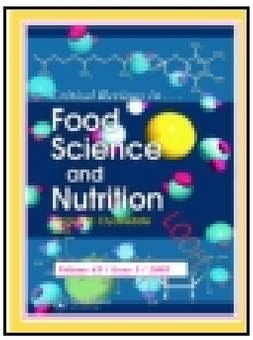

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REVIEW

A review of bacterial biofilm control by physical strategies

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ABSTRACT

Biofilms are multicellular communities of microorganisms held together by a self-produced extracellular matrix, which contribute to hygiene problems in the food and medical fields. Both spoilage and pathogenic bacteria that grow in the complex structure of biofilm are more resistant to harsh environmental conditions and conventional antimicrobial agents. Therefore, it is important to develop eco-friendly preventive methodologies to eliminate biofilms from foods and food contact equipment. The present paper gives an overview of the current physical methods for biofilm control and removal. Current physical strategies adopted for the anti-biofilm treatment mainly focused on use of ultrasound power, electric or magnetic field, plasma, and irradiation. Furthermore, the mechanisms of anti-biofilm action and application of different physical methods are discussed. Physical strategies make it possible to combat biofilm without the use of biocidal agents. The remarkable microbiocidal properties of physical strategies are promising tools for antimicrobial applications.

KEYWORDS

Bacterial biofilm; control; electric; physical method; plasma; ultrasound

Introduction

Microbes attached to a surface where they congregate in large numbers to form a film or layer known as a biofilm, which is a critical source of food contamination (Abebe 2020; Galié et al. 2018). Biofilms can be observed on every surfaces of equipment used in food processing and handling, even following cleaning and disinfection procedures (Yuan, Hansen, et al. 2020). Many outbreaks of pathogens have been attributed to biofilms, and biofilms also account for up to 80% of microbial infections (Alvarez-Ordóñez et al. 2019; Muhammad et al. 2020). The formation of biofilms on food products or the surfaces of equipment leads to serious hygienic problems and economic losses due to food spoilage (Coughlan et al. 2016). Owing to the problems associated with the presence of biofilms in the food industry, their removal and prevention are important to achieve sanitation goals.

Bacteria within biofilms, which may consist of both spoilage and pathogenic bacteria have shown to be more resistant (10–1000 times) to sanitizers and disinfectants than planktonic cells (Satpathy et al. 2016; Yuan, Hansen, et al. 2020). A greater resistance to conventional sanitizers is in biofilm forms. Several theories as to why biofilms are more resistant to the chemical treatments have been proposed. The possible mechanisms include: a reduced diffusion throughout the film due to production of extracellular polymeric substances (EPS) (Limoli, Jones, and Wozniak 2015); physiological changes of the organisms, a reduced bacterial respiratory rate, and protective enzyme production (Sharahi et al. 2019; Yuan, Sadiq, et al. 2020).

Biofilm formation is a dynamic process and different mechanisms are involved in their attachment and growth. EPS play an important role in the attachment and colonization of microorganisms to food-contact surfaces (Flemming et al. 2016; Karygianni et al. 2020). The complex components and structure of biofilms are highly capable of modifying their microenvironment both physically and chemically, so as to be quite distinct from their planktonic cells. Thus, various factors contribute to resistance of biofilms to chemical strategies employed for their control on food processing surfaces (Bridier et al. 2015). Some chemical biocides are ineffective against biofilm, leaving the biofilm intact to continue to recontaminate, corrode, and to build resistance against antimicrobial compounds. Other chemical agents can kill or reduce the microorganism population in the biofilm without removing them, providing a pre-conditioned surface (Iniguez-Moreno et al. 2018). The chemical approach tends to lose its prime position due to the evidence of persistent resistance. Instead, a toolbox of other approaches will gradually take up more space.

New and eco-friendly preventive methodologies to eliminate biofilms from foods and food contact equipment are continually being developed. Several mechanical approaches have been studied in order to minimize biofilm formation and/or to maximize its removal by avoidance of attachment of the bacteria to the surface, modification of surface charge and hydrophobicity, the optimization of the operating

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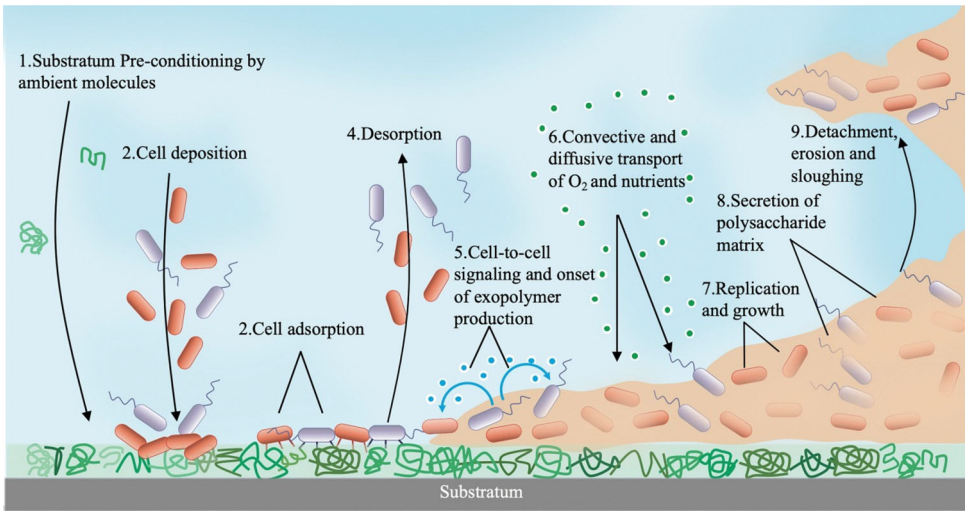

Figure 1. Process governing bacterial biofilm formation (Breyers and Ratner 2004).

conditions such as flow velocity and temperature gradient, induction of dispersion or degradation of the formed biofilm (Liu et al. 2019; Zhao, Zhao, et al. 2017; Masák et al. 2014; Tallawi, Opitz, and Lieleg 2017). Recently, some physical strategies were adopted for the anti-biofilm treatment such as use of ultrasound power (Vyas et al. 2019; Yu et al. 2020), electric (Wang and Ren 2017) or magnetic field (Gu et al. 2020) or the elimination of biofilm by plasma (Gilmore et al. 2018; Patange et al. 2019) and irradiation (Niemira et al. 2005, 2007; Jahid et al. 2014; Jung, Park, and Ha 2018). The combinations of existing physical technologies with other stressors are also currently being examined for biofilm control and removal.

The objective of this article is to give an overview of the current physical methods for biofilm control and removal. Special attention is paid to the mechanisms of anti-biofilm action and application of different physical methods.

Biofilm formation

The main stages in the formation of bacterial biofilms included: (1) attachment (reversible and irreversible); (2) biofilm growth or development and maturation; and (3) biofilm dispersion (Breyers and Ratner 2004; Cacciatore, Brandelli, and Malheiros 2020; Rabin et al. 2015) (Figure 1).

Attachment

The attachment in biofilm formation can be divided into reversible and irreversible adhesion, which included: (1) Preconditioning of the solid surface by macromolecules serving as binding ligands to receptors on colonizing bacteria; (2) Transport of planktonic cells to the surface; (3) Adsorption of cells at the surface; (4) Desorption of reversibly adsorbed cells; (5) Irreversible adsorption of bacterial cells at a surface (Figure 1, Steps 1–5). The initial adhesion of the organisms mediated by physical interaction forces (electrostatic, van der Waals, hydrogen bonding, etc.) is unstable and reversible, cells can detach from the surface (Srey, Jahid, and Ha 2013). If conditions are favorable, the bacterial cells can switch from reversible attachment to a more stable irreversible attachment (Kocot and Olszewska 2017; Dreeszen 2003). The organisms are attached firmly to the surface by secreting extracellular polymers in irreversible adhesion. Polymeric EPS fibrils form bridges between the microbial cells and the surface, enhancing bacterial adhesion and biofilm stability (Flemming et al. 2016). Forces responsible for this type of attachment act over short ranges including dipole–dipole interactions, hydrogen, ionic, and covalent bonding, as well as hydrophobic interactions (Ling et al. 2020; Mazza 2016). Attachment often occurs within several hours. Irreversible adhesion usually occurs within 5 to 30 s. The irreversible bonding usually occurs within a few hours of contact. Several studies indicate that irreversible attachment takes from 20 min to a maximum of 4 h at 4 to 20 °C (Chmielewski and Frank 2003). The process of bacterial adhesion is influenced by a number of variables. These include the species of bacteria, environmental factors, essential gene products, and the surface composition (Zhao, Zhao, et al. 2017). At the attachment stage, adhesion is relatively unstable. Biofilm can be removed by physical methods such as heating or washing.

