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Impact of needle-point bipolar ionization system in the reduction of bioaerosols in collective transport

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HIGHLIGHTS

- Collective transport is considered one of the environments with the highest rate of COVID-19 propagation.
- Air purification can reduce airborne transmitted diseases.
- Standard filters installed in trams present reduced efficiency for submicron particles.
- A bipolar ionization unit in a tram has been evaluated for the first time.
- Bipolar ionization can reduce environmental bioaerosols but not CFUs on surfaces.

ABSTRACT

The transmission rate of SARS-CoV-2 is higher in collective transport than in other public environments. Transport companies require preventive strategies to mitigate airborne risk of contagion which not imply responsible use at the individual level. Air purification systems, such as UV-C or needle-tip bipolar ionization, are attractive alternatives. However, only a few studies addressing the validation of this technology against bioaerosols in actual operation conditions have been published so far. In this work, the efficiency of a bipolar ionization unit in the Zaragoza Tram has been evaluated. Against environmental bioaerosols, ionization (~25.7 · 10^9 ions/m^3, on average) reduced the concentration of colony-forming units (CFU) by ~46 % and ~69 % after 30 and 90 min. No clear benefits were obtained against microorganisms on inner tram surfaces (seats, grab bars, walls, and windows). Pre-pandemic filtration equipment located in the HVAC based on a Coarse 45 %-type filter removed ~73 and ~ 92 % of aerial CFU by itself after 30 and 90 min. Microscopic visualization of the CFUs revealed that they were mostly >1 μm, much larger than the SARS-CoV-2 virion (~100 nm) and SARS-CoV-2-loaded bioaerosols (from 0.25 μm). Then, we studied the filter behavior under normalized laboratory methods. The filters efficiency against submicron particles was limited (between 5 and 12 % against 0.1 to 0.3 μm NaCl particles). Another ionization strategy was to generate aerosol agglomerates to enhance filtration performance, but the combined action of ionization and filtration did not improve substantially. The effect of these technologies was also characterized using the clean air delivery rate (CADR). Relative to untreated air (CADR = 0.299 m^3/min), ionization and filtration reduce ambient CFUs (CADR = 5.153 and 9.261 m^3/min, respectively; and CADR = 13.208 m^3/min, combined) which implies that it has a substantial impact on indoor bioaerosols.
1. Introduction

The airborne transmission model refers to the spread of a disease through aerosols up to 100 µm, which can be transmitted through the air over time and distance (Siegel et al., 2019). At the beginning of the COVID-19 pandemic, this route of infection was not considered relevant, and more attention was paid to the transmission through droplets and infected surfaces (Wang et al., 2021). The global acceptance of the spread of SARS-CoV-2 by aerosols allowed an improvement in the preventive approach, including new techniques for epidemiological management, such as the measurement of exhaled carbon dioxide (CO₂) as an indicator of the risk of contagion (Wang et al., 2021; Peng and Jimenez, 2021) and air purification technologies.

The term 'bioaerosol' refers to those particles of biological origin or active particles that can affect living organisms, causing them some allergy, toxicity, or infection (Rose, 1994; Sánchez-Monedero et al., 2006). Bioaerosols can include viruses, bacteria, spores, pollen, and, in general, any other microorganisms with an aerodynamic diameter between 0.5 and 100 µm (Cox and Wathes, 1995). In the context of airborne pathogen transmission, following mechanistic hypotheses about disease transmission, the spread of bioaerosols is associated with respiratory events (Hsiao et al., 2020), mainly coughs and sneezes (Asadi et al., 2019). Regarding the COVID-19 pandemic, bioaerosols released by a person infected with SARS-CoV-2 may contain some virus or traces of it, and the viral load contained in the particles is a crucial factor in determining the relative contribution of airborne transmission (Wang et al., 2021; Sattar and Ijaz, 1987). The modal distributions of aerosols acquire great relevance in infection transmission. Particle size determines the aerodynamic characteristics and deposition dynamics and the variability in the viral colonization model depending on the depth of the reached respiratory tract (Wang et al., 2021; Shinya et al., 2006; Guzman, 2021).

