



Effect of dry sanitization on dry surface biofilm of *Cronobacter sakazakii*

Raul Fernando Pereira^{a, }, Vinícius S.A. Vaz^a, Rafael Pimentel Maia^b, Jean-Yves Maillard^{c, }, Maristela S. Nascimento^{a, *}

^a Departamento de Engenharia e Tecnologia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, SP, Brazil

^b Departamento de Estatística, Instituto de Matemática, Estatística e Computação Científica, Universidade Estadual de Campinas, Campinas, SP, Brazil

^c School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

ARTICLE INFO

Keywords:

Biofilm
Cronobacter sakazakii
Sanitization
Hygiene
Sodium hypochlorite
UV-C
Ozone

ABSTRACT

Cronobacter sakazakii is an opportunistic foodborne pathogen that affects neonates, and has the ability to produce dry surface biofilm (DSB). However, little is known about its resistance to dry sanitizers. This study aimed to evaluate the efficacy of dry sanitizers compared with sodium hypochlorite (SH) on DSB of *C. sakazakii*. DSB were formed on stainless steel or polypropylene coupons (10 cm²) using two cycles of hydrated/dry phases at 25 °C: T1 (48/48 h) or T2 (24/120 h). At the DSB endpoint, the coupons were sanitized with 70% ethanol, a commercial product based on isopropyl alcohol (25%) and quaternary ammonium (0.015%), hot air (90 ± 2 °C), UV-C light (254 nm), gaseous ozone (45 ± 2 mg/L), or SH (200 mg/L, pH 6.5). All dry sanitizers showed no significant antimicrobial activity ($p > 0.05$), with reductions ≤ 0.8 log CFU/cm² over a 30-min exposure. In contrast, SH was effective against DSB regardless of the DSB formation protocol, with reductions ≥ 2 and > 5 log CFU/cm² after 10 and 30 min, respectively. Interestingly, DSB formed with a shorter hydrated phase and a longer dry phase (T2) had greater sensitivity to SH, but this was not observed with dry sanitizers. CLSM images suggested the presence of VBNC cells, particularly after SH treatment. In conclusion, under the conditions evaluated in the current study, SH exhibited more effective control of *C. sakazakii* DSB compared to the dry sanitization methods assessed.

1. Introduction

Cronobacter sakazakii is characterized as an opportunistic pathogen that affects vulnerable populations such as neonates and immunocompromised individuals (Farmer et al., 1980; Mousavi et al., 2023). Donaghy et al. (2025) classify this pathogen as posing a higher risk to children under 6 months, especially infants less than 60 days old. In recent years, two episodes of infection in newborns linked to powdered infant formula (PIF) contaminated with *C. sakazakii* have been reported in the US, one isolated case in 2021 and an outbreak in 2022, with four cases and two deaths (CDC - US department of health and human services/centers for disease control and prevention, 2025; Haston et al., 2023). Furthermore, around the world between 2022 and 2024, there were seven cases of recalls/withdrawals/outbreaks involving PIF contaminated with *C. sakazakii* (Donaghy et al., 2025). The worldwide prevalence of *C. sakazakii* in powdered milk and flours is approximately 8.4%; in Brazil, the prevalence in PIF (powdered milk) was 23.33%

(Ekundayo & Ijabadeniyi, 2024). Furthermore, the pathogen has already been isolated from chestnuts, rice, corn, wheat, and other cereals and flours (Lou et al., 2019; Samadpour et al., 2024). *C. sakazakii* has the ability to form biofilms on different surfaces, such as polyvinyl chloride (PVC), polypropylene (PP) and stainless steel (SS) (Gemba et al., 2025). According to Umeda et al. (2017), several strains of *C. sakazakii*, isolated from food and clinical samples in Brazil, are able to form biofilms on polystyrene plates. *C. sakazakii* is highly resistant to desiccation, and was recovered from PIF samples after 90 days (Hennekinne et al., 2018). This ability may be related to metabolic and physiological changes in biofilm cells (Lou et al., 2024). The physiological changes occur initially by the accumulation of substances that provide protection against external osmotic pressure, such as potassium and glutamate. In a second stage, the synthesis of solutes occurs, such as saccharides, free amino acids, small peptides, and sulfate esters; there is a five-fold increase in the trehalose concentration (Wang et al., 2024). This resistance not only facilitates the survival of *C. sakazakii*, but also hinders its eradication

* Corresponding author. Departamento de Engenharia e Tecnologia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Unicamp, Rua Monteiro Lobato n° 80, Campinas, 13083-862, SP, Brazil.

E-mail address: mnasci@unicamp.br (M.S. Nascimento).

<https://doi.org/10.1016/j.foodcont.2026.112687>

Received 18 December 2025; Received in revised form 2 October 2026; Accepted 7 October 2026

Available online 12 October 2026

0956-7135/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

from low moisture food (LMF) processing facilities (Breeuwer et al., 2003).

Stressful conditions such as desiccation, heat treatment, lack of nutrients or the presence of antimicrobials can trigger biofilm formation as an adaptive response of the microorganisms (Venkatesan & Sikkander, 2023). Biofilm adhesion is influenced by the type of surface and its characteristics, such as topography, texture, and the presence of cracks or crevices, as well as moisture content (Faille et al., 2018). Among biofilms, there is one type that requires greater attention from the LMF industry, the dry surface biofilm (DSB). This type of biofilm was first reported in the medical area by Vickery et al. (2012), and more recently has been studied in foodborne pathogens (Chaggar et al., 2024; Duggan et al., 2024; Lin et al., 2024). For DSB formation, alternating hydrated and dry phases are necessary. Bacteria need a stage with high moisture to enable their growth, followed by a dry step that activates their responses to desiccation stress. Studies have suggested that these biofilms may be resistant to sanitization and that some pathogens may reduce their metabolism and survive for months under this condition (Chaggar et al., 2024; Maillard & Centeleghe, 2023). In general, the extracellular polymeric substance (EPS) of DSB presents a high proportion of protein, a moderate amount of carbohydrate, and a little quantity of DNA (Ledwoch et al., 2019). Another bacterial adaptive response to stress such as starvation and desiccation is the viable but non-culturable (VBNC) state (Chen et al., 2021). This physiological state is characterized by the inability of cells to grow on conventional culture media while preserving metabolic activity, membrane integrity, and, in some cases, virulence-associated traits (Liu et al., 2023; Ma et al., 2024). VBNC cells are generally more tolerant to sanitizing agents, including chlorine-based compounds and quaternary ammonium compounds (Highmore et al., 2018). As a result, the presence of VBNC cells represents a challenge for current sanitization strategies, since routine microbiological analyses based on culturability may underestimate the actual bacterial population (Chen et al., 2021). Previous studies have reported VBNC induction under conditions of desiccation and sanitizer exposure, with a proportion of DSB populations remaining in this physiological state (Centeleghe et al., 2023; Lin et al., 2024). In addition, Vaz et al. (2026) demonstrated the occurrence of VBNC cells in *C. sakazakii* DSB.

To prevent biofilm formation, wet sanitization is routinely employed in the food industry. Among the main sanitizers used is sodium hypochlorite (SH), whose active principle is the hypochlorous acid (HOCl) (Gurtler et al., 2014). The concentration of this acid in its undissociated form is governed by pH, and it is predominant at values < 7.0. HOCl is a type of reactive oxygen species (ROS), i.e., a potent oxidizing agent capable of causing loss of cell membrane transport, disruption of the electron transport chain, dissipation of adenylate energy reserves, and suppression of DNA synthesis (Falk et al., 2020). SH at 250 mg/L caused reductions of around 3 log CFU/cm² in *C. sakazakii* hydrated biofilms formed on PP and SS (Bayoumi et al., 2012). Regarding its performance against *Candida auris* and *Staphylococcus aureus* DSB, reductions of 2 to >7 log CFU/cm² were obtained using concentrations of 500 mg/L and 10,000–20,000 mg/L, respectively (Almatroudi et al., 2016; Ledwoch & Maillard, 2018). However, wet cleaning is not recommended for the LMF industry. Water entry into the factory environment should be restricted, and dry sanitizing alternatives should be employed to prevent microbial growth (Codex Alimentarius, CXC 75-2018). Dry sanitization can be defined as sanitizing procedures performed without water addition to reduce the microbial load present on surfaces or in processing environments (Prestes et al., 2024). Dry sanitization methods include the application of dry heat, alcohol-based solutions, fumigation, and UV-C light (Green & Jackson, 2024; Prestes et al., 2024). Alcohol-based compounds act by denaturing and/or coagulating proteins and affecting cell membrane and enzymes (Kampf & Arbogast, 2020). Hot air chemically oxidizes and dehydrates the components of bacterial cells (Alonso et al., 2022), whereas UV-C radiation absorbed by the cell generates photoproducts that damage DNA (Blatchley III & Coohill, 2020). Ozone

is also a potent oxidizing agent, which can generate other short-lived oxidizing agents, such as hydroxyl radicals, resulting in degradation of genetic material (Weavers et al., 2020).

Due to the prevalence of *C. sakazakii* in powdered infant formula and its pathogenic potential in neonates, the FDA has established strategies to support the control and prevention of this pathogen (FDA, 2023). Expanding the understanding of *C. sakazakii* persistence and control in LMF is therefore relevant for food safety. Although DSB may contribute to the persistence of microorganisms in food processing environments, studies evaluating the resistance of *C. sakazakii* DSB to chemical and physical sanitizing treatments remain unavailable. Therefore, this study evaluated the effectiveness of dry sanitization methods in comparison with SH against *C. sakazakii* DSB formed on polypropylene (PP) and stainless steel (SS), providing data on the response of this pathogen to sanitization under low-moisture conditions.

