

Supplementary Material

Bacterial spores are microorganisms commonly used as bio-indicators in various sterilization processes. We have reviewed experimental data on spore inactivation by atmospheric pressure and reduced pressure plasmas operating in various conditions. The review is limited to the sterilization of spores deposited and dried on surfaces. The most significant experimental parameters are given, namely the type of microorganism, the strain, the initial load (N_0) and surface concentration in cfu/cm^2 , assuming homogeneous deposition, the material of the surface treated, the pressure, the exposure time, the operating gas mixture, and the operating conditions of the plasma source.

Table S1 - Review of spore inactivation results by plasma treatments reported in Fig. 5 of the main text. The D-value is given if it was determined from the phase decay of the spores population.

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	Comment
Halfman et al.	(1)	B. Atrophaeus	ATCC 51189	10 ⁶	–	Glass	RF ICP, 1kW	Ar-H ₂ /10 Pa	60 s	direct	Sprayed
								Ar-N ₂ /10 Pa	40 s	direct	
								Ar-O ₂ /10 Pa	60 s	direct	
								Ar/10 Pa	100 s	direct	
		G. Stearothermophilus	ATCC 7953	10 ⁶	–	Glass	RF ICP, 1kW	Ar-H ₂ /10 Pa	60 s	direct	Sprayed
								Ar-N ₂ /10 Pa	30 s	direct	
								Ar-O ₂ /10 Pa	30 s	direct	
								Ar/10 Pa	30 s	direct	
Stapelman et al.	(2)	B. Pumilus	SAFR-032	5x10 ⁸	~25 spore layers	Stainless steel screw	MW, 400W	H ₂ /5 Pa	242 s	direct	104°C after 300 s; D = 40.3 ± 3 s
		B. Subtilis	DSM 402					H ₂ /5 Pa	118 s	direct	66°C after 60 s; D = 19.7 ± 2.3 s
Moisan et al.	(3)	B. Atrophaeus	ATCC 9372	10 ⁷	10 ⁵	Polystyrene, 95 cm ²	MW, 200W, 50 L chamber	N ₂ -O ₂ /470 Pa	45 min	82 cm	<35-40°C after 30 min
							MW, 120W, 5.5 L chamber	N ₂ -O ₂ /670 Pa	60 min	25 cm	
		G. Stearothermophilus	ATCC 7953				MW, 120W, 5.5 L chamber	N ₂ -O ₂ /670 Pa	30 min	25 cm	

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	Comment
		B. Pumilus	ATCC 27142	10 ⁷			MW, 120W, 5.5 L chamber	N ₂ -O ₂ /670 Pa	60 min	25 cm	5-log
Nagatsu et al.	(4)	G. Stearothermophilus	ATCC 12980	2x10 ⁶	–	Stainless steel	MW, 300 W, 11 L chamber	Synthetic air/90 Pa	25-35 min	direct	95-100°C after 40 min
Lim et al.	(5)	B. Atrophaeus	ATCC 9372	2.10 ⁷	–	Glass	RF-APPJ, 130W,	Ar-O ₂ /1 Atm	30s	5 mm	85°C D=4.5 s
									72 s	10 mm	70°C; D = 12 s
									342 s	15 mm	50°C D = 57 s
Herrmann et al.	(6)	B. Globigii (Atrophaeus)	–	10 ⁷	5x10 ⁷	Glass, 20 mm ²	RF-APPJ, 300W, 92 slm	He-O ₂ /1 Atm	27 s	5 mm	D=4.5 s, 175°C
Venezia et al.	(7)	B. Atrophaeus	ATCC 9372	10 ⁶	1.1x10 ⁶	Stainless steel, 88 mm ²	DBD, PlasmaSol sterilizer, 30 W, 1slm, in closed container	N ₂ -O ₂ -ethylene (1%) / 1 atm	2 min	afterglow	Ambient temperature
		G. Stearothermophilus	ATCC 7953						10 min		
Akitsu et al.	(8)	B. Atrophaeus	ATCC 9372	2x10 ⁶	10 ⁶	Cellulose, 1.8 cm ²	DBD, 80W, 100 kHz	He-H ₂ O 3.2% / 1 atm	30 min	direct	~59°C
		G. Stearothermophilus	ATCC 7953	1.3x10 ⁶	6.5x10 ⁵	Cellulose, 1.8 cm ²	DBD, 80W, 100 kHz		30 min	direct	~59°C
		G. Stearothermophilus	ATCC 7953	1.5 x10 ⁶		Stainless steel	DBD, 13.56 MHz, 200W		3 min	direct	~108°C

