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Studies on the comparative effectiveness of X-rays, gamma rays and electron beams to inactivate microorganisms at different dose rates in industrial sterilization of medical devices

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ABSTRACT

The radiation resistance of *Bacillus pumilus* spores to gamma rays, X-rays, and electron beam (e-beam) was investigated using industrial irradiators operating at various dose rates. The dose rates were as follows: gamma 1 and 10 kGy/h; X-ray 10 and 200 kGy/h; e-beam 2000 kGy/h. The regression analysis showed that survivor curves were $\log_{10}$ linear for all three sources within the investigated absorbed dose range of 1–6 kGy, irrespective of the dose rate applied. All irradiation technologies were equally efficient to inactivate the spores, which is reflected in their comparable D-values ($p > 0.05$), and dose rate had no impact on the microbicidal efficacy. These results suggest that wherever a specified minimum dose is delivered, the sterilization dose can be transferred between irradiation technologies in industrial sterilization of medical devices without any impact on product sterility. These findings from a novel single study encompassing all available industrial radiation technologies for the purpose of medical devices sterilization, advance our understanding of microbial destruction as related to exposure to important sterilization modalities, which will help inform future applicability of these technologies for emerging industry opportunities.

1. Introduction

Sterilization by radiation is employed as the means of achieving required sterility assurance for some 50% of single use medical devices, with gamma irradiation being the most widely used technology (GIPA-Gamma Industry Processing Alliance, 2017). However, in the past number of years certain challenges regarding Cobalt-60, the raw material for gamma processing, have been identified (Dethier, 2016; BPSA, 2021). Such challenges have culminated in the advancement of accelerator based technologies, such as X-ray and electron beam (e-beam) (McEvoy et al., 2020). In particular, X-ray has been established as a sustainable supplement to gamma due to many similarities between the two photon-based technologies (McEvoy et al., 2020). Many potential changes are considered when migrating to X-ray, including the effect on materials, any potential induced radioactivity, the effect of dose rate, temperature impacts, and processing time. Considerable focus has been placed on material effects (Murray et al., 2012, 2014; Fifield et al., 2021) and induced radioactivity in materials processed with energy above 5 MeV (Michel et al., 2021). Currently, the Association for the Advancement of Medical Instrumentation (AAMI) is generating a Technical Information Report (TIR104) to provide guidance to users when considering a change of radiation technology (Montgomery et al., 2021). That entails performing a risk assessment to identify and quantify the potential impact on the functionality and performance of the medical devices following the terminal sterilization process (Montgomery et al., 2021). Among other factors to be considered when products are moved to X-ray, it is imperative to ensure that the sterility assurance level (SAL) is achieved. Dose rate is a key differential parameter between gamma, electron beam and X-ray, and its effect on sterilization efficacy should be considered (Kroc et al., 2017). Dose rate is defined as the quantity of radiation absorbed per unit of time, and while it can take hours to sterilize products with gamma, the treatment can be completed within

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minutes with an X-ray (Dethier, 2016) and seconds with an e-beam. Furthermore, a recent paper by the Irradiation Panel on gamma and electron irradiation (2020) has re-emphasised the need to consider the effect of higher dose rates. With regard to medical device sterilization, researchers have found that microbicidal efficacy is primarily a dose dependant activity. In their work, Tallentire et al. (2010) and Tallentire and Miller (2015) found that microbiological responses for water hydrated *B. pumilus* spores were the same for all types of industrial irradiators, while Hansen et al. (2020) demonstrated microbicidal equivalence of gamma and e-beam when microorganisms were irradiated in a dry state. However, such studies investigating the microbicidal efficacy of irradiation technologies under conditions typical for industrial sterilization of medical devices are scarce, and more research is needed to evaluate the possibility of transfer of minimum doses required for inactivation between irradiators of the same and/or different irradiation sources. Therefore, in this study, the microbicidal efficacy of all relevant industrial irradiators (gamma, X-ray, and e-beam), operating at different dose rates, have been directly compared in terms of decimal reduction time (D-value) utilizing a single biological indicator reference microorganism. A D-value can be defined as the time or dose required under given conditions to achieve inactivation of 90% (or 1 log) of a population of the test microorganisms (ISO 11139:2018) and is commonly used in sterilization microbiology to express the sterilization efficacy of the process. As a reference microorganism, *Bacillus pumilus* spores were selected due to its high radiation resistance and irradiated in a dry state, as being representative for industrial sterilization processes. Moreover, other sterilization parameters such as temperature and energy level were also assessed regarding their impact on microbial inactivation. The findings from this novel study herein are expected to further substantiate our understanding of the sterilization efficacy of industrial irradiators.