2. Material and methods

2.1. Origin of isolates and preparation of inoculum

A pool of five strains of *C. sakazakii* was used in this study. They were previously isolated from low moisture products (P4499 - isolated from pre-cooked milk flour; P4787 - isolated from pre-cooked rice-based cereal; P4791 - ground ginger; P4795 - oat and rice porridge; and P4798 - breadcrumbs) (Brandão et al., 2017). In addition, all strains had previously demonstrated the ability to form hydrated biofilms (Umeda et al., 2017). The strains were stored at −80 °C in an ultralow freezer (NuAire, NU-6518G, MN, USA) and recovered in brain and heart infusion (BHI, Difco, MD, USA) broth with incubation for 18–20 h at 37 °C. Subsequently, the strains were streaked on trypticase soy agar (TSA, Difco) and incubated for 24 h at 37 °C. To obtain the final inoculum, suspensions of each strain were prepared in saline solution (0.85%) (Synth, Brazil) to achieve turbidity equivalent to 0.5 McFarland. Then, 1 mL of each strain suspension was transferred to a sterile tube to form a stock suspension. After vortexing for 30 s at 30,000 rpm, the suspension was diluted in trypticase soy broth (TSB, Difco) to achieve a final concentration of ca. 6 log CFU/mL.

2.2. Surface preparation

PP and AISI 304 SS coupons measuring 2 × 5 cm were used as surface materials to form DSB. Initially, the coupons were washed with neutral detergent and rinsed with distilled water, followed by an ultrasonic bath at 40 kHz for 15 min (Ultrasonic, Brazil). Finally, the coupons were sterilized in an autoclave for 30 min at 121 °C and dried in an oven at 50 °C.

2.3. DSB formation

The study evaluated two DSB formation protocols, each consisting of two hydrated/dry cycles. Protocol T1 comprised 48-h hydrated phases and 48-h dry phases, and protocol T2 comprised 24-h hydrated phases and 120-h dry phases (adapted from Ledwoch et al., 2019). The coupons were placed in sterile Petri dishes (9-cm diameter) containing a circular polypropylene support (8.8-cm diameter) with a rectangular opening in the center (4.3 × 6.7 cm), so that only one face of the coupons remained in contact with the wet inoculum (TSB). Each plate with a support containing three coupons was inoculated with 10 mL of the inoculum, prepared as described in section 2.1. After incubation for 24 or 48 h at 25 °C (first hydrated phase), TSB was drained, and the coupons were transferred to another Petri dish and incubated at 25 °C for 48 or 120 h (first dry phase). The coupons were subsequently transferred to new Petri dishes containing sterile polypropylene supports, and 10 mL of TSB without inoculum was carefully added to allow contact with only one side of each coupon. The plates were then incubated at 25 °C for 48 or 120 h, corresponding to the second hydrated phase. This was followed

with a second dry phase as described above. At the end of the second dry phase (DSB endpoint), DSB on coupons were ready to be tested (Fig. 1).

2.4. Sanitization

Sanitization tests were performed in a biological safety cabinet. The sanitizers evaluated were: 70% ethanol (v/v), a commercial product based on isopropyl alcohol (25%) and quaternary ammonium compound (0.015%), hot air ($90 \pm 2^\circ\text{C}$), UV-C light (254 nm), gaseous ozone ($45 \pm 2\text{ mg/L}$), and sodium hypochlorite (SH 200 mg/L, pH 6.5). Only the coupon face on which DSB was formed was exposed to the sanitizers for 0 (positive control), 5, 10, 15, and 30 min (adapted from Harada & Nascimento, 2021a). Coupons without DSB formation were used as the negative control. Three independent tests were performed for each experiment (Fig. 1).

2.4.1. Alcohol-based sanitizers

The coupons were placed in a Petri dish covered with 400 μL of 70% ethanol or the commercial product. Fresh 70% ethanol solution was prepared by mixing sterile distilled water with absolute ethyl alcohol (Labsynth, Brazil). After each sanitization time, coupons of PP and SS were gently transferred to another Petri dish containing 20 mL of Lethen broth (Acumedia, MI, USA) supplemented with Tween 80 (Sigma Aldrich, Germany) and kept for 5 min to be neutralized.

2.4.2. Hot air

For hot air treatment, the coupons were placed on a glass Petri dish and transferred to a glass box (30 x 15 x 19 cm) with a small opening on the left side to which an air blower device was connected (Taiff, Brazil). The coupons were treated with a continuous flow (approximately 2.0 m/s) at $90 \pm 2^\circ\text{C}$. The temperature was monitored with a thermocouple (PT100 - Testo 112, Testo, Germany), and corresponded to the temperature of the air stream. After exposure for the appropriate contact time, coupons were removed for analysis (see 2.5 below).

2.4.3. Gaseous ozone

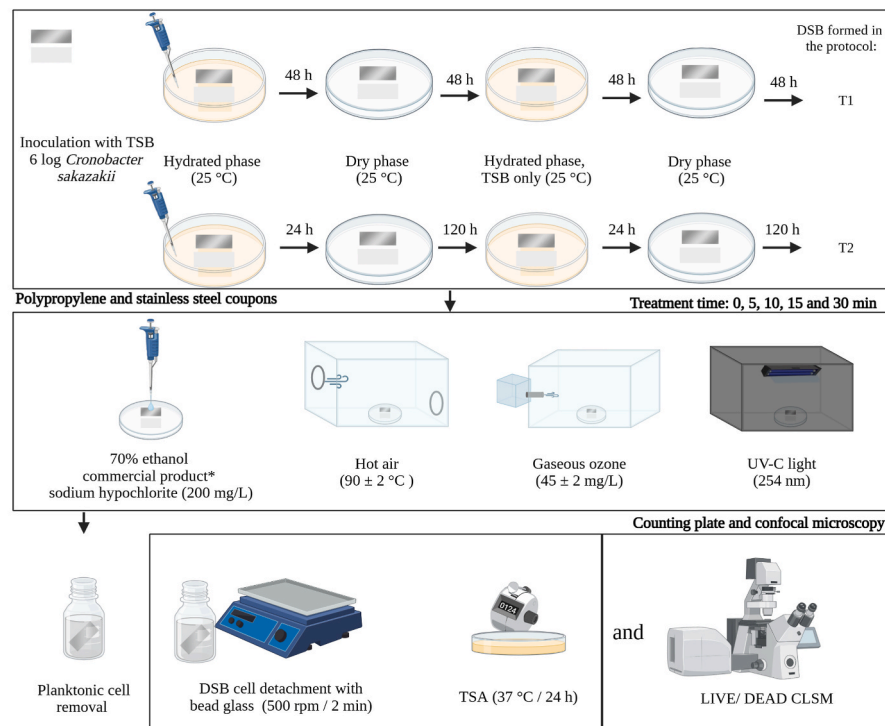
The coupons were placed inside a hermetic acrylic box (30 x 20.5 x 20 cm), which was previously sanitized with 70% ethanol. This box was connected to an ozone generator (Ozoxi, Brazil), an ozone meter (UV-100, EcoSensors, NM, USA) and an ozone destroyer (Ozoxi, Brazil) at the other outlet. The ozone concentration used was $45 \pm 2\text{ mg/L}$ at $\sim 20^\circ\text{C}$ and relative humidity of $\sim 50\%$. These environmental parameters were monitored using a sensor (AM2302/DHP22, Aosong, China) (Harada & Nascimento, 2021a). The box was placed inside an exhaust hood to prevent accidental exposure to the analyst (adapted from Nicholas et al., 2013). After exposure for the appropriate contact time, coupons were removed for analysis (see 2.5 below).

2.4.4. UV-C

For UV-C treatment, the coupons were arranged in a glass Petri dish and then placed in a dark box (30 x 15 x 15 cm) containing an 8 W, 254 nm UV-C lamp (OSRAM, Germany), 15 cm above the coupons, with a power of ca. 1.5 mW/cm^2 over the entire exposure time, measured with a radiometer (Maestro, Gentec, Canada). Prior to each experiment, the UV-C lamp was turned on for 30 min in advance to stabilize radiation emission and ensure consistent irradiance throughout the treatment. After exposure for the appropriate contact time, coupons were removed for analysis (see 2.5 below).

2.4.5. Sodium hypochlorite (SH)

The coupons were placed in a glass Petri dish and covered with 400 μL of SH (200 mg/L - pH 6.5). The sanitizer solution was prepared and titrated (ABNT NBR 9425, 2005) immediately before use, and the pH was adjusted with hydrochloric acid (0.1 N; Merck, Germany) to 6.5. After each exposure time, test coupons were gently immersed in 20 mL of a 0.85% saline solution + 1% sodium thiosulfate (Labsynth, Brazil) for 5 min to neutralize the sanitizer.



*commercial product based on isopropyl alcohol (25%) and quaternary ammonium (0.015%)

Fig. 1. Experimental design of *Cronobacter sakazakii* dry surface biofilm (DSB) forming on polypropylene (PP) and stainless steel (SS) and sanitizing tests.

2.5. DSB enumeration post-exposure

Prior to enumeration of surviving bacteria in DSB, loosely attached planktonic cells were removed. The neutralization process described above for liquid sanitizers served to remove planktonic cells. Furthermore, the effectiveness of those neutralizers was verified under the experimental conditions used. For other treatments, planktonic cells were removed by immersing the coupons in saline solution for 5 min (Harada & Nascimento, 2021a, 2021b).

After neutralization and planktonic cell removal, the coupons were transferred to tubes containing saline solution (0.85%) and glass beads and shaken at 500 rpm for 2 min to release adhered cells (adapted from Ziech et al., 2016). Then, serial decimal dilutions were performed in peptone water (0.1% w/v; Difco) and plated on TSA. After incubation at 37 °C for 24 h, colonies were counted. The results were expressed in log CFU/cm², and the detection limit was 0.7 log CFU/cm².

