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Plasma-activated liquids for mitigating biofilms on food and food contact surfaces

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Abstract

Plasma-activated liquids (PALs) are emerging and promising alternatives to traditional decontamination technologies and have evolved as a new technology for applications in food, agriculture, and medicine. Contamination caused by foodborne pathogens and their biofilms has posed challenges and concerns to the food industry in terms of safety and quality. The nature of the food and the food processing environment are major factors that contribute to the growth of various microorganisms, followed by the biofilm characteristics that ensure their survival in severe environmental conditions and against traditional chemical disinfectants. PALs show an efficient impact against microorganisms and their biofilms, with various reactive species (short- and long-lived ones), physiochemical properties, and plasma processing factors playing a crucial role in mitigating biofilms. Moreover, there is potential to improve and optimize disinfection strategies using a combination of PALs with other technologies for the inactivation of biofilms. The overarching aim of this study is to build a better understanding of the parameters that govern the liquid chemistry generated in a liquid exposed to plasma and how these translate into biological effects on biofilms. This review provides a current understanding of PALs-mediated mechanisms of action on biofilms; however, the precise inactivation mechanism is still not clear and is an important part of the research. Implementation of PALs in the food industry could help overcome the disinfection hurdles and can enhance biofilm inactivation efficacy. Future perspectives in this field to expand existing state of the art to seek breakthroughs for scale-up and implementation of PALs technology in the food industry are also discussed.

KEYWORDS
biofilm, cold plasma, decontamination, food, inactivation mechanism, plasma-activated liquid

1 | INTRODUCTION

In the last few decades, many countries have reported a significant increase in the number of safety-related illnesses caused by foodborne microorganisms. According to the World Health Organization (WHO), illnesses caused by foodborne agents (bacteria, parasites, and prions) have sickened more than 600 million people and led to the death of more than 420,000 people each year (WHO, 2022). Food contamination can occur at any stage of the food production process from "farm" to "table" and is caused by microorganisms (bacteria, fungi, viruses, parasites, and prions) that grow on food substrates and food contact surfaces/food processing equipment. Even though a series of operational norms and management systems for food hygiene and safety has been established, biofilm-related food safety incidents are still rising worldwide (Kamboj et al., 2020). This could be partly due to the lack of a management system and ineffective supervision of the disinfection procedures, and the lack of standardization of cleaning procedures (Carrascosa et al., 2021). The nature of the food and the food processing environment are major factors that contribute to the growth of various microorganisms, ultimately leading to the formation of a consortium of microorganisms in complex extracellular polymeric substances (EPS) called "biofilms" (Donlan, 2002). In the food industry, surfaces of processing equipment are susceptible to biofilm-forming microorganisms, acting as a source of cross-contamination, thus reducing the effectiveness of food processing strategies and compromising food quality and safety (Galié et al., 2018). In contrast, various biofilms formed by beneficial microorganisms have been explored to improve the quality and safety of the food processing process (Alvarez-Ordóñez et al., 2019).

Biofilms are formed by aggregations of multiple microbial species in extracellular matrices (containing exopolysaccharides, extracellular nucleic acids [eDNA and eRNA], proteins, lipids, and other biomolecules) of different compositions, and their characteristics are determined by the food production environment and the settled species that are adherent to each other and/or a surface. Through intercellular interactions, the biofilm lifestyle is clearly distinct from that of bacterial cells living outside the community, which means that the biofilm community has new properties that are not predictable from the study of free-living bacterial cells (Vert et al., 2012). It has been established that more than one bacterial species can be found in the biofilm matrix, which facilitates the formation and attachment to food contact surfaces. This is particularly true for some species that have been found to be able to form biofilms without the presence of specialized fimbriae (Galié et al., 2018). Biofilms are highly resistant to disinfection treatments using chemical disinfectants, such as chlorine, quaternary ammonium compounds, and other biocides (Giaouris et al., 2015).

2 | BIOFILMS AND THEIR CONTROL

The biofilms are complex clusters of microorganisms that may consist of diverse microbes including, fungi, algae, bacteria, protozoa, and yeast, attaching to each other by a self-produced matrix called the EPS. Biofilms have the ability to develop on any surface (biotic or abiotic surfaces) in different habitats through attachment. The ability of biofilm formation is said to be an adaptable survival mechanism of the planktonic cells against biocides and harsh environments. Thus, the biofilms are complex and secrete extracellular matrix after attaching to food contact surfaces, many of which are highly resistant to various bactericidal treatments.

2.1 | Biofilm formation

Biofilm formation is a dynamic and complex process involving different steps like attachment, proliferation, maturation, and dispersion (Figure 1). The biofilm-forming ability of microorganisms can significantly impact their capability of persistent colonization in food processing environments. Reversible or irreversible attachment of the biofilms is governed by the characteristics of the substrate/solid surface, cell surface (hydrophobicity and net charge), surrounding environmental conditions (pH, temperature, nutrient composition), and genetic regulation of bacteria (Shi & Zhu, 2009). All stages of biofilm development are influenced by quorum-sensing mechanisms or cell-to-cell communications that regulate a wide variety of functions such as control of virulence, genetic competence development, conjugative plasmid transfer, sporulation, biofilm formation, production of antimicrobial peptides, and symbiosis (Bai & Rai, 2011). In the quorum sensing system, fluctuations in cell density are detected by the recognition of small, secreted signaling molecules, the expression of specialized cell functions responsible for the initial attachment to the target surface, subsequent growth, and the maturation of biofilms (González & Keshavan, 2006). Therefore, quorum sensing enables bacteria to exhibit a coordinated response that is advantageous to the population and also improves nutrient uptake, the ability to occupy more hospitable environmental niches, and the ability to respond to environmental challenges and competing microorganisms (Annous et al., 2009). Quorum sensing in biofilms has been elaborately discussed in a recent review by Mukherjee and Bassler (2019). It is

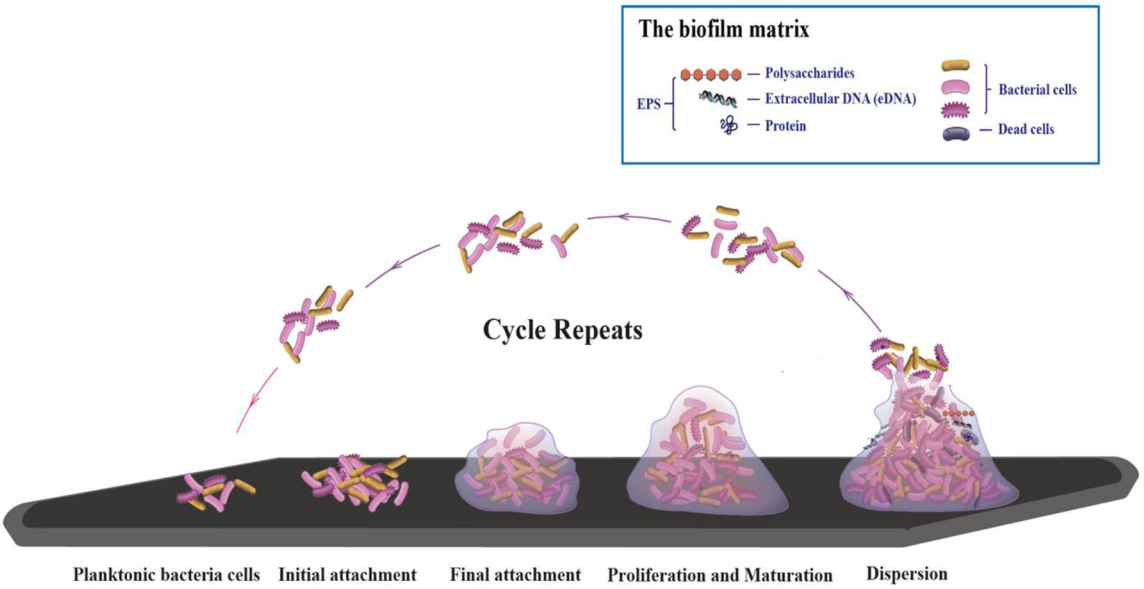

FIGURE 1 Graphical representation of biofilm formation on the food contact surfaces.

commonly accepted that biofilm formation is the result of a cooperative effort/synergy between strains and species, where they work together in formation and metabolism gaining mutual benefits (Oliveira et al., 2015). Microorganisms can activate niche-specific functions and form biofilm in response to food components; on the other hand, they can respond to processing condition changes during production are also those with the ability to survive and persistent on production surfaces through colonization (Alvarez-Ordóñez et al., 2019). Bacteria of various genetic backgrounds within the biofilm contribute to the formation of the complex consortia of biofilms. It is common for cells in biofilm to have competition and cooperation with other cells and such a relationship would further help maintain the biofilm structure (Giaouris et al., 2015). As a matter of fact, the biofilm-forming ability of bacteria is directly influenced by bacteria strains within the biofilm; even tiny genetic variations within one species can significantly impact the biofilm attachment capability and the structure of biofilm communities. However, as the key regulators of the complex microbial communities, bacteria demonstrate collaborative behavior in foraging, building, reproducing, dispersing, and communicating (Crespi, 2001).

Biofilm is a common source of contamination for food contact surfaces, food surfaces, and water. Extensive research has been conducted on the interaction between common foodborne pathogens and other food-related bacteria or microbiota that existed in the food processing environments based on dual-species and multispecies biofilm models (Maggio et al., 2021; Ripolles-Avila et al., 2022). Some bacterial pathogen strains with weak biofilm formation ability can multiply on food contact surfaces or food surfaces by interacting with bacteria that have strong biofilm production ability (i.e., synergistic effect) (Chitlapilly Dass et al., 2020). It should be noted that food processing environments may facilitate the proliferation of microorganisms and some persistent bacteria may be able to survive harsh conditions during food processing, further increasing the possibility of food product contamination (Larsen et al., 2014). Some food-related factors may have a considerable impact on the structure of biofilms, such as the concentration of nitrous components in food, oxygen availability, food substrates, the concentration of carbohydrates, hydrodynamic conditions, and so forth (Heir et al., 2018). However, these influences are changeable due to differences in the bacterial strains. It has been shown that mixed biofilm consortia such as *Listeria monocytogenes* and *Salmonella* Typhimurium can cross-contaminate food surfaces, whereas multidrug-resistant organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* can grow on medical equipment and are frequently quite resistant to conventional cleaning techniques, increasing the risk of chronic infections. This has inspired researchers to look for alternatives to traditional antibiotics that target and universally destabilize various biofilm communities and their matrix components, rendering the biofilms more vulnerable to antimicrobials (Roy et al., 2018).