2. Experimental

2.1. Pre-trial to determine the stability of irradiated spores

Prior to executing the experiment, a pre-trial was carried out to determine the stability of irradiated spores, i.e., to establish a time frame within which irradiated spores need to be microbiologically analysed. Spores (biological indicator Lot P102, Crosstex, USA) had a certified population of 2.2×10^6 and a D-value of 1.6 kGy. The manufacturer's spore population claim was verified, and samples were prepared and irradiated with e-beam at a nominal dose of 3.5 kGy. Irradiated samples were immediately (within 30 min) incubated at 2–8 °C and microbiologically analysed at designated time intervals: 0, 24, 48, 72 and 96 h. Grown colonies were enumerated and colony forming units (CFU) calculated. Viable counts (CFU) recovered at different time points were statistically compared to determine the population stability over time. Spore population claim verification, sample preparation, irradiation and microbiological analysis were carried out as described in the current paper.

2.2. Sample preparation

Commercial *Bacillus pumilus* (ATCC 27142) biological indicator (BI) paper strips were used in this study. Spores were supplied by Crosstex (USA, Lot P104) as a certified population containing 2.6×10^6 viable spores (colony forming units (CFU)) per paper strip with a D-value of 1.7 kGy based on the manufacturers test method. The spore population claim was verified following the manufacturer's instructions prior to running the experiment. In brief, the procedure was carried out as described for *B. pumilus* recovery with the addition of a heat shock treatment where biological indicators were first incubated at 65–70 °C for 15 min and then rapidly cooled to below 4 °C. *B. pumilus* samples for X-ray and gamma irradiation were prepared as described by Tallentire et al. (2010) and Tallentire and Miller (2015) with modifications. Briefly, individual *B. pumilus* spore strips were carefully secured in the middle of a Petri dish, without breaking the sterile barrier. Absorbed dose was measured with Alanine dosimeters (Harwell Dosimeters, UK), placed in a Petri dish next to each spore strip (Fig. 1). Samples were prepared differently for e-beam irradiation: two WINdose dosimeters (GEX, USA) were secured to a spore strip (each from one side) and then taped to the middle of a paper envelope (Fig. 1). Two new spore strips were then taped, one on the left and the other on the right side of the dosimeter. Duplicate spore strips were used for microbiological analysis, while the strip placed between two dosimeters was only used for reference dose measurement.

2.3. Irradiation of Bacillus pumilus spores

Dose mapping experiments were conducted for all technologies to determine the maximal and minimal dose zones, reproducibility, and dose rate of the process. Different configurations were designed and trialled to establish a set-up capable of precise dose delivery (data not shown). Once the configuration was established, spore strips were irradiated in duplicates, either placed in Petri dishes (X-ray and gamma) or as duplicate BI's placed in a paper envelope (e-beam). Petri dishes have been processed in static mode with a fix irradiation field dose rate for the X-ray or gamma at 1 kGy dose increments. Each set of duplicates received a nominal dose of 1, 2, 3, 4, 5 or 6 kGy. After exposure to the nominal dose for a particular set of duplicates was achieved, *B. pumilus* spore strips were retrieved for microbiological analysis, while Alanine dosimeters were retrieved for measurement of the absorbed dose. To evaluate the impact of dose rate on inactivation efficacy, samples were treated at different fixed dose rates: 1 or 10 kGy/h with gamma, and 10 or 200 kGy/h with X-ray. The X-ray dose rate is directly proportional to the electron beam current, and it was modified by changing this current. E-beam treatment was performed at 2000 kGy/h, and samples received a nominal dose (1, 2, 3, 4, 5 or 6 kGy) with a single conveyance through the electron field. All irradiated *B. pumilus* spore strips were kept at 2–8 °C and microbiologically analysed within 72 h of treatment. Spore samples were transported in temperature controlled boxes (2–8 C°; Peli Biothermal, UK).

