 1 minute) n number of replicates tested for each exposure time (in this case 20)

$\sum r_i$ the sum of the negative samples (not grown) between U_2 and U_{k-1} or between the first level where it is observed negative samples and the last level where it's observes samples growth

N_0 number of spores in each vial ($2.5*10^8$, and its $\log_{10}$ = 8,3979)

After the average sterilization time is obtained, it is possible to calculate the average time required to inactivate 90% of tested spores.

$$\overline{D} = U_{HSK} / \log_{10} N_0 + 0.2507$$

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2.4.2 Experimental tests

An initial series of tests was performed at 140°C, carrying out 7 sterilization cycles lasting between 1 and 7 minutes, with increments of 1 minute in duration for each subsequent cycle. For each cycle, 20 vials containing *Bacillus stearothermophilus* spores were introduced into the shredded waste. At the end of the cycle, the vials were recovered and transported to the laboratory for incubation at 58°C and subsequent observation to assess the growth or absence of microorganisms. Since all the spores had been inactivated after just 2 minutes of treatment, it was not possible to calculate the average sterilization time. The test was therefore repeated at 121°C, applying the following sterilization times: 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 minutes.

Figure 6 shows the 10 bottles into which the vials containing the spores were placed. Since the bottles were subsequently covered with shredded waste, the basket facilitated their recovery at the end of the sterilization cycles.

Table 1 shows the results obtained from performed tests.

Figure 6. Bottles containing spore vials before being covered with shredded waste.

Table 1. Results from sterilization tests after different increasing time.

Sterilization time (minutes)	Tested samples	No growth samples
2.5	20	0
5	20	0
7.5	20	3
10	20	10
12.5	20	20
15	20	20
17.5	20	20

In this case, U_k is 12.5 minutes and the set of samples to analyse are after 5, 7.5, 10, 12.5 and 15 minutes.

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The average sterilization time is:

U_{HSK} = 12.5 – 1.25 – (2.5/20 *13) = **9.625**

And the average D ($\overline{D}$) is:

$\overline{D}$= 9.625 / 8,3979 + 0.2507 = **1.113**

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3 WORKPLACE ENVIRONMENTAL ANALYSIS

The environmental investigation aimed to assess the Truster device's ability to contain biological contamination levels in the surrounding environment during operation. Additionally, the analysis sought to verify that the waste produced, whether solid (shredded waste) or liquid (leachate), was effectively free of microorganisms surviving the sterilization cycle.

3.1 Analyses of aerosol in working places

3.1.1 Experimental protocol

One of the innovative features of the Truster machine lies in the implementation of a fully enclosed cycle, aimed at preventing operator exposure to biological agents potentially hazardous to health.

To assess this feature, four cycles of sampling and microbiological analyses were performed, aimed at evaluating the following parameters:

- **Total aerobic bacterial load at 30°C (TBC):** parameter representing the general bacterial contamination potentially present in the waste. This category includes most existing bacteria, encompassing both generally harmless microorganisms and numerous pathogens.
- **Total anaerobic bacterial load at 30°C (TBC An):** parameter indicative of contamination by anaerobic bacteria potentially present in the waste. This category includes a wide range of anaerobic microorganisms, some of which are harmless while others are significant pathogens.
- **Molds:** microorganisms indicative of fungal contamination. Certain species are involved in allergic pathologies, while others may act as pathogens.
- **Yeasts:** microorganisms indicative of fungal contamination, including important pathogens such as *Candida albicans*.
- **Aerobic spore-forming microorganisms at 30°C (Spo):** parameter representing spore-producing bacteria, which generate spores resistant to adverse environmental conditions, including elevated temperatures. This parameter is particularly relevant as spores can maintain high concentrations during much of the sterilization cycle, even after most vegetative microorganisms have been inactivated. Pathogens in this category include *Bacillus anthracis*.
- **Anaerobic spore-forming microorganisms at 30°C (Spo An):** similar considerations apply to anaerobic spore-forming microorganisms. Important pathogens include *Clostridium tetani*, *Clostridium difficile*, *Clostridium botulinum*, and clostridia responsible for gas gangrene.

Aerosol sampling for microbiological analysis was conducted during all phases of the sterilization cycle, with particular attention to:

- **Background:** assessment of the background contamination present in the environment with the machine turned off, serving as a crucial reference for comparison with other phases. Significant deviations from these values would indicate the release of biological agents into the environment and a potential risk to operators.
- **Grinding phase:** a potentially high-risk phase, as the mechanical action on the waste could lead to the release of significant amounts of biological agents.
- **Sterilization phase:** at the beginning of this phase, the waste in the sterilization chamber is not yet sterile; any leaks of steam or pressurized gases could carry microorganisms into the environment.