2.2 | Biofilm control in industrial settings

Contaminations caused by foodborne pathogen-formed biofilms have posed considerable challenges and concerns

to the food industry in terms of food safety and quality. Biofilm-induced contamination can occur at any point in the lifecycle of food products, which makes prevention/control measures vital for keeping food safety (Tang et al., 2011). A number of methods have been employed during production or storage, including thermal treatment, chemical detergents, low-temperature storage, irradiation, and so forth in order to prevent microbial contamination (Amit et al., 2017; Brooks & Flint, 2008). In contrast with planktonic cells, biofilms have been regarded as more resistant to cleaning and sanitizing chemicals. Food safety may be compromised by inadequate sterilization operations when microorganisms attach to food contact surfaces in the form of biofilms (Tang et al., 2011). Chemical detergents can be very effective in controlling biofilms by breaking down the polysaccharide matrix instead of inactivating the bacterial cells (Brooks & Flint, 2008). The selection of disinfecting methods in the industry depends on the efficacy, safety, and instability of the disinfectants, the stage of application as well as where it is corrosive or affects the sensory values of the products manufactured (Kakurinov, 2014; Wirtanen & Salo, 2003). Extracellular DNA (eDNA) plays a vital role in the initial attachment and later aggregation of planktonic cells, both of which are critical steps for EPS production. Enzymes such as protease and DNase have been employed for inhibiting EPS production, both of them can be implemented alone or in combination with other sanitation strategies for achieving higher inactivation efficiency on microbial cells within biofilms (Fang et al., 2021; Kim & Kim, 2022). Studies have shown that enzymes such as proteinase K (Nguyen & Burrows, 2014), lipases (Seghal Kiran et al., 2014), and carbohydrate-degrading enzymes (such as β-glucans and α-amylase) (Araújo et al., 2017) are effective in biofilm removal by attacking the main components of the biofilm matrix. Furthermore, enzyme cleaning/treatment was significantly more effective than conventional cleaning or clean-in-place treatments in removing aerobic counts and biofilms from food contact surfaces (Delhelle et al., 2020; Lequette et al., 2010). Studies have shown that control methods such as the application of essential oils, organic acids, bacteriocins, bacteriophages, quorum sensing inhibitors, and photosensitizers (Table 1) have the potential to be used as antibiofilm agents in the food industry. Emerging/novel technologies such as gas plasma technology, magnetic fields, ultrasound, UV, ozone treatments, and so forth also have been found to be effective in inactivating microorganisms on food or food contact surfaces during food production (Table 2). An increasing number of bacterial strains that are not inactivated by antimicrobial agents (chemical detergents and antibiotics) continue to exist having higher resistance, leading to increased health and safety problems (Kampf, 2018; Rozman et al., 2021). Therefore, there is a need for a safe, easy-to-deliver elimination method that does not cause severe microbial resistance. Recent studies on plasma technology have shown good biofilm inactivation efficiency without developing significant resistance (Mai-Prochnow et al., 2021).

3 | PLASMA-ACTIVATED LIQUIDS

Plasma is referred to as "the fourth state of matter," where an increase in the material's energy levels converts its state from solid to liquid to gas and ultimately to an ionized state of the gas, "plasma," which exhibits a high reactive environment (Misra et al., 2016). Cold atmospheric plasma (CAP) is a nonthermal plasma, consisting of electrons at higher temperatures and heavy particles at room temperature, which is <40°C at the point of application (Hoffmann et al., 2013). It is a promising tool and has gained increasing interest in the food industry because it is green, has no residue after treatment, as well as does little harm to the sensory properties of food products (Niemira, 2012; Chen et al., 2020; Jiang et al., 2020). Plasma-activated liquids (PALs) are generated when atmospheric plasma encounters liquid such as water, known as plasma-activated water (PAW), or sodium chloride, sodium hypochlorite, buffers, and media (Bourke et al., 2017; Esua et al., 2022a,b,c; Ali et al., 2022). It is gaining interest in the field of agriculture and medicine due to its antimicrobial potential and its eco-friendly nature. Compared with CAP treatment, PALs can easily reach the samples in areas that are difficult for gas plasma to reach (Ali et al., 2021a; Esua et al., 2021; Liao et al., 2020;). Compared with other chemicals, PALs leave fewer residues after treatment, which lowers their impact on the environment. On the other hand, it is safer as it does not require the transportation and storage of potentially hazardous chemicals (Bourke et al., 2017).

3.1 | PALs generation

The generation mode of PAW can be divided into three categories: direct discharge of the electrode underneath the liquid surface, discharge above the liquid, and multiphase discharge, such as liquid mist or vapor in contact with the plasma. Over the years, researchers have developed various plasma generation systems for different applications, such as plasma jets, gliding arc discharge, plasma needles, plasma pencils, dielectric barrier discharge (DBD), and surface micro-discharge (Ali et al., 2021b; Pan et al., 2022; Scholtz et al., 2015; Wu et al., 2022). PALs have been found to be a promising antimicrobial agent that could effectively inactivate bacterial cells on various contact surfaces and food surfaces, resulting from the formation of

TABLE 1 Different control methods and their mechanism for the inactivation/eradication of biofilms.

Control methods		Mode of action	References
Enzymatic disruption	Cellulases	Degradation of cellulolytic bacteria that form biofilm on the substrate.	Deng & Wang, 2022; Li et al., 2022
	Proteinases	Breaking down peptide bonds within proteins causing substrates to break into shorter fragments, and eventually into amino acids.	Kim & Kim, 2022; Mahdi & Hasan, 2022
	DNAses	Extracellular DNA disruption	Fang et al., 2021; Hu et al., 2022
Biosurfactants (fengycin, viscosin, arthrofactin, surfactin, sophorolipids, and iturin)		Acting as cell envelope-modifying or anti-matrix molecules.	Gharaei et al., 2022; Tambone et al., 2021
Bacteriophages (UPF_BP1, UPF_BP2, UPF_BP3, asTrsa205,Trsa207, Trsa220, and Trsa222)		Prevent biofilm formation and disperse attached biofilms by penetrating into biofilms and generating depolymerases (enzymes that can degrade biofilm enzymes encoded by phage).	Stachler et al., 2021; Webber et al., 2022
Essential oils (carvacrol oil, citrus oil, clove oil, leaf essential oil, cinnamon essential oil, lemongrass oil, oregano oil, *Cymbopogon citratus* oil, limonene, and linalool)		Regulation of genes and proteins implicated in motility, Quorum sensing, and EPS matrix. Essential oils interact with different cellular constituents, affecting the bacterial membrane, leading to the losses of intracellular constituents, including DNA, protein, and ATP.	Campana et al., 2017; Cui et al., 2018
Plant extracts (phenolic acids [ferulic and salicylic acid])		Suppressing the expression of the genes within the biofilms in charge of its virulence that is important for motility, adhesion, and invasion.	Lahiri et al., 2021; Zhang et al., 2020
Organic acids (such as lactic acids, malic acids, acetic acids, and cinnamic acids)		Possible mechanism of act, metabolic activities inhibition, or chain length alternation; acidifying bacterial cells because of the penetration of weak organic acids; damaging outer membrane or cytoplasmic membrane, inhibiting macromolecule synthesis, and breaking DNA (Akbaş, 2016).	Panichikkal & Edayileveetil Krishnankutty, 2020; Sullivan et al., 2020)
Bacteriocins (nisin, nisin U, lactacin, pediocin PA-1/AcH, garvicin KS, and micrococcin P1)		Targeting cell envelope-associated processes, impeding initial cell adhesion, and biofilm formation.	Melian et al., 2022; Qiao et al., 2021
Coatings	Nanoparticles (such as Ag^{2+}, Fe_3O_4, TiO_2, ZnO, CuO, and MgO)	Bacterial cell damage caused by reactive species generated by nanoparticles.	Khelissa et al., 2021; Muraca et al., 2021
	Repelling surfaces (physical topography, chemical molecules, and their synergism)	Limiting the initial adhesion of bacteria to the surface.	Huang et al., 2020; Leulmi Pichot et al., 2020
Quorum sensing inhibition (furanone, patulin/clavin, rosmarinic acid, oxidoreductase, lactonase)		Enzymatic degradation or quorum sensing-controlled virulence factors inhibition; inhibition of quorum sensing signal cell syntheses; motility inhibition.	Luciardi et al., 2020; Nahar et al., 2021

(Continues)

TABLE 1 (Continued)

Control methods	Mode of action	References
Photosensitizers (riboflavin, curcumin, erythrosine B, caffeic acid, and phloxine B)	Generation of reactive oxygen species exerts a lethal effect on the microbe especially by disrupting the biofilm by damaging the cell membrane and DNA and by protein/enzyme inactivation.	Gulías et al., 2020; Kim et al., 2021
Chemicals	Disruption of the bacterial plasma membrane, which leads to metabolite leakage and cell lysis.	Kocot & Olszewska, 2020; Liu, Wu, et al., 2021
Emerging technologies		
Ultrasound	Cell membrane damage due to cavitation Hotspot formation DNA damage	Su et al., 2022; Wu et al., 2021
Irradiation	DNA damage Membrane permeability change	Pang et al., 2022; Zhang et al., 2021
Ultraviolet light	DNA damage Enzyme activity change Damage to call membrane integrity	Binns et al., 2020; Chen et al., 2020
Pulsed electric field	Cell membrane disorganization Alternation of protein channels	Huiszoon et al., 2021; Martins Antunes de Melo et al., 2021
Pulsed light	DNA damage Cell nuclei acids and proteins change Cell membrane disruption Cytoplasm shrinkage	Liu, Hu, et al., 2021; Shi et al., 2022
Electromagnetic field	Thermal destruction with high frequency (>100 kHz)	Bujňáková et al., 2022; Ciecholewska-Juśko et al., 2022
Ozone	Membrane damage Biofilm cell components oxidation DNA damage	Piletić et al., 2022; Sun et al., 2022
Cold plasma gas	Oxidation of lipid membrane Pore formation Enzyme activity change DNA damage	Okebiorun et al., 2022; Zipprich et al., 2022

reactive species when plasma interacts with water in the gas phase (Bourke et al., 2017; Esua et al., 2021; 2022d). For air plasmas, the resulting PALs contain high levels of reactive oxygen and nitrogen species (RONS) such as hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2), ozone (O_3), superoxide (O_2^-), nitric oxide (NO•), peroxynitrite (ONOOH), ammonium ion (NH_4^+), nitrites, and nitrates (Khlyustova et al., 2019). Plasma treatment of water can create an acidic environment that may contribute to the antimicrobial properties of microorganisms. PAW obtained by exposing water to air-based plasma discharge, containing multiple types of RONS, has exhibited their role in bacterial inactivation (Mai-Prochnow et al., 2021). During plasma discharge, various species are generated in the gaseous phase, and the generation of RONS is related to the energy generated by the collision between fast-moving electrons and neutral particles. Immediately after the collision, short-lived primary reactive species are generated in the gas phase, including electrons (e^-), ionized neutrals and gas (M^+), atomic nitrogen (N), atomic oxygen (O), atomic hydrogen (H), nitric oxide radical (·NO), and superoxide anion radical ($O_2^{\bullet-}$) (Lamichhane et al., 2020). When these reactive species come in contact with the liquids, numerous long-lived reactive species are formed and the gaseous RONS are transported through the plasma–liquid interface and induce the formation of secondary aqueous RONS in the water. The origin and generation of RONS in PAW have been thoroughly discussed in a recent review by Zhou et al. (2020). PALs have been proposed to be an alternative to conventional disinfectants such as chlorine solution, peracetic acid, and hydrogen peroxide in food industries for the clean-in-place procedure to reduce the contamination of processed foods (Kocot & Olszewska, 2020; Liu, Wu, et al., 2021).

TABLE 2 Emerging technologies for eradication of biofilms on different surfaces.