2.6. CLSM (LIVE/DEAD)

In order to compare the distribution of live and dead cells between the two DSB protocols, an untreated coupon (0 min) and a 30-min exposure coupon from each sanitizer were analyzed by CLSM. The PP and SS coupons were stained using the LIVE/DEAD Biofilm Viability Kit (Invitrogen, Eugene, OR, USA), composed of propidium iodide, a fluorophore that stains dead cells, and SYTO® 9, which stains living cells, both at a dilution of 1:1000. After 10 min contact, the coupons were washed with PBS solution. The images were acquired using a confocal microscope composed of an LSM 780-NLO coupled to a Axio Observer (Carl Zeiss AG, Germany) and a Zeiss EC Plan-Neofluar 20x/0.50 M27 objective lens. The images were processed using FIJI (ImageJ) software (<https://imagej.net/software/fiji/>) to measure the percentage of green and red cells, and the intensity of each channel.

2.7. Statistical analysis

To evaluate the effectiveness of sanitizers against *C. sakazakii* DSB, population reduction values (log N/N₀), calculated from viable counts (log CFU/cm²), were analyzed using one-way ANOVA followed by Tukey's post hoc test. For each sanitizer, comparisons were performed independently to assess: (i) the effect of exposure time within each experimental condition (surface material and DSB protocol), and (ii) the effect of surface type and DSB protocol under a given treatment condition (specific sanitizer and exposure time). Assumptions of normality and homoscedasticity were verified using the Shapiro–Wilk and Breusch–Pagan tests, respectively. All statistical analyses were performed in the R statistical environment (RStudio, R version 4.5.1, 2025), adopting a significance level of $p < 0.05$.

3. Results and discussion

3.1. Efficacy of sanitizers against *C. sakazakii* DSB

The FDA (2023) has identified the manufacturing environment as a potential source of PIF contamination by *C. sakazakii*. Due to the ability of this pathogen to persist in the environment long-term (Iversen & Forsythe, 2004), it is crucial to establish effective mitigation strategies. Although wet sanitization is not recommended as the first option for PIF processing plants (Codex Alimentarius, CXC 66-2008), SH was used for comparison purposes, as it is widely used in the food industry.

To date, no official method exists for evaluating the efficacy of dry sanitizers against biofilms, nor is there a standardized protocol for assessing sanitizer performance against DSB. Consequently, comparisons among studies remain limited due to differences in experimental design and reporting units, requiring caution when interpreting sanitizer performance. Ledwoch et al. (2019) adapted the ASTM E2967-15 method to evaluate the combined effect of chemical sanitizers and

mechanical action—using a wiperator—on DSB formed on stainless steel coupons by the sedimentation method. However, it is important to emphasize that no official methodology for in vitro DSB formation is available and that ASTM E2967-15 was withdrawn by ASTM International in 2024. The current study employed the sedimentation method adapted from Ledwoch et al. (2019) to form DSB on SS and PP coupons at 25 °C and the methodology of Harada and Nascimento (2021a, 2021b) to evaluate sanitizer efficacy. The initial population (positive control) of *C. sakazakii* DSBs formed by both protocols ranged from 7.5 to 8.4 log CFU/cm² on surfaces tested with dry sanitizers (Table 1). However, for coupons used in SH testing, there was a difference of 1.7 log CFU/cm² from the initial DSB population (endpoint) between PP and SS (8.0 and 6.3 log CFU/cm², respectively; Table 1). To reduce the influence of this difference, treatment performance was assessed using normalized results expressed as reduction values (log N/N₀). According to Nkemngong et al. (2020), a minimum DSB population density of 6.0 log CFU/coupon is required for sanitizer efficacy testing. All samples in the present study met or exceeded this threshold.

The five dry sanitizing methods showed limited action against *C. sakazakii* DSB on both PP and SS surfaces, none of them achieved reductions greater than 1 log. Overall, no significant difference ($p > 0.05$) was observed among exposure times or between DSB protocols when evaluating the same dry sanitizer. There was, however, a significant difference ($p < 0.05$) between surface materials for DSB from T1 when treated with 70% ethanol for 5 min or hot air for 10 min. According to some authors (Maillard & Centeleghe, 2023; Rahman et al., 2022) the desiccation stress experienced during DSB formation can alter stress-protein production, modify cell membrane composition, and promote the development of denser EPS matrices. These factors might be related to the high resistance observed here against dry sanitizing methods. In addition, Flemming and Wingender (2010), reported that the EPS of biofilms subjected to desiccation stress had a greater number of non-specific binding sites, which could hinder sanitizer diffusion and reduce antimicrobial effectiveness.

Alcohol-based sanitizers are widely used in the LMF industry (Codex Alimentarius, CXC 75, 2018). For 70% ethanol, the greatest numerical reduction in *C. sakazakii* viability (0.9 log CFU/cm²) was observed in DSB formed with longer hydrated phases and shorter dry phases (T1) on PP after 5 min of exposure (Table 2). In contrast, the commercial product achieved a maximum reduction of 0.6 log CFU/cm² after 15 min. To date, no studies have evaluated the effectiveness of alcohol-based sanitizers against *C. sakazakii* DSB. However, Jo et al. (2010) reported nearly 6-log CFU/coupon reductions in hydrated *C. sakazakii* biofilms using 70% ethanol, while Lin et al. (2024) observed a 1.5-log reduction in *Salmonella* DSB after a 1-min exposure to 70% isopropanol. In addition, Chaggar et al. (2022) reported a 5.9-log reduction in *S. aureus* biofilms after a 1-min exposure to a commercial formulation containing alcohol and quaternary ammonium compounds. Alcohol-based sanitizers act mainly through protein denaturation/coagulation and disruption of cell membranes and enzymatic systems (Kampf & Arbogast, 2020). Their reduced efficacy in the presence of organic matter may be associated with decreased availability of the active compound (Boyce, 2018). In DSB, the EPS matrix may further restrict sanitizer penetration by acting as a diffusion barrier (Rutala & Weber, 2008), potentially requiring longer contact times for deeper action within the biofilm (Kane et al., 2016), although these effects remain hypothetical in the present context. The limited reductions observed in this study, together with previous reports (Harsent et al., 2026; Ledwoch et al., 2021), suggest that effective control of DSB likely requires the combination of chemical sanitizers with mechanical action. Although mechanical effects were not evaluated here, the present study provides a controlled assessment of the intrinsic antimicrobial efficacy of the tested treatments. Future studies should therefore investigate the interaction between chemical and mechanical factors to better reflect real-world sanitation conditions.

Hot air achieved reductions of ≤ 0.6 log CFU/cm², even after 30 min

Table 1

Initial population (log CFU/cm²) of *C. sakazakii* dry surface biofilm (DSB) formed on polypropylene (PP) and stainless steel (SS). 70% ethanol (OH), commercial product based on isopropyl alcohol (25%) and quaternary ammonium (0.015%) (CP), hot air 90 ± 2 °C (HA), gaseous ozone 45 ± 2 mg/L (OZ), UV-C light 254 nm (UV), and sodium hypochlorite 200 mg/L, pH 6.5 (SH). Each DSB protocol consisted of two hydrated/dry cycles: T1 (48 h/48 h) and T2 (24 h/120 h).

Protocol	Surface	Initial population (0 min)					
		OH	CP	HA	OZ	UV	SH
T1	PP	8.0 ± 0.2	8.0 ± 0.2	8.4 ± 0.4	7.7 ± 0.4	7.9 ± 0.4	7.9 ± 0.5
	SS	7.5 ± 0.2	7.7 ± 0.3	7.8 ± 0.3	7.7 ± 0.3	7.9 ± 0.3	6.2 ± 0.1
T2	PP	8.1 ± 0.0	8.1 ± 0.0	8.2 ± 0.2	8.1 ± 0.1	8.1 ± 0.4	8.0 ± 0.3
	SS	7.8 ± 0.2	7.8 ± 0.2	7.8 ± 0.1	7.6 ± 0.4	8.0 ± 0.3	6.4 ± 0.4

Table 2

Reductions in log CFU/cm² of dry surface biofilm (DSB) of *C. sakazakii* formed on polypropylene (PP) and stainless steel (SS) after sanitization for up to 30 min using: 70% ethanol (OH), a commercial product based on isopropyl alcohol (25%) and quaternary ammonium (0.015%) (CP), Hot air 90 ± 2 °C (HA), gaseous ozone 45 ± 2 mg/L (OZ), UV-C light 254 nm (UV), and sodium hypochlorite 200 mg/L, pH 6.5 (SH). Protocols were made with 2 cycles wet/dry phases: T1 (48/48) and T2 (24/120 h).