- **Drying phase:** as this phase involves aspiration of vapours and their discharge, it could result in the release of biological agents, particularly in the event of insufficient efficiency of the burner located downstream of the sterilization chamber outlet.

Aerosol sampling was performed during three separate sterilization cycles under different waste load conditions (low, medium, and high).

Aerosol sampling was performed using the **SAS Super 100** (Pbi International, Italy), a device that aspirates air through a perforated head and directs it onto an agar-based plate, allowing the deposition of airborne microorganisms. Depending on the phase of the operational cycle, the sampler was positioned in various locations around the machine, for example: above the unit during the grinding phase, in front of the loading door during the sterilization and opening phases, etc. (Figure 7). At the end of each sampling, the agar plate was removed from the sampler, kept under refrigeration, and transported to the laboratory for the determination of microbiological parameters. The results were expressed as **Colony Forming Units** (CFU), indicative of the number of viable microorganisms present, normalized per cubic meter of ambient air (**CFU/m³**).

Figure 7. Device for aerosol sampling (SAS) positioned near the opening door of the sterilization chamber.

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3.1.2 Results

Table 2 reports the results of the microbiological analyses performed during the first sterilization cycle assessed for environmental compatibility, corresponding to a cycle with a low waste load. Analysis of the data presented in Table 2 shows that, for all microbiological parameters investigated, no significant deviations were observed compared to the results obtained from the control sample (blank) collected with the machine turned off. Some microorganisms were absent (e.g., yeasts) or present at very low levels; in any case, all indicators of environmental contamination remained within fully acceptable limits throughout the entire cycle.

Table 2. Results of environmental compatibility of cycle n. 1 (low load)

	Results (CFU/m^3)					
Phase	TBC	TBC An	Molds	Yeast	Spo	Spo An
Blank	430	30	100	<10	20	<10
Shredding	600	60	110	<10	10	10
Sterilization	440	80	120	<10	10	<10
Drying	700	70	120	<10	10	<10

Table 3 shows the results of microbiological tests performed during the second cycle of sterilization (medium waste load cycle). Similarly, during the second sterilization cycle assessed for environmental compatibility, the microbiological values seem to fluctuate randomly, with no significant deviations from the background levels.

Table 3. Results of environmental compatibility of cycle n. 2 (medium load).

	Results (CFU/m^3)					
Phase	TBC	TBC An	Molds	Yeast	Spo	Spo An
Blank	360	<10	90	<10	<10	<10
Shredding	480	<10	90	<10	<10	<10
Sterilization	230	<10	70	<10	10	<10
Drying	440	<10	80	<10	10	<10

Table 4 presents the results of microbiological analyses conducted on samples collected during a full-load sterilization cycle. Even in this case, the values recorded do not show significant fluctuations in microbial concentrations.

Table 4. Results of environmental compatibility of cycle n. 3 (high load).

	Results (CFU/m^3)					
Phase	TBC	TBC An	Molds	Yeast	Spo	Spo An
Blank	300	20	110	<10	<10	<10
Shredding	500	30	60	<10	<10	10
Sterilization	300	10	150	<10	40	<10
Drying	350	60	120	<10	<10	<10

A final test to evaluate the environmental impact at the biological level was carried out in parallel with the validation cycle of liquid waste sterilization at 121°C. In this case, the cycle was simpler since the shredding of the waste and the drying phase at the end of the sterilization process were not included; consequently, environmental sampling was limited to the control blank and the sterilization phase. The results are reported in Table 5 below. Also in this case, no significant differences were observed between the results obtained from the control blank and those from the aerosol sampled during the sterilization phase.

Table 5. Results of environmental compatibility of cycle n. 3 (liquid waste).

	Results (CFU/m^3)					
Phase	TBC	TBC An	Molds	Yeast	Spo	Spo An
Blank	530	<10	90	<10	<10	<10
Sterilization	630	<10	100	<10	<10	<10

In order to facilitate the correct interpretation of the results shown in the previous tables, it should be noted that aerosol samples were collected at different locations during the various experimental phases by repositioning the SAS sampler in areas considered at higher risk of microbiological contamination. Airborne contamination is inherently heterogeneous due to multiple influencing factors (such as air currents and dust acting as a carrier). This explains the minor variations observed among the different sampling points. In contrast, the presence of a true source of contaminated aerosol would have resulted in substantially greater deviations in microbial concentrations.