Biofilm growth and maturation

Following irreversible attachment, bacterial cells continue to proliferate and divide into microcolonies until a mature biofilm is formed on the surface. Once attached to surfaces, bacteria begin cell-to-cell signal communications (Figure 1, Step 5) that may control cell growth, replication, and EPS production (Figure 1, Steps 6, 7, and 8). EPS gradually increases within the biofilm and accounts for 90% of the dry weight of the mature biofilm. EPS sticks bacteria in the biofilm together to maintain the three-dimensional structure of the biofilm, protecting them from the unfavorable environment (Yin et al. 2019). Simultaneously, EPS can accumulate signal molecules, extracellular enzymes, bacterial secondary products, providing a place for bacteria to exchange information (Toyofuku et al. 2015; Flemming 2016).

Biofilm dispersion

As biofilms mature, the bacteria escape from the mature biofilm and become planktonic bacteria which may be transported to new locations and restart the biofilm process (Sharma, Misba, and Khan 2019). This phenomenon has been collectively termed dispersion, redistribution, or recolonization (Step 9). Both biological and physical factors influence this process, such as EPS, quorum-sensing signals, nutrients levels, growth status of the microbes, generated gas bubbles, and fluid shear stress (Hunt et al. 2004). Biofilm dispersion is not only the end of the previous biofilm formation cycle, but also the beginning of a next biofilm cycle.

Physical methods for biofilm control and removal

There has been a broad spectrum of theoretical and experimental works on biofilm control by applications of physical strategies in the past. Ultrasound (US), electric (magnetic) field, plasmas, and irradiation are commonly used physical methods, which are summarized in Table 1.

Ultrasound

Anti-biofilm mechanism by ultrasonic waves

Ultrasound (US) is a mechanical energy that can generate shear forces to disrupt biofilm from a surface. US were able to eliminate various bacterial biofilms from foods and food contact equipment (Oulahal-Lagsir et al. 2000; Bigelow et al. 2008; Yu et al. 2020). The biofilm disruption by ultrasonic waves seems linked to bubble and fluid flow phenomena (Figure 2) taking place during sonication. When ultrasonic waves propagate in liquid media, longitudinal waves are generated, forming alternating compression and expansion zones. The changes in the pressure can cause cavitation and stimulate tiny bubble nuclei. The activated cavitation bubble nucleus undergoes a series of dynamic processes such as oscillation, growth, and contraction. The implosive collapse of cavitating bubbles produces high and localized temperatures and pressures, high shear forces, accompanied by strong shock waves, which can fragment the biofilm on the surface (Verhaagen and Rivas 2016; Vyas et al. 2019). When a cavitation bubble near a rigid boundary implodes, a liquid jet may occur which exerts a localized high-shear force onto the solid surface and help to dislodge attached microbes and biofilm (Verhaagen and Rivas 2016). Clusters of cavitating bubbles will generate the cavitation clouds, within which the high-velocity micro-jets and shock waves help to remove biofilm (Van Wijngaarden 2016).

Acoustic streaming, microstreaming, and microstreamers are other major contributors to biofilm removal from surface. Acoustic streaming is generated during the momentum transfer from the acoustic wave to the liquid (Nowak et al. 2015). The acoustic streaming can increase the permeability of cell membrane and reduce the boundary layer thickness for mass transfer and hence help to remove biofilm from the surface (Ananta et al. 2005). Microstreaming is fluid flow occurring alongside growing and collapsing cavitation bubbles, generating drag forces sufficient to lift biofilms off the surface (Halford et al. 2012). Microstreamers are ribbons of cavitating microbubbles, which may aid in the biofilm removal (Reuter et al. 2017).

Applications of ultrasound on biofilm control

There are differences in anti-biofilm activity when ultrasound was acting under different conditions. High intensity US seems to deliver enough mechanical energy to obtain a high degree of biofilm structure destruction and cell detachment while low intensity appears to stimulate bacterial metabolism with the formation of a biofilm which is more resistant and strongly adherent to surfaces (Erriu et al. 2014). There was a statistically significant difference between the amounts of biofilm removed at different power level. More biofilm was removed at medium power compared to low power as shown in Figure 3 (Vyas et al. 2016). It was reported that relatively low power intensity (10–100 mW/cm) did not show a noticeable reduction in viability of biofilm. A higher cavitation intensity ($>10\,W/cm^2$) is required to remove biofilms attached to a surface (Erriu et al. 2014). Recently, it was found US irradiation can decrease the amount of biofilm when the irradiation time is sufficiently long even if the intensity is low (Koibuchi et al. 2018). Pulsed ultrasound treatment is more effective in removing biofilms compared to a continuous ultrasound treatment at the same power intensity (Rediske et al. 2000; Ma et al. 2015; Ma and Wu 2016). It may indicate that the bacteria respond to the maximum or peak ultrasound intensity and not to the average or the total amount of energy delivered.

Usually, the higher the frequency of US, the more energy it produces, and the more obvious the effect of sonodynamic sterilization. However, the increase of the ultrasonic frequency will also be accompanied by the attenuation of ultrasonic propagation. It has been demonstrated that lower frequency sonication is significantly more effective than higher frequency in reducing bacteria viability within a biofilm (Erriu et al. 2014; Chemat et al. 2017; Izadifar, Babyn, and Chapman 2019; Peterson and Pitt 2000; Kirzhner et al. 2009). Low-frequency ultrasound (<500 kHz) produces more intense cavitation and larger cavitating bubble because the energy needed to induce cavitation in a liquid (cavitation energy threshold) decreases when frequency decreases (Erriu et al. 2014). The powerful cavitation implosion at a lower frequency may help to more effectively remove biofilms (Chemat et al. 2017; Izadifar, Babyn, and Chapman 2019). Ultrasound at 20 kHz removed more biofilm than ultrasound at 33 kHz and 150 kHz (Vyas et al. 2019). Torlak and Sert (2013) evaluated the effectiveness of ultrasound at a low-frequency level (35 kHz) in eliminating *Listeria monocytogenes* biofilm formed on a polystyrene surface, more than 90% of *L. monocytogenes* in the biofilm was inactivated after the ultrasonic treatment. However, in another study, bactericidal effect of ultrasound on its biofilm was not significant (37 kHz, 1200 W for equipment) compared with the control (Na-Young, Seok-Won, and Sang-Do 2014). Inconsistent results of bactericidal effects in *L. monocytogenes* biofilm treated by ultrasound may be mainly attributed

Table 1. Biofilm control by different physical strategies.