Biofilm-producing pathogens	Antibiofilm method	Biofilm forming surface	Reduction efficiency	Reference
Photosensitizers				
Streptococcus mutans	Fagopyrin F-rich fraction (FFF)	Well plates	FFF (5 μg/mL), 10 min, blue light (450 nm; 10 J/cm^2), 97.6% of reduction	Kim et al., 2021
Ceftazidime-resistant *Pseudomonas aeruginosa* and *Escherichia coli*	Meso-imidazolyl porphyrins (TMPyP)	24-well flat-bottom polystyrene plates	5 nM TMPyP and 5 J/cm^2 treatment achieved 6.9-log reduction of biofilm counts.	Vinagreiro et al., 2020
Escherichia coli ATCC 25922 and ATCC 35218	Methylene blue (MB)	96-well polystyrene plates	78 μM methylene blue, red light (625 nm, 18 J/cm^2), *Escherichia coli* ATCC 25922 achieved a 3-log reduction.	Gulías et al., 2020
Ultrasound				
Pseudomonas aeruginosa	High-intensity focused ultrasound	Petri dish		Bharatula et al., 2020
Staphylococcus epidermidis	Low-intensity continuous and pulsed wave ultrasound (US)	Six-well plates	US with pulsed waves for 20 min treatment twice inhibited 33.6% of cells 20 min of single US treatment caused 17.9% reduction.	Koibuchi et al., 2021
Listeria monocytogenes 10403S	Ultrasound	Stainless chip	Power ultrasound (20 kHz, 100% amplitude, 120 W) achieved 3.8-log CFU/mL reduction after 60 s.	Baumann et al., 2009
Streptococcus mutans	280 kHz, 1 MHz, and 2 MHz; intensity output 0—3.00 W	Polypropylene mesh	280 kHz, removed >80% of biofilm after 30 min of US exposure.	Bigelow et al., 2017
Ozone				
Listeria monocytogenes	Gaseous ozone	Six-well polystyrene culture plates	50 ppm of gaseous ozone, 3.7 ± 0.4- and 3.9 ± 0.4-log CFU/mL after 10 and 30 min, respectively.	Panebianco et al., 2021
Listeria monocytogenes Scott A and 10403S	Aqueous ozone	Chips	Strain Scott A: 4.00 ppm O_3 achieved complete elimination at 8.07-log reduction CFU per chip for planktonic cells. A 16-fold increase in sanitizer concentration was required to destroy biofilm cells of *L. monocytogenes* versus planktonic cells of Strain 10403S: a concentration of 4.00 ppm O_3 led to a 7.47-log reduction CFU per chip.	Robbins et al., 2005
Plasma gas				
Candida albicans SC5314	kINPen09 plasma jet	96-well plate	A maximum of 2.0-log reduction after 300 s of plasma treatment, a 39% reduction after 30 s of plasma treatment; 41% of reduction at 30 s and by 89% of reduction at 60 s on the metabolic activity of biofilms.	Handorf et al., 2018

(Continues)

TABLE 2 (Continued)

Biofilm-producing pathogens	Antibiofilm method	Biofilm forming surface	Reduction efficiency	Reference
Listeria monocytogenes and *Salmonella* Typhimurium	Dielectric Barrier Discharge (DBD) plasma system	Polystyrene petri dishes	DBD configuration results in the highest log-reduction (up to 3.6-log CFU/cm^2). Without oxygen, the highest log-reduction is obtained (up to 3.6-log CFU/cm^2). Increasing the input voltage results in increased log-reduction (up to 3.6-log CFU/cm^2). Cold atmospheric plasma treatment induces sublethal injury to biofilm cells.	Govaert et al., 2019
Irradiation				
Pseudomonas fluorescens	Low-energy X-ray irradiation	Stainless steel	Low-energy X-ray irradiation at 125 Gy inactivated 4.21-log CFU/cm^2 for *P. fluorescens* biofilm cells.	Pang et al., 2022
Salmonella Typhimurium	X-ray, X-ray + aqueous chlorine dioxide	Quail egg shells	The synergistic reduction of biofilms after X-ray/ClO_2 treatment on the egg shells was 0.1–1.1-log CFU/egg. The largest reduction was 1.1-log CFU/cm^2 after a 2.0 kGy X-ray and a 20 ppm ClO_2 treatment.	Park et al., 2018
Salmonella Enteritidis, *Pseudomonas fluorescens, Salmonella* Enteritidis + *Pseudomonas fluorescens*	Low-energy X-ray	Shell eggs	5-log reduction, 225 and 150 Gy of irradiation was required for *S. enteritidis* and *P. fluorescens*, respectively. Co-culture with *S. enteritidis*: *P. fluorescens* ratio of 1:1 and 1:100 biofilms, only 200 and 175 Gy were required to achieve a 5-log reduction.	Zhang et al., 2021
Electromagnetic fields				
Staphylococcus epidermidis and *Pseudomonas aeruginosa*	Extremely low-frequency electromagnetic field (ELF-EMF)	96-well plates	Some reduction of biofilm formation compared to the nonexposed cultures both at 24 and 48 h for 50 Hz. Not much change observed for 100 Hz treatment.	Karaguler et al., 2017
Escherichia coli (UPEC), *Pseudomonas aeruginosa*, and *Staphylococcus aureus*	Programmable electromagnetic field	Poly(dimethylsiloxane) (PDMS)	*E. coli* (UPEC), *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, with up to 3.7-log of biomass reduction.	Gu et al., 2020
Ultraviolet light				
Candida albicans	254-nm ultraviolet light	Poly(methylmethacrylate) resin	UV-254 nm optical irradiation treatment for 5 s (3.5 mJ/cm^2) decreased 73% reduction of *C. albicans*.	Binns et al., 2020
Chlorine-resistant *Phaeobacter caeruleus*	Ultraviolet light	Polyvinylidene fluoride (PVDF) MF/UF and polyamide RO membranes	Viable *P. caeruleus* was significantly reduced as much as 99.8%.	Cho et al., 2018
Pseudomonas aeruginosa	Ultraviolet LED	Polycarbonate coupons and quartz coupons	UV LED with peak emission at 270 nm achieved <1.5–2.5-log reduction; 6-log reduction for resuspended biofilm bacteria in aqueous solution.	Ma et al., 2022

(Continues)

TABLE 2 (Continued)

Biofilm-producing pathogens	Antibiofilm method	Biofilm forming surface	Reduction efficiency	Reference
Electrochemical treatment				
Streptococcus mutans and *S. aureus*	Direct current (DC)	Acrylic coupons	28 μA/cm^2 of DC for 1 h, the viability of biofilm cells was reduced by about 4- and 5-log for *S. mutans* and *S. aureus*, respectively.	Wang & Ren, 2017
Acidic electrolyzed water				
Listeria innocua	Acidic electrolyzed water	Polypropylene, stainless steel, rubber, and glass surfaces	87.82%, 89.91%, 95.04%, and 97.33% reduction after exposing to concentration of 30, 50, 70, and 120 ppm for 15 min.	Jeon et al., 2018
Nanoparticles				
Multidrug-resistant *Klebsiella pneumoniae*	Silver nanoparticles (AgNPs)	96-well microtiter plates	Minimum inhibition concentration: 62.5 and 125 μg/mL. Minimum bactericidal concentration: 250 and 500 μg/mL. *K. pneumoniae* strain inhibition percentage (AgNP concentration 100 μg/mL): For strain MF953600: 64% For strain MF953599: 86%	Siddique et al., 2020
Pseudomonas aeruginosa	Alginate lyase immobilized chitosan nanoparticles of ciprofloxacin (AgLase-CIPRCH-NPs)	96-well microtiter plates	AgLase-CIPRCH-NPs inhibited around 97% of biofilm at 0.25 μg/mL after 24 h	Patel et al., 2019
E. coli ATCC 25922, *P. aeruginosa* PAO1, and *S. aureus* MTCC 3160	Titanium dioxide nanoparticles (TiO_2-NPs) synthesized using *Carum copticum* extract	Glass surface	*E. coli* ATCC 25922, *P. aeruginosa* PAO1, and *S. aureus* MTCC 3160 were inhibited by 60.09%, 64.14%, and 48.30%, respectively.	Altaf et al., 2021
Escherichia *coli* and methicillin-resistant *Staphylococcus aureus* (MRSA)	CPP nanozyme (mimicking substrate-specific peroxidase, from CDs@PtNPs [CPP] nanoflare by integrating recognition/transduction carbon dots [CDs] with platinum nanoparticles [PtNPs])	Eight-well plates	MRSA-against antibacterial CPP nanozyme increased from 37.4% to 91.1%; *Escherichia coli* was reduced by 92%.	Liang et al., 2020
Hydrodynamics				
Chlorella vulgaris (microalgae)	Hydrodynamics	A pilot-scale flat-panel photobioreactor and laboratory tubular system	Completely removal with the wall shear stress of 53 Pa.	Belohlav et al., 2020
Surface waves				
E. coli (ATCC) 25922GFP	Surface waves	Six-well microplates	Biofilm formation is strongly affected by the flows in thin layers of bacterial suspensions controlled by surface waves.	Hong et al., 2020

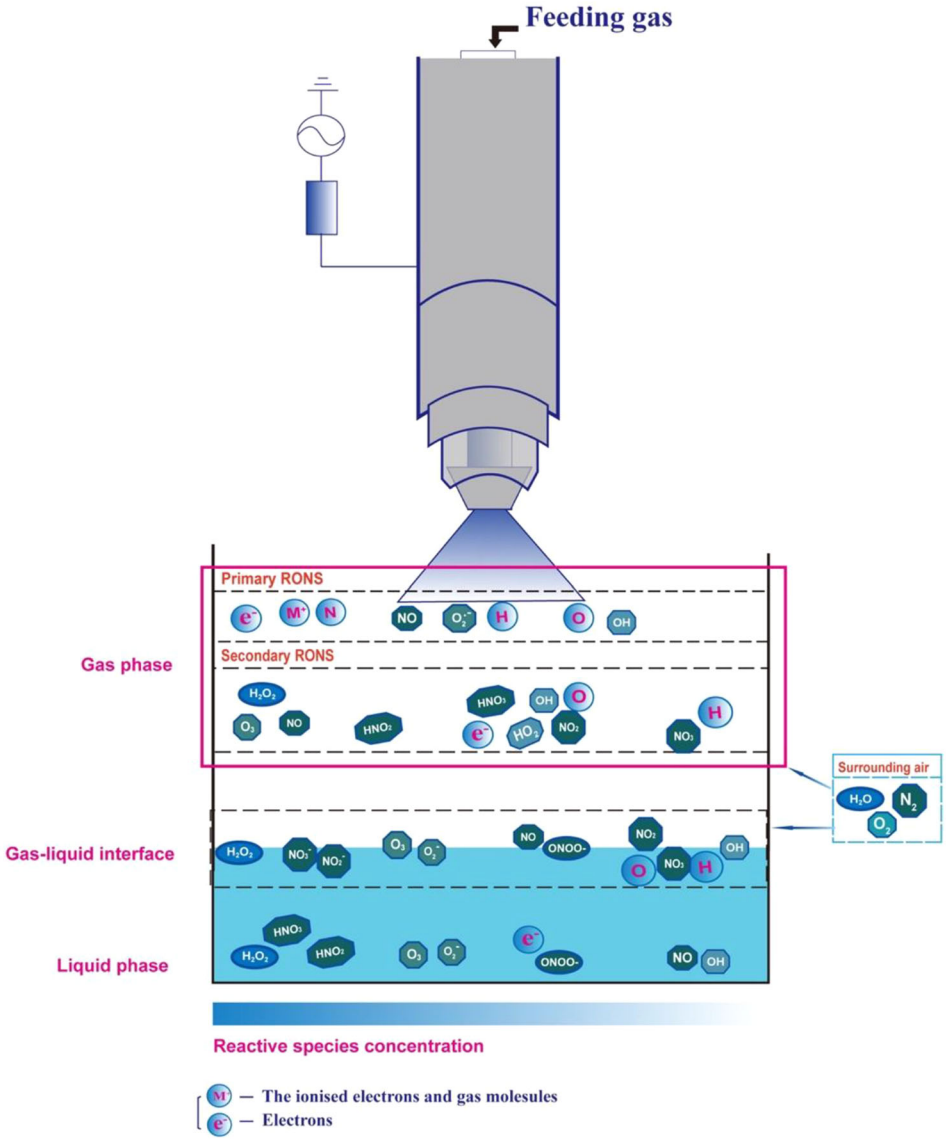

FIGURE 2 Formation of reactive species in plasma activated liquid solutions.