Protocol	Surface	Exposure time (min) [statistical comparison]			
		5	10	15	30
OH					
T1	PP	0.9 ± 0.4 [Ba]	0.3 ± 0.3 [Aa]	0.6 ± 0.5 [Aa]	0.5 ± 0.1 [Aa]
	SS	0.1 ± 0.4 [Aa]	−0.2 ± 0.2 [Aa]	−0.0 ± 0.4 [Aa]	0.3 ± 0.1 [Aa]
T2	PP	0.3 ± 0.1 [ABa]	−0.0 ± 0.1.0 [Aa]	−0.0 ± 0.1 [Aa]	0.1 ± 0.3 [Aa]
	SS	0.1 ± 0.2 [Aa]	0.3 ± 0.3 [Aa]	0.1 ± 0.2 [Aa]	0.2 ± 0.2 [Aa]
CP					
T1	PP	0.1 ± 0.1 [Aa]	0.1 ± 0.2 [Aa]	0.2 ± 0.3 [Aa]	0.4 ± 0.3 [Aa]
	SS	−0.0 ± 0.2 [Aa]	−0.0 ± 0.2 [Aa]	0.6 ± 0.3 [Aa]	0.5 ± 0.3 [Aa]
T2	PP	−0.0 ± 0.2 [Aa]	−0.0 ± 0.1 [Aa]	0.1 ± 0.4 [Aa]	0.1 ± 0.2 [Aa]
	SS	−0.0 ± 0.3 [Aa]	−0.0 ± 0.2 [Aa]	−0.1 ± 0.4 [Aa]	−0.1 ± 0.2 [Aa]
HA					
T1	PP	0.3 ± 0.2 [Aa]	0.6 ± 0.1 [Ba]	0.5 ± 0.2 [Aa]	0.5 ± 0.2 [Aa]
	SS	0.1 ± 0.4 [Aa]	−0.1 ± 0.1 [Aa]	0.1 ± 0.3 [Aa]	0.2 ± 0.5 [Aa]
T2	PP	0.1 ± 0.3 [Aa]	0.1 ± 0.3 [ABa]	0.1 ± 0.3 [Aa]	0.3 ± 0.2 [Aa]
	SS	−0.1 ± 0.1 [Aa]	−0.0 ± 0.2 [Aa]	0.1 ± 0.3 [Aa]	−0.1 ± 0.1 [Aa]
OZ					
T1	PP	−0.0 ± 0.3 [Aa]	0.1 ± 0.1 [Aa]	0.1 ± 0.4 [Aa]	0.2 ± 0.1 [ABa]
	SS	0.3 ± 0.3 [Aa]	0.2 ± 0.2 [Aa]	−0.0 ± 0.4 [Aa]	−0.1 ± 0.4 [Aa]
T2	PP	0.2 ± 0.3 [Aa]	0.4 ± 0.1.0 [Aa]	0.1 ± 0.3 [Aa]	0.6 ± 0.4 [Ba]
	SS	0.1 ± 0.3 [Aa]	0.3 ± 0.5 [Aa]	0.3 ± 0.5 [Aa]	0.1 ± 0.1 [ABa]
UV					
T1	PP	0.4 ± 0.9 [Aa]	0.1 ± 0.3 [Aa]	0.7 ± 0.5 [Aa]	0.6 ± 0.7 [Aa]
	SS	0.1 ± 0.3 [Aa]	0.2 ± 0.3 [Aa]	0.2 ± 0.5 [Aa]	0.3 ± 0.6 [Aa]
T2	PP	0.1 ± 0.5 [Aa]	0.3 ± 0.4 [Aa]	0.4 ± 0.1 [Aa]	0.6 ± 0.1 [Aa]
	SS	0.2 ± 0.4 [Aa]	0.3 ± 0.5 [Aa]	−0.0 ± 0.2 [Aa]	0.3 ± 0.4 [Aa]
SH					
T1	PP	1.3 ± 0.5 [Aa]	2.6 ± 1.6 [ABa]	3.4 ± 1.7 [Aa]	>7.2 ± 0.5 [Bb]
	SS	1.5 ± 1.3 [Aa]	2.4 ± 0.7 [Aa]	4.8 ± 0.4 [Ab]	>5.5 ± 0.1 [Ab]
T2	PP	1.5 ± 0.2 [Aa]	2.0 ± 0.6 [Aa]	>7.3 ± 0.3 [Bb]	>7.3 ± 0.3 [Bb]
	SS	1.9 ± 0.6 [Aa]	4.9 ± 0.1 [Bb]	5.3 ± 0.9 [ABb]	5.5 ± 0.7 [Ab]

Identical capital letters indicate that there was no statistical difference between averages in each column (exposure time) Identical lowercase indicates that there was no statistical difference between averages in each row (DSB protocol and surface).

exposure (Table 2). The increased thermal resistance of DSB compared with wet surface biofilms (WSB) has been previously documented by Almatroudi et al. (2018). The authors reported reductions of 7.2 log CFU/coupon for WSB but only 0.9 log CFU/coupon for *S. aureus* ATCC 25923 DSB formed on polycarbonate after 10 min exposure to 100 °C. These findings indicate that desiccation stress enhances the thermal tolerance of DSB. Bacterial thermal resistance is closely associated with the production of heat shock proteins (HSPs), particularly chaperone proteins, which help stabilize and refold damaged proteins under combined heat and low-moisture stress conditions. Recent studies have shown that pre-exposure to desiccation increases the expression of these chaperones and results in greater thermal tolerance in *C. sakazakii* (Wang et al., 2021; Zhu et al., 2023). This phenomenon could be hypothesized as one of the factors contributing to the low effectiveness of hot air observed in the present study.

Gaseous ozone and UV-C achieved maximum reductions of 0.6 and 0.7 log CFU/cm² after 30 and 15 min exposure, respectively (Table 2). Epelle et al. (2024) studied UV light (185 nm, 3.2 mW/cm²), gaseous

ozone (50 mg/L), and the combination of both against DSB of *C. auris* and *S. aureus* formed on polystyrene. After 20 min exposure, the authors noted significant reductions, > 4 and > 6 log CFU/mL for *S. aureus* and ca. 6 and 8 log CFU/mL on *C. auris*, for ozone and UV, respectively. This finding suggests that *C. sakazakii* DSB exhibits greater resistance to UV-C and ozone compared with other clinically relevant pathogens. In addition, Harada and Nascimento (2021a) evaluated the same sanitizers tested in the present study, on semi-hydrated biofilms of *Bacillus cereus* formed on PP and SS. The authors obtained better performance with UV-C and ozone compared to our results, with reductions of 2 log CFU/cm² after 30 min. The main mechanism of action of ozone is associated with its strong oxidative activity, which generates hydroxyl radicals and promotes the degradation of genetic material (Weavers et al., 2020). The enzyme superoxide dismutase exhibits antioxidant activity, particularly against superoxide radicals (Sun et al., 2022). Genes encoding this enzyme have been identified in some *C. sakazakii* strains (Bao et al., 2017; Jin et al., 2024), and their activity might partially explain the reduced susceptibility of this microorganism to

ozone. Mechanisms underlying resistance to UV-C light have not yet been fully elucidated; however, some plausible hypotheses may be raised to explain the limited performance of UV-C observed in the present study. Arroyo et al. (2012) suggested that high tolerance to UV-C could be associated with the production of yellow pigments by *C. sakazakii*, which would limit the action of the radiation on the bacterial cell. In addition, UV-C light shows low penetration capacity (Montgomery & Banerjee, 2015) and is influenced by the presence of organic material (Rutala & Weber, 2008). In DSB presenting a dense EPS matrix, both factors may restrict UV-C penetration and limit its interaction with nitrogenous bases, thereby reducing DNA damage and subsequent cell inactivation.

The effect of SH was remarkably greater than that of dry treatments, with reductions above 1.0 log CFU/cm² after 5 min exposure. In addition, both exposure time and DSB protocol significantly influenced SH performance ($p < 0.05$), with reductions > 5.0 log CFU/cm² after 15 min when DSB were formed according to T2 and after 30 min for T1 (Table 2). These results suggest that the shorter wet-phase exposure (24 h) and/or the longer desiccation period (120 h) applied in the T2 protocol increased the sensitivity of *C. sakazakii* to SH. In contrast, Chaggar et al. (2022) reported greater resistance of *Pseudomonas aeruginosa* dried hydrated biofilms with 72-h dehydration compared to 24-h dehydration, with reductions of 2.7 log CFU/coupon versus 3.8 log CFU/coupon after a 1-min exposure to 0.39% SH. Interestingly, the initial action of SH on PP appeared to be slower than on SS, but over time, the reduction in microbial load on both surfaces reached the limit of detection (0.7 log CFU/coupon). Most available studies on DSB resistance to SH are focused on *S. aureus*. Ledwoch et al. (2019) reported a reduction of 5.8 log CFU/coupon after treatment with SH (1000 mg/L/2 min) in combination with mechanical action (10 s and 500 g pressure), using a wiperator. In contrast, other authors obtained a lower reduction in *S. aureus* DSB, ca. 3 log CFU/coupon, using the same SH concentration for 2-5 min exposure (Chowdhury et al., 2019; Ledwoch et al., 2021). A similar reduction was observed for *Salmonella* DSB after 1 min exposure to SH (Lin et al., 2024). In fact, our results indicate the need for longer exposure to SH to achieve reductions > 4 log CFU/cm² in *C. sakazakii* DSB. This might be related to the dense and compact DSB EPS (Centeleghe et al., 2023; Rahman et al., 2022), which may limit SH penetration into bacterial cells, thereby requiring longer contact times. This factor should be taken into account by the LMF industry when validating sanitation procedures. It is also important to highlight that, according to the recommendations of the Codex Alimentarius (CXC 66, 2008) the use of wet sanitization in PIF processing environments requires strict humidity control measures, with immediate drying of equipment and surrounding areas to ensure that any remaining water is removed immediately. Overall, further studies are needed to fully understand the mechanisms involved in DSB resistance to sanitizers, especially dry sanitizers, to enhance product performance in practice. However, it is important to note that, although the findings provide relevant insights, their interpretation is limited to the experimental conditions investigated in this study.