A higher transmission rate of SARS-CoV-2 has been observed in means of transport compared to other public and shared spaces. Thus, Lan et al., 2020 analyzed the relationship between the transmission of COVID-19 and the type of work, pointing to an incidence of 18 % of cases in the transport sector, only behind the health sector (22 %). Zhao et al., 2020 determined an association between the number of infected patients and the domestic transportation route of Wuhan by train, private car, and flights, finding a significant relationship between the infection rate and transportation by train, but not in flights and private vehicles. This trend was also studied using other infectious disease models (Herna et al., 2010, Troko et al., 2011, Goscé and Johansson, 2018).

The risk of contagion in public transport is determined by the seat's proximity to the infected person, the number of passengers, the number of interactions with other passengers, the duration of the trip, and the capacity performance of air renewal (Nasir et al., 2016). Generic measures have been adopted and have proven effective to reduce the virus spreading: surfaces sanitation, social responsibility measures (such as the use of a mask, social distancing, and hand hygiene), and ventilation measures (Moreno et al., 2021; Di Carlo et al., 2020). Regarding basic interior sanitation, solutions based on chlorine and isopropyl alcohol effectively disinfect (hardly characterizable using the previous tests). Finally, the combined use of two daily cleanings and suggest having particular account high-touch areas (American Public Transportation Association, 2020). The survival of SARS-CoV-2 varies depending on the material on which it is deposited. SARS-CoV-2 remains active for up to 72 h on plastic and stainless steel, up to 24 h on cardboard, and <4 h on copper (Van Doremalen et al., 2020), therefore it would be necessary to consider heterogeneity within the different areas of the same public transport unit.

HEPA filtration is very effective eliminating of microorganisms present in the ambient air in the means of transport since it allows efficient elimination of microorganisms reaching efficiencies close to 99.997 % (Askar et al., 2012; Leder and Newman, 2005). However, the performance of this technology will depend on the number of air changes and the feasibility of incorporating these filters to the installed air conditioning system. The percentage of recirculated air is another aspect to consider in the air quality in transport (Zitter et al., 2002). Additionally, natural air renovation, such as opening windows, can considerably reduce the concentration of CO₂ inside a vehicle as well as the number of airborne containing microorganisms (Matose et al., 2019). Concerning the COVID-19 pandemic, the preventive strategy to reduce the risk of contagion by aerosols has been managed along two different lines: on the one hand, based on the measurement of CO₂ as an indicator of air renovation. On the other hand, by purifying or cleaning the air by different means including ultraviolet C (UV-C) radiation technology, dry fumigation with hydrogen peroxide (H₂O₂) or technology based on non-thermal plasma, among others.

Technology based on the UV spectrum between 200 and 280 nm (UV-C) has been widely used in disinfection processes. The intracellular components of microorganisms (DNA, RNA, and proteins) present a high and variable sensitivity to absorb UV-C photons, producing fatal damage to their genome and inhibiting their correct replication (Bolton and Cotton, 2008). The efficiency of UV disinfection varies depending on the distance from the surface and the application time (Li et al., 2017). This technology has proven helpful against the SARS-CoV-2 virus (Li et al., 2021; Blasini et al., 2021; Ruetalo et al., 2021), reducing the viral load between 0 and 6 log orders of magnitude in culture media (Raeiszadeh and Adeli, 2020). Although the performance of the technology is limited against bioaerosols loaded with SARS-CoV-2, where the required UV₂₅⁴ dose is high (Raeiszadeh and Adeli, 2020), some studies highlight its effectiveness for air purification (Bowen et al., 2021) and especially if combined with HEPA filtration (Barnewall and Bischoff, 2021).

Non-thermal plasma-based technology has positioned itself as one of the leading air purification strategies in the context of the pandemic caused by the SARS-CoV-2 virus. Plasma inactivation of microorganisms has been attributed to cell wall rupture and damage of the genetic material (Liang et al., 2012). Its mechanism of action is multiple. The presence of charged particles, ions, reactive oxygen species (ROS) and oxygen-containing radicals, UV-C, vacuum ultraviolet (VUV), and localized heating events stand out, acting exclusively or in combination (Gallagher et al., 2007; Laroussi, 2007; Sakudo and Shintani, 2010; Nehra et al., 2008). The antimicrobial effect of this technology has been widely exploited in the health sector to sterilize surgical instruments (Klämp et al., 2012). Moreover, it has been tested in controlled environments reporting excellent efficiency in the inactivation of specific bacterial species (Liang et al., 2012) and enveloped/non-enveloped viruses (Sakudo et al., 2013; Sakudo et al., 2017; Sakudo et al., 2016). However, a limited number of studies support its efficiency in other settings.