In conclusion, although the tests were conducted using simulated materials, the evidence indicates that the **shredding/sterilisation cycle performed with the Truster device does not result in measurable adverse effects with respect to biological contamination of the surrounding environment**.

Furthermore, **no correlation was observed between the volume of waste processed and the levels of environmental contamination**, thereby reinforcing the conclusion that the device ensures effective performance throughout all stages of the sterilisation cycle.

3.2 Monitoring of waste and leachate

3.2.1 Experimental protocol

An additional aspect considered for the evaluation of the sterilization cycle's efficacy was the microbiological analysis of both the shredded waste and the percolate generated during the sterilization process. Regarding the percolates, it should be noted that they also receive gases aspirated from the sterilization chambers during the vacuum creation phase, which precedes sterilization. To prevent the release of potential microbial contaminants into the environment, the gases pass through a dedicated burner, exposing them to very high temperatures. The analysis of the percolates was therefore aimed not only at evaluating the effectiveness of the sterilization cycle but also that of the gas treatment: the presence of microorganisms in the samples could indicate either a failure of the sterilization cycle or an ineffective treatment of the contaminated gases.

Samples were collected from three separate test cycles corresponding to low, medium, and high loads of simulant material, in order to assess the potential presence of surviving microorganisms.

The microbiological parameters analysed were the same as those assessed in the aerosol studies, in order to identify any potential correlations between the treated waste and the generated aerosol. Specifically:

- Total aerobic count at 30°C (**TBC**)
- Total anaerobic count at 30°C (**TBC An)**
- Molds
- Yeast
- Spores of aerobic microorganisms at 30°C (**Spo**)
- Spores of anaerobic microorganisms at 30°C (**Spo An**)

To collect the percolate, which is normally discharged through a drainage tube, a dedicated device was employed. This device consisted of a stainless-steel cylinder equipped with a control valve to regulate the inflow of percolate and a vent valve that allowed gas to escape, thereby facilitating the filling of the cylinder. Once the percolate was collected, the tube was sealed at both ends and disconnected. The percolate was then rapidly transferred into sterile containers for subsequent analysis (Figure 8).

Figure 8. The collection system of leachates.

3.2.2 Results

Table 6 and Table 7 summarize the results obtained for the waste and the percolates, respectively.

Table 6. Results of microbiological analysis on final waste.

	Results (CFU/gram)					
	TBC	TBC An	Molds	Yeast	Spo	Spo An
Waste - Cycle 1 (low load)	< 10	< 10	< 10	< 10	< 10	< 10
Waste - Cycle 2 (medium load)	< 10	< 10	< 10	< 10	< 10	< 10
Waste - Cycle 3 (full load)	< 10	< 10	< 10	< 10	< 10	< 10

Table 7. Results of microbiological analysis on leachates.

	Results (CFU/ml)					
	TBC	TBC An	Molds	Yeast	Spo	Spo An
Waste - Cycle 1 (low load)	< 1	< 1	< 1	< 1	< 1	< 1
Waste - Cycle 2 (medium load)	< 1	< 1	< 1	< 1	< 1	< 1
Waste - Cycle 3 (full load)	< 1	< 1	< 1	< 1	< 1	< 1

Table 8 shows the results obtained from the analysis of liquid waste and leachate from the validation cycle at 121°C for 15 minutes. The difference in magnitude between the results for the waste and the percolates is attributable to a methodological aspect: solid materials must be resuspended in a liquid diluent at a 1:10 ratio in order to undergo microbiological analysis. As expected, all materials sampled at the end of the examined cycles tested negative in microbiological analyses.

Table 8. Results of microbiological analysis on liquid waste and leachate.

	Results (CFU/ml)					
	TBC	TBC An	Molds	Yeast	Spo	Spo An
Liquid waste	< 1	< 1	< 1	< 1	< 1	< 1
Leachates	< 1	< 1	< 1	< 1	< 1	< 1

Consistent with the findings obtained using *Geobacillus stearothermophilus* biological indicators, **the waste and percolates produced by the Truster device were free of viable microorganisms or spores**, under both aerobic and anaerobic conditions.

4 CONCLUSIONS ON TASK 1 - MICROBIOLOGICAL TESTS TO ASSESS THE EFFICIENCY OF STERILIZATION

The experimental results indicate that the Truster autoclave is capable of effectively sterilizing the waste it grinds, both under different load conditions (low, medium, high) and homogeneously within a single workload, as demonstrated by the mapping tests.