Physical treatment	Biofilm type	Results	References
Ultrasound			
	E. coli	US was able to completely destroy biofilm with a short irradiation time.	Bigelow et al. (2008)
	E. coli	Micro-streaming can increase the permeability of cell membrane to induce cell death.	Ananta et al. (2005)
	S. mutans	The biofilm was removed from the cavitation bubbles generated by the ultrasonic scaler.	Vyas et al. (2016)
	S. epidermidis	A long US irradiation (24 h) can decrease the biofilm amount even with low acoustic intensity	Koibuchi et al. (2018)
	L. monocytogenes	More than 90% of L. monocytogenes in the biofilm was inactivated after the ultrasonic treatment	Torlak and Sert (2013)
	L. monocytogenes	Bactericidal effect of ultrasound on its biofilm was not significant. The viable *L. monocytogenes* was only decreased from 6.9 to 5.5 Log_{10} CFU/mL	Na-Young, Seok-Won, and Sang-Do (2014)
	E. coli	Low-intensity ultrasound with gentamicin can significantly enhance killing of *E. coli* biofilm	Peterson and Pitt (2000)
	E. coli, S. aureus	The combination of ultrasound with enzymes showed a higher efficacy in removing biofilms by destroying heterogenic matrix of EPS	Oulahal et al. (2003, 2004, 2007)
	L. monocytogenes	a significant *L. monocytogenes* biofilm removal on stainless steel food contact surfaces by combining the use of ozonation and sonication	Baumann, Martin, and Feng (2009)
	S. typhimurium	Combined US and disinfectants on the cherry tomatoes achieved a higher biofilm reduction	José and Vanetti (2012).
	S. enterica	A 5 min combined treatment of US and peracetic acid showed the best performance in biofilm inactivation	do Rosário et al. (2017).
	C. sakazakii	The combined US and peracetic acid disinfection treatments resulted in synergistic benefits for reducing *C. sakazakii* biofilms on the surface of fresh cucumber	Bang et al. (2017).
	E. coli, P. pastoris, A. pullulans	The combined of power ultrasound and neutralized electrolyzed water showed greater antibacterial effect than using either the electrolyzed water or the ultrasound.	Zhao, Zhang, and Yang (2017).
	B. cereus	The highest cleaning efficacy of *B. cereus* biofilm was achieved with the combination of US and a commercial cleaning agent	Fink et al. (2017)
	S. aureus, *Salmonella* spp.	Combination of acidic electrolyzed water (AEW) and ultrasound produced an obvious synergistic effect on biofilm reduction	Shao et al. (2020)
Electrical field			
	S. epidermidis	Blocking current of 100 μA is better than direct current for detaching *S. epidermidis* from stainless steel surface.	Van der Borden, Van der Mei, and Busscher (2005)
	MRSA, *E. coli*, *P. aeruginosa*	The morphology of bacteria is not greatly affected by the strength of the current, while the motility of different bacteria is greatly affected.	Berthelot and Neethirajan (2017)
	S. aureus, S. epidermidis, P. aeruginosa	Longer-term (up to 7 days) exposure to low-intensity DC current (20, 200, 2000 μA) can effectively inhibit biofilms.	Del Pozo, Rouse, Mandrekar, Steckelberg, et al. (2009)
	P. aeruginosa, MRSA,*S. epidermidis*	enhancement of the activity ofantimicrobial agents against biofilm organisms by EC is not a generalizable phenomenon across microorganisms and antimicrobial agent	Del Pozo, Rouse, Mandrekar, Sampedro, et al. (2009)
	P. aeruginosa	80-100% of bacteria were eradicated with 300 pulses, 80% threshold eradication was not achieved with 100 pulses.	Khan, Lee, et al. (2016)
	P. aeruginosa	Low-duty ratio pulses had a positive effect on preventing biofilm. Frequency and applied voltage were observed to have less influence on biofilm	Perez-Roa et al. (2006)
	S. epidermidis	The electric current did not significantly affect the percentage detachment, but initial detachment rates increased with increasing current	Van der Borden, Van der Mei, and Busscher (2004)
	S. gordonii	Biofilm killing increased to a 4.3 log reduction when 2 mA DC was applied during antibiotics treatment.	Wattanakaroon and Stewart (2000)
	E. coli	The energy of the electrical signal, not the type of electrical signal (AC or DC or SP), is the key to determine the efficacy of the biofilm treatment.	Kim et al. (2015)
	S. epidermidis, *S. aureus, E.coli*, *C. albicans*	Biofilm formation can be reduced using low dose direct current	Ruiz-Ruigomez et al. (2016)
	S. epidermidis, *S. aureus, E.coli*, *C. albicans*	Time- and dose-dependent biofilm killing was observed with all amperages and durations of DC administration	Voegele et al. (2016)
	S. epidermidis	Electrical enhancement of biofilm killing by gentamicin in *in vitro* models	Pickering, Bayston, and Scammell (2003)
	E. coli	Electrical enhancement of biofilm killing by gentamicin in *in vitro* models	Caubet et al. (2004)
	S. epidermidis		Sandvik et al. (2013)

		Electrolysis reactions generating hypochlorous acid from chloride are likely a main contributor to the anti-biofilm efficacy of direct current application.	
	S. aureus	The killing efficacy of the bacteria within the biofilm by gentamicin was greatly enhanced by the direct current treatment	Zhang et al. (2014)
	E. faecalis	The highest bioelectric effect occurred with the use of high-frequency alternating electric current with chlorhexidine	Froughreyhani et al. (2018)
	S. aureus, S. epidermidis, P. aeruginosa	Low-amperage current or 4 h a day of intermittent current reduced biofilms on a variety of surface materials.	Schmidt-Malan et al. (2015)
	A. baumannii, P. aeruginosa	Wireless electroceutical dressing (WED) can generate weak electric field to prevent biofilm formation and disrupt established biofilm by blunting biofilm-induced expression.	Barki et al. (2019)
Plasma			
	E. coli, MRSA, *S. epidermidis*	These biofilms were removed in less than 20 s, whereas planktonic bacteria were inhibited in 5 s by microwave-induced argon plasma	Lee et al. (2009)
	C. albicans, S. aureus P. aeruginosa	78% population reduction was reached within 3 min. for *S. aureus* to an argon plasma jet	Taghizadeh et al. (2015)
	E. coli, C. albicans S. epidermidis, S. aureus, C. glabrata	Biofilms were inactivated after 3 min of exposure, whereas the planktonic cells were within 1 min.	Poor et al. (2014)
	P. aeruginosa, E. coli, S. aureus, S. epidermidis	Gram-negatives were found to be more susceptible to plasma treatment than Gram-positives	Ermolaeva et al. (2011)
	B. cereus, S. aureus, E. coli, P. aeruginosa	biofilms of Gram-positive bacteria (4 min treatment) were more easily eradicated than Gram-negatives (10 min treatment).	Alkawareek et al. (2012)
	S. aureus, E. coli	mechanism of ionomers acting on Gram-positive and Gram-negative microorganisms is different.	Han et al. (2015)
	S. aureus	compared with Gram-positive bacteria with thicker membrane structure, Gram-negative bacteria with thinner cell walls is easier to inactivate by erosion	Traba and Liang (2011)
	P. aeruginosa	The efficiency of the argon plasma on biofilms decreases with biofilm thickness	Zelaya, Vandervoort, and Brelles-Mariño (2012)
	P. gingivalis	All the bacteria were inactivated in the 15 μm thick biofilm, but most of the cells in the deeper layers of biofilm were not damaged	Xiong et al. (2011)
	P. aeruginosa	the penetration thickness in biofilm reduction during plasma exposure within DBD treatment.	Ziuzina et al. (2014)
	-	there is only about 5% of ROS can penetrate biological tissue with a thickness of 500 μm, but more than 80% of RNS can penetrate biological tissues up to 500 μm.	Duan, Lu, and He (2017)
	E. coli	Compared with 100% He carrier gas, adding 1% O_2 to He carrier gas will cause more serious damage to the structure of E. coli biofilm	Dezest et al. (2017)
	S. enterica	The mixed application of NO and H_2O_2 in the inactivation of *Salmonella* will get a better sterilization effect compared to using NO or H_2O_2 alone	Gabriel et al. (2018)
	S. aureus	membrane damage was observed in 94% of bacteria with 0.5% O_2 in He carrier gas.	Xu et al. (2017)
	S. aureus	the inactivation rate of *Staphylococcus aureus* biofilm is the highest at 8 mm	Taghizadeh et al.(2015)
	S. enterica	no significant difference in the sterilization effect of *Salmonella* biofilms cultured for 1, 2 and 3 days under the treatment time	Niemira, Boyd, and Sites (2014)
	L. monocytogenes, S. typhimurium	with the extension of the biofilm incubation time, the resistance of the two biofilms to the atmospheric cold plasma increased	Govaert et al. (2018)
	S. enterica	both metal type and surface finish were observed to affect the efficacies of atmospheric pressure plasma jet treatment.	Gabriel et al. 2018
	P. aeruginosa	Under the plasma treatment, the porosity of the biofilm increases, allowing CHX to penetrate deeper inside the porous biofilm and kill all bacterial cells.	Gupta et al. (2017)
	E. coli	The sodium chloride (NaCl) solution combined with plasma resulted in a complete bacteria inactivation after 15 mins of exposure.	Oehmigen et al. (2011)
	S. epidermidis, E. coli, P. aeruginosa	A significantly stronger bactericidal effect was observed when bacterial biofilms are pretreated with vitamin C for 15 min before exposure to plasma jet	Helgadóttir et al. (2017)
Magnetic field			
	S. epidermidis	both DC and AC fields is capable of biofilm removal	McLeod and Sandvik (2010)
	P. aeruginosa	After being exposed to a static magnetic field for 6h, release of some compounds (rhamnolipids) of the biofilm matrix were considerably enhanced	Raouia et al. (2020)
	S. aureus, P. aeruginosa	Antimicrobials resulted in fifty percent greater reduction in biofilm growth and biomass after exposure to rotating magnetic fields	Junka et al. (2018)
	S. aureus	Magnetic nanoparticles allowed deeper penetration into a mature biofilm by magnetic field, leading to an effective eradication of biofilm	Wang, Deng, et al. (2018)

(*continued*)

Table 1. Continued.