Non-thermal plasma-based technology has been tested for other applications regarding the current pandemic, obtaining variable and lower results than those described by the manufacturers (Licht et al., 2021). One of the major concerns and limitations of this technology is the generation of by-products in harmful concentrations (Steiman and Fisk, 2009), where some authors suggest increases in actual conditions (Zeng et al., 2021). However, this aspect is not discussed in this article. This work provides information on possible systems to reduce the risk of infectious diseases in public transport. First, the efficacy of needle-point bipolar ionization against eliminating environmental bioaerosols in the Zaragoza Tram (Zaragoza, Spain) is evaluated. Given biosafety and ethical constraints, we decided to perform the assays using environmental bacteria (not artificially dusted). Afterward, the efficiency of the isolated filtration media is tested against non-biological particles. These tests carried out in the laboratory allow the efficiency of the filter to be measured against submicron matter (hardly characterizable using the previous tests). Finally, the combined action of both strategies is studied. In parallel, the antimicrobial performance of ionization for surface disinfection is evaluated. This work evaluates the air purification systems for their implementation in local public transport.
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2. Materials and methods

2.1. Needle-tip bipolar ionization system

As shown in Fig. 1, two PA604 ionization units of the 600 Series (Tayra SA, Spain) were installed in the suction vertices of the delivery fan of the two air conditioning units arranged in the Zaragoza Tram Unit (Model Urbos 3 CAF, ES). The two HVAC (Heating, Ventilation, and Air Conditioning) units installed in the Tram drive a total flow of 2800—3300 m³/h, with a fresh air ratio of 1:3 fresh/return air. The selected ionizers were of the needle tip brush type (PA604, Tayra SA Spain). They produce an equal amount of positive and negative ions. They have a maximum treated flow of 4100 m³/h and a ±5 kV DC voltage between brushes. In the case of the tests where ionization was evaluated, an Air Ion Counter COM-3200PRO II (Com System INC, Tokyo JA) was used to ensure the correct generation of ions.

2.2. Conditions and preparation of the tram units

The Urbos 3 tram model (CAF, Spain) has a total length of 33 m, a width of 2.65 m, and a height of 3.2 m. It has a capacity of 200 seats, of which 146 are standing seats (3.5 people per m²) and 54 are seats. It operates in Zaragoza, the fifth biggest city in Spain, with a population close to 700 k inhabitants, located midway between Madrid and Barcelona. The tram units included in the study were in operation for a full day, and the usual protocol was not carried out night cleaning. During the morning of the following day, the tram unit provided partial service lasting approximately 4 h. During this time, the hydroalcoholic gel dispensers were removed to avoid disinfection of the tram surfaces included in the study, and the windows were closed during the journeys. Travelers wore masks. Upon arrival at the depot, all the tram doors were opened for 15 min to renew the interior air and replace it with fresh ambient air. Doors opening were performed to maximize the homogeneity and reproducibility of the assays. The conditioning and visualization of the bacterial species found have been carried out using MALDI-TOF mass spectrometry technology (MALDI Biotyper, Bruker Massachusetts USA), comparing the results to databases (Bruker, Massachusetts USA). The morphology of the bacterial strains fibers was observed using a Scanning Electron Microscopy (SEM) JEOL 6360-LV (Deben UK Ltd., Edmunds, United Kindom). For sample preparation, a sample of the corresponding CFU plate was placed on a slide, fixed with carbon tape to a SEM microscope holder and sputtered with Au/Pd to promote electron conduction. The average of CFUs diameters and standard deviations (SD) were obtained from manual measurements with the free software Image-J (v1.52; Schindelin et al., 2012) for n = 50.

Table 1

| Sampling conditions | Dampers | Filter | HVAC conditions | AFNPBI |
|---------------------|---------|--------|-----------------|--------|
| Without ionization and filtration (stability test) | Closed | No | 22 °C | No |
| Ionization | Closed | No | 22 °C | Yes |
| Filtration | Closed | Yes | 22 °C | No |
| Ionization and filtration | Closed | Yes | 22 °C | Yes |