3.2 | Physiochemical properties of PALs

A wide variety of complex chemical reactions happen at the gas–liquid interface when gas plasma comes in contact with liquid (Figure 2), creating various RONS and significant alternations in the pH, oxidation–reduction potential (ORP), and electrical conductivity (EC) of the plasma-treated solutions. A more detailed and elaborate discussion has been made in a recent review by Zhao et al. (2020). Briefly, in PALs, RONS are produced in three phases. First in the gas phase, short-lived reactive species are formed (•OH, •NO, •H, •O, and •N). These radicals can further interact with each other or combine with surrounding gases, which leads to the formation of secondary species (including H_2O_2, NO_2, NO_3, and O_3). At the gas–liquid interface, the reactive species generated in the gas phase come in contact with the evaporated water molecule, leading to the generation of •NO, HNO_3, HNO_2, •OH, O, H, O_3, H_2O_2, and so on (Samukawa et al., 2012). In the liquid phase, reactive species generated at the gas–liquid interface dissolve in the liquid solution, and some of them interact with water molecules leading to the formation of reactive species, for example, hydrogen peroxide (H_2O_2), nitrate (NO_2), nitrite (NO_3), ozone (O_3), peroxynitrite anion (ONOO), and peroxynitrous acid (ONOOH), and the primary gaseous RONS are transported through the plasma–liquid interface and induce the formation of secondary aqueous RONS in the water and so on (Machala et al., 2019). The generating mechanism/reactions of these are presented in Table 3. It should be noted that the plasma apparatus, the feeding gas, and the liquid solution all contribute to the composition and concentration of the

TABLE 3 Generation mechanism of reactive species in plasma-activated water (PAW).

Reactive species	Generation mechanisms
Singlet oxygen (O)	$e^- + O_2 \rightarrow 2O + e^- O_2 + H \rightarrow \cdot OH + O$
Hydroxyl radicals ($\cdot OH$)	$e^- + H_2O \rightarrow \cdot OH + \cdot H + e^- e^- + H_2O \rightarrow H_2O^+ + 2e^- H_2O^+ + H_2O \rightarrow \cdot OH + H_3O^+ O_2 + H \rightarrow \cdot OH + O$
Superoxide (O_2^-)	$e^- + O_2 + M \rightarrow O_2^- + Me^- + O_2 \rightarrow O + O + e^-$
Nitric oxide (NO)	$e^- + N_2 \rightarrow N + N + e^- N + O \rightarrow NON + \cdot OH \rightarrow NO + HN + O + M \rightarrow NO + MN + O_2 \rightarrow NO + O_2N + O_3 \rightarrow NO + O_2$
Peroxynitrite ($ONOO^-$)	$O_2 + NO \rightarrow ONOO^- NO_2^- + H_2O_2 \rightarrow ONOO^- + H_2O$
Hydrogen peroxide (H_2O_2)	$\cdot OH + \cdot OH \rightarrow H_2O_2$
Ozone (O_3)	$O + O_2 \rightarrow O_3 + M$
Nitrite (NO_2^-)	$NO + O_3 \rightarrow O_2 + NO_2NO + OH \rightarrow HNO_2HNO_2 \rightarrow NO_2^- + H^+2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + H^+NO + NO_2 + H_2O \rightarrow 2NO_2^- + H^+$
Nitrate (NO_3^-)	$NO_2 + \cdot OH \rightarrow NO_3^- + H^+HNO_3 \rightarrow NO_3^- + H^+NO_2^- + O_3 \rightarrow NO_3^- + O_2NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + H^+ONOOH \rightarrow NO_3^- + H^+$

reactive species generated in PALs. When plasma comes in contact with water, the evaporation of water supports the formation of OH radicals and H_2O_2 in the gas phase. Smet et al. (2019) reported that H_2O_2 was abundantly detected in the PALs, and its concentration increased with the presence of oxygen in the working gas. Additionally, the composition of the reactive species also varies greatly depending on the type of plasma source used (Baik et al., 2013; Thati et al., 2021). According to Smet et al. (2019), during PAW generation with the presence of O_2, more OH radicals could be generated through electron-induced dissociation of atomic oxygen (O), further resulting in an increased H_2O_2 concentration in the liquid phase. During plasma treatment of liquids, NO abundantly exists in the gas phase above the liquid, which can be easily oxidized to form NO_2 and further converted to NO_2^- and NO_3^- in the liquid phase (Lu et al., 2017). In addition, if NO_2^- reacts with H_2O_2 under acidic conditions, nitrate can also be formed. It was also reported that NO_2^- was barely undetectable in the PAL samples and NO_3^- can only be observed at higher concentrations, and different plasma processing factors (i.e., gas composition, generation time, and storage) appeared to have a limited impact on NO_3^- concentrations (Smet et al., 2019).

3.2.1 | Temperature and pH

Plasma activation does not significantly increase solution temperatures (Ercan et al., 2013; Qian et al., 2019; Tian et al., 2015). It has been reported by Ercan et al. (2013) that the temperature of the DBD plasma system was maintained between 23 and 26°C after 3-min treatment in deionized water, phosphate-buffered saline (PBS), or 5 mM *N*-acetylcysteine (NAC) solution. Tian et al. (2015) found that the temperatures of the PAW generated above and beneath the water surface by a plasma microjet for 20 min increased slowly over time, from 25 to 31.5 and 38.1°C, respectively. The thermal effect of the plasma discharge during PAW preparation could be prevented by using a circulating water jacket, which makes PAW an ideal antibiofilm strategy for heat-sensitive foods (Tian et al., 2015).

The pH value of PALs was found to be decreased from neutral to acidic. For example, the pH value of PAW decreased dramatically to 2.5–2.9 after the plasma treatment, and the concentrations of NO_2^- and H_2O_2 were reduced during the 48-h storage, while an increased concentration was observed for NO_3^- (Zhao et al., 2021). The pH reduction is mainly due to the interactions between reactive nitrogen species (RNS), reactive oxygen species (ROS), and the hydrolyzed water molecules, resulting in the formation of nitric and nitrous acids, protons, hydroxyl

radicals, and ONOOH, generated from the NO, NO_2, and NO_x formed in the plasma phase (Oehmigen et al., 2010). In addition, water molecules interacting with H_2O_2 resulted in the generation of acidic hydronium ions (H_3O) in the gaseous or aqueous phase, which might also contribute to the decrease in the pH value. In most cases, in PALs, acidification levels vary depending on the plasma generation system used and are inversely proportional to the treatment time (Los et al., 2020; Smet et al., 2019; Sysolyatina et al., 2020; Tan & Karwe, 2021; Xu et al., 2020). The formation of RONS results in the acidic pH of PAW, and the environmental pH leads to decreased intracellular pH value. When the intracellular pH is below a specific threshold value, almost all the metabolic activities in living microorganisms will be stopped, leading to cell damage and death. Therefore, acidic pH is assumed to play a critical role in microbial inactivation (Oehmigen et al., 2010). Xu et al. (2020) reported that after 60 min of plasma inducement, the concentrations of H_2O_2, NO_2^-, and NO_3^- increased significantly, and a significant drop in the pH value of the solution from 6.86 to 2.56 was observed with increased plasma treatment time (Xu et al., 2020).

3.2.2 | EC and ORP

EC indicates the ability of an aqueous solution to conduct electricity, which depends on the types of ions, their concentrations, and the solution temperature. Many researchers have reported that the EC value of PAW increased dramatically with the activation time, indicating the generation of specific reactive ions as a result of plasma interaction with water (Sysolyatina et al., 2020; Tan & Karwe, 2021; Wu et al., 2017; Zhang et al., 2016). Wu et al. (2017) revealed that the conductivity of plasma-activated H_2O_2 solution simultaneously increased with H_2O_2 concentration. The EC value of PAW is closely related to the feeding gas for plasma discharge. For example, Vlad and Anghel (2017) revealed that the EC of PAW produced by air discharge plasma is higher than that of PAW produced by helium or argon. EC tends to increase as ions accumulate in the solution, which may contribute to the inactivation of microorganisms by perforating their cell membranes.

ORP reflects the oxidization and reduction abilities of solution and is considered a primary factor influencing microbial inhibition (Shen et al., 2016). The ORP of PAW significantly increased with the activation time (An et al., 2019; Sysolyatina et al., 2020). Sysolyatina et al. (2020) revealed that the conductivity of PAW mist condensates increased from 13 to 2730 μS, and the acidizing of water and the increase of ORP and EC can be explained by the accumulation of plasma–chemical reaction products in water, specifically HNO_2, HNO_3, H_2O_2, and HO_2. As Tian et al. (2015) observed, PAW generated beneath the water surface exhibited a higher ORP value than that produced above the water surface. Furthermore, H_2O_2 supplementation could increase the ORP of plasma-activated distilled water due to the formation of various reactive chemical species, such as H_2O_2, O_3, NO_3, NO_2, and ONOOH (Wu et al., 2017). As a result of higher ORP values and reactive species in PAW, damage can occur to the bacterial cell membrane, thereby inactivating the microorganism.

4 | FACTORS INFLUENCING MICROBIAL INACTIVATION EFFICIENCY OF PALs

Many extrinsic and intrinsic factors associated with processing factors and characteristics of microorganisms, respectively, have been reported to influence the antimicrobial efficiency of biofilms by PALs (İbiş & Ercan, 2020; Kelar Tučeková et al., 2021; Los et al., 2020).

4.1 | Processing factors

The inactivation of microbes is influenced by the type of plasma discharge used. Various plasma systems have been used to generate PALs, including DBD, corona discharge, gliding arc discharge, plasma jet, microwave discharge plasma, and so on (Tendero et al., 2006). Among all the plasma sources, DBD and plasma jet are the two most commonly used plasma systems in the research field due to their relatively simple construction and low cost of maintenance. The discharges differ in their electrical characteristics and emissions, inducing various chemical and biological effects that play a role in biofilm decontamination. It is important to know that except for the plasma-generating systems, the characteristic of plasma is also influenced by many other plasma source-related processing factors, such as input power, treatment voltage, mode of treatment, the distance between the plasma plume and the liquid surface, and so forth, all of which make it hard to directly compare the difference when applying to planktonic bacteria or biofilms (Zhao et al., 2020). However, it is possible to draw some general conclusions regarding the effect of processing factors on the efficacy of PALs for the removal of biofilms. In addition to the plasma system itself, other factors like the generation method of PAW (plasma discharge from the gas phase or directly in the liquid), the volume, temperature and water source (such as tap water and distilled water), and the storage time of PAW also affect the inactivation efficiency of biofilms (Wang & Salvi, 2021).

4.1.1 | Voltage, working gas, and relative humidity of air

Plasma generation parameters, including plasma voltage, input power, frequency, and so forth, directly influence the input energy of the plasma system, thus affecting the inactivation efficacy of PALs. Input power is an important factor that is directly related to the physiochemical characteristics of PALs, that is, the composition of reactive species generated in PAW, further contributing to the inactivation of biofilm cells. Kelar Tučeková et al. (2021) used a peak-to-peak voltage and discharge current change with increasing input powers of 30, 50, and 100 W to investigate the inactivation ability of PAW and water vapor on various bacterial biofilms formed on polypropylene nonwoven textile coupons. Results indicated that increased input power reduced the *S. aureus* biofilm, with only 0.5-log more than that of non-plasma-treated water vapor.