Physical treatment	Biofilm type	Results	References
	P. aeruginosa	Combination of magnetic nanoparticles and ciprofloxacin demonstrates superior properties in eliminating biofilms	Bandara et al. (2015)
	MRSA	The combination of magnetic iron oxide nanoparticle (MNPs) and a magnetic field destroyed the biofilm matrix of MRSA, achieving up to a nearly 5 log10 reduction in biofilm bacteria.	Li, Nickel, et al. (2019)
	S. aureus	Biofilm eradication was significantly (4–6-fold) improved through enhanced penetration of antibiotic through artificial channels	Quan et al. (2019)
Irradiation	*S. enterica*	Biofilm-associated cells were significantly more sensitive to ionizing radiation than the respective planktonic cells	Niemira et al. (2005)
	E. coli	Culture maturity had a more significant influence on the irradiation efficacy of planktonic cells but not on biofilm-associated cells of *E. coli* O157:H7.	Niemira et al. (2007)
	L. monocytogenes, *E. coli*	UV-C light can effectively reduce the bacteria population on the surface of fruits and berries, but this effect was affected by the surface characteristics.	Adhikari et al. (2015)
	L. monocytogenes	Ultraviolet-C (UV-C) and cold oxygen plasma (COP) can effectively reduce *L. monocytogenes* biofilms formed on lettuce and cabbage, and have no effect on quality.	Srey et al. (2014)
	S. typhimurium	Mixed culture of *S. typhimurium* and cultivable indigenous microorganisms biofilm has a significantly higher resistance to UV-C treatment	Jahid et al. (2014)
	S. enterica	a significantly biofilm reduction of *Salmonella* strains on eggshells by treatment with X-ray irradiation.	Mahmoud et al. (2015)
	L. monocytogenes	UV-C and NaOCl has a synergistic inhibitory effect on *L. monocytogenes* biofilms formed on the surface of stainless steel and eggshell surface	Kim, Park, and Ha (2016)
	S. typhimurium	Combined X-ray/NaOCl treatment showed the highest synergistic reduction value on biofilms	Jung, Park, and Ha (2018)

to a different frequency and intensity/power of ultrasound, and different resistances among microbial strains. Ultrasonic cleaning operation conducted between 10 and 200 kHz needs further investigation to find the correlation between biofilm removal and this range of frequency (Fuchs 2015).

Ultrasound alone at low power intensity was not significantly detrimental to biofilm viability, combining ultrasound with other lethal stressors were recommended (Yu et al. 2020). Ultrasound was reported to increase the effectiveness of antibiotics against biofilm cells on various bacteria (Peterson and Pitt 2000; Liu, Liu, et al. 2016, Liu et al. 2020; Cai et al. 2017; Wang, Wen, et al. 2018). The amount of the killing observed when ultrasound and antibiotic were applied simultaneously was between one and two orders of magnitude greater than the killing by antibiotic alone. Ultrasound can increase cell membrane permeability and improve the transport of antibiotics into the biofilm matrix which induce the disruption of cell membranes and biofilm (Erriu et al. 2014; Yu, Chen, and Cao 2012).

The combination of ultrasound with biological or chemical sanitizer for biofilm removal was also reported (Baumann, Martin, and Feng 2009; José and Vanetti 2012; Bang et al. 2017; do Rosário et al. 2017; Duckhouse et al. 2004; Stanley et al. 2004; Zhao, Zhang, and Yang 2017; Fink et al. 2017; Shao et al. 2020). The combination of ultrasound with enzymes showed a higher efficacy in removing biofilms (Oulahal et al. 2003, 2004, 2007) by destroying heterogenic matrix of EPS. Baumann, Martin, and Feng (2009) showed a significant *L. monocytogenes* biofilm removal on stainless steel food contact surfaces by combining the use of ozonation and sonication. Reductions were significantly higher than either treatment alone. The microorganisms are eradicated by the disruption or breakdown of the cell envelope, which in turn leads to the leakage of the cell contents. José and Vanetti (2012) evaluated the effectiveness of combined ultrasonication (45 kHz) and four disinfectants, i.e., sodium dichloroisocyanurate, hydrogen peroxide, chlorine dioxide, and peracetic acid, during the decontamination of natural contaminant microbiota and *Salmonella typhimurium* biofilm on minimally processed cherry tomatoes. A higher biofilm reduction was achieved by combined US and disinfectants. Fink et al. (2017) found the highest cleaning efficacy of *Bacillus cereus* biofilm was achieved with the combination of US and a commercial cleaning agent 2% P3-Tresolin ST® (10% alcohols, 2.5% benzakolium chloride, 2.5% didecyldimethylammonium chloride). The combined US and peracetic acid disinfection treatments resulted in synergistic benefits for reducing *Cronobacter sakazakii* biofilms (Bang et al. 2017) and *Salmonella enterica* biofilm (do Rosário et al. 2017). US or peracetic acid treatment alone was not greatly effective in reducing *C. sakazakii* biofilms. The cavitation phenomenon generated through US enhanced the penetration of peracetic acid to biofilm matrix, resulting in the increase of cell wall permeability and denaturation of proteins and enzymes due to oxidizing capacity of peracetic acid (Hilgren et al. 2007; Bang et al. 2017; Krolasik et al. 2010; Marques et al. 2007; Tote et al. 2010). Zhao, Zhang, and Yang (2017) examined the efficacy of power ultrasound

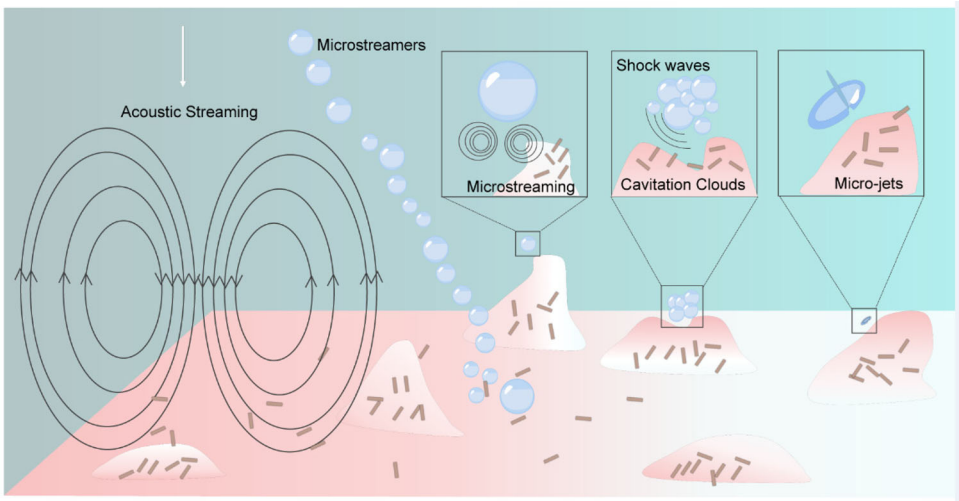

Figure 2. Phenomena involved in biofilm disruption by ultrasonic waves (Vyas et al. 2019).

and neutralized electrolyzed water used individually, and in tandem, for the inactivation and detachment of *Escherichia coli*, *Pichia pastoris*, and *Aureobasidium pullulans*. The combined method showed greater antibacterial effect than using either the electrolyzed water or the ultrasound. The ultrasound promoted bacterial detachment, improved the dispersion of HClO in the aqueous media, and facilitated the penetration of HClO into the detached cells. Furthermore, Shao et al. (2020) found the combination of acidic electrolyzed water (AEW) and ultrasound produced an obvious synergistic effect on biofilm reduction in *Staphylococcus aureus and Salmonella* spp.

Electrical field

The effect of electric field on biological cells was evident, electric fields and currents can influence the organization of biological membranes, metabolic processes, cell behavior, and the various cellular responses within both prokaryotic and eukaryotic cells (Ravikumar, Basu, and Dubey 2019; Sabelnikov et al. 1991). Repulsive electrostatic forces can mediate the adhesion of the microorganisms to the surface (Rijnaarts et al. 1993; Jucker, Harms, and Zehnder 1996). The application of electrical current can increase the repulsive forces, facilitating the surface detachment of bacterial biofilms, a phenomenon labeled as "electricidal effect" (Van der Borden, Van der Mei, and Busscher 2005). The electrical current can also act synergistically with biocides or antimicrobial agents, which is termed as "bioelectric effect."