Tram to homogenize the sampling and collect microorganisms at points characterized by different ionic concentrations. The manufacturers initially modeled the ion concentration throughout the Tram Unit, guaranteeing a sufficient concentration for the inactivation of microorganisms in any of their locations. In each test, three samplings were carried out with a frequency of 30 min to evaluate the system's efficiency for 90 min, corresponding to the travel time in each tram line. In addition, an initial sampling was carried out to calculate the relative efficiency. Once the sampling was finished, the volume corresponding to 450 l of air sample aspiration was seeded on a Plate Count Agar (PCA) plate (Scharlab, Spain), for 72 h at 30 °C. The final sampled solution was variable depending on the climatological conditions of the sampling. Thus, the counts were normalized to be comparable, taking the environmental sampling prior to the intervention as a reference. Five replicates of each sample were made. Colony-forming units (CFU) were manually counted after the incubation period (72 h at 30 °C). The CFU counted in each replica were averaged and normalized. Efficiency in CFU inactivation was calculated by comparing the percentage of surviving CFUs with the reference sample.

2.4. Identification and visualization of the bacterial species found

Among the CFUs, the 15 most representative specimens collected during the samplings were analyzed. The identification of the microorganisms has been carried out using MALDI-TOF mass spectrometry technology (MALDI Biotyper, Bruker Massachusetts USA), comparing the results to databases (Bruker, Massachusetts USA). The morphology of the bacterial strains fibers was observed using a Scanning Electron Microscopy (SEM) JEOL 6360-LV (Deben UK Ltd., Edmunds, United Kindom). For sample preparation, a sample of the corresponding CFU plate was placed on a slide, fixed with carbon tape to a SEM microscope holder and sputtered with Au/Pd to promote electron conduction. The average of CFUs diameters and standard deviations (SD) were obtained from manual measurements with the free software Image-J (v1.52; Schindelin et al., 2012) for n = 50.

2.5. Sampling conditions of surfaces and cultivation

Rodac-PCA plates (Plate Count Agar-Scharlab, Spain) were used for surface sampling. At least 30 surface samples per test were taken at different points of the Tram, at different levels, and considering different types of

![Schematic representation of the bipolar ionization units and the HVAC systems ubicacion in the studied in the Urbos 3 tram model studied. Where the implementation of the BIU systems in the two HVAC is observed.](Image)
surfaces. Seats, backrests, grab bars, intermediate supports, walls, and windows were included. Despite being carried out initially, the soil samples were excluded due to their high variability associated with residual contamination and dirt. The final surface sample was compared with a nearby (5 cm proximity) reference sample, and the ionization efficiency was calculated following the same method as for air sampling. Samples collected by contact in the Rodac-PCA plates were cultivated for 72 h at 30 °C.

2.6. Determination of filtration efficiency against submicron particles

As shown in Fig. 2-a, aerosols were produced using a Topas-ATM226 generator with a saline solution of sodium chloride (3 % NaCl in ddH2O). The obtained microdroplets pass through a tubular silica gel air dryer to evaporate the water and produce solid particles. The particle size distribution (Fig. 2-b) inside the cabin was measured using an SMPS TSI 3936 composed of an electrostatic classifier (DMA TSI 3081) and a condensation particle counter (CPC TSI 3782). The particles were dragged at a 0.6 l/min flow rate. The filter was placed between bronze discs sealed with Teflon tape, with 30 × 20 mm Teflon washers on each side. The exposed filter area was variable (2.05, 4.1, and 8.1 mm) to adjust the desired flow rate. The measurements lasted 2 min and were made in duplicate. Due to the concentration of particles variation, measurements were made passing through a free tube between measurements to calculate relative efficiency according to Eq. (1). Where \(C_{up}\) stands for concentration upstream and \(C_{down}\) stands for concentration downstream. The retention efficiency is expressed in global efficiency as ‘number of particles’.

\[
n = 100 \times \frac{C_{up} - C_{down}}{C_{up}}
\]

Pressure drop testing was carried out using alcohol columns based on Bernoulli’s principle. The free ends of the tubes have been inserted into the two quick couplings located on both sides of the filter sample holder. Measurements were made with a volumetric flow rate of 0.6 l/min.

