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Review

# Application of electric field treatment (EFT) for microbial control in water and liquid food

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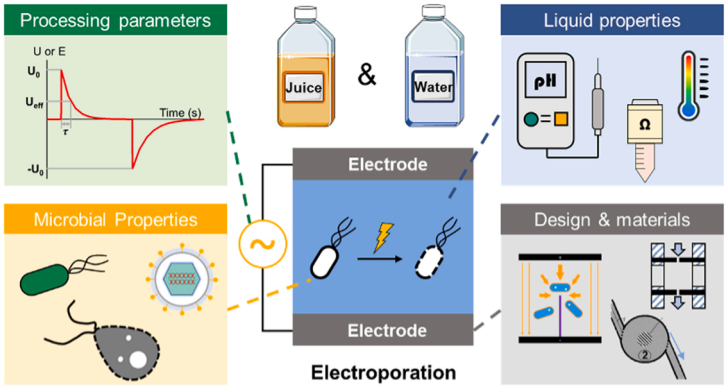
## HIGHLIGHTS

- The similarities and differences of EFT in water and food are compared.
- Latest advances of the impact of operation parameters and liquid properties are reviewed.
- EFT on bacteria, viruses, and protozoa are systematically reviewed.
- Three future directions to promote the applications of EFT are proposed.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Water disinfection and food pasteurization are critical to reducing waterborne and foodborne diseases, which have been a pressing public health issue globally. Electrified treatment processes are emerging and have become promising alternatives due to the low cost of electricity, independence of chemicals, and low potential to form by-products. Electric field treatment (EFT) is a physical pathogen inactivation approach, which damages cell membrane by irreversible electroporation. EFT has been studied for both water disinfection and food pasteurization. However, no study has systematically connected the two fields with an up-to-date review. In this article, we first provide a comprehensive background of microbial control in water and food, followed by the introduction of EFT. Subsequently, we summarize the recent EFT studies for pathogen inactivation from three aspects, the processing parameters, its efficacy against different pathogens, and the impact of liquid properties on the inactivation performance. We also review the development of novel configurations and materials for EFT devices to address the current challenges of EFT. This review introduces EFT from an engineering perspective and may serve as a bridge to connect the field of environmental engineering and food science.

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## 1. Pathogen inactivation in water and liquid food

Waterborne and foodborne diseases have been a pressing public health issue globally. More than 3.4 million people die every year due to water-related diseases (Organization and UNICEF, 2017). The 2010 cholera outbreak in Haiti caused more than 665,00 confirmed cases with 8183 deaths due to sanitary deficiencies and direct use of contaminated Artibonite River water (Piarroux et al., 2011). Not only developing countries (regions) but also developed countries (regions) face challenging situations against waterborne diseases. According to the U.S. Centers for Disease Control and Prevention (CDC), from the 32 drinking-water-associated outbreaks in 2011–2012, 28 cases were caused by viral, bacterial, or parasitical contamination (Beer et al., 2015). Foodborne diseases also pose threats to human health and are estimated to cause 800 outbreaks and 15,000 illnesses from 2009 to 2015 in the U.S (Dewey-Mattia et al., 2018). In 2009, approximately 1.8 million cases of diseases and 233 deaths were reported in the Netherlands, while one-third of them were attributed to foodborne pathogens (Havelaar et al., 2012).

Pathogenic microorganisms of primary health concern, either waterborne or foodborne, include bacteria, protozoa, and viruses. The pathogens of concerns in water and liquid food are similar (Table 1). Among them, Norovirus is the leading cause of illness and disease outbreaks due to its relatively high infectivity, long persistence in water, and various transmission routes. (Maunula et al., 2005). In the U.S., Norovirus was responsible for 38 % of the outbreaks, followed by *Salmonella* and Shiga toxin-producing *Escherichia coli* (*E. coli*), according to a 2011–2012 surveillance report for waterborne disease outbreaks from the Environmental Protection Agency (EPA) (Beer et al., 2015). In 2009, a food crisis of peanut products containing *Salmonella* caused 8 deaths and more than 500 illnesses, resulting in one of the most significant food recall events. (2012) *Legionella* is also the common cause of waterborne disease outbreaks (States et al., 1998). Recently, 1 death, 12 confirmed diagnoses, and 63 probable cases were reported in a single *Legionella* outbreak at a hotel located in Georgia, U.S., due to the aging plumbing system (Oliviero, 2019).