Electricidal effect

Bacterial detachment from stainless steel (Van der Borden, Van der Mei, and Busscher 2004) and indium tin oxide surfaces (Poortinga, Bos, and Busscher 2000) by low-intensity electric direct current (DC) have been described, which may be related to the electricidal effect. Van der Borden, Van der Mei, and Busscher (2004) demonstrated that more than 75% of the initially adhering *Staphylococci* could be stimulated to detach from stainless steel and prevention of re-deposition of detached bacteria by the application of an electrical current. In a follow-up study (Van der Borden, Van Der Werf, et al. 2004), the effect of DC electrical currents and block currents against biofilms in the late stages of formation was studied. The block currents yielded higher detachment percentages than DCs due to the electro-osmotic fluid flow directed to and from the surface under electrical current, causing an extra force that stimulates detachment (Poortinga, Bos, and Busscher 2000; Van der Borden, Van Der Werf, et al. 2004). Time- and dose-dependent biofilm killing was observed with durations of DC administration (Voegele et al. 2016). Biofilm formation can be reduced using low dose DC (Ruiz-Ruigomez et al. 2016). Schmidt-Malan et al. (2015) found low-amperage current or 4 h a day of intermittent current can reduce *S. aureus, S. epidermidis,* and *P. aeruginosa* biofilms on a variety of surface materials. Exposure time is another important factor influencing anti-biofilm activity in addition to the electric field intensity. Electrical current is not able to eradicate bacteria in biofilms in the short term exposure (Berthelot and Neethirajan 2017; Weaver et al. 2012). Del Pozo, Rouse, Mandrekar, Steckelberg, et al. (2009) found prolonged exposure to low-intensity electrical current resulted in a marked decrease in the viability of *S. aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* biofilms.

In another application, electroporation created by pulsed electric fields (PEF) has been shown to create non-thermal permanent damage to biofilm structure (Khan, Lee, et al. 2016; Perez-Roa et al. 2006). Perez-Roa et al. (2006) found that low voltage PEF reduced the growth of *P. aeruginosa* biofilm development. Low-duty ratio pulses have more influence on biofilm eradiation than frequency and applied voltage. Khan, Lee, et al. (2016) investigated the eradication of multidrug-resistant *Pseudomonas* biofilm with PEF and found bacterial eradication efficacy was closely related with number of pulses and electrical field strength. Recently, Barki et al. (2019) applied wireless electroceutical dressing (WED) to generate weak electric field to prevent biofilm formation and disrupt established biofilm by blunting biofilm-induced expression.

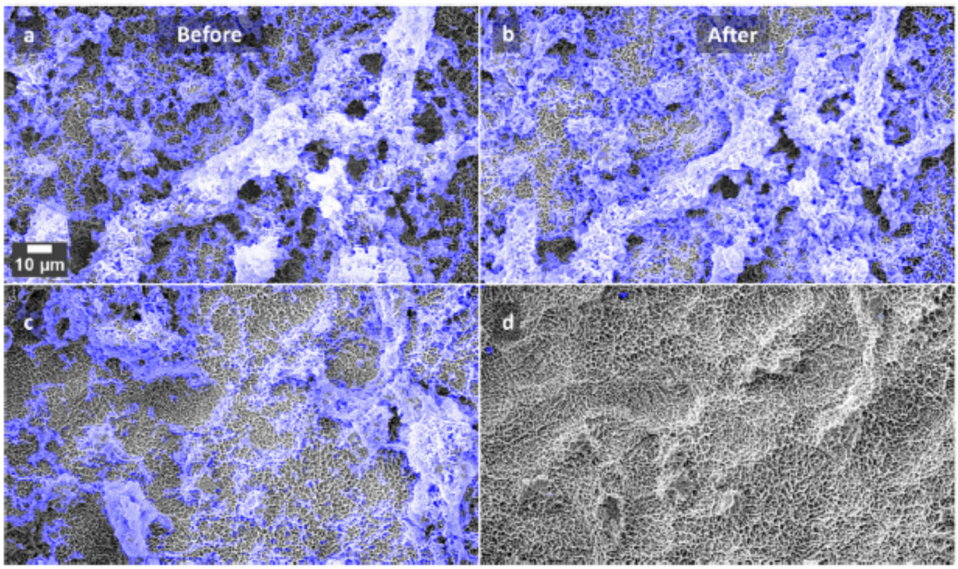

Figure 3. Scanning electron microscopy images of *Streptococcus mutans* biofilm before and after treatment with an ultrasonic scaler at low power (a, b) and medium power (c, d). The blue overlay shows the automatic detection of bacteria (Vyas et al. 2016).

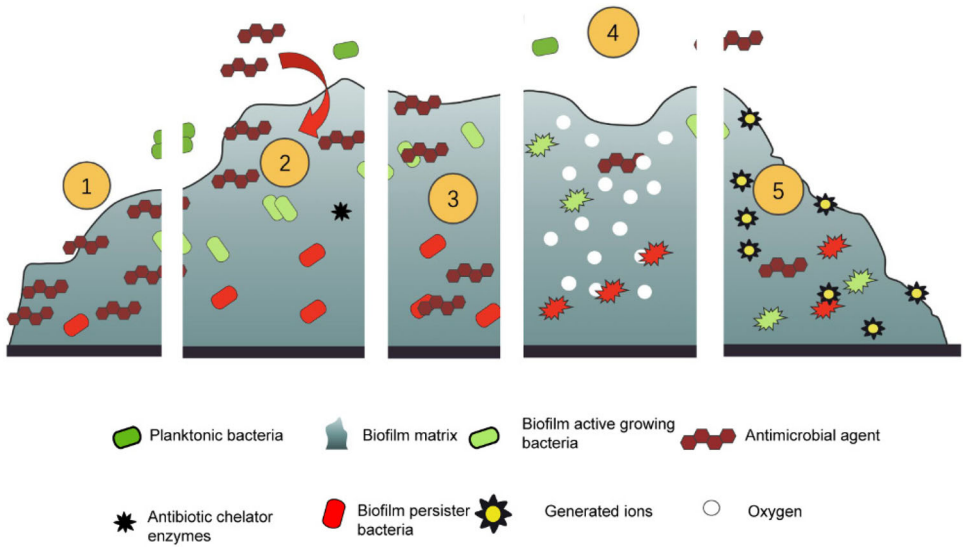

Figure 4. Some proposed bioelectric effect mechanisms: (1) reduction of the biofilm capacity for binding to the antimicrobial agent; (2) electrophoretic augmentation of the antimicrobial agent transport; (3) membrane permeabilization; (4) electrolytic generation of oxygen; (5) electrochemical generation of potentiating oxidants (Del Pozo, Rouse, and Patel 2008).

Bioelectric effect

Bioelectric effect generated by the combined use of antimicrobial agents with electric current proved more effective in controlling the biofilms (Del Pozo, Rouse, and Patel 2008). The combination of tobramycin and direct current increased the biofilm control (Costerton et al. 1994; Stewart et al. 1999). Electrical enhancement of *Streptococcus gordonii* (Wattanakaroon and Stewart 2000), *S. epidermidis* (Pickering, Bayston, and Scammell 2003) *and E. coli* (Caubet et al. 2004) biofilm killing by gentamicin in in vitro models were also confirmed. Zhang et al. (2014) found the killing efficacy of the bacteria within the *S. aureus* biofilm by gentamicin was greatly enhanced by the direct current treatment. The highest bioelectric effect on *Enterococcus faecalis* biofilm was achieved with the use of high-frequency alternating electric current with chlorhexidine (Froughreyhani et al. 2018). In another study, Del Pozo, Rouse, Mandrekar, Sampedro, et al. (2009) found the activity of linezolid or minocycline against *Methicillin-resistant Staphylococcus aureus (*MRSA*)* biofilms was not enhanced by electrical current, indicating that the enhanced activity of antimicrobial agents by electrical current against biofilm organisms may not be a generalizable phenomenon across microorganisms and antimicrobial agents. Furthermore, Kim et al. (2015) found the correlation between the electrical energy and the treatment efficacy of the bioelectric effect on *E. coli* biofilms. The energy of the electrical signal is the key to determine the efficacy of the biofilm treatment instead of the type of electrical signal. This observation will facilitate the understanding of the mechanism of the bioelectric effect treatment.