2.7. Determination of air purification efficiency

System performance has been characterized using different approaches. Firstly, the calculation of the relative efficiency in the air and surface samples was carried out according to Eq. (2). The determination of the final CFU (CFUf) was given as the average between the counts of the five replicates of each sampling. The initial CFU data (CFU1) was taken from the previous test to assess performance every 30 min. Those cultures plates replicates with CFUs with values higher than three times those of the rest of the plates of the same sample were eliminated as they were considered non-representative extreme values. Secondly, clean air delivery rate (CADR) and first order loss rates using a simple linear regression against ln-transformed mean concentration values was determined as described by Stephens et al. (2022).

\[
n = 100 \times \frac{\text{CFU}_1 - \text{CFU}_2}{\text{CFU}_1}
\]

3. Results and discussion

3.1. Effect of filtration and needle-tip brush bipolar ionization on air

Airborne microorganisms are significantly affected by weather and environmental conditions (Fang et al., 2018; Zhang et al., 2017; Ehrlich et al., 1970). To guarantee a stable concentration of environmental bacteria during the tests, air samples were taken every 30 min without filtration and ionization. As depicted in Fig. 3, the results of this test suggest that the environmental microbiota is stable over time during the study hours on the same day. An average of 40.4 ± 1.5 CFU/m³ of air was obtained during the first test and 53.8 ± 3.0 CFU/m³ during the second test. CFU counts were averaged using all five replicates of each sample. In the plates at 30 and 90 min from the first test, 40.0 CFU/m³ were counted in each one
with respect to the initial 42.5 CFU/m³. In the plates of the second test, 52.5 and 53.1 CFU/m³ were found at 30 and 90 min, compared to the initial 57.5 CFU/m³. To determine the stability of the CFUs, the Relative Standard Deviation (RSD) of each set of plates was calculated. The obtained RSD of ~0.04 in the first test and ~0.06 in the second test suggests that the bacterial environmental contamination is stable in air during the 90 min of sampling, so that the rest of the tests presented below are performed under robust and reproducible conditions throughout each test. Loss-rate regression of CFU has been fitted to a polynomial to characterize stability (Fig. 3c). The resulting CADR of 0.299 m³/min and the loss rate constant of 0.0012 per minute during the stability control condition reinforces the previous conclusion about the stability of environmental CFUs.

3.1.2. Bacterial species identification

As enumerated in Table 2, the 15 most frequent colonies were isolated and identified. The bacterial identification is of interest since the efficiency of the BIU is evaluated predominantly for this subset of strains, despite the existence of others in a smaller proportion. In addition, its visualization allowed to evaluate its morphology and size, which is relevant for filtration studies. As depicted in Fig. 4, most bacterial strain (12/15) have sizes >1μm in its longest dimension. The measured dimension represents the minimum size in which each strain of CFUs can be found in the air.

Table 2

| Ref | Strain                        | Score  | Gram | Average ± SD | Probable origin                                      |
|-----|-------------------------------|--------|------|--------------|-----------------------------------------------------|
| S1  | Roseomonas mucosa             | 1.76   | Neg  | 3.5 ± 0.7 μm | Mucous (Romano-bertrand et al., 2016)               |
| S2  | Micrococcus luteus            | 2.13   | Pos  | 1.2 ± 0.8 μm | Soil, dust, cutaneous (Kooken et al., 2014)        |
| S3  | Tsukamurella paurometabola    | 1.71   | Pos  | 1.0 ± 0.1 μm | Pathogen (Murak et al., 2011)                      |
| S4  | Kocuria rhizophila            | 1.77   | Pos  | 0.9 ± 0.2 μm | Cutaneous (Takarada et al., 2008)                   |
| S5  | Not identified                |        |      |              |                                                     |
| S6  | Dermacoccus nishinomiyaensis  | 1.75   | Pos  | 1.1 ± 0.1 μm | Cutaneous (Williams and Maclea, 2019)              |
| S7  | Microbacterium paladicola     | 2.02   | Pos  | 4.2 ± 1.3 μm | Not defined                                         |
| S8  | Dermabacter hominis           | 1.91   | Pos  | 1.2 ± 0.2 μm | Cutaneous (Jones and Collins, 1988)                |
| S9  | Roseomonas mucosa             | 2.6    |      | 1.3 ± 0.2 μm | Mucous (Romano-bertrand et al., 2016)              |
| S10 | Not identified                |        |      |              |                                                     |
| S11 | Staphylococcus haemolyticus   | 2.1    | Pos  | 0.9 ± 0.1 μm | Cutaneous (Bliswity et al., 2020)                  |
| S12 | Deinococcus wulamugensis      | 2.36   | Pos  | 1.5 ± 0.2 μm | Not defined                                         |
| S13 | Not identified                | 2.0    |      | 1.0 ± 0.1 μm | Not defined                                         |
| S14 | Bacillus cereus               | 1.95   | Pos  | 1.5 ± 0.2 μm | Not defined                                         |
| S15 | Bacillus sp.                  |        | Pos  | 3.5 ± 0.9 μm | Not defined                                         |

* The score indicates the effectiveness of the identification. Scores >2 indicate an excellent identification of the bacteria. Scores <2 indicate that the spectrum is related to the indicated bacterium but its concordance may not be exactly, so it could not be the indicated strain.