**Table 1**
Typical waterborne and foodborne pathogens, and their associated diseases (Centers for Disease Control and Prevention, 2020a, 2020b).

| Pathogens | Waterborne | Foodborne | Diseases |
|---|---|---|---|
| **Bacteria** | | | |
| *Bacillus cereus* | | Y | Nausea, vomiting, and diarrhea |
| *Brucella* | | Y | Brucellosis |
| *Campylobacter jejuni, C. coli* | Y | Y | Gastroenteritis |
| *Clostridium* | | Y | Botulism |
| *Escherichia coli* – pathogenic | Y | Y | Gastroenteritis |
| *E. coli* O157:H7 (enterohaemorrhagic) | Y | Y | Gastroenteritis and hemolyticuremia |
| *Legionella* spp. | Y | | Legionnaires' disease |
| *Leptospira interrogans* | Y | | Liver damage and kidney failure |
| *Listeria monocytogenes* | | Y | Listeriosis |
| *Pseundomonas aeruginosa* | Y | | Pulmonary disease and skin infection |
| *Salmonella typhi* | Y | Y | Typhoid fever |
| *Salmonella enterica* | Y | Y | Salmonellosis |
| *Shigella* | Y | Y | Shigellosis |
| *Staphylococcus* | | Y | Staph infections |
| *Vibrio cholerae* | Y | Y | Cholera |
| *Yersinia enterocolitica* | Y | Y | Gastroenteritis |
| **Protozoa** | | | |
| *Acanthamoeba* spp. | Y | | Keratitis and encephalitis |
| *Cryptosporidium parvum* | Y | Y | Cryptosporidiosis |
| *Cyclospora cayetanensis* | Y | Y | Gastroenteritis |
| *Entamoeba histolytica* | Y | | Amoebic dysentery |
| *Giardia intestinalis* | Y | Y | Giardiasis |
| *Naegleria fowleri* | Y | | Primary amoebic meningoencephalitis |
| *Toxoplasma gondii* | Y | Y | Toxoplasmosis |
| **Viruses** | | | |
| Adenoviruses | Y | | Gastroenteritis and respiratory infection |
| Astroviruses | Y | Y | Gastroenteritis |
| Clostridium perfringens | | Y | blisters, tachycardia, swelling, and jaundice |
| Enteroviruses | Y | | Gastroenteritis |
| Hepatitis viruses A and E | Y | Y | Hepatitis |
| Noroviruses | Y | Y | Gastroenteritis |
| Rotavirus | Y | Y | Gastroenteritis |
| Sapoviruses | Y | | Gastroenteritis |