The possible mechanism for the bioelectric effect (Figure 4) has been suggested to include reduction of the biofilm

capacity for binding to the antimicrobial agent (Blenkinsopp, Khoury, and Costerton 1992), electrophoretic augmentation of the antimicrobial agent transport (Sadekuzzaman et al. 2015), membrane permeabilization (Barki et al. 2019), electrolytic generation of oxygen (Stewart et al. 1999; Zhang et al. 2014), electrochemical generation of potentiating oxidants (Costerton et al. 1994; Sandvik et al. 2013).

Plasmas

Plasma is the fourth state of matter after solid, liquid, and gas. When the energy increases, solid molecules move freely and form a liquid state. As the energy level increases, free molecules are dispersed into a larger space and transformed into a gaseous state. Finally, enough energy can decompose gas molecules into free electrons and ions (Liao et al. 2017). Physically speaking, plasma is a partially or fully-ionized gas. The gas mixture is mainly composed of photons, ions, ultraviolet rays, free electrons, and an element with a net neutral charge in the ground or excited state (Zhu et al. 2020). In general, according to the thermodynamic balance between electrons and ions, plasma can be divided into thermal and non-thermal plasma (NTP, also low-temperature or cold plasma) (Gilmore et al. 2018). In thermal plasma, the plasma is in a local equilibrium state and its temperature reaches the values of several thousands of Kelvins. In non-thermal plasma (NTP), most of the coupled energy is transmitted into the electrons and only their temperature reaches the high value, the neutral particles and ions have only negligible energy and stay cold (Múgica-Vidal et al. 2019). Because of the simple design and easy maintenance, NTP is usually generated by electric discharges in gases under either low or atmospheric pressure.

It requires low energy consumption, fast sterilization speed, and can effectively maintain the advantages of food color, texture, and nutrient content, thus attracting widespread attention in the food field.

Mechanisms of anti-biofilm action

The possible microbiocidal mechanism for plasma action (Figure 5) is mediated mainly by reactive oxygen species (ROS) and reactive nitrogen species (RNS), sometimes summarized as RONS, arising from the complex plasma-chemical reactions (Graves 2012). Depending on the composition of the input gas and the parameters of the plasma discharge, different active components will be produced. There exists various species such as ions, radicals, stable (hydrogen peroxide H_2O_2, ozone O_3 and nitrogen oxides NO_x) or unstable (superoxide anion O_2^-, singlet oxygen $^1O_2$, hydroxyl radical·OH), nitric oxide radical·NO_2, peroxynitrite $ONOO^-$ and others) molecules in the surrounding gas. The presence of particular particles depends on the nature of discharge, surrounding gas, and other parameter. The significance of reactive charged particles is proved to cause physical damage of microbial cells (Liu, Yin, et al. 2016). The details and mechanisms of plasma microbiocidal effects can be explained by oxidative damage, etching effect, and regulation of quorum sensing signals.

(1) Oxidative damage. Cold plasma can destroy biological membranes from different aspects, such as causing oxidation of lipids and membrane components in biological membranes, promoting the collapse of biofilm substrate, the decrease of the biofilm adhesion, and the disintegration of the biofilm (Gupta et al. 2018). Active species in plasma, such as ROS and RNS has a strong oxidizing effect on the external structure of cells, which can cause oxidative damage of cell membrane or intracellular components (such as DNA, protein, carbohydrate compound) (Cheng et al. 2016). Some researchers have also found that ROS can oxidize the extracellular polysaccharides in the biofilm matrix, so that the area of the biofilm matrix is relatively reduced. This will lead to a decrease in the adhesion of the biofilm, and eventually disintegrate the three-dimensional structure of the biofilm (Khan, Lee, et al. 2016).

(2) Etching effect. The charged particles generated by the plasma can break the chemical bond, and at the same time destroy the biofilm matrix and the EPS structure, making the biofilm lose its protective function against microorganisms, and this process is called etching (Zhu et al. 2020). As the damage intensifies, the biofilm will fall off from the solid surface. The microorganisms are released in a floating form, so that the plasma can directly act on the surface of microorganism. The perforation of the cell membrane caused by etching will promote the diffusion of the secondary active substances formed in the plasma discharge (Gupta and Ayan 2019). Excited atoms, molecules, and free radicals can cause breakdown of chemical bonds, which can fragment cells and form volatile compounds, resulting in morphology changes, from the shrinkage of the cell to the appearance of deep channels in the cell, until the cell is completely destroyed and loses activity (Vandervoort and Brelles-Marino 2014). In addition, charged particles will aggregate on the surface of microorganisms. When generated electrostatic stress exceeds the tensile strength of the cell membrane, the membrane structure will undergo morphological changes. The perforation of the cell membrane caused by etching will also promote the transport of active substances generated during the plasma discharge process. The penetration of the substance into the cell membrane causes the erosion and destruction of bacterial cells, causing serious damage to the cell membrane or DNA.

(3) Regulation of quorum sensing signals. Quorum sensing (QS) systems can regulate multiple physiological activities, such as biofilm formation, the expression, and spread of microbial virulence. Plasma can deactivate quorum sensing signal molecules and interfere with expression of virulence genes. Flynn et al. (2016) found that cold plasma can interfere with the release of QS toxic factors in Gram-negative bacteria. And under the cold plasma treatment, acyl homoserine lactones (AHL, signaling molecule in Gram-negative bacteria) will change rapidly and produce secondary

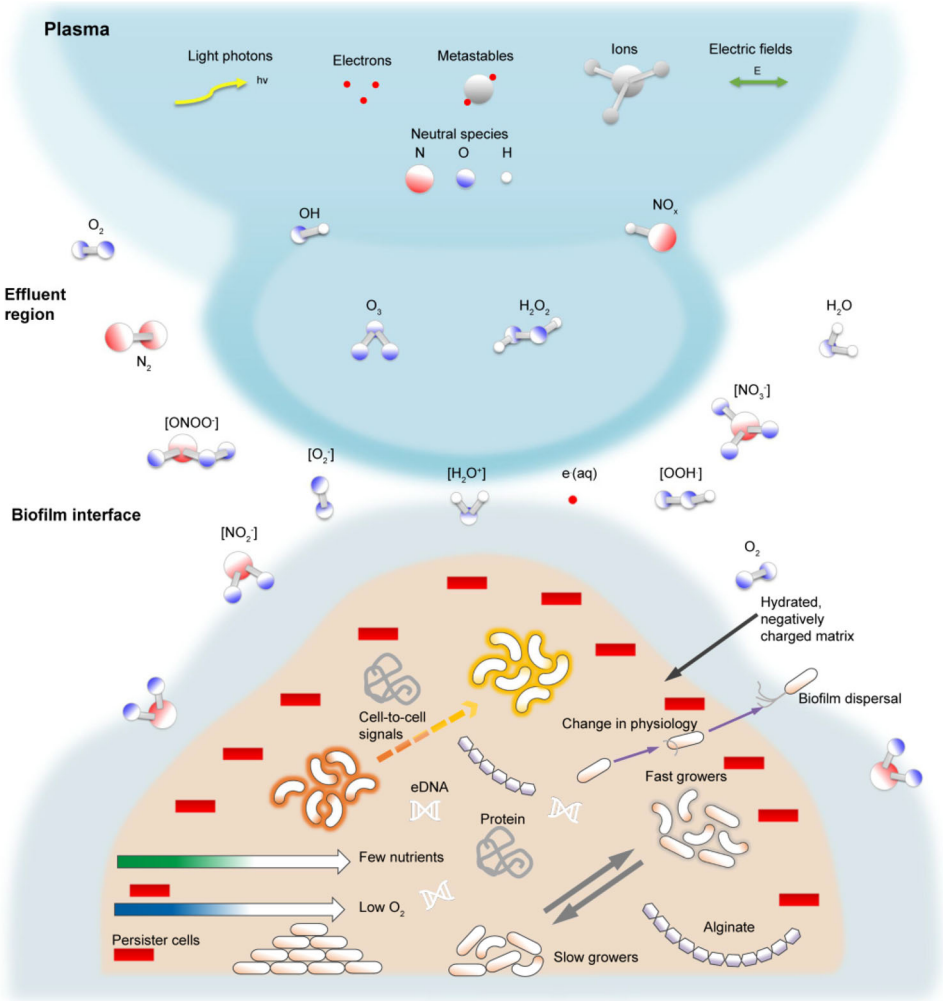

Figure 5. Microbiocidal mechanism for plasma action (Gilmore et al. 2018).

derivatives. After a long time treatment, AHL will eventually lose its activity. Similarly, Li, Nickel, et al. (2019) found in the study of *E. faecalis* biofilm that plasma activated water (PAW) can effectively inhibit the formation of *E. faecalis* biofilm, while reducing the expression of quorum sensing-related virulence genes.