Tests conducted with commercial biological indicators confirmed the system's efficacy against both solutions containing *Geobacillus stearothermophilus* spores and spores adhered to a solid material carrier.

The determination of the average sterilization time, carried out at 121°C on indicators with a high microbial load (2.5×10^8 CFU), showed an average sterilization time of 9.625 minutes and a mean D-value (time required to achieve a 90% reduction of the tested spores) of 1.113 minutes.

Environmental compatibility tests demonstrate that, from a biological standpoint, the autoclave operates safely during all operational phases, including potentially critical ones such as waste shredding. The grinding of contaminated material could potentially generate significant amounts of bioaerosol containing microorganisms, but tests conducted with simulated materials suggest that this risk is negligible.

Except for minor fluctuations due to differences in sampling point locations and the inherent heterogeneity of airborne dust and aerosol dispersion, environmental contamination levels remain essentially unchanged throughout all stages of the sterilization cycle.

Microbiological analyses performed directly on waste and percolates from cycles carried out under different load conditions confirm the absence of viable microorganisms at the end of the cycle, further validating the effectiveness of the sterilization process.

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TASK 2
CHEMICAL VALIDATION OF ENVIRONMENTAL COMPATIBILITY

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5 EXPERIMENTAL ACTIVITIES FOR VALIDATION OF ENVIRONMENTAL COMPATIBILITY

The activities have the aim to identify the characteristics of the final waste coming out from the machine, for its classification according to the current regulations. In addition, the measurement of the environmental impact resulting from the treatment done from the prototype wants to be performed during the different cycles. The activity consists of the following stages:

- Definition of the analytical protocol for waste characterization;
- Characterization and classification of waste;
- Definition of the sampling plan for environmental monitoring;
- Environmental assay;
- Interpretation of results according to current environmental legislation.

Dealing with the characterization and classification of waste, the treatment cycles are carried out using a simulating material as input waste to be treated, shredded and sanitized. The mixture simulant is constituted by:

- SMS type fabric used for the production of tissue "disposable" in operating rooms
- Pieces of different types of tissue
- Plastics: syringes, bottles, plastic containers
- Glass and laboratory glassware
- Metal made materials (eg. Needles)
- Packaging material (plastic, paper and cardboard)

This material has simulated a real load, comprising the solid material from different sources (latex / nitrile gloves, cloth, woven non-woven, gauze, pieces of meat) so that it can more closely simulate the real situation that could be processed by the system. So, the material coming out from the process during these trials is not characterized nor classified.

Regarding the characterization and classification of the real waste, in this report a complete analytical protocol is presented for the entire information. From a biological point of view, the determination of complete sterility determined at the end of the entire process, allows to assert that the waste presents no infectious characteristics (so it is not HP9 statement, according to Regulation CE 1272/2008).

In the course of experiments conducted, materials of different types have been used, in order to simulate the actual waste that could go to feed the machine: different plastics packaging, packaging paper and cardboard, food material and the hospital materials (syringes, tissues ...).

Figure 9 shows two images of the mixture of input materials used during the cycles. The final waste coming from the treatments (trituration and sterilization) is presented as shown in Figure 10: the material has a very small size and a reduced moisture content as result of the drying process.

Figure 9. The input material to be treated by the prototype.

Figure 10. The final material after the overall treatment in the prototype.

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5.1 Waste classification

The material coming from the machine can be codified in the European Waste Catalogue (EWC code), according to the macro-activity:

18 WASTES FROM HUMAN OR ANIMAL HEALTH CARE AND/OR RELATED RESEARCH (except kitchen and restaurant wastes not arising from immediate health care)

And according to the activity:

18 01 wastes from natal care, diagnosis, treatment or prevention of disease in humans between the next list of EWC code:

180103* wastes whose collection and disposal is subject to special requirements in order to prevent infection

180104 wastes whose collection and disposal is not subject to special requirements in order to prevent infection (for example dressings, plaster casts, linen, disposable clothing, diapers)

180106* chemicals consisting of or containing dangerous substances

180107 chemicals other than those mentioned in 18 01 06

The choice of the EWC code should be carried out as a function of the loaded material:

- ✓ 180104 -> in the case of mainly biological waste, effectively sterilized by the machine
- ✓ 180106* -> in the case of waste mainly of chemical source (gauze, cutting materials, contaminated and / or containing dangerous chemicals packaging) also if effectively sterilized from the biological point of view
- ✓ 180107-> in the case of waste mainly of chemical nature (gauze, cutting materials, clean or not containing hazardous chemicals packaging) and effectively sterilized from a biological point of view.