Another processing parameter that determines the inactivation efficacy of PALs for biofilm removal is the selection of feeding gases. A number of gases have been applied for the generation of PALs, such as ambient air (humid or dried air), oxygen (O_2), nitrogen (N_2) (Seo et al., 2019), carbon dioxide (CO_2), noble gases like helium (He) (Chen et al., 2016) and Argon (Ar) (Vlad & Anghel, 2017), or a mixture of different gases (Hong et al., 2021; Smet et al., 2019). Several studies reported that feeding gases impact the PAW and RONS composition, where the concentrations of H_2O_2, NO_3^-, and NO_2^- and pH values determine the antimicrobial efficacy of PAW (Machala et al., 2019; Vlad & Anghel, 2017). In a study conducted by Vlad and Anghel (2017), the chemical properties of PAW were compared using He, Ar, and air as working gases, and the results indicated that argon-discharged PAW contained higher concentrations of H_2O_2, while air-discharged PAW contained higher levels of RNS, acidic pH, and greater EC. Although it is difficult to conclude which working gas is most effective in PALs inactivation of biofilms, many studies have demonstrated that the addition of small amounts of oxygen to noble gases could enhance the antimicrobial activity of PALs, and when the ambient air is used as working gas, greater inactivation efficacy can usually be achieved as compared to noble gases (Li et al., 2015). Yang et al. (2021) used synthetic air (79% N_2 + 21% O_2)-produced plasma-activated saline (PAL) for the inactivation of methicillin-resistant *S. aureus* (MRSA) biofilms formed on silica films. The treatment of PALs alone achieved an approximately 1.2-log reduction of MRSA cells in the biofilm. Zhang et al. (2012) reported that a higher inactivation rate on *S. aureus* biofilm cells was achieved when using $Ar/O_2(2\%)$, followed by $Ar/O_2(2\%)/N_2(10\%)$ and pure Ar. In addition, the SEM images of PAW-treated *S. aureus* showed that the morphological damage of *S. aureus* was dependent on the working gas, which was consistent with the inactivation effect.

In addition to gas composition, the effect of relative humidity has also been investigated and it was observed that the humidity of input gas can be a factor that influences the antimicrobial efficiency of PALs (Kamgang-Youbi et al., 2009; Kelar Tučeková et al., 2021; Sysolyatina et al., 2020). It can be found that humid air can be used to improve the effect of plasma-activated gaseous media and the generation of OH radicals and higher relative humidity may lead to the formation of a higher amount of OH radicals that have been proven to be able to cause oxidative damage to nucleic acids and proteins. Kelar Tučeková et al. (2021) developed a device for plasma activation of flowing gas mixtures with high water vapor concentrations, which enables plasma chemical splitting of pure water vapor molecules for the generation of OH radicals and their products, for example, H_2O_2, without stabilizing gas admixtures and toxic byproducts.

4.1.2 | Plasma treatment time, distance, and exposure time

In addition to the plasma system itself, plasma treatment time for PAW generation is closely related to the biofilm inactivation efficacy of PAW. Higher bacterial biofilm inactivation rates were achieved by longer treatment time due to greater physicochemical changes of PALs, including acidity and higher concentration of reactive species (Ercan et al., 2013; Handorf et al., 2020; Hong et al., 2021; Joshi et al., 2010; Los et al., 2020; Seo et al., 2019; Smet et al., 2019; Zhou et al., 2019). Smet et al. (2019) mentioned that the highest PALs efficacy was obtained for a generation time of 30 min for both *S.* Typhimurium biofilms (3.9-log reduction) and *L. monocytogenes* biofilms (3.2-log reduction), which was higher than those PALs with 10 and 20 min activation time. In another study, different plasma treatment times (1–5 min) were applied to PBS and saline solutions resulting in increased H_2O_2 concentration from 10 to 30 and 25 to 41 mg/L for plasma-activated PBS and PALs, respectively (Hong et al., 2021). Ghimire et al. (2017) investigated the effect of various distances (from 1 to 4 mm) on the formation of RNS and OH species close to the water surface, and results showed that the OH concentration was reduced (from 6.10×10^{15} to 1.35×10^{15} cm^{-3}), when the gap distance was increased (from 1 to 4 mm).

Another factor affecting the antimicrobial effectiveness of PAW is the exposure time of biofilms to PAW. Similar to plasma treatment time, the longer exposure time achieved a higher level of biofilm removal rate (Handorf et al., 2020, 2021; Kelar Tučeková et al., 2021; Kim et al., 2018; Kovalova et al., 2016; Los et al., 2020; Smet et al., 2019;

Sysolyatina et al., 2020). Longer exposure time often achieves a higher antimicrobial rate due to the continuous generation of long-living reactive species in PAW. For instance, Smet et al. (2019) studied the influence of exposure times (5, 10, 25, or 30 min) of PALs on single strains of *L. monocytogenes* and *S.* Typhimurium biofilms. They found that although increased exposure time would result in higher inactivation efficacy, this increase stopped after a certain time; beyond this point, gradually increased bacterial counts were observed. The reason could be the difference in biofilm resistance to reactive species generated in PAW contributing to biofilm inactivation only within a specific (short) exposure time (Smet et al., 2019).

4.1.3 | PALs post storage conditions (before biofilm treatment)

The PALs age, the storage time after generation, is another important factor that influences its efficacy on bacterial biofilm removal. Smet et al. (2019) used four different poststorage times (0, 3, 10, and 30 days) after PAW generation for biofilm removal. The results indicated that an increase in PAW age reduces its microbial inactivation efficacy for biofilm, inferring that the solutions tended to become less effective with increasing age. Apart from PAW poststorage time after generation, poststorage temperature also played role in influencing its efficiency (Seo et al., 2019). PAW stored for up to 90 days at six different conditions of temperature showed that the antimicrobial activity of PAW was well retained when stored at −80°C at least for 3 months, or at −4°C for 3 weeks (Seo et al., 2019). An et al. (2019) studied the effect of storage conditions on the biofilm inactivation efficiency of *Escherichia coli* biofilms inoculated on stainless steel coupon after being exposed to three different PALs, that is, distilled water, NaCl, or NaOCl, for 10 min. The results showed that plasma-activated NaOCl solution showed higher bactericidal efficiency in the close-lid storage condition than in the open-air condition, whereas no significant difference was found between the open and closed storage conditions in PAW and plasma-activated NaCl solution (An et al., 2019).

4.1.4 | Different PALs and their characteristics

In recent studies, differences in inactivation efficiency toward plasma-treated solutions in treating different types/forms of microorganisms have been reported. Many studies have used various solutions to generate PALs to investigate their inactivation efficiency toward biofilm cells, including PBS (Hong et al., 2021; İbiş & Ercan, 2020; Joshi et al., 2010; Kwandou et al., 2018; Seo et al., 2019), saline solution (0.9%) (Bhatt et al., 2018; Chen et al., 2016; Hong et al., 2021; Yang et al., 2021), NAC (İbiş & Ercan, 2020), citrate solution (Chen et al., 2016), and a number of organic solutions, including glucose, cysteine, glycine, proline, methionine, threonine, glutamate, arginine, and heparin (Ercan et al., 2014). In the study of Ercan et al. (2014), it was reported that the plasma-treated methionine solution was more efficient and significant in inhibiting the biofilms of all the bacterial strains tested (carbapenem-resistant *Acinetobacter baumannii*, MRSA, metallo-β-lactamase (NDM1)-positive *Klebsiella pneumoniae*, and *Enterococcus faecalis*) by preventing the formation of biofilms by 70% as compared to untreated ones. Different PALs including deionized water, saline, and citrate solution showed different antibacterial activities against *E. coli* and *S. aureus*, with PAW and plasma-activated saline being much more potent than plasma-activated citrate solution, due to the buffering capacity of the citrate solution (Chen et al., 2016). In contrast, Kwandou et al. (2018) observed that plasma-activated PBS and PAW did not affect the *E. coli* biofilms significantly after exposure with less than 2% of cells being inactivated, which were similar to the levels in untreated biofilms.

Differences in inactivation efficiency toward various water forms activated by plasma have been reported in recent studies (Ibiş & Ercan, 2020; Kovalova et al., 2016; Sysolyatina et al., 2020; Vlad & Anghel, 2017). For instance, Sysolyatina et al. (2020) reported around 44%, 77%, and 71% reductions were achieved for *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 biofilms, respectively, after being exposed to plasma-activated water mist (PAWM) generated by plasma treatment and air–water vapor mixture for 2 min. These results suggest the importance of short-lived radicals because PAWM condensate is not bactericidal (Sysolyatina et al., 2020). Water electrospray has also been treated with plasma to generate PAW electrospray and the impact of water electrospray and polarity of the air corona discharge on 48 h *E. coli* biofilm was investigated (Kovalova et al., 2016). The decontamination of *E. coli* biofilms was significantly enhanced by water electrospray with 5.3- and 5.4-log reduction for positive and negative corona within 15 min of exposure time, respectively, with thinner and patchy biofilm cellular structure (Kovalova et al., 2016). İbiş and Ercan (2020) reported the antimicrobial activity of nebulized PALs (distilled water, PBS, and NAC solution) on pathogenic biofilms. They found that nebulized plasma-activated NAC solution seems to have the strongest antibiofilm efficacy among all PALs with complete inactivation (~7-log inactivation) of *P. aeruginosa*, *A. baumannii*, and *S. aureus* biofilms, whereas it only led to a 3.4-log inactivation of *Candida albicans*. This superior

antibiofilm effect of plasma-activated NAC solution could be attributed to the formation of peroxynitrite in NAC during plasma treatment (İbiş & Ercan, 2020). In addition, it is worth mentioning that the volume of water for PAW generation also affects the antimicrobial efficiency of PALs to biofilm and higher antibiofilm efficiency was achieved by a smaller volume for PAW generation (Vlad & Anghel, 2017).

4.1.5 | Treatment surface

The removal efficiency of PALs on bacterial biofilms grown on different types of surfaces was significantly different. PALs have been applied by many researchers to remove bacterial biofilms on various types of surfaces, including stainless steel (An et al., 2019; Kamgang-Youbi et al., 2008, 2009; Kim et al., 2018; Zhou et al., 2019); glass cover slides (Kovalova et al., 2016); polypropylene surfaces, including HDPE (high-density polyethylene) surfaces (Kamgang-Youbi et al., 2008, 2009), petri dishes (Smet et al., 2019), FluoroDish™ cell culture dishes (Kwandou et al., 2018), and polypropylene nonwoven textile coupons (Kelar Tučeková et al., 2021); plates, including 48-well plates (Hong et al., 2021), 96-well plates (Ercan et al., 2014; Handorf et al., 2020, 2021; Joshi et al., 2010), six-well plates (Los et al., 2020), 24-well polystyrene plates (Chen et al., 2016; Hozák et al., 2018; Xu et al., 2020), and 12-well PVC (polyvinylchloride) plates (Seo et al., 2019); piping systems, including internal surfaces of a polypropylene pipe (Tan & Karwe, 2021), silicone catheter (Bhatt et al., 2018), and endotracheal tubes (polyvinylchloride/PVC) (İbiş & Ercan, 2020); and silica gels (Yang et al., 2021). Stainless steel and HDPE are the most commonly used surfaces in industry; many studies have been conducted to explore how effective it is to apply PALs for the removal of bacteria attached to these surfaces. Kamgang-Youbi et al. (2008) used stainless steel and HDPE in contact with the PAW for 10, 20, or 30 min to remove the adherent *Hafnia alvei* cells after 3 h of adhesion. Results showed that viable cell counts on surfaces gradually decline with longer exposure time during treatment, with 5.4- and 5.9-log reduction for stainless steel and HDPE surfaces after 20 and 30 min of exposure, respectively (Kamgang-Youbi et al., 2008). Furthermore, Kamgang-Youbi et al. (2009) investigated the inactivation efficiency of PAW obtained by gliding electric discharges on biofilm cells of *Staphylococcus epidermidis*, *Leuconostoc mesenteroides*, *H. alvei*, and *Saccharomyces cerevisiae* formed on stainless steel and HDPE. More than 5-log reduction was obtained for *H. alvei* and *S. epidermidis* on both surface types after being in contact with PAW for up to 30 min. However, *S. cerevisiae* cells exhibited higher resistance compared to other strains, with a reduction of 3- and 3.5-log after 30 min of exposure adhering to stainless steel and HDPE, respectively. This difference in the reduction for *S. cerevisiae* could be the influence of the initial level of adhesion to materials, that is, greater adhesion to stainless steel correlating to the poorer disinfection efficiency (Kamgang-Youbi et al., 2009).