Factors influencing anti-biofilm activity of cold plasma

The inhibitory effect of cold plasma on biofilm is closely related to its generating device, microbial characteristics, biofilm thickness, the composition of the input gas, the distance between the ion source and the biofilm, biofilm age, surface properties of the material, etc.

Generating device

Commonly used cold plasma sources are corona discharge, dielectric barrier discharge (DBD), radio frequency plasma, microwave plasma, plasma jet, and sliding arc discharge. Lee et al. (2009) applied the microwave-induced argon plasma on *E. coli*, *S. epidermidis,* and MRSA biofilms. These biofilms were removed in less than 20 s, whereas planktonic bacteria were inhibited in 5 s. Taghizadeh et al. (2015) exposed *Candida albicans*, *P. aeruginosa,* and *S. aureus* biofilms to an argon plasma jet and found bacterial biofilms were more susceptible to plasma than planktonic organisms. The treatment of *S. aureus* was the most effective: 78% population reduction was reached within 3 min. Excited nitrogen species was proved to be the important carriers of excitation energy to the biofilms. Poor et al. (2014) demonstrated that the argon NTP exhibited strong biocidal properties, biofilm of pathogens *Acinetobacter baumannii*, *E. coli*, *S. aureus*, *S. epidermidis*, *C. albicans,* and *Candida glabrata* were inactivated after 3 min of exposure, whereas the planktonic cells were within 1 min. The argon plasma was applied on biofilm removal by Ermolaeva et al. (2011). Bacteria in biofilms were susceptible to the argon plasma.

Microbial characteristics

In the study of Ermolaeva et al. (2011), Gram-negative bacteria were found to be more susceptible to plasma treatment than Gram-positive bacteria. However, in the study of Alkawareek et al. (2012), a plasma jet was applied on biofilms of *B. cereus*, *S. aureus*, *E. coli,* and *P. aeruginosa*, the biofilms of Gram-positive bacteria (4 min treatment) were more easily eradicated than Gram-negatives (10 min treatment). Han et al. (2015) found that the mechanism of

ionomers acting on Gram-positive and Gram-negative microorganisms is different. For Gram-negative *E. coli*, The ROS induced by cold plasma mainly act on the peroxidation of membrane lipids. For Gram-positive *S. aureus*, ROS can be actively transported inside the membrane, causing oxidative damage to the intracellular components. Studies have shown that, compared with Gram-positive bacteria with thicker membrane structure, Gram-negative bacteria with thinner cell walls is easier to inactivate by erosion (Traba and Liang 2011).

Biofilm thickness

The inactivation of biofilm cells is also dependent on the biofilm thickness and the depth into which the reactive species penetrate. In the study of Zelaya, Vandervoort, and Brelles-Mariño (2012), the possibility of biofilms removal by gas discharge plasma was evaluated. The reduction of adhesiveness and the thickness of the biofilms were observed and almost 100% of the *P. aeruginosa* cells were inactivated after a 5 min exposure. The efficiency of the argon plasma on biofilms decreases with biofilm thickness, which possibly due to the limit of plasma penetration (Ermolaeva et al. 2011). Xiong et al. (2011) also confirmed the influence of plasma penetration on the *Porphyromonas gingivalis* biofilms. All the bacteria were inactivated in the 15 μm thick biofilm, but most of the cells in the deeper layers of biofilm were not damaged. Ziuzina et al. (2014) observed the penetration thickness in biofilm reduction during plasma exposure within DBD treatment. In addition, Duan, Lu, and He (2017) measured the tissues thickness that the ROS and RON generated by the plasma can penetrate. It was found that ROS was consumed a lot during the penetration process. The data shows that there is only about 5% of ROS can penetrate biological tissue with a thickness of 500 μm, but more than 80% of RNS can penetrate biological tissues up to 500 μm. In addition, under certain experimental conditions, some RON can penetrate biological tissue with a thickness of 1.25 mm. However, these studies are limited by the final thickness limit of the cultured biofilm.

Cold plasma gas composition

The damaging effect of cold plasma on the biofilm mainly depends on the active substances in the mixed gas. The types of active substances can be determined by the type of the input gas. Inert gases have better stability, such as helium and argon, and are often used in cold plasma inactivation. Recent studies have shown that adding O_2 to an inert gas can help the production of active substances and improve the sterilization ability. Compared with 100% He carrier gas, adding 1% O_2 to He carrier gas will cause more serious damage to the structure of *E. coli* biofilm (Dezest et al. 2017). A large amount of intracellular protein is oxidized, and the inactivation effect is the strongest. This is due to the formation of reactive oxygen species in the He-O_2 cold plasma environment, such as singlet O and O_3. The mixed application of NO and H_2O_2 in the inactivation of *Salmonella* will get a better sterilization effect compared to using NO or H_2O_2 alone (Gabriel et al. 2018). Furthermore, Xu et al. (2017) proved the increased deactivation efficacy of a plasma jet against *S. aureus* by the admixture of oxygen into the working gas. Membrane damage was observed in 94% of bacteria with 0.5% O_2 in He carrier gas. The reactive oxygen species or oxygen free radicals generated by the plasma can pass through the biofilm matrix and cause oxidative damage to the cells, proteins, and DNA chains in the membrane (Niemira, Boyd, and Sites 2014), demonstrating a stronger sterilization effect.

Other factors

In addition, the distance between the plasma source and the biofilm will also affect the inactivation efficiency of the biofilm. Taghizadeh et al. (2015) found that compared with the use distance (such as 9, 10 and 11 mm), the inactivation rate of *S. aureus* biofilm is the highest at 8 mm. This may be because changing the distance between the plasma nozzle and the sample under a fixed gas flow rate can direct different doses of photons and reactive substances on the target sample, causing different damage effects on the biofilm (Gupta and Ayan 2019). The age of the biofilm is another important factor affecting the antibacterial effect of cold plasma. Govaert et al. (2018) cultivated five biofilms of *L. monocytogenes* and *S. typhimurium* of different ages (1, 2, 3, 7, 10 days). It was found that with the extension of the biofilm incubation time, the resistance of the two biofilms to the atmospheric cold plasma increased. Compared with the 1-day control group, the inactivation efficiency was significantly reduced. However, in another study of *Salmonella* biofilms, the sterilization effect of *Salmonella* biofilms cultured for 1, 2 and 3 days under the treatment time were similar, and there is no significant difference. This may because the biofilm was air-dried for 10 min before the cold plasma sterilization, leading to the increased resistant power of *Salmonella* biofilm (Niemira, Boyd, and Sites 2014).

Under certain conditions, the surface properties of the material will affect biofilm structure. For example, the appearance of *P. aeruginosa* on the surface of stainless steel has a mushroom-like structure with a thin interlayer. The biofilm formed on polycarbonate has a thick cell layer connection, which is denser than that formed on stainless steel (Liao et al. 2017). In addition, the inhibitory effect of cold plasma on the biofilm will be affected by the different attachment surface characteristics. Gabriel et al. (2018) studied the effect of atmospheric cold plasma on the sterilization effect of *S. enterica* biofilm on two types of stainless steel (304 and 316) with three different surface finishes, both metal type and surface finish were observed to affect the efficacies of atmospheric pressure plasma jet treatment.

Plasma was reported to increase the anti-biofilm effectiveness by combining with other stressors. The sodium chloride (NaCl) solution combined with plasma resulted in a complete bacteria inactivation after 15 min of exposure (Oehmigen et al. 2011). Gupta et al. (2017) examined the combined action of chlorhexidine (CHX) and plasma jet on

treatment of *P. aeruginosa* biofilms on titanium coupons. A complete sterilization of a biofilm was observed with plasma prior to CHX treatment. A significantly stronger bactericidal effect was observed when *S. epidermidis*, *E. coli,* and *P. aeruginosa* bacterial biofilms are pretreated with vitamin C for 15 min before exposure to plasma jet (Helgadóttir et al. 2017).