3.2. CLSM images

Fig. 2 shows the CLSM images obtained for the DSB protocols after 0 and 30 min exposure to all sanitizers evaluated on both surfaces. Analysis of the images of the positive control samples (time 0) indicated DSB with heterogeneous coverage and a percentage of green-stained cells (live), ranging from 37 to 75% (Fig. 3). Lin et al. (2024) found a high percentage of red-stained cells (dead) in *Salmonella* DSB when compared to WSB, reaching 87% in DSB aged 96 h. According to these authors, the outermost layers of DSB have a higher concentration of dead cells due to the greater desiccation stress to which they are exposed. In addition, these layers might serve as a protective barrier against antimicrobial agents for the innermost layers, which have a higher percentage of living cells. The same phenomenon of

heterogeneity was reflected in the samples after 30 min exposure to sanitizers, which may have hindered the quantitative and qualitative analysis of CLSM images (Rumbaugh & Sauer, 2020). For 70% ethanol, there was a reduction in the percentage of green-stained cells in T1, but an increase or no change was noted for T2 (Fig. 3). On the other hand, the commercial product and ozone caused a reduction in the percentage of green-stained cells on both surfaces for DSB formed following either T1 or T2 protocols. For hot air, the same trend was observed for both DSB protocols—on SS there was an increase in the percentage of green-stained cells and the opposite was observed on PP. Interestingly, in most scenarios evaluated for UV-C, there was an increase in the percentage of cells stained green after 30 min exposure (Fig. 3). For SH, there was a difference between the DSB protocols. For T1, the percentage of green-stained cells remained stable on SS and increased on PP (from 58% to 75%), whereas for T2, it decreased on both surfaces—from 62% to 54% on SS and from 71% to 61% on PP (Fig. 3). These observations align with the plate count results (Table 2), where T2 exhibited greater sensitivity to chlorine. However, since the counts were reduced to values close to or below the detection limit, a high proportion of red-stained (dead) cells was expected in SH-treated samples. In fact, the high proportion of green-stained cells noted after SH treatment may suggest two feasible scenarios: overestimation of bacterial survival or the presence of viable but non-culturable (VBNC) cells.

The live/dead staining method is based on membrane integrity - Syto 9 permeates intact cells, whereas propidium iodide enters only cells with damaged membranes (Berney et al., 2007; Tchatchiashvili et al., 2025). For SH, inactivation of cellular metabolism (loss of culturability) occurs before significant membrane damage (Virto et al., 2005), meaning that, in this case, dead cells may fluoresce green, as observed in the current study. Luo and Raval (2025) reported that there is no direct correlation between the physiological state of cells indicated by live/dead dyes and cell morphology observed by scanning electron microscopy, suggesting an overestimation of bacterial survival when using these dyes. Furthermore, according to Nocker et al. (2007), the concentration of propidium iodide required to obtain red-stained cells after exposure to chlorine may be higher than usual. Given that CLSM analysis was conducted on a single coupon per condition at 0 and 30 min, these observations should be interpreted cautiously and within the scope of the experimental design. Thus, the CLSM findings should be considered indicative of possible trends in biofilm behavior rather than definitive evidence. Therefore, complementary methods should be considered for a more accurate assessment of cell viability in DSB studies. Regarding VBNC cells, previous studies have demonstrated that *C. sakazakii* can enter a VBNC state in response to desiccation stress, including when in DSB (Jameelah et al., 2018; Vaz et al., 2026; Yan et al., 2025). Centeleghe et al. (2023) also reported higher viability than culturability in *Klebsiella* DSB aged 2 and 4 weeks, suggesting the presence of VBNC cells. As observed in the current study, Almatroudi et al. (2016) also noted a high proportion of green-stained cells after exposure of *S. aureus* DSB to 1000–20,000 mg/L SH (pH 6.5–7.0) for 10 min, despite observing > 7 -log CFU reductions by plate count. Therefore, further studies based on molecular detection (PMA-qPCR), flow cytometry, determination of metabolic activity or assessment of membrane integrity would be needed to confirm this hypothesis.

4. Conclusion

This study showed that DSB formed by *C. sakazakii* exhibited strong resistance to dry sanitization procedures, resulting in reductions of less than 1 log CFU/cm² over 30 min exposure. Indeed, from a risk management perspective, the control of *C. sakazakii* through dry sanitization remains a challenge for the LMF industry, especially for PIF processing plants. In contrast, SH achieved reductions greater than 5 log CFU/cm², highlighting its potential as an effective option for controlling *C. sakazakii* DSB in such environments, provided that strict humidity control and immediate drying of the production line after sanitization

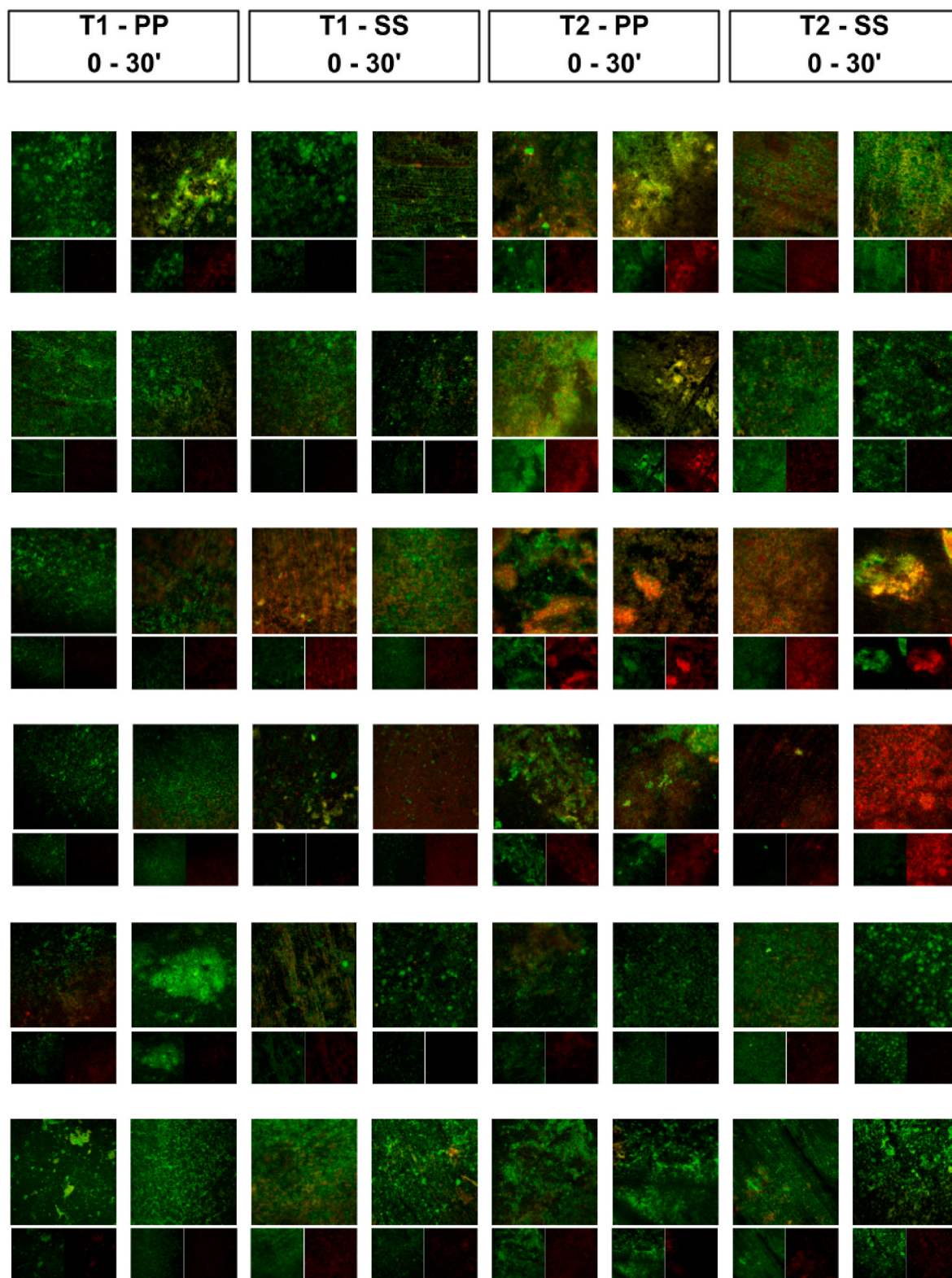


Fig. 2. Confocal laser scanning microscopy (CLSM) images of *Cronobacter sakazakii* dry surface biofilm (DSB) formed on polypropylene (PP) and stainless steel (SS) before (0 min) and after 30-min sanitization. 70% ethanol (OH), a commercial product based on isopropyl alcohol (25%) and quaternary ammonium compound (0.015%) (CP), hot air ($90\text{ }^{\circ}\text{C} \pm 2$) (HA), gaseous ozone (OZ, 45 mg/L), UV-C light (254 nm) (UV), and sodium hypochlorite (SH, 200 mg/L pH 6.5). DSB protocols: T1 (48 h hydrated phase/48 h dry phase) and T2 (24 h hydrated phase/120 h dry phase). Green-stained cells (live) and red-stained cells (dead). Cells were stained using propidium iodide (dead) and SYTO® 9 (live), both at a dilution of 1:1000.