2.4. X-ray, gamma, and e-beam irradiation systems

STERIS AST Radiation Technology Center (RTC) in Däniken (Switzerland), Tullamore (Ireland) and in Bradford (UK) were utilized for X-ray, e-beam, and gamma treatment, respectively. With X-ray, *B. pumilus* spore strips were treated with photons achieving a maximal energy of 7 MeV (560 kW) using a Rhodotron TT1000 (IBA, Belgium) electron accelerator. Radiation source for gamma was Cobalt-60, with an activity of approximately ~330 kCi. E-beam treatment was performed using a 10 MeV (5 kW) electron accelerator (Mevex, Canada) with a horizontal beam delivery. All treatments were carried out at ambient atmosphere and temperature. Temperature indicators (GEX, USA) with a detection range of 27.5 °C–65 °C were used to measure the maximal temperature achieved during treatment.

2.5. Evaluation of the absorbed dose

For gamma and X-ray, Alanine dosimeters (detection range 0.1–100 kGy) were analysed using an electron spin resonance spectroscopy dosimetry system (Aerial/Bruker MS5000). For e-beam, GEX B3 WINdose radiochromic thin film dosimeters were measured using a dosimetry system based on a visible spectrophotometer (Thermo Fisher Genesys 20). Dosimetry systems were calibrated for condition of use against the National Physics Laboratory (NPL, UK). Dosimetry system uncertainty has been assessed at 4% ($k = 2$) for Alanine and at 6% ($k = 2$) for GEX dosimetry.