4.2 | Characteristics of microorganisms

Bacterial strain, cell type, cell population, and mode of living affect the inactivation efficacy of PALs, and a summary of the biofilm removal studies using PALs is represented in Table 4.

4.2.1 | Cell type

Studies have reported higher resistance of fungal biofilms toward PALs treatments compared to the vegetative bacteria (İbiş & Ercan, 2020). They investigated the antibiofilm efficacies of nebulized plasma-activated distilled water, PBS, and NAC solution on biofilms of *P. aeruginosa*, *A. baumannii*, *S. aureus*, and *C. albicans*. They found that fungal cells of *C. albicans*-formed biofilms seem to be the most resistant pathogen against PALs that can be mainly attributed to the chitin fungal cell wall and eukaryotic structure of *C. albicans* (İbiş & Ercan, 2020). Los et al. (2020) also observed that the fungal biofilm of *Aspergillus flavus* was resistant to PAW compared to bacteria, which could be due to the reason that the differences in the structures and compositions of prokaryotic and eukaryotic microbial cells led to a more sophisticated cell structure of fungi than the bacterial one. Similarly, Kamgang-Youbi et al. (2009) evaluated the microbial disinfection efficacy of PAW against a range of bacteria (*S. epidermidis*, *L. mesenteroides*, and *H. alvei*) and a yeast (*S. cerevisiae*) model and found that inactivation was more effective for bacteria than for the yeast. Xu et al. (2016) investigated the effects of soaking on the postharvest preservation of button mushrooms (*Agaricus bisporus*) in PAW over 7 days of storage at 20°C. They observed that PAW reduced the microbial counts by 1.5 and 0.5 log for bacteria and fungi, respectively, during storage.

4.2.2 | Bacterial strains

PALs have been reported to be effective in removing biofilms formed by various bacterial strains, including *H. alvei* (Kamgang-Youbi et al., 2008, 2009), *Enterobacter aerogenes* (Tan & Karwe, 2021), *Chromobacterium violaceum* (Flynn et al., 2016), *Agrobacterium tumefaciens*

TABLE 4 Application of PALs for the inactivation of biofilm.

Bacterial strain	Plasma type and parameters (voltage, frequency, power, working gas, and flow rate)	Initial cell load and age	Liquid solutions and time for activation	Substrate for biofilm formation and exposure time	Inactivation Yield	Reference
Staphylococcus epidermidis, methicillin resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*	Multi-hollow surface dielectric barrier discharge (MSDBD), pure water vapor at different input power 30, 50, and 100 W using ambient air at 25 L/min	1×10^8 CFU/mL	Water vapor	Polypropylene nonwoven textile surface (distances of 20, 30, and 40 cm for 30 or 150 s)	*E. coli*: 4.8-log reduction *P. aeruginosa*: 3.1-log reduction *S. epidermidis*: 2.6-log reduction *S. aureus*: 2.0-log reduction	Kelar Tučeková et al., 2021
Enterobacter aerogenes	Glow-discharge plasma jet, 295 V, 22.5 kHz, dry air, 42.5 L/h	1×10^8 CFU/cm^2	Distilled water (400 mL, 5 min), flow velocity: 0.11 and 0.22 m/s 7.7 cm from water surface	Polypropylene internal surfaces of a model piping system containing tees, elbows, and tubing with liquid solutions circulating (15 min)	PAW flowing at 0.11 m/s reduced cell counts by 3.0-, 3.2-, and 3.8-log CFU/cm^2 on different parts of the system	Tan & Karwe, 2021
Streptococcus mutans	Nonthermal atmospheric plasma brush, 10 W, 6 mA, and Ar/O_2 (2%) at 39 L/min	1×10^7 CFU/mL	PBS and 0.9% saline (5 min)	48-well plate (60 min)	PA-PBS: Planktonic bacterial cells: 38% reduction; biofilms: 25% reduction. PALs: Planktonic bacterial cells: 22%; biofilms: 18% reduction	Hong et al., 2021
Listeria monocytogenes (ATCC 15313)	Microwave plasma (MidiPLexc), air at 1 SLM	N/A	Deionized water (100, 300, and 900 s)	96-well plate (1, 3, and 5 min)	A maximum reduction of 4.7-log after 300 s	Handorf et al., 2021
Candida albicans	DBD-Pencil plasma jet, 0.24 kW, 40 kHz, 2.8 kV, Air and N_2 at 5 LFM	1×10^8 CFU/mL	Distilled water (5, 10, and 15 min)	Polyethylene strip	1.1-log reduction	Rathore et al., 2021
Pseudomonas aeruginosa, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Candida albicans*	DBD (jet), 31.4 kV, 1.5 kHz, air	N/A	Nebulized deionized water, phosphate-buffered saline, and *N*-acetylcysteine (NAC) (4 mL, flowrate: 6 SLM, 3 min), 2 mm from the liquid surface	5-cm-long lumen of endotracheal tubes (ETs) fragments (14 min)	NAC solution: *P. aeruginosa*, *A. baumannii*, and *S. aureus* biofilms: ~7-log reduction *C. albicans*: 3.4-log reduction	İbiş & Ercan, 2020

(Continues)

TABLE 4 (Continued)

Bacterial strain	Plasma type and parameters (voltage, frequency, power, working gas, and flow rate)	Initial cell load and age	Liquid solutions and time for activation	Substrate for biofilm formation and exposure time	Inactivation Yield	Reference
Staphylococcus aureus (NCTC-8325)	Corona discharge plasma, 10 kV, 50 mA, air	1×10^9 cells/mL	Distilled water (0, 15, 30, 45, and 60 min [fixed exposure time: 30 min])	Petri dish (0, 15, 30, 45, and 60 min [fixed pretreatment time of 30 min])	Pre- 60 min, post- 30 min: 4.74-log CFU/mL reduction Pre- 30 min, post- 60 min: 5.52-log CFU/mL reduction	Xu et al., 2020
Listeria monocytogenes, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7	DBD, 3 W, 30 kV, 15 kHz, humid air, 1.6 CFM	1×10^7 CFU/mL	Distilled water (15 s), 220 mm from liquid surface	96-well plates (2 and 5 min)	72-h biofilm biomass reduction by 44%, 77%, and 71%, respectively, after being treated for 2 min.	Sysolyatina et al., 2020
Aspergillus flavus	High-voltage-DBD, 80 kV and 50 Hz, air	1×10^6 and 1×10^7 CFU/mL	Deionized water (10 mL, 5 and 20 min) 40 mm from water surface	Six-well flat-bottom polystyrene plates (0, 2, and 24 h)	Spore cells in biofilm: For PAW: 20 min, 24 h, 0.6-log CFU/mL reduction. For gas plasma: 20 min, 24 h, 2.2-log CFU/mL reduction.	Los et al., 2020
Pseudomonas fluorescens	Microwave-driven plasma source (MidiPLexc), forward power of 80 W and reverse power of 20 W, air, 1 SLM	N/A	Deionized water (10 mL, 100, 300, and 900 s)	96-well plates (1, 3, and 5 min)	Plasma treatment of 100 s, 1 min exposure: 3-log reduction Plasma treatment of 300 s, 1 min exposure: 6-log reduction	Handorf et al., 2020
Escherichia coli	DBD generated from micro-plasma bubbles, 40 W, 4.0 kV, 8.0 kHz, Air, 2.0 SLM	1×10^8 to 1×10^9 CFU/mL	Sterilized water (200 mL, 15 min), 10 mm from water surface	Stainless steel piece (1, 2, and 3 min)	Biomass reduction: >60% after treatment time of 15 min	Zhou et al., 2019
Listeria monocytogenes and *Salmonella* Typhimurium	DBD, 15 kHz and He or He/O_2 (1%) at He: 4 L/min; He: 4 min/L + O_2: 0.04 L/min	1×10^7 CFU/mL	Sterile demineralized water (10, 20, and 30 min)	Polystyrene Petri dish (5, 10, 25, and 30 min)	5.3- and 5.8-log CFU/mL reduction for planktonic cells. 3.2- and 3.9-log CFU/mL reduction for biofilms.	Smet et al., 2019
Pseudomonas syringae pv. *tomato* DC3000 (PstDC3000)	Micro-plasma jet, 15 kV at 15 kHz, N_2	1×10^8 to 1×10^9 CFU/mL	PBS solution (1 mL, 1, 3, and 5 min), 10 mm from liquid surface	12-well PVC plates (20 min)	Strong inhibition on the target biofilm by N_2 plasma solution generated after 3- and 5-min treatments.	Seo et al., 2019

(Continues)

TABLE 4 (Continued)

Bacterial strain	Plasma type and parameters (voltage, frequency, power, working gas, and flow rate)	Initial cell load and age	Liquid solutions and time for activation	Substrate for biofilm formation and exposure time	Inactivation Yield	Reference
Escherichia coli O157:H7	15 kHz and 250 W, atmospheric air	~1×10^7 CFU/cm^2	Distilled water (DW), 100 ppm of sodium chloride solution (NaCl)	Stainless steel coupons with open or closed of a container-type plasma apparatus (10 min)	PA-NaCl: 2.06-log reduction; PAW: 1.95-log reduction.	An et al., 2019
Escherichia coli	DBD, 60 kHz, 6 kV, 2 kW, Air	1×10^7 CFU/mL	PBS (1 m), 6 mm from the liquid surface	FluoroDish™ cell culture dish (0, 10, 20, 30, 40, 50, and 60 s)	Less than 2% of biofilm cell reduction, with increased cell aggregation from 3% to 6%.	Kwandou et al., 2018
Lactococcus lactis, *Brevundimonas naejangsanensis*, and *Delftia lacustris*	Model EP purification micro-plasmas, 15 W, Air	1×10^4 CFU/mL	Sterile distilled water (5 s)	Stainless steel coupons (0, 10, 30, and 40 min)	After 40 min of treatment: *Brevundimonas naejangsanensis*: 4.8-log CFU/coupon reduction; *Delftia lacustris*: 4.2-log CFU/coupon reduction; *Lactococcus lactis*: no viable count was detected.	Kim et al., 2018
Escherichia coli (wild strain, Gram-negative), *Staphylococcus epidermidis* (wild strain, Gram-positive), *Escherichia coli* (DBM, Gram-negative), *Staphylococcus epidermidis* (DBM, Gram-positive), viridans streptococci (Gram-positive)	Point-to-plane DC corona discharge, 50 mW, 9 kV, 300 μA, air	1×10^6 CFU/mL	Demineralized water (1 mL, 30 min), 10 mm from the water surface	24-well polystyrene plate (2, 4, 6, 8, 10, 12, and 14 min)	Gram-positive bacterial biofilms were inactivated within minutes, while Gram-negative ones took longer time to inactivate.	Hozák et al., 2018
Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans*	3.2 W, 9.4 kV, and 23 kHz, air	1×10^7–10^8 CFU/mL	Saline (5 mL, 2 min)	Sterile silicone lumen catheter and glass slides (0, 30, and 60 min)	Sterile silicone catheter: Exposure to PALs for 60 min led to 6- to 8-log reduction of viable bacterial and fungal biofilms without regrowth (24 h incubation).	Bhatt et al., 2018