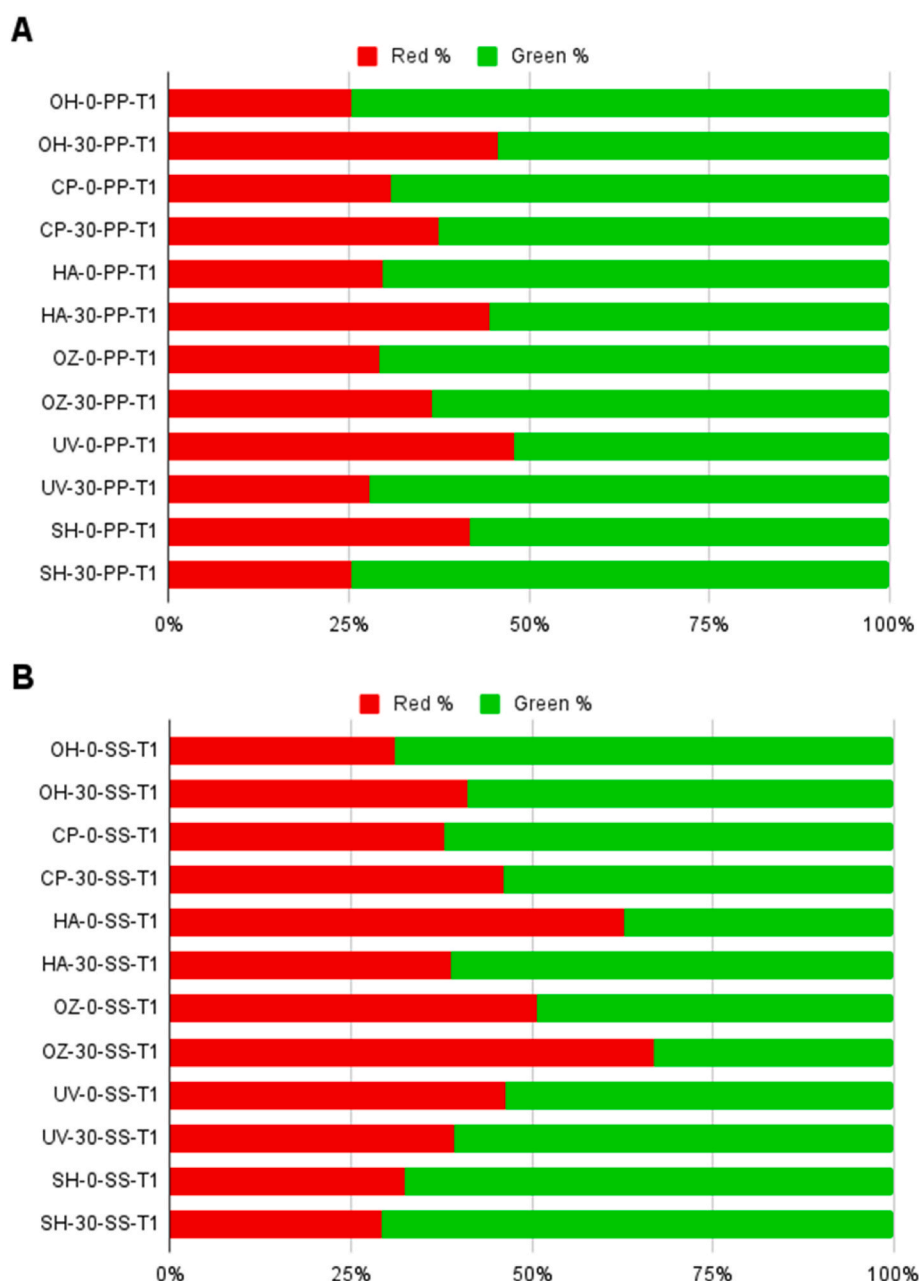


Fig. 3. Percentage of green-stained cells (live) and red-stained cells (dead) obtained from CLSM using the LIVE/DEAD biofilm viability kit (composed of propidium iodide (dead) and SYTO® 9 (live), both at a dilution of 1:1000) of *Cronobacter sakazakii* dry surface biofilm (DSB) formed on polypropylene (PP) and stainless steel (SS) before (0 min) and after 30-min sanitization. 70% ethanol (OH), commercial product based on isopropyl alcohol (25%) and quaternary ammonium compound (0.015%) (CP), hot air (90 °C ± 2) (HA), gaseous ozone (OZ, 45 mg/L), UV-C light (254 nm) (UV), and sodium hypochlorite (SH, 200 mg/L pH 6.5). DSB protocols: T1 (48 h hydrated phase/48 h dry phase) and T2 (24 h hydrated phase/120 h dry phase). A: T1+PP; B: T1+SS; C: T2+PP; D: T2+SS.

are ensured. However, the conclusions of this study are limited to the experimental conditions evaluated and require prior validation before being extrapolated to industrial settings. Furthermore, CLSM images suggest the presence of cells in the VBNC state after chlorine sanitization, representing an additional challenge that must be considered when establishing environmental monitoring and control measures. Therefore, future studies should investigate the combined effects of different dry sanitization methods and their interaction with mechanical action. Additionally, studies focused on characterizing the metabolic and transcriptomic responses induced by desiccation stress, and on determining how these responses contribute to increased tolerance to dry sanitizers are essential to improve the understanding and control of these biofilms in industrial settings.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used ChatGPT and Gemini in order to improve the language, grammar, and fluency of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the manuscript.

CRediT authorship contribution statement

Raul Fernando Pereira: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Vinicius S.A. Vaz:** Methodology, Investigation. **Rafael Pimentel Maia:** Formal analysis,

Data curation. **Jean-Yves Maillard**: Writing – review & editing, Methodology. **Maristela S. Nascimento**: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp, 2021/06809-2), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES; Finance Code 001). The authors also thank the National Institute of Science and Technology on Photonics Applied to Cell Biology (INFABIC) at the State University of Campinas (FAPESP, 2014/50938-8 and the Conselho Nacional de Desenvolvimento Científico e Tecnológico, 465699/2014-6). Lastly, the authors thank the National Institute of Quality Control in Health at the Oswaldo Cruz Foundation for donating the *C. sakazakii* strains.

Data availability

Data will be made available on request.