To inactivate waterborne and foodborne pathogens, various technologies have been developed and implemented (Table 2). Boiling the water, *i.e.*, a thermal treatment, before drinking used to be the most prevalent practice at the household level (Hall and Dietrich, 2000). However, the high energy consumption hinders its application in large-scale water treatment plants. The thermal treatment has also been widely studied and used in the dairy, wine, and other liquid food processing industries (Richardson, 2001). With the adoption of thermal pasteurization, food safety is significantly enhanced with extended product shelf-life. However, the high temperature destroys proteins and vitamins, as well as the original taste of the liquid food, which leads to the development of non-thermal treatment methods (Vega-Mercado et al., 1997). Adding chemical disinfectants is currently the most popular water disinfection approach, due to its great effectiveness, low cost, and easy operation. Most disinfectants kill pathogens through oxidation, such as free chlorine, chloramine, chlorine dioxide, and ozone (Crittenden and Harza, 2005). Due to their various oxidation power and stability in water, different disinfectants are chosen for different scenarios. For example, highly active free chlorine and ozone are usually applied in the centralized water treatment plants, while chloramines serve as excellent residual disinfectants as they react more slowly but stay active longer (Crittenden and Harza, 2005) However, all the above chemical disinfection methods suffer from the generation of carcinogenic disinfection by-products (DBPs) (Richardson and Postigo, 2011). The application of chemical disinfectants in liquid food has more restrictions. The oxidation processes could easily ruin the taste and nutrients of the food, and potentially generate secondary toxicity substances (Alexander et al., 1954). Although natural substances like bacteriocins and essential oils have been used for food preservation, large-scale applications are restricted due to high cost and the development of antibiotic resistance (Cleveland et al., 2001; Ju et al., 2019). Alternative non-thermal physical methods, such as ultraviolet (UV), high-pressure processing, and non-thermal plasma, have also been developed, which could significantly reduce the thermal damage to the product during treatment (Hijnen et al., 2006; Martín-Belloso and Sobrino-López, 2011; Scholtz et al., 2015). Specifically, UV has been successfully adopted in water treatment and food processing with little concerns of DBP formation (Hijnen et al., 2006; Nelson et al., 2013; Shah et al., 2011). Nevertheless, it is still limited by the microbial regrowth, no residual effect, and poor performance against certain microorganisms, such as Adenoviruses and bacterial spores (Crittenden and Harza, 2005; Hijnen et al., 2006).

## 2. Overview of EFT for pathogen inactivation

Electric field treatment (EFT) has emerged as a non-thermal pathogen inactivation method. In typical EFT, strong electric pulses with short durations are applied between the electrodes, so that the pathogens in the media are inactivated by electroporation (Weaver and Chizmadzhev, 1996). When a bacterial cell is placed in an electric field, an electric potential across the cell membrane, *i.e.*, transmembrane potential (TMP), is generated (Fig. 1) (Kotnik et al., 2015). For a spherical cell, the relationship between the TMP and the strength of the external

**Table 2**
Summary of pathogen inactivation technologies. Technologies with grey, yellow, and green shadings refer those are applied in water only, both water and liquid food, and liquid food only (Aboud et al., 2019; Peanut Butter Outbreak, 2009; Awad et al., 2012; Balasubramaniam et al., 2004; Daher et al., 2017; Flemming and Trevors, 1989; Haas and Aturaliye, 1999; Hashemi et al., 2019; Huo et al., 2021; Khadre et al., 2001; Koseki and Yamamoto, 2007; Liao et al., 2017; Loo et al., 2012; Madaeni, 1999; Mikš-Krajnik et al., 2017; Ngwenya et al., 2013; Park et al., 2004; Park and Ha, 2019; Perinban et al., 2019; Plazas-Tuttle et al., 2018; Priyadarshini et al., 2019; Rahman et al., 2010; Ross et al., 2003; Tian et al., 2018; Vincent et al., 2016; C. Wang et al., 2020; Wang et al., 2018).