(Continues)

TABLE 4 (Continued)

Bacterial strain	Plasma type and parameters (voltage, frequency, power, working gas, and flow rate)	Initial cell load and age	Liquid solutions and time for activation	Substrate for biofilm formation and exposure time	Inactivation Yield	Reference
Escherichia coli	Needle-to-plane electrode corona discharges, Treatment conditions: Positive corona (PC): +9 kV, 10 to 20 kHz, and 10 mA, 100 mW. Positive corona water electrospray: 10 kHz, 50 mA, 200 mW. Negative corona (NC): −9 kV, 0.5–2 MHz, and −1 mA, 300 mW. Negative corona with water electrospray: 200–500 kHz and −10 mA, 400 mW.	$3.11 \pm 6.59 \times 10^6$ CFU/mL	Deionized water (15 min), 5 mm from the water surface	Glass cover slides (2.5, 5, 10, and 15 min)	Without electrospray: 4.76- and 4.99-log reduction for positive and negative corona within 15 min. With water electrospray: 5.3-log for positive and 5.4-log reduction for negative corona.	Kovalova et al., 2016
Escherichia coli, *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus aureus* (ATCC 29213, penicillin resistant)	Micro-hollow cathode discharge (MHCD), 1100 V and 90 mA, He at 4 L/min, 5.0 mm above the surface	2×10^8 CFU/mL	Deionized water, saline, and citrate solution (60 min)	24-well polystyrene cell culture plates (5, 10, 20, 30, and 45 min)	*S. aureus* biofilms: 99.5% reduction (3 h); *E. coli* biofilms: 99.5% reduction (1 h).	Chen et al., 2016
E. coli, *S. aureus*, multidrug-resistant methicillin-resistant *S. aureus* (MRSA) –95 (clinical isolate), -USA300, and -USA400	Floating-electrode dielectric-barrier discharge (FE-DBD), 50 mW, 120 V, 0.13 W/cm^2, energy levels: 0, 0.39, 0.78, 1.56, 1.95, 3.12, 3.9, 7.8, 11.7, 15.6, and 19.5 J/cm^2, air	1×10^9 CFU/mL	PBS (100 μL, 3, 6, 12, 15, 24, 30, 60, 90, 120, and 150 s), 10 mm from the liquid surface	96-well plates	MRSA-USA400 biofilm cells was comparable with -USA300 but less susceptible than MRSA95 (clinical isolate), *S. aureus, and E. coli* to FE-DBD plasma. Plasma was able to kill >60% reduction of MRSA biofilm cells within 15 s (1.95 J/cm^2). Complete inactivation of all bacterial biofilms within 120 s (15.6 J/cm^2).	Joshi et al., 2010

(Continues)

TABLE 4 (Continued)

Bacterial strain	Plasma type and parameters (voltage, frequency, power, working gas, and flow rate)	Initial cell load and age	Liquid solutions and time for activation	Substrate for biofilm formation and exposure time	Inactivation Yield	Reference
Hafnia alvei	Gliding arc plasma, humid air, distance 13 cm	2×10^4 to 8×10^6 CFU/mL	Sterile distilled water 20 mL (5 min)	Stainless steel and high-density polyethylene (HDPE) surface (10, 20, and 30 min)	5.36- and 5.90-log reduction for stainless steel and HDPE surfaces at 30 min	Kamgang-Youbi et al., 2008
Staphylococcus epidermidis, *Leuconostoc mesenteroides*, *Hafnia alvei*, and *Saccharomyces cerevisiae* (yeast)	Gliding arc discharges, humid air, 550 L/h	Yeast: 4×10^7 CFU/mL, Bacteria: 8×10^8 CFU/mL	Sterile distilled water (20 mL, 5 min)	AISI 304 stainless steel and HDPE (0, 5, 10, 15, 20, 25, and 30 min)	After 30 min of exposure: *Hafnia alvei* and *Staphylococcus epidermidis* on both surfaces: >5-log reduction *L. mesenteroides* adhered on HDPE surface: slightly lower reduction For *Saccharomyces cerevisiae* cells: 3- and 3.5-log reduction on two surfaces.	Kamgang-Youbi et al., 2009
Bacillus cereus ATCC 11778-3	Plasma Jet, 650 W, 39 L/min	Spores: 1×10^6 CFU/mL	Distilled water (50, 75, and 100 mL) (60 s)	Well plates	1.62 ± 2.96-log CFU/mL reduction at 55°C	Liu et al., 2019
Methicillin-resistant *Staphylococcus aureus* ATCC33591	Surface discharge plasma jet, power density 0.2 W/cm^2, gap 8 cm, 79% N_2 + 21% O_2 flows at 4 L/min	3 days, 1×10^8 CFU/mL	Normal saline (10 min)	1 cm^2 silica gel	Plasma-activated saline (PALs) alone: 1.2-log reduction PALs + rifampicin: more than 6-log reduction PALs + vancomycin: around 6-log reduction	Yang et al., 2021

(Flynn et al., 2016), *L. mesenteroides* (Kamgang-Youbi et al., 2009), *L. monocytogenes*, MRSA (Bhatt et al., 2018; Ercan et al., 2014; Kelar Tučeková et al., 2021; Yang et al., 2021), *S. epidermidis* (Bhatt et al., 2018; Kamgang-Youbi et al., 2009; Kelar Tučeková et al., 2021), *S. aureus* (Chen et al., 2016), *Streptococcus mutans* (Hong et al., 2021), *S.* Typhimurium (Smet et al., 2019; Sysolyatina et al., 2020), *P. aeruginosa* (Bhatt et al., 2018; Handorf et al., 2020; Kelar Tučeková et al., 2021), *Pseudomonas syringae* pv. *tomato* DC3000 (Seo et al., 2019), carbapenem-resistant *Acinetobacter baumannii* (Ercan et al., 2014), metallo-β-lactamase (NDM1)-positive *K. pneumonia* (Ercan et al., 2014), *E. coli* (Charoux et al., 2020; Flynn et al., 2016; Kelar Tučeková et al., 2021; Sysolyatina et al., 2020), and fungal biofilms *S. cerevisiae* (Kamgang-Youbi et al., 2009), *E. faecalis* (Ercan et al., 2014; Li et al., 2019; Pan et al., 2017), and *C. albicans* (Bhatt et al., 2018). The antibiofilm efficacy of PALs is highly depended on the microbial strains, and Gram-positive bacterial biofilms tend to be more susceptible to PALs than Gram-negative bacterial biofilms. Ercan et al. (2014) explored the inactivation efficacy of a range of plasma-activated antibiofilm solutions on five bacterial biofilms and found that after 3 min of exposure to different PALs, Gram-positive bacteria such as MRSA and *E. faecalis* exhibited higher resistance than Gram-negative bacterial species-formed biofilms. In addition, plasma-activated threonine, glucose, and cysteine solutions were more efficient in removing Gram-positive biofilm cells compared to other solutions. Hozák et al. (2018) reported that PAW inhibits rapidly both planktonic Gram-positive (*S. epidermidis* and viridans streptococci) and Gram-negative bacteria (*E. coli*), where *E. coli* showed more resistance to PAW than those of *S. epidermidis* and viridans streptococci, and in contrast, no inactivation was observed in their biofilm form after exposing to PAW. The inactivation effect of PALs biofilm was also affected by different bacterial isolates and phenotypes (Hozák et al., 2018; Joshi et al., 2010). Joshi et al. (2010) evaluated the efficacy of the floating-electrode dielectric-barrier discharge (FE-DBD) plasma system on *E. coli* and MRSA collections (–95, -USA300, and -USA400 isolates). They found that MRSA-USA400 and MRSA-USA300 had similar susceptibility to FE-DBD plasma treatment and PAW was able to inactivate MRSA more than 60% within 15 s (1.95 J/cm^2). Biofilm exposure time and initial cell loads are key factors for biofilm inactivation and the PALs were able to disinfect surfaces in less than 120 s (Joshi et al., 2010).

4.2.3 | Mode of living

Microorganisms can exist in both plankton and biofilm forms, and their mode of living has a great influence on the antibacterial effect of PALs. It is generally accepted that biofilm has higher resistance than cells in a planktonic state (Mah & O'Toole, 2001). The increased resistance of biofilm to various antibiofilm treatments was reported in various studies. Hong et al. (2021) studied the ability of plasma-activated PBS and 0.9% saline solution to disinfect *S. mutans* in both planktonic and biofilm forms. The overall results showed that PALs could inactivate 38% of the planktonic bacteria and induce 25% biofilm reduction. The structure and unique properties of biofilm make the bacterial biofilms substantially tougher to eradicate as they are surface attached and matrix encapsulated (Hong et al., 2021). Similar results were also obtained by Smet et al. (2019); the cell population of planktonic *L. monocytogenes* and *S.* Typhimurium was decreased by 5.3- and 5.8-log CFU/mL after being exposed to PAW for 20 and 30 min, respectively, while in biofilms it showed much lower inactivation rate (3.2- and 3.9-log CFU/mL reduction, respectively) under the same condition. Kamgang-Youbi et al. (2008) compared the inactivation efficiency of PALs when treating *H. alvei* with three different living modes, that is, planktonic, adherent, and detached states. In addition to nutrient depletion and starvation during adhesion, the limited diffusion of reactive species in cell aggregates may also contribute to the slower inactivation of adherent cells in *H. alvei*. According to the study, *H. alvei* adherent cells are slower to inactivate than planktonic cells. Some possible reasons include nutrient depletion or starvation during adhesion, and limited diffusion of reactive species within aggregates of cells (Mah & O'Toole, 2001). Apart from *H. alvei* cells growing in a planktonic state and adherent to surfaces, detached cells also exhibited a similar resistance level to PAW treatment indicating that adhesion induced physiological tolerance and this response was sustained after detachment, which may be due to nutrient-limited stationary phase. Furthermore, various strategies such as limited diffusion/penetration of antibiotics through the biofilm matrix altered environment within the matrix, genetic adaptation, slow growth, efflux pumps, enzyme-mediated resistance, and low metabolism of bacteria in biofilms, accounting for the resistance of biofilm bacteria to antimicrobial agents (Kamgang-Youbi et al., 2008; Sharma et al., 2019).

4.2.4 | Initial microbial load (initial cell concentration)

The initial cultivable population is an important factor that affects the disinfection rate of PALs and it has been reported by several studies that the inactivation rate of PALs decreases when the initial microbial increases. Kamgang-Youbi et al. (2008) studied the kinetics of

inactivation with PAW (5–30 min) on *H. alvei* planktonic cells with various initial populations, ranging from 2×10^4 to 8×10^6 CFU/mL. A rise in initial cell levels results in a decrease in the efficiency of microbial inactivation, and the inactivation kinetics (k_{max}) were of the first order and dependent on the initial cultivable cell population (N_0). Possible explanations for weakened PALs inactivation efficiency could be that the molecules produced by cultivable cells quenched reactive species of PAW and the protective mechanism of bacterial cells reaggregation due to high initial cultivable cell loads (Kamgang-Youbi et al., 2008).

5 | PALs ACTIONS ON BIOFILMS

The antimicrobial effect of PALs can be attributed to the synergistic effect of high ORP and low pH, but the exact mechanism or mode of action for the antibiofilm efficacy of PALs is still unclear and requires more investigation for better understanding. There are limited studies on the combination of PALs with other technologies for the removal of biofilms. However, a few studies have shown that combination treatment such as mild heat treatment and ultrasound along with PALs significantly reduced the bacterial cells in the planktonic state due to the synergistic actions.