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5.2 Definition of sampling plan for environmental monitoring

In order to check the environmental impact resulting from the start-up of the prototype, three environmental campaigns for the characterization of the air in working places are performed. In the next paragraphs, the criteria adopted for investigation, the used methods for sampling and analyses, the results and interpretation are described.

The environmental monitoring is based on the sampling technique in fixed position: it is a technique used to characterize the working environment near areas frequented intensely or potential sources of contaminants through these "fixed points" to quantify the concentration of pollutants, even if there is no presence of workers. This measure serves mainly to define the "levels of environmental pollution" and it can be correlated with the exposure of operators in function of the time of permanence of the individual subject area.

To assess the potential release of chemical compounds hazardous to the health of workers in the area where the sterilizer is located during operation, quantification investigations have been conducted for the following parameters using the relevant methods:

- Active sampling with a vial for VOC quantification: ISO 16200-2:2001.
- Active sampling with a vial for ALDEHYDES quantification: EPA 8315A 1996.
- Air sampling with bags for olfactometric analysis and quantification of odorant units: UNI EN 13725:2022.
- Air sampling with bags for qualitative/semi-quantitative SPME-GC-MS analysis using a customised method.

Specifically, it is planned to perform duplicate sampling for VOCs and aldehydes during the high-load (worst-case) test, and single sampling for the low- and medium-load tests.

Sampling for olfactometric and SPME-GC-MS analyses involves three bags taken during the loading, sterilization, and unloading phases, for each loading cycle performed (low, medium, and high).

All results are then compared with the limit of exposure fixed by the Italian Regulation (D.Lgs.81/08) or with the threshold limits defined from ACGIH (American Conference of Governmental Industrial Hygienists, 2024).

The experimental campaign for the characterization of working places from Health and Safety assessment are performed on September 09th – 10th, 2025.

5.3 Volatile organic compound (VOC) analyses and results

The environmental measurements are conducted to evaluate the health of the local and the exposure of workers, using methods of sampling and analysis required by the relevant technical sector.

The active carbon used in the sampling with radial symmetry samplers are desorbed with carbon disulphide and then subjected to gas chromatographic analysis. The analysis is conducted with a Gas-chromatograph equipped with FID and mass detectors, according to the method ISO 12600-2:2000. This method provides two different operational phases: the extraction and determination mediated GC.

The analytical procedure: opening the vial absorbent and transfer the main and safety adsorbent layers in two different vials; then the vials are closed and weighed on an analytical balance. Cooling the vials containing activated carbon in the freezer for a time of 10 minutes. Extraction of the vials from the freezer and removing the volatile organic substances by means of pipettes adding a quantity of carbon disulphide equal to 2 ml for the main layer and 1 ml for the layer of security. Place the vials in the ultrasonic bath for 10 minutes, taking care to keep the temperature below 10°C. Extraction of the vials from the ultrasonic bath, weighed to determine the volume of carbon disulfide recovered and separate the coals by decantation (or by centrifugation, in the presence of the particulate in suspension). Transfer

the extracts centrifuged or decanted into vials for GC autosampler and proceed to the analysis, according to the following experimental setup for the used equipment:

TRACE 2000 GC (Gas-chromatograph):

- Column: ZB 1701 to 30 m; 0.25 mm ID; 1 micron film
- Injector: Split; Split flow = 60 ml / min; T = 250 ° C
- Carrier: He; Steady stream to 1.3 ml / min
- Detector: FID: T = 300 ° C; H2 flow = 35 ml / min; Air flow = 350 ml / min; Make-up flow (N2) = 30 ml / min
- Oven: T1 = 35 °C; t1 = 6 min; r1 = 10 °C / min; T2 = 130 °C; t2 = 0 min; r2 = 30 °C / min; T3 = 260 °C; t3 = 2 min

Auto-sampler AS 2000:

- Injection volume = 1.0 µl
- Injection speed = 20 l / sec
- Air volume = 2.0 µl
- Injection delay = 0 sec
- Syringe extraction delay = 2 sec

Thanks to the instrument software, it's possible to integrate the area of the chromatographic peak relative to each compound and determine the amount of compound present in the vial through the calibration line, after correcting (by weighing the vials before and after the solvent addition) for the actual volume of carbon disulphide (related to the volume of 2 ml which represents the theoretical volume of extraction). If the amount of the compounds present in the layer of security does not exceed 5% of that present in the main layer, the sample is valid. The results are expressed as concentration of the single compound in mg / m^3, taking into account the sucked air volume for each sample.