References

- Almatroudi, A., Gosbell, I. B., Hu, H., Jensen, S. O., Espedido, B. A., Tahir, S., Glasbey, T. O., Legge, P., Whiteley, G., Deva, A., & Vickery, K. (2016). *Staphylococcus aureus* dry-surface biofilms are not killed by sodium hypochlorite: Implications for infection control. *Journal of Hospital Infection*, 93(3), 263–270. <https://doi.org/10.1016/j.jhin.2016.03.020>
- Almatroudi, A., Tahir, S., Hu, H., Chowdhury, D., Gosbell, I. B., Jensen, S. O., Whiteley, G., Deva, A. K., Glasbey, T., & Vickery, K. (2018). *Staphylococcus aureus* dry-surface biofilms are more resistant to heat treatment than traditional hydrated biofilms. *Journal of Hospital Infection*, 98(2), 161–167. <https://doi.org/10.1016/j.jhin.2017.09.007>
- Alonso, V. P. P., Gonçalves, M. P. M. B. B., de Brito, F. A. E., Barboza, G. R., Rocha, L. O., & Silva, N. C. C. (2022). Dry surface biofilms in the food processing industry: An overview on surface characteristics, adhesion and biofilm formation, detection of biofilms, and dry sanitization methods. *Comprehensive Reviews in Food Science and Food Safety*, 22(1), 688–713. <https://doi.org/10.1111/1541-4337.13089>
- Arroyo, C., Gayán, E., Pagán, R., & Condón, S. (2012). UV-C inactivation of *Cronobacter sakazakii*. *Foodborne Pathogens and Disease*, 9(10), 907–914. <https://doi.org/10.1089/fpd.2012.1178>
- Bao, X., Yang, L., Chen, L., Li, B., Li, L., Li, Y., & Xu, Z. (2017). Analysis on pathogenic and virulent characteristics of the *Cronobacter sakazakii* strain BAA-894 by whole genome sequencing and its demonstration in basic biology science. *Microbial Pathogenesis*, 109, 280–286. <https://doi.org/10.1016/j.micpath.2017.05.030>
- Bayoumi, M. A., Kamal, R. M., Aal, S. F. A. E., & Awad, E. I. (2012). Assessment of a regulatory sanitization process in Egyptian dairy plants in regard to the adherence of some food-borne pathogens and their biofilms. *International Journal of Food Microbiology*, 158(3), 225–231. <https://doi.org/10.1016/j.ijfoodmicro.2012.07.021>
- Berney, M., Hammes, F., Bosshard, F., Weilenmann, H.-U., & Egli, T. (2007). Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight kit in combination with flow cytometry. *Applied and Environmental Microbiology*, 73(10), 3283–3290. <https://doi.org/10.1128/AEM.02750-06>
- Blatchley III, E. R., & Coohill, T. P. (2020). Ultraviolet disinfection. In G. McDonnell, & J. Hansen (Eds.), *Block's disinfection, sterilization, and preservation* (6th ed., pp. 171–190).
- Boyce, J. M. (2018). Alcohols as surface disinfectants in healthcare settings. *Infection Control & Hospital Epidemiology*, 39(3), 323–328. <https://doi.org/10.1017/ICE.2017.301>
- Brandão, M. L. L., Umeda, N. S., Jackson, E., Forsythe, S. J., & Filippis, I. d. (2017). Isolation, molecular and phenotypic characterization, and antibiotic susceptibility of *Cronobacter* spp. from Brazilian retail foods. *Food Microbiology*, 63, 129–138. <https://doi.org/10.1016/j.fm.2016.11.011>
- Brazilian National Standards Organization. ABNT NBR 9425. (2005). *Sodium hypochlorite - Determination of active chlorine - Volumetric method*. Rio de Janeiro: ABNT.
- Breeuwer, P., Lardeau, A., Peterz, M., & Joosten, H. M. (2003). Desiccation and heat tolerance of *Enterobacter sakazakii*. *Journal of Applied Microbiology*, 95(5), 967–973. <https://doi.org/10.1046/j.1365-2672.2003.02067.x>
- CDC - US department of health and human services/centers for disease control and prevention. (2025). *Cronobacter outbreak linked to powdered infant formula*. . (Accessed 3 November 2025).
- Centeleghre, I., Norville, P., Hughes, L., & Maillard, J.-Y. (2023). *Klebsiella pneumoniae* survives on surfaces as a dry biofilm. *American Journal of Infection Control*, 51(10), 1157–1162. <https://doi.org/10.1016/j.ajic.2023.02.009>
- Chaggar, G. K., Bryant, D. B., Chen, R., Fajardo, D., Jules-Culver, Z. A., Drolia, R., & Oliver, H. F. (2024). Development of *Salmonella enterica* serovar typhimurium, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* multi-species in vitro dry surface biofilm models: Insights into resilience and persistence in low-moisture environments. *Food Control*, 166, Article 110703. <https://doi.org/10.1016/j.foodcont.2024.110703>
- Chaggar, G. K., Nkemngong, C. A., Li, X., Teska, P. J., & Oliver, H. F. (2022). Hydrogen peroxide, sodium dichloro-s-triazinetrienes and quaternary alcohols significantly inactivate the dry-surface biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* more than quaternary ammoniums. *Microbiology*, 168(3), Article 001140. <https://doi.org/10.1099/mic.0.001140>
- Chen, G., Lin, M., Chen, Y., Xu, W., & Zhang, H. (2021). Induction of a viable but nonculturable state, thermal and sanitizer tolerance, and gene expression correlation with desiccation-adapted biofilm and planktonic *salmonella* in powdered infant formula. *Journal of Food Protection*, 84(7), 1194–1201. <https://doi.org/10.4315/JFP-20-402>
- Chowdhury, D., Rahman, A., Hu, H., Jensen, S. O., Deva, A. K., & Vickery, K. (2019). Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms. *Journal of Hospital Infection*, 103(1), 33–41. <https://doi.org/10.1016/j.jhin.2018.10.019>
- Codex Alimentarius Commission. (2008). *Code of hygienic practice for powdered formulae for infants and young children*. CXC, 66-2008.
- Codex Alimentarius Commission. (2018). *Code of hygienic practice for low-moisture foods*. CXC, 75-2015 - Revision 2016 – Amended in 2018.
- Donaghy, J. A., Zwietering, M. H., & Farber, J. M. (2025). A hazard does not always equate to a risk: *Cronobacter* is a rare opportunistic pathogen and the greatest risk is only for a small sub-population of infants and only associated with powdered infant formula or human breast milk. *Trends In Food Science & Technology*, 157, Article 104890. <https://doi.org/10.1016/j.tifs.2025.104890>
- Duggan, K., Shepherd, M., & Maillard, J.-Y. (2024). Susceptibility of *Salmonella enterica* typhimurium dry surface biofilms to disinfection. *Journal of Food Safety*, 44(2), Article e13117. <https://doi.org/10.1111/jfs.13117>
- Ekundayo, T. C., & Ijabadeniyi, O. A. (2024). Global and regional prevalence of *Cronobacter sakazakii* in powdered milk and flour. *Scientific Reports*, 14(1), Article 6865. <https://doi.org/10.1038/s41598-024-57586-x>
- Epelle, E. I., Amaeze, N., Mackay, W. G., & Yaseen, M. (2024). Dry biofilms on polystyrene surfaces: The role of oxidative treatments for their mitigation. *Biofouling*, 40(10), 772–784. <https://doi.org/10.1080/08927014.2024.2411389>
- Faille, C., Cumault, C., Dubois, T., & Bénézech, T. (2018). Hygienic design of food processing lines to mitigate the risk of bacterial food contamination with respect to environmental concerns. *Innovative Food Science & Emerging Technologies*, 46, 65–73. <https://doi.org/10.1016/j.ifset.2017.10.002>
- Falk, N. A., Macnaughtan, M., Taheri, A., & McCormick, W. (2020). Chlorine and chlorine compounds. In G. McDonnell, & J. Hansen (Eds.), *Block's disinfection, sterilization, and preservation* (6th ed., pp. 280–305).
- Farmer, J. J., Asbury, M. A., Hickman, F. W., & Brenner, D. J. (1980). *Enterobacter sakazakii*: A new species of “enterobacteriaceae” isolated from clinical specimens. *International Journal of Systematic Bacteriology*, 30, 569–584. <https://doi.org/10.1099/00207173-30-3-569>
- FDA. (2023). *Summary of FDA's strategy to help prevent Cronobacter sakazakii illnesses associated with consumption of powdered infant formula*. . (Accessed 26 September 2025).
- Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews Microbiology*, 8(9), 623–633. <https://doi.org/10.1038/nrmicro2415>
- Gemba, M., Rosiak, E., & Kotošyn-Krajewska, D. (2025). Development of predictive models of biofilm formation by *C. sakazakii*, *E. cloacae* on surfaces used in the food industry and medicine. *International Journal of Food Microbiology*, 434, Article 111131. <https://doi.org/10.1016/j.ijfoodmicro.2025.111131>
- Green, H., & Jackson, L. S. (2024). Dry cleaning and sanitization technologies. In G. W. Smithers (Ed.), *Encyclopedia of food safety* (2nd ed., pp. 732–738). Academic Press. <https://doi.org/10.1016/B978-0-12-822521-9.00225-2>
- Gurtler, J. B., Doyle, M. P., & Kornacki, J. L. (2014). The microbiological safety of spices and low-water activity foods: Correcting historic misassumptions. In J. Gurtler, M. Doyle, & J. Kornacki (Eds.), *The microbiological safety of low water activity foods and spices* (pp. 3–14). https://doi.org/10.1007/978-1-4939-2062-4_1
- Harada, A. M. M., & Nascimento, M. S. (2021a). Effect of dry sanitizing methods on *Bacillus cereus* biofilm. *Brazilian Journal of Microbiology*, 52, 919–926. <https://doi.org/10.1007/s42770-021-00451-0>
- Harada, A. M. M., & Nascimento, M. S. (2021b). Efficacy of dry sanitizing methods on *Listeria monocytogenes* biofilms. *Food Control*, 124, Article 107897. <https://doi.org/10.1016/j.foodcont.2021.107897>
- Harsent, R., Cattori, V., Pascoe, M., Pertusati, F., Westwell, A. C., & Maillard, J.-Y. (2026). *Enterococcus* spp. ability to form a dry surface biofilm: A route to persistence on environmental surfaces. *Journal of Hospital Infection*, 167, 23–33. <https://doi.org/10.1016/j.jhin.2025.09.008>
- Haston, J. C., Miko, S., Cope, J. R., McKeel, H., Walters, C., Joseph, L. A., Griswold, T., Katz, L. S., Andújar, A. A., Tourdot, L., Rounds, J., Vagnone, P., Medus, C., Harris, J., Geist, R., Neises, D., Wigginton, A., Smith, T., Im, M. S., ... Lee, C. C. (2023). *Cronobacter sakazakii* infections in two infants linked to powdered infant Formula and breast pump equipment — United States, 2021 and 2022. *MMWR - Morbidity and*