3.1.3. Bipolar ionization reduces aerial CFUs

The effect of ionization was considered exclusively to evaluate the single efficiency of the bipolar ionization unit. The objective of this study was to isolate the efficiency of the ion system and quantify the improvement that it supposes by itself in the absence of other perturbations, such as the filtration of the air conditioning system. An increasing efficiency was observed from the initial sampling to the final samplings. Ion concentration varied (19.9 · 10⁹ — 31.5 · 10⁹ ions/m³) in all the samplings carried out due to the heterogeneity in the air distribution at the different points of
the Tram. On average, during the first 30 min of the experiment, CFUs were reduced by 45.9 % (54.1 % persisted). As shown in Fig. 5, an efficiency of 61.8 % (38.2 % persisted) was estimated after 60 min. The efficiency increased to 69.2% (30.8 % persisted) after 90 min. Licht et al., 2021 evaluated the efficiency of needle-point bipolar ionization systems (~10—20 kV) in airplanes, obtaining a reduction of CFU (Staphylococcus epidermidis) variable between 20.7 % and 60.0 % after 1 h of ionization, which is consistent with the results obtained in the present study. The resulting loss rate constant is 0.018 per minute under ionization conditions, and the estimated CADR was 9.261 m$^3$/min. These parameters imply that filtration is more effective than ionization. CFU amount upon filtration suggests a reduced efficiency considering the number of air changes inside the tram unit. On average, CFUs were reduced by 73.4 % (26.6 % persisted), 84.0 % (16.0 % persisted), and 92.0 % (8.0 % persisted) during the first 30, 60, and 90 min, respectively. In this sense, the CFUs are large enough to favor the filtration mechanisms since they are, for the most part, >1 μm.

Faced with submicron particles, smaller in size than the bacteria tested, the efficiency of the filter is limited according to the conditions studied (Table 3). The filter complies with the UNE-EN 16890 standard and refers to a 75 % filtration for particles greater than ~10 μm. This filter is efficient for the retention of dust or pollen. However, it is inefficient against fine particles and viruses. The tests carried out are limited. Due to the conditions of the equipment used for the determination of filtration efficiency against submicron particles, the filter may have been ‘clogged’ by the concentration of NaCl particles, assuming a notable increase in pressure drop and an ornament in the efficiency result. The Coarse 45 % filter was evaluated in a more compact format than natural: 2—3 mm thick instead of 20—30 mm. The results suggest that the speed at which the air passes through the filter medium retains the larger particles. However, no notable difference is observed in the flows studied. The curves could not be very representative of this filter’s actual efficiency (in working conditions in the air conditioning system of the Tram).

**Table 3**

| Laboratory conditions used in the filtration tests. |
|-----------------------------------------------------|
| **Area (cm$^2$)** | **Flow (m$^3$/h)** | **Speed (cm/s)** |
|-------------------|-------------------|------------------|
| Coarse 45 % filter | 3740              | ~261.2           | 19.4             |
|                    |                   | ~1019.2          | 75.7             |
|                    |                   | ~4079.6          | 303.0            |
3.1.5. Effect of combined ionization and filtration efficiency against aerial CFUs

Once the effects of ionization and filtration were evaluated separately, the combination’s performance was intended to study. However, no clear advantage was obtained from the combination of mechanisms. The first 30 min reduced the CFU concentration by 74.2 % (25.8 % persisted), while at 60 min, the efficiency increased to 82.8 % (17.2 % persisted), and at 90 min, 94.1 % was eliminated (5.9 % persisted) (Fig. 8). While these data are promising, the filtration alone reduced the CFU concentration by 73.4 % and 92.0 %, respectively, after 30 and 90 min. The maintenance of filtration efficiency can be explained because the highest rate of particle agglomeration due to electrostatic phenomena occurs in the finest particles (<0.15 μm). Then, there is a higher concentration of particles >0.3 μm (Zeng et al., 2021), which does not favor the performance of filtration (Yeh and Liu, 1974a, 1974b).