The analyses for the determination of **Volatile Organic Compounds** have provided the results in Table 9, which lead to finalize that:

- The presence of traces of compounds such as METHYL ETHYL KETONE, TOLUENE, iso- and n-BUTYLACETATE and (m+p)-XYLENES. All these Volatile Organic Compound are less concentrated than 1% of their specific TLV.
- All other Volatile Organic Compounds are below the quantification Limit of the technique.

Table 9. VOCs concentrations.

		22525493/1	22525496/1	22525494/1	22525495/1		
Method: ISO 16200-1:2001	**UM**	**Entire process LOW - 25KG**	**Entire process MEDIUM - 50KG**	**Next to the loading door HIGH - 100KG**	**Close to the electrical device HIGH - 100KG**	**LQ**	**TLV limit (D.Lgs. 81 / ACGIH)**
PENTANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,02	2000
ETHYL ALCOHOL	mg/m³	< LQ	< LQ	< LQ	< LQ	0,9	1884
ACETONE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,07	1210
METHYL ACETATE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,11	606
ISOPROPYL ALCOHOL	mg/m³	< LQ	< LQ	< LQ	< LQ	0,11	492
HEXANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,02	72
DICHLOROMETHANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,22	175
CYCLOHEXANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,02	350
ETHYL ACETATE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,1	734
METHYL ETHYL KETONE	mg/m³	0,142	< LQ	< LQ	0,111	0,08	600
CHLOROFORM	mg/m³	< LQ	< LQ	< LQ	< LQ	0,72	10
HEPTANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,06	2085
BENZENE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,02	0,66
TRICHLOROETHYLENE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,09	54,7
1,2-DICHLOROPROPANE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,05	46
TOLUENE	mg/m³	< LQ	< LQ	0,031	0,03	0,02	192
METHYL ISOBUTYL KETONE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,03	82
iso-BUTYLACETATE	mg/m³	< LQ	< LQ	0,017	0,011	0,01	241
PERCHLOROETHYLENE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,07	138
n-BUTYLACETATE	mg/m³	< LQ	< LQ	0,062	0,064	0,02	241
ETHYLBENZENE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,02	442
(m+p)-XYLENES	mg/m³	< LQ	< LQ	0,033	0,04	0,01	221
o-XYLENE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,01	221
TOTAL VOCs	mg/m³	< LQ	< LQ	0,148	0,256	0,5	-
OTHER VOCs	mg/m³	< LQ	< LQ	< LQ	< LQ	0,5	-

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5.4 Aldehydes analyses and results

The analyses of the **Aldehydes** were carried on according to EPA 8315A 1996 standard method. This method primarily focuses on measuring formaldehyde, acetaldehyde, and other volatile aldehydes that may pose health risks to workers. The procedure involves collecting air samples through a sorbent tube or cartridge, typically containing a derivatizing agent such as 2,4-dinitrophenylhydrazine (DNPH). The derivatization process converts aldehydes into stable hydrazone derivatives, which are more amenable to analysis.

Sampling begins with drawing a known volume of air through the sorbent tube using a calibrated pump at a controlled flow rate. The air sample interacts with the derivatizing agent on the sorbent, capturing and converting aldehydes into hydrazones during collection. After sampling, the cartridge is sealed and transported to the laboratory for analysis. In the laboratory, the hydrazone derivatives are extracted using an appropriate solvent, such as acetonitrile or methanol.

The extracted solution is then analyzed using high-performance liquid chromatography (HPLC) equipped with a UV detector. The HPLC separates the hydrazone derivatives, and their concentrations are determined by comparing the sample peaks to those of calibration standards prepared with known aldehyde concentrations. The method includes quality control measures such as calibration verification, method blanks, and replicate analyses to ensure accuracy and precision.

EPA 8315A provides detailed instructions on sample collection, handling, storage, and analysis, ensuring consistent and reliable quantification of aldehyde levels in occupational settings. The method's sensitivity allows detection of aldehyde concentrations at low parts-per-billion levels, facilitating effective exposure assessment and ensuring compliance with occupational health standards.