- Mortality Weekly Report, 72(9), 223–226. <https://doi.org/10.15585/mmwr.mm7209a2>
- Hennekinne, R. B., Guillier, L., Fazeuilh, L., Ellis, T., Forsythe, S., Jackson, E., Meheut, T., & Besse, N. G. (2018). Survival of *Cronobacter* in powdered infant formula and their variation in biofilm formation. *Letters in Applied Microbiology*, 66(6), 496–505. <https://doi.org/10.1111/lam.12879>
- Highmore, C. J., Warner, J. C., Rothwell, S. D., Wilks, S. A., & Keevil, C. W. (2018). Viable-but-nonculturable *Listeria monocytogenes* and *Salmonella enterica* serovar thompson induced by chlorine stress remain infectious. *mBio*, 9(2). <https://doi.org/10.1128/mbio.00540-18>
- Iversen, C., & Forsythe, S. (2004). Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. *Food Microbiology*, 21(6), 771–777. <https://doi.org/10.1016/j.fm.2004.01.009>
- Jameelah, M., Dewanti-Hariyadi, R., & Nurjanah, S. (2018). Expression of rpoS, ompA and hfq genes of *Cronobacter sakazakii* strain Yrt2a during stress and viable but nonculturable state. *Food Science and Biotechnology*, 27(3), 915–920. <https://doi.org/10.1007/s10068-018-0313-5>
- Jin, T., Pang, L., Yue, T., Niu, L., Li, T., Liang, Y., Zhang, Y., Yan, C., Yang, B., & Zhang, C. (2024). The role of DsbA and PcpP genes in the environmental tolerance and virulence factors of *Cronobacter sakazakii*. *Food Research International*, 190, Article 114555. <https://doi.org/10.1016/j.foodres.2024.114555>
- Jo, S.-H., Baek, S.-B., Ha, J.-H., & Ha, S.-D. (2010). Maturation and survival of *cronobacter* biofilms on silicone, polycarbonate, and stainless steel after UV light and ethanol immersion treatments. *Journal of Food Protection*, 73, 952–956. <https://doi.org/10.4315/0362-028x-73.5.952>
- Kampf, G., & Arbogast, J. W. (2020). Alcohols. In G. McDonnell, & J. Hansen (Eds.), *Block's Disinfection, sterilization, and preservation* (6th ed., pp. 384–407).
- Kane, D. M., Getty, K. J. K., Mayer, B., & Mazzotta, A. (2016). Sanitizing in dry-processing environments using Isopropyl alcohol Quaternary ammonium formula. *Journal of Food Protection*, 79(1), 112–116. <https://doi.org/10.4315/0362-028X.JFP-15-257>
- Ledwoch, K., Magoga, M., Williams, D., Fabbri, S., Walsh, J., & Maillard, J.-Y. (2021). Is a reduction in viability enough to determine biofilm susceptibility to a biocide? *Infection Control & Hospital Epidemiology*, 42(12), 1486–1492. <https://doi.org/10.1017/ice.2021.42>
- Ledwoch, K., & Maillard, J.-Y. (2018). *Candida auris* Dry Surface Biofilm (DSB) for disinfectant efficacy testing. *Materials*, 12(1), 18. <https://doi.org/10.3390/ma12010018>
- Ledwoch, K., Said, J., Norville, P., & Maillard, J.-Y. (2019). Artificial dry surface biofilm models for testing the efficacy of cleaning and disinfection. *Letters in Applied Microbiology*, 68(4), 329–336. <https://doi.org/10.1111/LAM.13143>
- Lin, Z., Liang, Z., He, S., Chin, F. W. L., Huang, D., Hong, Y., Wang, X., & Li, D. (2024). *Salmonella* dry surface biofilm: Morphology, single-cell landscape, and sanitization. *Applied and Environmental Microbiology*, 90(11), Article e0162324. <https://doi.org/10.1128/aem.01623-24>
- Liu, J., Yang, L., Kjellerup, B. V., & Xu, Z. (2023). Viable but nonculturable (VBNC) state, an underestimated and controversial microbial survival strategy. *Trends in Microbiology*, 31(10), 1013–1023. <https://doi.org/10.1016/j.TIM.2023.04.009>
- Lou, X., Liu, T., Zhang, W., Yu, H., Wang, H., Song, S., Chen, Q., & Fang, Z. (2019). The occurrence and distribution characteristics of *Cronobacter* in diverse cereal kernels, flour, and flour-based products. *Food Microbiology*, 84, Article 103269. <https://doi.org/10.1016/j.fm.2019.103269>
- Lou, X., Wu, Y., Huang, Z., Zhang, W., Xiao, X., Wu, J., Li, J., & Fang, Z. (2024). Biofilm formation and associated gene expression changes in *Cronobacter* from cereal related samples in China. *Food Microbiology*, 118, Article 104409. <https://doi.org/10.1016/j.fm.2023.104409>
- Luo, J., & Raval, R. (2025). Correlative Imaging and super resolution microscopy studies reveal complexities in determining live-dead state of bacteria. *Biofilm*, 10, Article 100302. <https://doi.org/10.1016/j.biofilm.2025.100302>
- Ma, Z., Xu, W., Li, S., Chen, S., Yang, Y., Li, Z., Xing, T., Zhao, Z., Hou, D., Li, Q., Lu, Z., & Zhang, H. (2024). Effect of RpoS on the survival, induction, resuscitation, morphology, and gene expression of viable but non-culturable *Salmonella* Enteritidis in powdered infant formula. *International Journal of Food Microbiology*, 410, Article 110463. <https://doi.org/10.1016/J.IJFOODMICRO.2023.110463>
- Maillard, J.-Y., & Centeleghe, I. (2023). How biofilm changes our understanding of cleaning and disinfection. *Antimicrobial Resistance and Infection Control*, 12(1), Article 95. <https://doi.org/10.1186/s13756-023-01290-4>
- Montgomery, N. L., & Banerjee, P. (2015). Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in biofilms by pulsed ultraviolet light. *BMC Research Notes*, 2015, 8(1), 235. <https://doi.org/10.1186/s13104-015-1206-9>, 8:1.
- Mousavi, Z. E., Hunt, K., Koolman, L., Butler, F., & Fanning, S. (2023). *Cronobacter* species in the built food production environment: A review on persistence, pathogenicity, regulation and detection methods. *Microorganisms*, 11(6), Article 1379. <https://doi.org/10.3390/microorganisms11061379>
- Nicholas, R., Dunton, P., Tatham, A., & Fielding, L. (2013). The effect of ozone and open air factor on surface-attached and biofilm environmental *Listeria monocytogenes*. *Journal of Applied Microbiology*, 115(2), 555–564. <https://doi.org/10.1111/jam.12239>
- Nkemngong, C. A., Voorn, M. G., Li, X., Teska, P. J., & Oliver, H. F. (2020). A rapid model for developing dry surface biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* for in vitro disinfectant efficacy testing. *Antimicrobial Resistance and Infection Control*, 9(1), Article 134. <https://doi.org/10.1186/s13756-020-00792-9>
- Nocker, A., Sossa, K. E., & Camper, A. K. (2007). Molecular monitoring of disinfection efficacy using propidium monoazide in combination with quantitative PCR. *Journal of Microbiological Methods*, 70(2), 252–260. <https://doi.org/10.1016/j.mimet.2007.04.014>
- Prestes, F. S., Yotsuyanagi, S. E., Alonso, V. P., & Nascimento, M. S. (2024). Dry sanitization in the food industry: A review. *Current Opinion in Food Science*, 57, Article 101166. <https://doi.org/10.1016/J.COFS.2024.101166>
- Rahman, M. A., Amirkhani, A., Parvin, F., Chowdhury, D., Molloy, M. P., Deva, A. K., Vickery, K., & Hu, H. (2022). One step forward with dry surface biofilm (DSB) of *Staphylococcus aureus*: Tmt-based quantitative proteomic analysis reveals proteomic shifts between dsb and hydrated biofilm. *International Journal of Molecular Sciences*, 23(20), Article 12238. <https://doi.org/10.3390/ijms232012238>
- Rumbaugh, K. P., & Sauer, K. (2020). Biofilm dispersion. *Nature Reviews Microbiology*, 18(10), 571–586. <https://doi.org/10.1038/s41579-020-0385-0>
- Rutala, W. A., & Weber, D. J. (2008). *Guideline for disinfection and sterilization in healthcare facilities*, 2008. CDC - Centers for Disease Control and Prevention. <http://www.cdc.gov/infection-control/hcp/disinfection-and-sterilization/index.html>
- Samadpour, M., Benoit, L., Myoda, S., Hans, B., Nadala, C., Kim, S. H., Themeli, E., Cantera, R., Nguyen, T., & Richter, H. (2024). Microbiological survey and genomic analysis of *Cronobacter sakazakii* strains isolated from US households and retail foods. *Applied and Environmental Microbiology*, 90(7). <https://doi.org/10.1128/aem.00700-24>. e00700-24.
- Sun, Y., Li, J., Yang, Y., Yang, G., Shi, Y., Wang, S., Wang, M., & Xia, X. (2022). The role of ptiH in stress adaptation and virulence in *Cronobacter sakazakii* BAA-894. *Foods*, 11(17), Article 2680. <https://doi.org/10.3390/foods11172680>
- Tchatchiashvili, T., Jundzill, M., Marquet, M., Mirza, K. A., Pletz, M. W., Makarewicz, O., & Thieme, L. (2025). CAM/TMA-DPH as a promising alternative to SYTO9/PI for cell viability assessment in bacterial biofilms. *Frontiers in Cellular and Infection Microbiology*, 14, Article 1508016. <https://doi.org/10.3389/fcimb.2024.1508016>
- Umeda, N. S., Filippis, I., Forsythe, S. J., & Brandão, M. L. L. (2017). Phenotypic characterization of *Cronobacter* spp. strains isolated from foods and clinical specimens in Brazil. *Food Research International*, 102, 61–67. <https://doi.org/10.1016/j.foodres.2017.09.083>
- Vaz, V. S. A., de, A. F. F., Finger, J., Pereira, R. F., Derami, M. S., Maillard, J.-Y., & Nascimento, M. S. (2026). Dry surface biofilm of *Salmonella* and *Cronobacter sakazakii*: A real concern for the low moisture food industry. *Food Microbiology*, 136, Article 105013. <https://doi.org/10.1016/J.FM.2025.105013>
- Venkatesan, N., & Sikkander, S. (2023). Chapter 1 - Microbial biofilms: Issues and challenges. In H. Sarma, S. Joshi, D. Lahiri, R. R. Ray, & M. Davoodbasha (Eds.), *Microbial biofilms* (pp. 1–17). <https://doi.org/10.1016/b978-0-323-95715-1.00003-0>
- Vickery, K., Deva, A., Jacombs, A., Allan, J., Valente, P., & Gosbell, I. B. (2012). Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. *Journal of Hospital Infection*, 80(1), 52–55. <https://doi.org/10.1016/j.jhin.2011.07.007>
- Virto, R., Mañas, P., Alvarez, L., Condon, S., & Raso, J. (2005). Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and Environmental Microbiology*, 71(9), 5022–5028. <https://doi.org/10.1128/AEM.71.9.5022-5028.2005>
- Wang, L., Forsythe, S. J., Yang, X., Fu, S., Man, C., & Jiang, Y. (2021). Invited review: Stress resistance of *Cronobacter* spp. affecting control of its growth during food production. *Journal of Dairy Science*, 104(11), 11348–11367. <https://doi.org/10.3168/jds.2021-20591>
- Wang, X.-y., Li, P., Du, X.-j., & Wang, S. (2024). Effect of glutathione-transport-related gene gsiD on desiccation tolerance of *Cronobacter sakazakii* and its related regulatory mechanism. *Applied and Environmental Microbiology*, 90(2). <https://doi.org/10.1128/aem.01562-23>. e01562-23.
- Weavers, L. K., Wickramanayake, G. B., Shifrin, M., & McDonnell, G. (2020). Disinfection and sterilization using ozone. In G. McDonnell, & J. Hansen (Eds.), *Block's disinfection, sterilization, and preservation* (6th ed., pp. 684–701).
- Yan, Y., Cao, M., Ma, J., Suo, J., Bai, X., Ge, W., Lü, X., Zhang, Q., Chen, J., Cui, S., & Yang, B. (2025). Mechanisms of thermal, acid, desiccation and osmotic tolerance of *Cronobacter* spp. *Critical Reviews in Food Science and Nutrition*, 65(29), 6569–6591. <https://doi.org/10.1080/10408398.2024.2447304>
- Zhu, D., Fan, Y., Wang, X., Li, P., Huang, Y., Jiao, J., Zhao, C., Li, Y., Wang, S., & Du, X. (2023). Characterization of molecular chaperone GroEL as a potential virulence factor in *Cronobacter sakazakii*. *Foods*, 12(18), Article 3404. <https://doi.org/10.3390/foods12183404>
- Ziech, R. E., Perin, A. P., Lampugnani, C., Sereno, M. J., Viana, C., Soares, V. M., Pereira, J. G., Pinto, J. P. A. N., & dos Santos Bersot, L. (2016). Biofilm-producing ability and tolerance to industrial sanitizers in *Salmonella* spp. isolated from Brazilian poultry processing plants. *LWT-Food Science and Technology*, 68, 85–90. <https://doi.org/10.1016/j.lwt.2015.12.021>