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	Comment
Muranyi et al.	(9)	B. Atrophaeus	ATCC 51189	10 ⁶	6.2x10 ⁴	PET, 16 cm ² , spray	CDBD, 130 W	Synthetic air / 1 atm	1 s	direct	Sprayed
Muranyi et al.	(10)	B. Subtilis	DSM 4181	10 ⁶	6.2x10 ⁴	PET, 16 cm ² , spray	CDBD, 170 W	Air, 70% RH / 1atm	1 s	direct	Sprayed
Patil et al.	(11)	B. Atrophaeus	ATCC 9372			Strip, 1.8 cm ² , inside polypropylene container	DBD, 40 W, 20 mm gap, 70 kV _{RMS}	Air, 50 % RH / 1 atm	60 s	direct	
								Air, 50% RH / 1 atm	120 s	direct	
Schnäbel et al.	(12)	B. Atrophaeus	–	10 ⁶	~2.5 x10 ⁴	Glass bottle, 250 mL (about 40 cm ²)	MW, 1.2 kW, 13 slm, plasma on for 7 s	Air, 20% RH / 1 atm	25 min	25 cm	26.9°C on glass surface;
Klämpfl et al.	(13)	B. Subtilis	ATCC 6633	10 ⁶	5x10 ⁵	Stainless steel, 2cm ²	SMD, 4W, 35 mW/cm ²	Air / 1 atm	5 min	5 mm	Sporicidal effect limited by bacterial density and additional burden
Klämpfl et al.	(14)	G. Stearothermophilus	ATCC 7953	2x10 ⁶	10 ⁶	stainless steel, 1.80 cm ²	SMD, 35 mW/cm ²	Air / 1 atm	5.7 min	8mm	Ambient temperature heat: +0.2°C/min D = 0.9 min
		B. Atrophaeus	ATCC 9372						3.4 min		D =0.6 min
		B. Subtilis	DSM 13019						1.7 min		D = 0.3 min
		B. Pumilus	ATCC 7142						3.2 min		D = 0.5 min

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	Comment
Shimizu et al.	(15)	B. Atrophaeus	ATCC 9372	10 ⁷	10 ⁷	Aluminium, 95 mm ²	SMD, 0.4W/cm ² , in 15x12x12 cm ³ chamber	Ambient air (50% RH)/ 1 atm	90 min	21 cm	D = 15 min

Table S2 - Review of spore inactivation results by standard sterilization techniques reported in Fig. 5 of the main text.

Author	Ref	Microorganism	Strain	N _O	N _O /cm ²	Surface	Method	Model	Temperature	Time	Comment
Kempf et al.	(16)	B. Atrophaeus	ATCC 9372	10 ⁸		Stainless steel vessel	Dry heat (ambient air)		115°C	555 min	D values
									170°C	2 min	
Alfa et al.	(17)	B. Atrophaeus	ATCC 9372	10 ⁶	5x10 ⁴	Lumen, 20cm ²	H ₂ O ₂ plasma	Sterrad	40°C	75-85 min	in Tyvek package
Rogers et al.	(18)	B. Subtilis	ATCC 19659	10 ⁸	7x10 ⁶	Glass, 14.2 cm ²	Formaldehyde 1200ppm, RH: 70-75%		22-23°C	10h	
		G. Stearothermophilus	ATCC 12980								
Shintani	(19)	B. Atrophaeus	ATCC 9372				EtO, 1000 mg/L, 50% RH		54.4 °C	9 min	
							EtO, 200 mg/L, 50% RH		54.4 °C	32 min	
Klämpfl et al.	(14)	G. Stearothermophilus	ATCC 7953				H ₂ O ₂ , 6 mg/L, saturated stream		60 °C	25.2 min	data from Simicon Gmbh
Rogers et al.	(20)	B. Subtilis	ATCC 19659	10 ⁸	7x10 ⁶	Glass, 14.2 cm ²	H ₂ O ₂ , 1000 ppm		ambient	20 min	
Rutala et al.	(21)	G. Stearothermophilus	PB49T	10 ⁶	4x10 ⁴	Stainless steel lumens, 25 cm ²	H ₂ O ₂ plasma	Sterrad 100	41°C	73 min	Failed 74% of times
								Sterrad 100S		52 min	Complete

Note: ^a humidified, and 1% ethylene admixture; ^b EtO tests were always followed by 13-15h of aeration

Table S3 - Review of the spore inactivation results by plasma treatments that were not included in Fig. 5.