The results for the determination of **Aldehydes** have provided the results in Table 10Table 9, which lead to finalize that:

- The presence of FORMALDEHYDE and ACETALDEHYDE is detected during the high loading test. In only one condition, the quantified concentration of the formaldehyde is slightly above 1% of the specific TLV, correspondent to the sampling close to the loading door.
- All other sampling activities with low and medium loading setting are below the quantification Limit of the technique for all aldehydes.

Table 10. Aldehydes Concentrations.

		22525493/1	22525496/1	22525494/1	22525495/1		
Method: EPA 8315A 1996	**UM**	**Entire process LOW - 25KG**	**Entire process MEDIUM - 50KG**	**Next to the loading door HIGH - 100KG**	**Close to the electrical device HIGH - 100KG**	**LQ**	**TLV limit (D.Lgs. 81 / ACGIH)**
FORMALDEHYDE	mg/m³	< LQ	< LQ	0,00498	0,00395	0,002	0,37
ACETALDEHYDE	mg/m³	< LQ	< LQ	0,00255	0,00214	0,002	46
PROPIONALDEHYDE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,002	48,3
ACROLEIN	mg/m³	< LQ	< LQ	< LQ	< LQ	0,002	0,23
BUTYRALDEHYDE	mg/m³	< LQ	< LQ	< LQ	< LQ	0,002	64

5.5 Odour analyses and results

The UNI EN 13725:2022 standard specifies a standardized method for the determination of odour concentration in ambient air using dynamic olfactometry. This method is essential for ensuring consistent and reliable measurement of odours, which are often subjective and difficult to quantify. The primary goal of the standard is to establish a procedure that provides reproducible and comparable results across different laboratories and contexts.

The test method involves collecting air samples containing odours and analysing them using a panel of trained assessors, known as panellists. The process begins with the sampling of ambient air through a specialized sampling device that minimizes contamination and preserves the integrity of the Odour sample. The collected sample is then diluted with Odor-free air in a dynamic olfactometer, a device designed to generate controlled Odor concentrations by mixing the sample with odourless air.

The olfactometer presents a series of diluted samples to the panelists in a randomized order. Each panelist evaluates the samples using a forced-choice method, typically a triangle test, where they are asked to identify the sample with a higher odour concentration or to detect the presence of an odour. The panelists' responses are used to determine the odour detection threshold, which is then used to calculate the odour concentration in the original sample, expressed in European Odour Units (ouE/m³).

Olfactometric analysis information:

- Olfactometer: Four-port inhalation device, model OLFASENSE TO8, internal serial number OLF03.
- Panel presentations: Presentation mode: yes/no (UNI EN 13725:2022 § 9.4.1.2).
- Number of panel members: 4.
- Number of measurement cycles: 3.
- Set-point temperature: 23.0°C
- Reference odorants: primary reference odorant: 1-Butanol (CAS No. 71-36-3) in nitrogen at various certified concentrations, in cylinders
- Overall sensory accuracy: Overall sensory quality variables as of 08/09/2025: Aod = 0.0534; r = 0.1571
- Panel threshold for reference odorants
- For the primary reference odorant: 0.0397 µmol/mol

The obtained results for the determination of **Odor** have been provided in Table 11, which lead to finalize that:

- During loading step, odours are generally more noticeable. As waste gets shredded, there's often an initial release of odours because of increased surface area exposing volatile compounds. During the unloading step, the odour may either increase or decrease, depending on how thoroughly waste has been processed.
- As waste amount increases (from 25kg to 100kg), odour concentration raises because there's simply more material releasing odorous compounds into the air.
- However, considering the treatment system as effective, odour levels stay relatively stable despite increasing waste loads.

Table 11. Odour Concentration.

Sample number	***22525456/1***	***22525457/1***	***22525458/1***	***22525462/1***	***22525463/1***	***22525464/1***	***22525459/1***	***22525460/1***	***22525461/1***
Sample name	LOADING	SHREDDING	UNLOADING	LOADING	SHREDDING	UNLOADING	LOADING	SHREDDING	UNLOADING
Loaded amount of waste	LOW - 25KG			MEDIUM - 50KG			HIGH - 100KG		
Odor concentration (ouE/m³)	**30**	**23**	**24**	**34**	**28**	**31**	**46**	**34**	**34**

5.6 Qualitative/semi-quantitative SPME-GC-MS analysis

The qualitative/semi-quantitative SPME-GC-MS analysis involves sampling volatile or semi-volatile compounds from a sample of air collected during the processing steps, by using Solid-Phase Microextraction (SPME). The SPME fiber, coated with an adsorbent, is exposed to the air sample to absorb target analytes. After extraction, the fiber is inserted into the GC-MS injection port, where thermal desorption releases the analytes into the gas chromatograph for separation. The mass spectrometer then detects and identifies compounds based on their mass spectra. Qualitative analysis involves identifying compounds by comparing spectra to libraries, while semi-quantitative analysis estimates relative concentrations using peak areas or heights, often normalized to internal standards or total ion current.