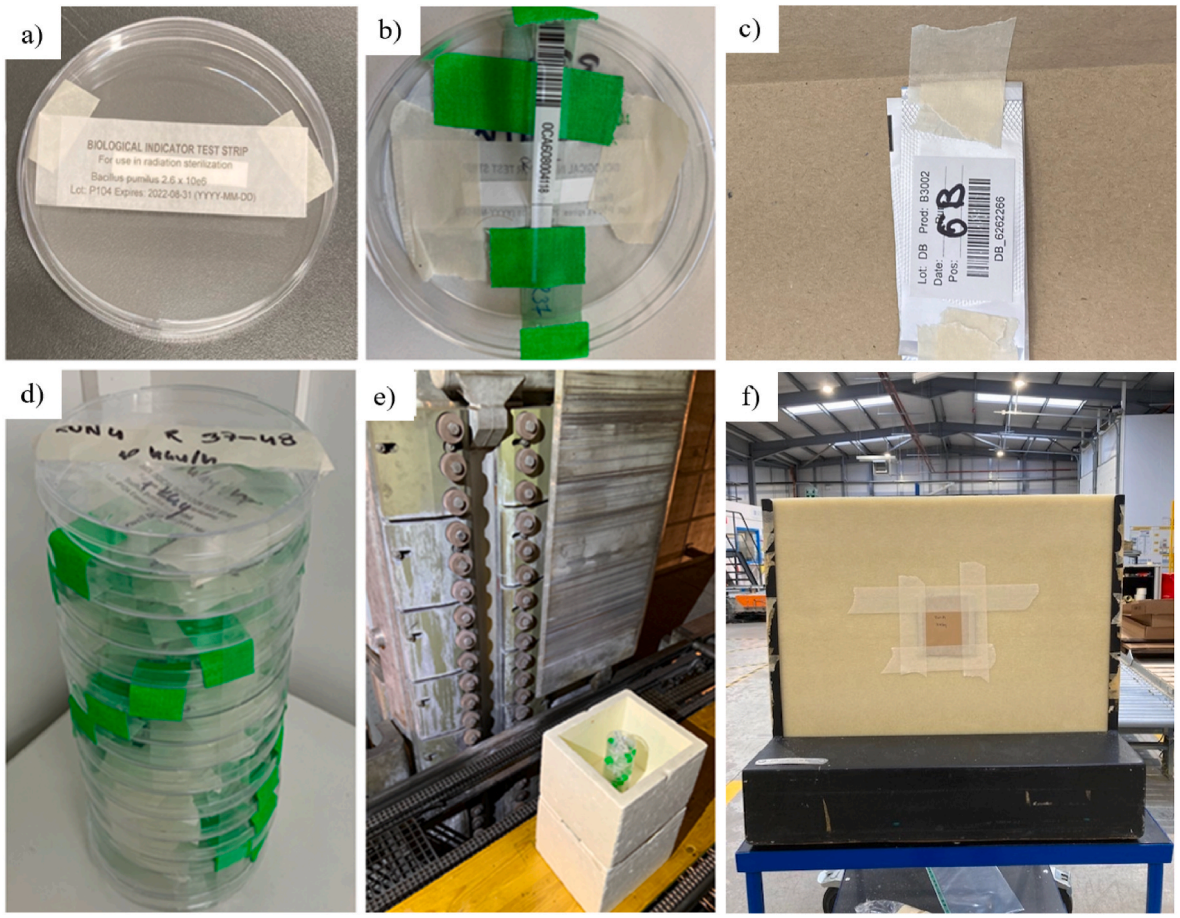

Fig. 1. Experimental set-up. Sample preparation (configuration) was identical for X-ray and gamma: biological indicators were placed in a Petri dish (a) together with dosimeters (b), and stacked Petri dishes were irradiated (d). Placement at X-ray in static mode in front of the beam is shown in panel e. For e-beam irradiation, one biological indicator was placed in between two dosimeters and taped in the middle of a paper envelope (c), while two individual biological indicators used for microbiological analysis were then taped, one on the left and the other on the right side of the dosimeter (not shown in the figure). Sample placement on the carrier (paper envelope fixed to the middle of the carrier) for e-beam treatment is shown in panel f.

2.6. Bacillus pumilus recovery, plotting a survivor curve and D-value calculation

Untreated *B. pumilus* (N_o) and surviving fraction of treated spores (N) were recovered from spore strips and cultured in Tryptic soy agar (TSA, Biokar, France). First, the spore strips were transferred into a sterile test tube containing 5 mL of sterile dH_2O and 10 sterile 6-mm glass beads. The tube was vortexed until the strip was pulped, and another 5 mL of water was added. Then the tube was vortexed again until a homogeneous suspension was achieved. Serial 1:10 dilutions were aseptically prepared using sterile water and 1 mL of the appropriate dilution was inoculated in TSA agar, in duplicates. Plates were incubated at 30–35 °C, enumerated after 48h of incubation, and colony forming units (CFU) were calculated to quantify the viable bacteria. The survivor curve was generated by plotting the logarithm of the survivor fraction ($\log_{10} N/N_o$) against the absorbed dose. Regression analyses were performed, and average D-values calculated from the slope of the obtained curve (ISO 11138-7:2019), for each technology and dose rate combination.

2.7. Statistical analysis

Student's T-test was used to determine the statistical significance between the population recovered immediately after irradiation (0 h) and after incubation at 2–8 °C (24, 48, 75 and 96 h). Analysis of variance (one-way ANOVA) was used to determine the statistical significance between the obtained D-values. Tests were performed at confidence level (or intervals) alpha of 0.05, using Minitab Statistical Software.

3. Results

3.1. Stability of irradiated spores

The stability of irradiated spores was tested over a time period of 96 h. Spore samples were irradiated with e-beam, at an average absorbed dose of 3.4 ± 0.08 kGy. No statistical difference was found when population recovered immediately after irradiation (CFU at 0 h) was compared with population obtained after designated incubation period at 2–8 °C (CFU at 0, 24, 48, 72 and 96 h). The population was approximately at 4.4 log CFU up to 96 h of incubation, when it increased to 4.6

Table 1
A viable population (CFU) of spores recovered immediately after irradiation (0 h) and after 24, 48, 72 and 96 h of treatment. Spores were incubated at 2–8 °C.