5.1 | PAL mediation mechanisms

Biofilm EPS is primarily composed of polysaccharides, eDNA, and proteins, each of which performs different functions (Flemming et al., 2007). Thus, processes aiming to control the biofilm formation and decompose the EPS structure are effective antibiofilm methods. It has been reported by Traylor et al. (2011) that the antimicrobial property of PAW can be attributed to the synergistic impact of the reactive species it generates and the acidic environment it creates. Bacterial cells suffer from oxidative stress generated by reactive species generated in PALs (Wu et al., 2017) and with longer PALs exposure time, more reactive species could permeate into bacterial cells within biofilms, thus interfering and weakening the functional enzyme systems on the cell membrane, which changes the bacterial metabolic capacity (Wang et al., 2018). Xu et al. (2020) reported significantly decreased metabolic capacity of *S. aureus* biofilms during the longer plasma treatment time and confocal microscopic analysis indicated severely damaged bacterial membrane structures causing cell death. The reactive species in the aqueous solution can not only etch the cell structure from the outer layer of the bacterial membrane structure, but also disturb the balance of reactive species in biofilms by diffusing into the biofilm matrix via membrane channels, resulting in the production of endogenous reactive species (Xu et al., 2018). With its production, it also makes it possible for endogenous reactive species to accumulate in cells within the biofilm matrix. Marinho et al. (2014) reported that the extracellular H_2O_2 concentration is nearly 100-fold higher than that inside the cell. The overly high concentration of H_2O_2 results in the destruction of the normal physiological function of microbial cells, stopping cell growth and metabolic activities, and leading to cell death (Marinho et al., 2014).

The reactive species in PALs could act as signal molecules that trigger the intrabacterial antioxidative response system within biofilms, then more endogenous ROS scavengers and enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase can be produced by the expression of specific genes, thereby turning high-toxicity reactive species into low-toxic intermediates, keeping the intracellular reactive species concentration in a relatively stable level, thus protecting the bacterial cells from potential damage (Li et al., 2019). However, when the intracellular reactive species concentration reaches its antioxidative threshold, it causes irreversible oxidative damage to DNA, protein, lipids, enzyme systems, and other crucial components in cell (Cabiscol Català et al., 2000), causing severe damages to the physiological functions of bacterial cells (cell cultivability and metabolic capacity) and further cell death. For example, Xu et al. (2020) found that longer PAW exposure or treatment time to *S. aureus* biofilms led to raised intracellular ROS concentrations and they hypothesized that it may exert synergistic effects in deactivating the biofilm *S. aureus*.

Based on the results of the total organic carbon measurement, the hypothesis has been further validated, indicating that the presence of ROS may play an important role in inhibiting the formation of EPS and damaging the membranes of biofilms. The porosity, density, water content, sorption properties, hydrophobicity, and mechanical stability of the EPS and biofilm cells were significantly changed by reactive oxygen species, making them susceptible to the host immune system (Li et al., 2019). On the other hand, it has been reported that intracellular reactive species are crucial for maintaining normal cellular function and signal transduction. Among the various active substances produced in PALs, H_2O_2 is considered to be very suitable for redox signal transduction because of its strong reduction ability (Marinho et al., 2014). According to Li et al. (2019), PAW-contained reactive species could severely damage the physiological functions of *E. faecalis* cells within the biofilm. ROS are considered to be capable of interacting with the membrane components of biofilm cells and later the cytoplasmic components and organelles within the cell, which can further lead to cell functional damage and physiological imbalance (Li et al., 2019). H_2O_2

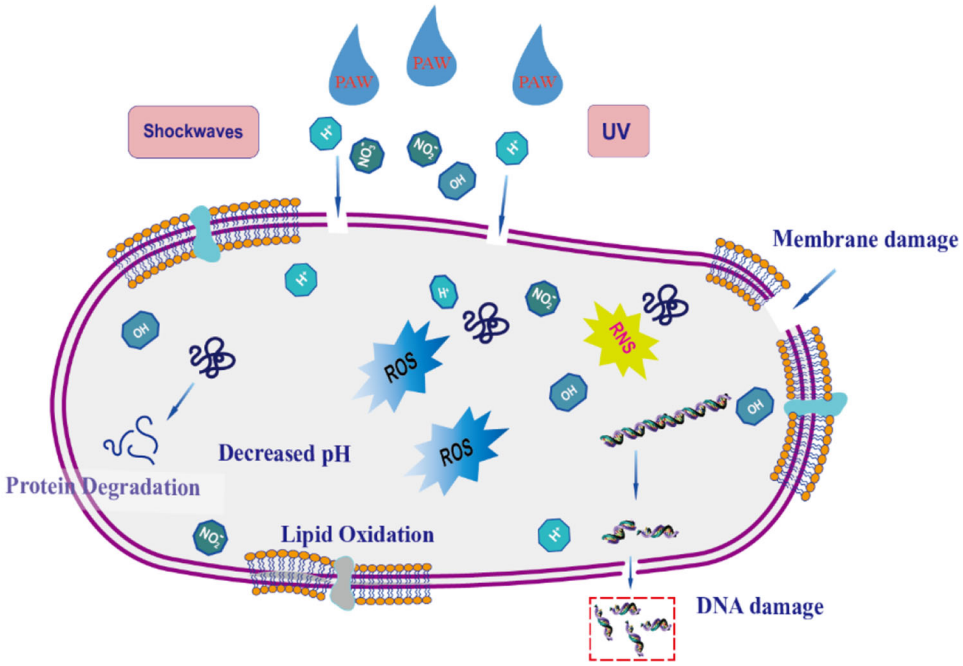

FIGURE 3 Mechanism of plasma activated water in inactivating the microbial biofilms.

is a strong oxidant with strong antimicrobial properties that cause oxidative damage to cell walls and membranes, structurally damaging DNA and proteins within bacterial cells (Hozák et al., 2018; Royintarat et al., 2019; Xu et al., 2020). Cell walls could be damaged when PAL-produced O_3 interacts with C–H bonds in bacterial cells (Li et al., 2019). Moreover, $ONOO^-$ could diffuse into the cell without damaging membrane integrity, resulting in the rupture of bacterial walls (Xiang et al., 2022). Therefore, the oxidative stress on the cell membranes of the bacterial cells, and the interaction of various biofilm components with different reactive species generated by PALs (Figure 3), may lead to lipid peroxidation in the cell membrane, cell leakage, cell structural damage, DNA damage, and structure and function changes of proteins.

5.2 | Synergistic effects of PALs with other technologies

There are very few reports on the combinational treatments with PAW on biofilm removal. However, recent studies have demonstrated potential applications of combined treatments for PALs with other technologies for the inactivation of biofilms. In the study conducted by Yang et al. (2021), synthetic air (79% N_2 + 21% O_2)-produced PALs were used as an antibiotic adjuvant in the combined treatment with antibiotics (rifampicin and vancomycin) to promote the inactivation of MRSA biofilms formed on silica films. The treatment of PALs alone achieved an approximately 1.2-log reduction of MRSA cells in the biofilm, while the combined treatment of PALs and antibiotics (vancomycin or rifampicin) reduced ~≥6- log reduction of MRSA cells in the biofilms. The synergistic effect of PALs and antibiotics may be contributed to the reactive species in PALs diffused into the biofilms and directly acting on the MRSA cells at the beginning. Then, the RONS entered MRSA cells, causing oxidative damage to the cell components, such as proteins and DNA, ultimately leading to their death. Gradually, PALs containing reactive species were depleted and unable to further inactivate MRSA but could sensitize it to help antibiotics kill it (Yang et al., 2021). Charoux et al. (2020) investigated the synergistic effects of airborne acoustic ultrasound and PALs from both cold and thermal plasma systems for inactivating *E. coli K12* biofilms. The results showed that airborne acoustic ultrasound treatment for 15 min followed by treatment with PAW for 15 min significantly reduced the *E. coli* biofilm count by 2.2- to 2.6-log CFU/mL when compared to control treated with distilled water. It is noteworthy that the combined treatments of PAW and airborne acoustic ultrasound showed better efficiency in the activation of *E. coli* biofilms compared to the treatments applied individually (Charoux et al., 2020). In addition, Schnabel et al. (2020) used a combination of plasma-processed air (PPA) and PAW generated from different plasma treatment times (5, 15, and 50 s) and reported a synergistic effect of PPA (50 s) and PAW (3 min) on *C. albicans* biofilms by a reduction of 5.57-log CFU/mL (Schnabel et al., 2020). Surprisingly, they also found that the combined treatment exhibited an antagonistic effect

by decreasing the inactivation efficacy. Hence, a prolonged exposure time to PAW could significantly reduce the antagonistic effect on *C. albicans* biofilms. However, all the studies have not used fractional inhibitory concentration index or isobolograms in their studies, which is a measure/predictor of synergy. Thus, there is a wide scope to comprehensively assess the synergies of different novel methods along with PALs in mitigating biofilms.

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, scholars in different fields have carried out extensive research on the effect of PALs on various microorganisms and biofilms formed by them. However, further research is still needed for the practical applications of PALs in food processing. Gas plasma interaction with the aqueous solution is a cutting-edge topic of theoretical research, and it is also a crucial factor that affects the effect of PALs application. The current research can only partially reflect the interaction between plasma and aqueous solution; the main technical bottlenecks are as follows: (a) a wide variety of reactive particles can be produced in the interactions between plasma and aqueous solution, but the current experimental technology can only quantitatively test a few particles. (b) The interaction of plasma and water solution is a dynamic process, and the current experimental diagnosis method is generally performed after the plasma processing, which means that it is difficult to quantitatively describe the evolution process of reactive species. (c) For gas–liquid two-phase mass transfer, the existing theories are aimed at uncharged particles, but there are still some theoretical gaps for charged particles. (d) It is well known that the chemical reactions in plasma-treated aqueous solutions are very complicated, so it should be noted that some auxiliary reagents might react with these reactive particles, which in turn affects the measurement accuracy of specific particles. (e) The current research mainly seeks answers to the problems of how the reactive particles produced by the gas plasma are transferred into the aqueous solution, how they are transformed in the aqueous solution, which kind of the reactive particles can act on the treated substance, and how to control the compositions and concentrations of reactive particles to optimize the application performance. The PALs field still faces many problems that demand comprehensive research, which requires researchers in this field to expand research fields and seek breakthroughs. (1) Although many PALs generation systems have been developed recently, most are costly and less efficient. Therefore, future research should be devoted to developing energy-efficient, relatively safe, economic plasma generation systems with high potential and good adaptability. Future research should also develop regulatory rules for applying PALs under different scenarios and conduct comprehensive safety assessments for the system before practical application. (2) In future work, research can be carried out on the synergistic effect of PALs in combination with other technology, promoting coordinated development across different technologies and providing better solutions to potential problems that may arise during the biofilm removal process. (3) Further study in exploring the biofilm mitigation mechanisms of PALs should focus on finding a convincing theoretical basis for various hypotheses, thereby laying a solid foundation for subsequent research.

AUTHOR CONTRIBUTIONS

Yunlu Zhao: Writing – original draft; formal analysis; investigation. **Mysore Lokesh Bhavya**: Investigation; formal analysis. **Apurva Patange**: Investigation; formal analysis. **Da-Wen Sun**: Writing – review and editing; supervision; funding acquisition. **Brijesh K. Tiwari**: Writing – review and editing; supervision; resources; funding acquisition.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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