The loss rate constant was 0.047 per minute, and the estimated CADR was 13.208 m³/min. The value of CADR of ionization and filtration together was higher than filtration and ionization separately by 3.947 and 8.055 m³/min, respectively.

3.2. Effect of the bipolar ionization in the elimination of CFUs on surfaces

3.2.1. Evaluation of the CFUs stability on surfaces

To guarantee the uniformity of the tests, four surface samples have been carried out, with a total of at least 60 Rodac PCA plates per test: 30 at t = 0 (CFU₁) and 30 at t = 2 h (CFU₂). Ideally, the CFU₁ should be equal to the CFU₂ (i.e., the percentage of CFU₁ = CFU₂ would ideally be 100%). However, we have considered it relevant to analyze whether there are more or fewer CFU at the end of the experiment to determine if it is a matter of stability or simply variability across the surface (i.e., CFU₁ < CFU₂ or CFU₂ > CFU₁). If the CFUs had deteriorated over time, a trend greater than CFU₂ > CFU₁. This has not been observed, so it is assumed that the CFUs are stable during the time of the experiment (2 h) but there is a non-negligible need between the neighboring test surfaces. To quantify this dispersion, the RSD of each set of plates has been calculated. RSD close to zero suggests high uniformity in the samples. The variability of the results (Table 5) required an increase in the sample to obtain representative conclusions.

3.2.2. Bipolar ionization efficiency on surface samples

On the one hand, “effectiveness” refers to the percentage of PCA plates where final CFU number was lower than the initial (CFU₁ > CFU₂). That is, in this column the percentage of CFU plates that showed a potential action of ionization has been studied. On the other hand, “efficiency” represents the percentage of “removed” bacteria in these plates potentially due to ionization effect, which is calculated according to Eq. (2). It is relevant to differentiate between the efficiency for plates with CFU > 50, CFU > 20, and CFU > 10 to avoid distorting the results obtained. However, the global efficiency does not discriminate between values obtained (Table 6).

Table 5

| Test   | RSD (average) | CFU₁ = CFU₂ | CFU₁ < CFU₂ | CFU₂ > CFU₁ |
|--------|---------------|--------------|--------------|--------------|
| Test 1 | 0.39          | 39.3 %       | 21.4 %       | 39.3 %       |
| Test 2 | 0.64          | 17.2 %       | 58.6 %       | 24.2 %       |
| Test 3 | 0.39          | 23.1 %       | 30.6 %       | 46.1 %       |
| Test 4 | 0.61          | 13.5 %       | 33.3 %       | 53.4 %       |

CFU₁ = Initial CFU; CFU₂ = Final CFU.

In experimental sampling tests, SARS-CoV-2 viral RNA has been mainly found in particle sizes from 0.25 μm (Kenarkoohi et al., 2020; Liu et al., 2020). Therefore, determining the efficiency of the filter against submicron sizes and above is relevant to quantify its performance. As shown in Fig. 7, the Coarse 45 % filter presented an approximate retention efficiency of ~30 % for 0.5 μm particles at a flow rate of ~4079 m³/h. For flow rates lower than this, the efficiency of the filter decreases to values <7 %. These tests suggest that larger particles have more inertia at high speeds (high flow rates) and, therefore, a higher retention rate in the filter medium (Yeh and Liu, 1974a, 1974b). However, the clogging of NaCl particles observed in the head loss tests may have overestimated these results. In the tests where the highest speed is simulated, clogging is observed, which translates into an increase up to 380 Pa of pressure drop (Table 4).

### Table 4

| Velocity in filter (cm/s) | Pressure drop (Pa) |
|---------------------------|--------------------|
| 19.4                      | ~6                 |
| 75.7                      | ~40                |
| 303.0                     | ~380               |

Fig. 7. Coarse 45 % filter retention efficiency depending on the particle diameter at different velocity in filter (flow rates). Where it is observed that the efficiency is dependent on the flow rate and the particle size.

Fig. 8. a) Average percentage of survivor CFU. b) CFU/m³ count of the three samples in simultaneous ionization and filtration tests from the initial reference value. c) First-order loss rate constant regression in both ionization and filtration inactivation tests.
the predominance of agglomerates is close to 300 nm. Nevertheless, after the pandemic, preventive strategies are relaxed and important, the development and validation of new effective air purification systems may be essential for minimizing the spread of infectious diseases in the future and this field merits further research.