Runs	Log CFU at investigated time points				
	0 h	24h	48h	72 h	96 h
Run 1	4.48 ± 0.05	4.39 ± 0.17	4.48 ± 0.15	4.41 ± 0.07	4.43 ± 0.01
Run 2	4.42 ± 0.20	4.44 ± 0.03	4.37 ± 0.01	4.53 ± 0.20	4.65 ± 0.14
Run 3	4.42 ± 0.18	4.37 ± 0.01	4.41 ± 0.11	4.43 ± 0.08	4.74 ± 0.06
Average	4.44	4.40	4.42	4.46	4.61

log CFU (Table 1). Although the increase in CFU was not statistically significant, for the purpose of this study a time point of 72 h was selected as the last stable point. Therefore, further microbiological analysis was carried out within 72 h of irradiation.

3.2. Absorbed doses

Considering that accurate dose delivery is crucial for obtaining reliable results in inactivation experiments, the absorbed dose of each sample was monitored in the study. The absorbed doses during treatment at each nominal dose are shown in Table 2.

3.3. Temperature

During treatment with gamma and e-beam, the temperature was below the detection limit for GEX temperature indicators (27.50 °C). During X-ray processing at 10 kGy/h, the temperature was observed to increase from an average starting temperature of 32 ± 2 °C to 36 ± 2 °C at the end of irradiation. Similarly, at a dose rate of 200 kGy/h, the temperature increased from 29 ± 1 °C to 34 ± 3 °C.

3.4. B. pumilus survivor curves and resistance to irradiation with regards to irradiation technology and dose rate

The regression analysis indicated that all survivor curves were $\log_{10}$ linear (ISO 11138-7:2019) within the investigated dose range, irrespective of the dose rate applied, with all $R^2 \geq 0.95$. The curves with corresponding R^2 values are shown in Fig. 2. Based on the slope of the obtained curves, the D-values were calculated and compared (Fig. 3). No statistical difference ($p > 0.05$) was found between irradiation sources, irrespective of the dose rate applied.

4. Discussion

To ensure that the microbiological growth response is a valid representation of the sterilization efficacy of the process, there should be adequate control over the biological indicator recovery system (Caputo et al., 1980). Namely, there should be a control over the length of time elapsed between exposure to a sterilant and growth testing, and the temperature at which microorganisms are incubated before the microbiological analysis, as these factors are known to impact the recovery of treated microorganisms (Caputo et al., 1980). In this study, the recovery of irradiated spores (CFU) was comparable for all tested time points. A slight increase in CFU was observed at 96 h post treatment, and although this change was not statistically significant, the previous time point (72 h) was selected as a timeframe for carrying out microbiological analysis. Therefore, all spore samples in this study were incubated at 2–8 °C immediately after irradiation (within 30 min of treatment), transported at temperature-controlled conditions and microbiologically analysed within 72 h of irradiation.

Table 2
Absorbed doses of samples during irradiation with gamma-rays, X-rays, and electron beam radiation at different dose rates. Data are shown as means of three independent runs ±1 standard deviation.

Radiation technology	Dose rate (kGy/h)	Absorbed dose during treatment at each nominal dose (kGy)					
		1	2	3	4	5	6
Gamma	1	1.00 ± 0.02	2.00 ± 0.03	2.95 ± 0.06	3.91 ± 0.08	4.86 ± 0.11	5.86 ± 0.11
	10	1.02 ± 0.02	2.07 ± 0.03	3.06 ± 0.08	4.03 ± 0.05	5.09 ± 0.06	6.15 ± 0.10
X-ray	10	1.01 ± 0.01	2.00 ± 0.01	2.97 ± 0.04	3.99 ± 0.06	5.04 ± 0.06	6.10 ± 0.12
	200	0.98 ± 0.04	2.09 ± 0.20	2.87 ± 0.08	3.92 ± 0.04	4.97 ± 0.15	6.34 ± 0.22
E-beam	2000	0.92 ± 0.10	2.05 ± 0.10	3.02 ± 0.08	4.12 ± 0.13	5.10 ± 0.10	6.0 ± 0.00