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	RF	Comment
Levif et al.	(22)	B. Atrophaeus	ATCC 9372	10 ⁶	–	Metallic forceps	MW, 5.5 L chamber, 120 W, 2.45 GHz	N ₂ -O ₂ / 666 Pa	60 min	afterglow	4.5	Effect of packaging considered
				10 ⁷	–	Polystyrene, 9 cm ²			30 min		5	T<50°C
Lerouge et al.	(23)	B. Subtilis	ATCC 9372	10 ⁷	2x10 ⁶	Borosilicate glass vial	MW, 2.45 GHz, 200W	O ₂ -CF ₄ / 80 mTorr	5 min	direct	4	
							RF, 13.56 MHz, 200 W				2.5	Vial on powered electrode
											0.8	Vial on rounded electrode
							MW, 2.45 GHz, 200W	O ₂			2.2	
Brandenburg et al.	(24)	B. Atrophaeus	ATCC 9372	10 ⁶	≈10 ⁷	Polyethylene strip, 0.2 cm ²	RF-APPJ, 27.12 MHz, 20W, 20 slm	Ar / 1 atm	7 min	22 mm	4.3	80-90°C
Van Bokhorst–van de Veen et al.	(25)	B. Cereus	ATCC 14579	10 ⁶	5.9 x10 ⁴	GSWP filter (0.22 µm), cellulose, 17 cm ²	AC-APPJ, CP121 plasma demonstrator, 50 Hz, 3 kV, 15 slm	N ₂ / 1 atm	20 min	–	3.5	
		B. Atrophaeus	ATCC 9372								5	
		G. Stearothermophilus	ATCC 7953								4.1	

Author	Ref	Microorg.	Strain	N _O	N _O /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	RF	Comment			
Boudam et al.	(26)	B. Subtilis	–	10 ⁶	10 ⁶	Polystyrene, 1 cm ²	DBD (glow)	N ₂ -N ₂ O (40 ppm) /1 atm	7 min	direct	5.1				
						Petri dish in 20 L pyrex afterglow chamber	MW, 100W, 2.45 GHz	N ₂ -O ₂ (0.7 %) /5 torr	40 min	afterglow	6				
Heise et al.	(27)	B. Subtilis	ATCC 51189	10 ⁶	2.6x10 ⁴	PET, sprayed on 38.5 cm ²	DBD, 7 W/cm ²	Ar /1 atm	15 s	direct	5				
								N ₂ /1 atm	20 s		4				
								Dry air /1 atm	25 s		1				
								CDBD with 282 nm excimer lamp, 7 W/cm ²	10 s		6				
		A. Niger	DSM 1957				DBD, 7 W/cm ²	Ar/1 atm	12 s		6				
								N ₂ /1 atm	23 s		2.5				
								Dry air /1 atm	23 s		4				
								CDBD with 222 nm excimer lamp, 7 W/cm ²	8 s		6				
Lai et al.	(28)	B. Cereus	ATCC 1178	10 ⁶	5x10 ⁶	Glass slide, 0.2 cm ²	MW torch, 2.45 GHz, 700 W, 25 slm	Air / 1 atm	10 s	3 cm	5	Temperature unknown			
									14 s	4 cm	5				

Author	Ref	Microorg.	Strain	N ₀	N ₀ /cm ²	Surface	Source	Gas/ Pressure	Time	Distance	RF	Comment
Kuo et al.	(29)	B. Cereus	ATCC 1178	10 ⁶	10 ⁶	Paper coupon inside envelope, 1 cm ²	arc-seed MW torch, 245 GHz, 0.4 l/s	Air / 1 atm	3 s	4 cm	1	< 40 °C inside envelope
Kovalova et al.	(30)	B. Cereus	—	—	—	Polypropylene foil, 12.5 cm ²	Pulsed negative corona	Ambient air	10 min	direct	2.2	
							Pulsed positive corona				1.5	
Pointu et al.	(31)	G. Stearothermophilus	CIP 52.82	2x10 ⁴	1.1 x10 ⁴	Glass, 1.8 cm ²	ns pulsed DC, 15 W	N ₂ / 1 atm	50 min	10 cm	2.5	
Ben Gadri et al.	(32)	G. Stearothermophilus	—	10 ⁶		Nitrocellulose	DBD (glow)	Air / 1 atm	5.5 min		>5	
		B. Pumilus	—			Paper			2.4 min		>4	
		B. Subtilis niger	—								>4	

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