Other methods

Magnetic field

A magnetic field is defined as a region of space in which a magnetic body is capable of magnetizing the surroundings (Gu et al. 2020). The inactivation of microorganisms with pulsed magnetic field has been conducted with limited effects. Benson et al. (1994) reported that static magnetic fields may enhance the activity of gentamicin against a *P. aeruginosa* biofilm. McLeod and Sandvik (2010) designed a system of magnetic fields applied to biofilms, which is capable of applying magnetic fields, both DC and AC fields (up to about 1 kHz), from ten to several thousand milligauss. Relatively low level magnetic fields, in conjunction with the appropriate antibiotic, may be able to help control and eventually clear bacterial biofilm (McLeod and Sandvik 2010; Bandara et al. 2015). Application of rotating magnetic fields increases the activity of antimicrobials (gentamycin, ciprofloxacin, octenidine, chlorhexidine, polihexanidine, and ethacridine lactate) against *S. aureus* and *P. aeruginosa* biofilms (Junka et al. 2018). Recently, Raouia et al. (2020) investigated the effect of static magnetic field on biofilm formation and other virulence factors in *P. aeruginosa* and its isogenic sod mutants. The release of some compounds of the biofilm matrix such as rhamnolipids has been considerably enhanced after 6 h of exposure in the wild type. On the other hand, the pyocyanin and biofilm production was increased significantly in all strains compared to controls. Furthermore, the pslA and ppyR gene expressions were confirmed in the biofilm formation. Besides, some microenvironment -responsive magnetic nanoparticles were fabricated to enhance anti-biofilm activity in the presence of magnetic field (Wang, Deng, et al. 2018; Li, Pan, et al. 2019; Quan et al. 2019). Magnetic nanoparticles allowed deeper penetration through the biofilm with the assistance of a magnetic field and establish an artificial channel in the biofilm matrix. Biofilm eradication was significantly (4–6-fold) improved through enhanced penetration of antibiotic through artificial channels (Quan et al. 2019).

Ionizing irradiation

Another physical strategy for anti-biofilm is ionizing irradiation. Ionizing irradiation has the ability to damage microbial DNA in order to ensure the food safety and quality when applied at an appropriate irradiation dose (CFR 2016; Diehl 2002). Irradiation has been approved by the FDA as a method to control microorganisms on the surface of food products . Ionizing irradiation has many advantages over other existing sanitation methods because it does not require the use of heat or chemicals and induces fewer changes in the nutritional quality of food (Zehi et al. 2020). The effects of ionizing irradiation on *Salmonella* biofilms was studied by Niemira and Solomon (2005). Three serovars of *S. enterica* were examined for sensitivity to ionizing radiation as planktonic cells and as biofilms. The populations of both planktonic and biofilm-associated *L. monocytogenes* were reduced effectively after ionizing radiation. Biofilm phenotype was found to be more sensitive to radiation than the planktonic phenotype in contrast to chemical antimicrobial treatments. Niemira (2007) also examined the effect of ionizing radiation on *E. coli* O157:H7 strains and, as was observed for *Salmonella*, the effect varied depending on the strain isolate and the culture maturity. Organisms in biofilms may respond to ionizing radiation differently than planktonic cells, which will depend on the specific strain of organism encountered.

UV-C (Ultraviolet C) is also effective at reducing bacterial biofilm populations (Adhikari et al. 2015; Jahid et al. 2014; Srey et al. 2014). The antimicrobial effect of UV-C on microorganisms occurs as a consequence of DNA damage, specifically crosslinking of neighboring pyrimidine bases in the DNA strands (Lopez-Malo and Palou 2005). *L. monocytogenes* biofilm populations on cabbage, pear, strawberry, and cantaloupe surfaces were significantly reduced after treatment with UV-C irradiation (Adhikari et al. 2015; Srey et al. 2014). Additionally, it has been reported that after treatment with UV-C, there were reductions of biofilms of *S. typhimurium* and cultivable indigenous microorganisms, respectively (Jahid et al. 2014). Combination treatment of UV-C irradiation and NaOCl resulted in a synergistic effect in reducing *L. monocytogenes* biofilms on stainless steel and eggshell surfaces (Kim, Park, and Ha 2016). However, there are some questions about the ability of UV radiation to control biofilm due to its poor penetrating power. X-ray irradiation with deeper penetration depth has been used as a useful alternative method for biofilm control. There have been several studies regarding biofilm control using X-ray irradiation, or the combined treatment of X-rays and chemical disinfectants (Jung, Park, and Ha 2018; Mahmoud et al. 2015, 2016). Mahmoud et al. (2015) noted a significantly biofilm reduction of *Salmonella* strains on eggshells by treatment with X-ray irradiation. Jung, Park, and Ha (2018) found combined treatment with X-ray irradiation and NaOCl resulted in a synergistic anti-biofilm effect on *S. typhimurium*. Combined X-ray/NaOCl treatment showed the highest synergistic reduction value on biofilms

The primary mode of action of irradiation is to create highly reactive species, including free radicals such as the hydroxy radical, and hydrogen peroxide (Niemira et al. 2003; Sommers 2003). These radicals damage cellular and biochemical structures that they come in contact with, such as nucleic acid strands, cell membranes, and genetic material (microbial DNA) structures. This not only disrupts the cellular function randomly, but also incapacitates the ability of microbes to replicate or regenerate (Ravindran and Jaiswal 2019; Seifert et al. 2016). Additionally, other components of the cell are also subjected to interactions with ionizing

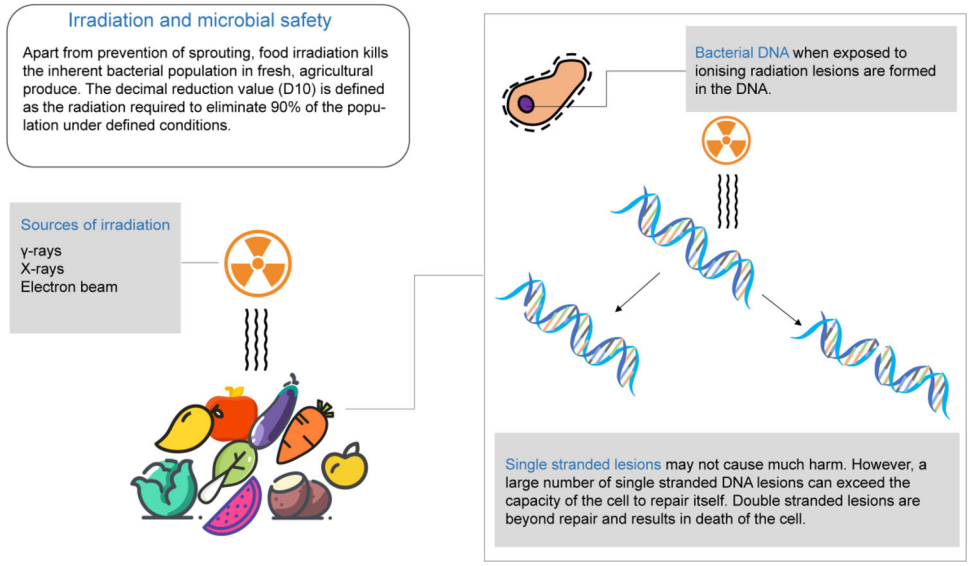

Figure 6. The effect of radiation on the bacterial DNA (Ravindran and Jaiswal 2019).

radiations. Even with an undisturbed DNA, damaging effects on cellular proteins and membrane may impede the survival chances of the injured cell (Figure 6).

It was observed that radical formation is most likely not hindered in a biofilm, but mobility of the radicals may be hindered in EPS matrix, leading to self-quenching and reduced efficacy (Niemira et al. 2003). The structural elements of the EPS matrix may themselves be the primary targets of the radicals, thereby facilitating the biofilm removal.

Conclusions and future trends

Microbial control in food processing involves reduction/eradication of microbes and the prevention/control of biofilm formation on the process equipment. In general, the microorganisms in the form of biofilm are considerably more resistant to sanitizers and disinfectants in comparison with their planktonic forms. Due to the increased resistance of biofilms to conventional disinfection processes, novel means for their control are continually being developed. Different physical approaches including ultrasound, electric/magnetic field, plasma, and ionization for the removal and control of biofilm have been reviewed in this paper. The mechanisms of anti-biofilm action and application of different physical methods were summarized. From the point view of the food industry, the physical strategies for biofilm control seem especially promising because it avoids the main problems that may arise with other alternatives. Physical strategies make it possible to combat biofilm without the use of biocidal agents, which is very desirable for preserving the quality of food.

The physical methods are a great alternative for biofilm removal, but they face the difficulties in killing the bacteria within the biofilm. Physical methods alone were not significantly detrimental to biofilm viability, combining physical and chemical methods to realize biofilm control and removal were recommended. It can be concluded that the remarkable microbiocidal properties of physical strategies are interesting tools for antimicrobial applications.

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