Considering the same samples as collected for the odour analyses (paragraph 5.5), no chemical compounds with odorous characteristics were detected in the collected samples (Table 12).

Table 12. Qualitative / semi-quantitative analyses.

Sample number	***22525456/1***	***22525457/1***	***22525458/1***	***22525462/1***	***22525463/1***	***22525464/1***	***22525459/1***	***22525460/1***	***22525461/1***
Sample name	LOADING	SHREDDING	UNLOADING	LOADING	SHREDDING	UNLOADING	LOADING	SHREDDING	UNLOADING
Loaded amount of waste	LOW - 25KG			MEDIUM - 50KG			HIGH - 100KG		
GC-MS analytes	**no analytes were detected**								

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5.7 Workplace noise measurements

To assess the noise due to the sterilizer during operation, noise measurements have been conducted using a sound level meter compliant with ISO 10012, ANSI S1.4-1983, IEC 651-1979 type 1, IEC 804-1985 type 1, IEC 1260-1995 Class 1, and ANSI S1.11-1986 type 1D standards.

Dealing with health and safety aspects for workers in terms of the **NOISE as physical risk**, the Italian regulation D.Lgs. 81/08 (transposition of the EU Directive) reports the following thresholds and measurements for noise risk (Table 13).

Table 13. Criteria for noise risk evaluation (thresholds and measurements defined by Italian regulation D.Lgs. 81/08).

< 80DB	80-85 DB	> 88 DB
No specific obligations	Training for workers on risks of exposure to noise  Making PPE available to workers 	Health surveillance of all exposed workers  Delimitation of places with levels above 85 dB (A) 	 Technical measures to reduce the exposure of workers  Mandatory use of PPE

For the **noise,** considering the obtained results as shown Table 14, the main processes provide noise values lower than 80 Db, and no action is needed for these processes in terms of noise risk.

Table 14. Noise measurements during process phases.

	Loading	Noise (dB(A))/1
Loading	25 kg	61,8
Shredding		71,2
Conditioning		72,2
Sterilization		60,3
Drying		68,0
Unloading		66,3
Shredding	100 kg	70,5
Conditioning and Sterilization		70,5
Unloading		73,8

6 CONCLUSIONS ON TASK 2 - CHEMICAL VALIDATION OF ENVIRONMENTAL COMPATIBILITY

The choice of the EWC code of the final waste depends on the loaded material and could be represented by:

- EWC 180104: for waste mainly biological source, effectively sterilized by the machine
- EWC 180106*: in the case of waste mainly of chemical nature (gauze, cutting materials, contaminated and / or containing dangerous chemicals packaging) also if effectively sterilized from the biological point of view
- EWC 180107 in the case of waste mainly of chemical nature (gauze, cutting materials, clean or not containing hazardous chemicals packaging) and effectively sterilized from a biological point of view

The determination of VOCs has been developed in order to determine the "exposure level" of workers during the operation process of the prototype: the results of the VOCs concentrations shows the total compliance with the limit given by the Legislative Decree no. 81/08 (for Italy) and with the TLV from ACGIH (2014) for all the investigated parameters during all tested condition of the machine. The presence of traces of compounds such as Methyl-Ethyl-Ketone, Toluene, Iso- and N-Buthylacetate and (m-,p-)Xylenes has been detected with concentration lower than 1% of their specific TLV. All other Volatile Organic Compounds are below the quantification Limit of the technique.

The results obtained for the determination of the aldehydes provide very low concentrations for the main selected molecules. The presence of Formaldehyde and Acetaldehyde is detected during the high loading test. In only one condition, the quantified concentration of the formaldehyde is slightly above 1% of the specific TLV, correspondent to the sampling close to the loading door. All other sampling activities with low and medium loading setting are below the quantification Limit of the technique for all aldehydes.

Regarding the odorimetric analyses carried out with the use of the Olfactometry, any critical situations occur during the overall treatment cycles tested under different loading conditions.

For the noise as physical risk, the main processes provide noise values lower than 80 Db, and no action is needed for these processes in terms of noise risk.

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