The resistance ofB. pumilus was examined under the following experimental conditions: gamma 1 kGy/h; gamma 10 kGy/h; X-ray 10 kGy/h; X-ray 200 kGy/h and e-beam 2000 kGy/h. The configuration used in the experiment supported accurate and uniform dose delivery, which is evidenced by the absorbed dose results (Table 2), but also in the clear clustering of data points when recovered microorganisms were plotted against the absorbed dose (Fig. 2, panel f). The regression analysis indicated that all inactivation curves were $\log_{10}$ linear ($R^2 \geq 0.95$) within the investigated dose range of 1–6 kGy. A $\log_{10}$ linear correlation between bacterial inactivation and the treatment dose of ionizing radiation was previously reported by others (Tallentire et al., 2010; Zhang et al., 2020). A non-linear (biphasic) inactivation curve for *B. pumilus* has been reported by Tallentire and Miller (2015); however, the inactivation still followed the first-order kinetics up to about 6 kGy, whereas for doses above 6 kGy, inactivation occurred at an increased rate. Findings from this study support first order model, where a plot of the logarithm of surviving fraction against time yields a straight line, and the inactivation rates are expressed in terms of decimal reduction time, or D-value, which is the reciprocal of the specific inactivation rate at a particular dose of the agent. However, there are many exceptions to the simple first-order type kinetics, especially when microorganisms are exposed to relatively mild inactivation that frequently yield a low number of log reductions (Rowan, 2019) that can produce non-log linear inactivation curves; moreover, these inactivation curves may exhibit pronounced initial shoulders, extended tails, or sigmoid curves that are challenging to fit to the primary data (Rowan et al., 2015). Effective modelling of microbial inactivation arising from physical, chemical or gaseous treatment modalities typically requires the plot to encompass a 6 log microbial count (or survival ratio) versus time data (Buzrul, 2017) for several reasons. Firstly, such a dose-response curve is necessary to address the potential occurrence of microbial variance and resistance to the applied stress that may take different inactivation shapes interpreted through a mathematical best-fit (Garre et al., 2020). A large number of log-reductions are required to effectively interpret and fit inactivation plots (Rowan, 2019), and 6 log reduction has also been shown to support and enable the irreversible destruction of treated microorganisms by way of demonstrating simultaneous occurrence of cellular and molecular damage through 'lethal hits' (Farrell et al., 2011; Hayes et al., 2013; Gérard et al., 2019; Franssen et al., 2019; Fitzhenry et al., 2021). As evident from these findings, when microorganisms are exposed to irradiation, the concentration of surviving *B. pumilus* spores decreases exponentially with dose. This infers the inactivation process reflects a first-order reaction where lethal events occur at random over time with a defined population of spores, which are similarly susceptible to the agent (Klotz et al., 2007). As reported in this study, the first order kinetics are aligned with the physical nature of the process. Thus, when a uniform suspension of microorganisms is irradiated, quanta of radiant energy interact with spores in a random stochastic, which from first principles, implies that lethal 'hits' are distributed in a Poissonian manner (Klotz et al., 2007). These findings suggest that spores in a pre-determined population are equally susceptible to death resulting from a single hit in a dried treatment state. In contrast to radiation, moist heat may differ where treated microorganisms do not all receive the same dose of energy per unit time, as the kinetic energy of water molecules are distributed according to the Mazwell-Botzmann distribution (Klotz et al., 2007).

No significant statistical difference was detected between the obtained D-values, indicating that all radiation technologies (gamma, X-ray, and e-beam) were equally effective at inactivating the challenge microorganism, regardless of the dose rate applied. D-values were