4. Conclusions

The increasing understanding of the mechanisms behind COVID-19 infection transmission should drive a shift in the way we address the prevention of the transmission of this disease and other respiratory infections. A higher transmission rate of SARS-CoV-2 has been observed in transport compared to other public and shared spaces. It is required to implement preventive measures for different transportation settings and countries. In previous studies we found that the Zaragoza Tram did not represent a high risk of contagion (for more information see Baselga et al., 2022). Nevertheless, after the pandemic, preventive strategies are relaxed and the potential risk of contagion increases, so it is necessary to find long-term solutions for the improvement of the quality of the air we breathe. Although masks are very efficient in expelling fewer potentially pathogenic microorganisms into the air, it is a temporary measure that probably will not be used indefinitely.

Studies on environmental bioaerosols in air samples suggest that BIU systems have a beneficial effect on eliminating CFU. The main limitation for the implantation of ionization systems is a possible generation of by-products due to the electrostatic interaction between elements, although this aspect is not discussed here, and it is out of the scope of this work. Under the conditions studied, which are favorable (including closed tram unit and long exposure times), efficiencies close to 69.2% were obtained after 90 min of ionization. The combined ionization system offers better results in the same period against microorganisms present in the air (92.0%). The combined action of both systems slightly improved the ionization performance (94.1%), not representing a major improvement. According to the literature, the predominance of agglomerates is close to 300 nm. However, the Tram filters tested in this study did not perform well in laboratory analyses against those particle sizes (>10 μm). We cannot exclude a synergy of bipolar ionization with other filter types that could retain these agglomerates.

The filtration tests assayed in the studied Tram units are not very efficient for submicron particles. The Coarse 45 μm-type filters are designed to eliminate dust, pollen, and larger particles. However, considering the number of air changes per hour (~25 ACH, ~1450 m3/h) approximately 6% of the CFUs are retained each time the air passes through the filter leading to a ~75% efficiency observed during the first half-hour (note that no CFU sources existed during the tests). In addition, the main bacterial families found almost always presented sizes >1 μm, so the filtration of submicron matter would be more representative in the case of viruses. A limited filtration of fine particles is observed in the tests carried out with submicron particles, where the retention of 0.1 μm particles was <5%. Faced with these sizes, HEPA filters are an effective strategy. However, it must be considered that the quality of the filter is as important as its performance (measured in m3/h). This is why the implementation of HEPA filters in the HVAC system of the Tram is unthinkable due to its pressure drop. Installation of stand-alone HEPA purification systems to reduce airborne CFUs and viruses could be considered, although this would require separate studies.

Through the technical feature recently agreed upon by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE), it was possible to quantify the impact of the technologies (ionization and filtration) on the interior air of the Tram. It was performed using the loss rate value and the CADR parameter. Combined action of ionization and filtration (CADR = 13.208 m3/min) present a substantially advantage over untreated air (CADR = 0.299 m3/min). Even so, the filtration was also effective on its own (CADR = 9.261 m3/min). Exclusive ionization did not have such a notable effect (CADR = 5.153 m3/h), although also reduced the aerial CFU concentration.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 6

| Test | Effectiveness (CFU > CFU) | Global efficiency | Efficiency (CFU > 50) | Efficiency (CFU > 20) | Efficiency (CFU > 10) |
|------|---------------------------|------------------|----------------------|----------------------|----------------------|
| Test 1 | 60 % | 65.1 % | 65.9 % | 72.1 % | 66.6 % |
| Test 2 | 53 % | 44.0 % | 43.4 % | 36.3 % | 40.9 % |
| Test 3 | 30 % | 54.6 % | 38.6 % | 47.7 % | 46.3 % |
| Test 4 | 53 % | 73.0 % | 65.9 % | 78.4 % | 73.0 % |
| Test 5 | 42 % | 78.1 % | 73.4 % | 70.5 % | 78.1 % |
| Test 6 | 40 % | 81.4 % | 69.2 % | 83.6 % | 81.4 % |

CFU = Initial CFU; CFUf = Final CFU.

According to our findings, in this experimental set-up, the ionization did not present a major role on the inactivation of microorganisms on surfaces. The average ionization effectiveness was 46%, while the average CFU > CFU in the stability study was 36%. We cannot exclude that the difference between initial and final CFUs could be due to effects other than ionization.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