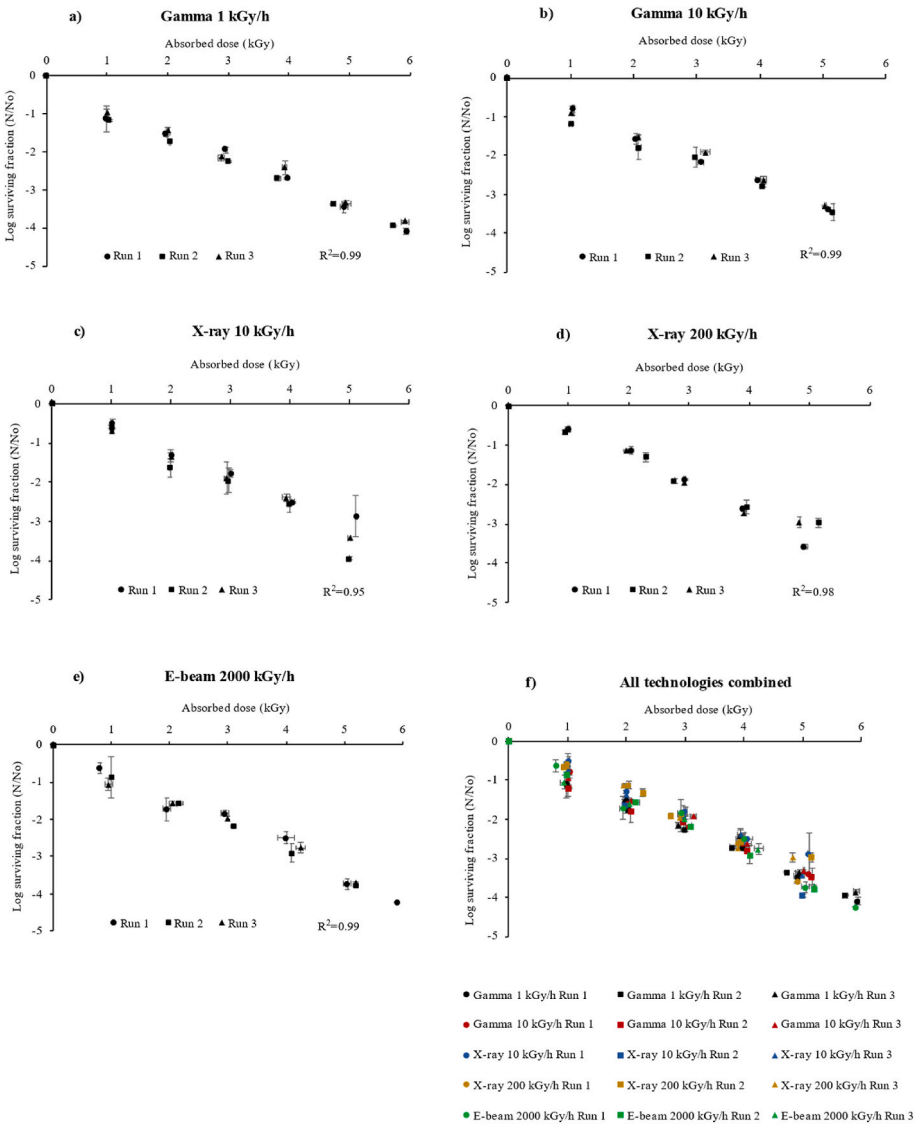

Fig. 2. Inactivation of *B. pumilus* spores treated with (a) Gamma 1 kGy/h, (b) Gamma 10 kGy/h, (c) X-ray 10 kGy/h, (d) X-ray 200 kGy/h and (e) e-beam 2000 kGy/h. Data points for all investigated technologies are plotted together in panel f. Error bars represent the standard deviations for absorbed dose (horizontal) and counts of microorganisms (log CFU; vertical). R^2 values are calculated as means of three independent runs (panels a–e).

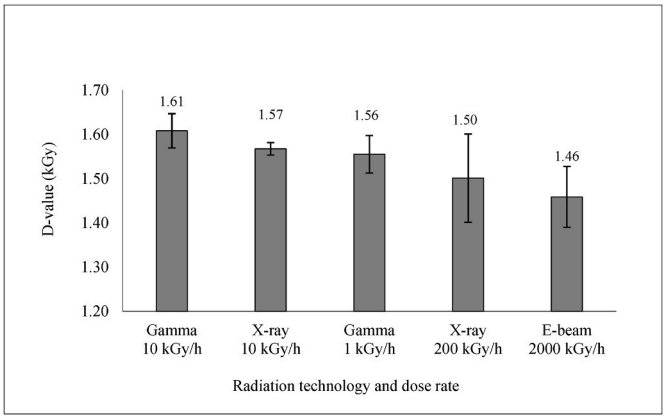

Fig. 3. D-values of *B. pumilus* treated with gamma, X-ray, and e-beam at different dose rates. Data are shown as means ± standard deviation.

within a range of 1.46–1.61 kGy. The finding that dose rate had no impact on microbial inactivation supports the possibility of dose transfer between technologies. Other researchers have obtained similar results for B. pumilus *treated with industrial irradiators (gamma and* 10 MeV *e-beam)*; for example, Tallentire et al. (2010) reported a D-value of 1.5 for both technologies, while Hansen et al. (2020) also found the two technologies to be comparable and reported D-values within a range of 1.2–1.5 kGy. On the other hand, some authors have reported results showing variance when investigating the dose-rate effect and microbicidal efficacy of radiation technologies (Jung et al., 2015; Song et al., 2016; Kyung et al., 2019; Begum et al., 2020). However, such results are often difficult to compare as studies have been carried out under differing test conditions (not always applicable to industrial sterilization settings) and product types.

Although dose rate is a critical parameter, other factors, including temperature and energy level, may also differ between the technologies. The bactericidal effects of ionizing radiation may be enhanced at elevated temperatures (usually above 45 °C), however, this synergistic effect is characteristic for vegetative cells, while spores are impacted to a much lesser extent, as pointed out by Emborg (1974) and Silva Aquino (2012). To evaluate the potential impact of such parameters on the

radiation resistance of *B. pumilus*, spores were treated with gamma and X-ray operating at the same dose rate, and temperature was monitored during treatment. However, it is not possible to determine the exact temperature difference between the two technologies due to the limitations of the measuring system. A higher temperature was detected in the X-ray bunker, where spores were exposed to at least ~5 °C higher temperature than gamma at the beginning and at least ~9 °C higher temperature at the end of the treatment. More importantly, the maximum temperature detected in this study was 36 °C which is unlikely to influence the rate of inactivation of the spores. Regarding the differences in the energy level of the two radiation sources, gamma emits two wavelengths of high energy rays (1.17 and 1.33 MeV), while 5–10 MeV X-ray emits a spectrum of photon energies with a peak occurring at approximately 0.3 MeV, as highlighted by Meissner et al. (2000) and McEvoy et al. (2020). Considering that comparable D-values ($p > 0.05$) were obtained for both technologies operating at 10 kGy/h, the results suggest that when the potential impact of the dose rate was excluded, the variability in temperature and energy levels used in this research had no impact on microbicidal effectiveness of the source.

As previously pointed out by Tallentire et al. (2010), in industrial sterilization of medical devices the microorganisms are commonly irradiated in a "dry" state, although "dryness" is not precisely defined, and it is often a function of the ambient relative humidity. The goal of this study was to evaluate biological indicator spores in a 'dry' physiological state, as being representative of a significant microbiological challenge in industrial sterilization processing. Hence, based on the experimental conditions, results reported in this study suggest that the sterilization dose can be transferred between modes of irradiation in industrial sterilization of medical devices, without causing any impact on the sterility assurance level (SAL), as long as the specified dose is delivered. This finding is particularly relevant to the ecosystem of industry, but also regulators and academia, who seek evidence-based findings to further enable and advance a transition from gamma to X-ray.

5. Conclusions

All investigated technologies (gamma, X-ray, and e-beam) showed log-linear inactivation kinetics ($R^2 \geq 0.95$) and were equally efficient to inactivate *B. pumilus*, which is indicated in comparable D-values ($p > 0.05$), regardless of the dose rate applied.

Considering that dose rate had no impact on sterilization efficacy, the data suggests that an easier transition can be obtained within different ionizing radiation technologies without extensive work related to the sterilization effects as a function of the dose rate. That is, the results reported herein suggest that transfer of minimum doses required for inactivation is possible between irradiators of the same and/or different irradiation source without impacting the sterility assurance level (SAL), in accordance with ISO11137-2:2013.

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Authors contribution statement

Brian McEvoy participated in designing the experiment, interpreting the results and took the lead in writing the manuscript. Ana Maksimovic carried out microbiological analysis and participated in interpreting the results and writing the manuscript. Daniel Howell, Pierre Reppert, Damien Ryan, and Hervé Michel carried out irradiation experiments. Neil Rowan assisted in writing and reviewing the manuscript and supported the research through all stages. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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.

Data availability

Data will be made available on request.

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