| | *Technology* | *Introduction* | *Current stage* | *Advantages* | *Disadvantages* | *References* |
|---|---|---|---|---|---|---|
| **Water disinfection only** | Chlorine and other chemical disinfectants | Oxidation of pathogenic microorganisms to damage their cell membrane and intracellular macromolecules. | The most well-studied and prevalent method in water disinfection. No food applications because some disinfectants are toxic and oxidize organic nutrients in food. | Easy to operate, low cost, providing residual disinfection power. | Production of toxic and carcinogenic DBPs, limited effect on bacterial spores. | (Crittenden and Harza, 2005) |
| | Membrane filtration | Physically screening out the microorganisms by size exclusion and other mechanisms. | A popular water disinfection method with no concerns of DBPs. No food applications because the membrane will also exclude other nutritional components in food. | No concerns of DBP, high efficiency, universal removal of all microorganisms larger than the membrane pore size. | Expensive, requiring periodically maintenance and cleaning, decreasing throughput due to membrane fouling | (Crittenden and Harza, 2005) |
| | Heavy metals | Altering the physiological and biochemical properties of microorganisms. | An ancient passive method widely used in food processing and water disinfection as the utensils. Some applications in water (e.g., swimming pool by Cu disinfection) and limited food processing since metals also react with organic molecules. | High efficiency, easy to use, rapid process. | Bringing in secondary contaminants, which may need additional step to remove. | (Flemming and Trevors, 1989; Vincent et al., 2016) |
| **Both water and liquid food** | Heating | The heat is generated through an electric resistance (Joule heating) or by the electromagnetic energy with a wavelength of 1.3-4.0 μm (infrared heating). | The most primary and well-studied method in food processing. Only household-level water disinfection in individual families. | Thermal process, high thermal efficiency, rapid treatment, lower cost than other thermal methods. | Limited by heat transfer, destroy the nature of the product, corrosion, non-uniform, not sensitive to reflective properties of coatings (infrared only). | (Hashemi et al., 2019; Tian et al., 2018) (Aboud et al., 2019; Priyadarshini et al., 2019) |
| | **Electric field treatment (EFT)** | Pathogens are inactivated by the strong electric pulses through electroporation. | Fundamental research and several pilot- and full-scale applications in food processing. Much less investigation in water disinfection. | Non-thermal physical process, rapid treatment, no chemical use, no resistance or regrowth. | Relatively high cost, unfavorable heat generation and electrochemical reactions. | (Gusbeth et al., 2009; Jeyamkondan et al., 1999; Mizuno et al., 1990; Toepfl et al., 2014) |
| | Microwave | The use of electromagnetic energy to inactivate pathogens by thermal and non-thermal effects. | Fundamental research and limited real-world applications in both fields. | Thermal physical process, rapid treatment with high throughput. | Radiation concerns, high cost, may destroy the nature of the food product. | (Hashemi et al., 2019; Plazas-Tuttle et al., 2018; Wang, C. et al., 2020) |
| | Ultrasonication | The application of high-power sound waves (20 kHz) inactivates | Fundamental research and limited real-world applications in both fields. | Thermal/non-thermal process, safe and easy operation, less complex equipment. | Relatively high cost (than thermal treatment), potential change in | (Awad et al., 2012; Park and Ha, 2019; Ross et al., 2003) |

(*continued on next page*)

**Table 2** (*continued*)

| | | | | | | |
|---|---|---|---|---|---|---|
| | | pathogens by producing cavitation. | | | material characteristics. | |
| | Ozone | Ozone inactivates the pathogens by oxidation of cellular structures. | Limited study in food processing due to the universal oxidation of other nutrients, but much more investigation and applications in water disinfection. | Non-thermal chemical process, broad spectrum effectiveness, rapid treatment, remove unfavorable color, taste, and odor. | High cost, require on-site generation. | (Crittenden and Harza, 2005; Khadre et al., 2001; Ngwenya et al., 2013) |
| | UV | UV irradiation denatures the nucleic acids and proteins to disable the function of microbial proliferation. | Limited study in food processing due to the low light transmission, but much more investigation and applications in water disinfection. | Non-thermal physical process, rapid treatment with high throughput, abundant engineering experience. | Microbial regrowth, less effective on spores and certain viruses, affected by the matrix. | (Crittenden and Harza, 2005; Loo et al., 2012; Madaeni, 1999; Ngwenya et al., 2013) |
| | Cold plasma | The cold plasma generates antimicrobial agents (ions, electrons, reactive oxygen and nitrogen species (ROS/RNS)) and UV, which collaboratively contributes to the inactivation. | Fundamental research and limited real-world applications in both fields. | Non-thermal physiochemical process, perform at room temperature and pressure, rapid treatment. | Constrained by the size and shape of the product, high cost, limited ROS/RNS penetration into the product. | (Liao et al., 2017; Perinban et al., 2019) |
| **Liquid food pasteurization only** | High pressure processing (HPP) | High pressure (usually>400MPa) inactivates pathogens by disrupting cell membrane and enzymes. | At the R&D stage for food processing. Not suitable for water disinfection due to the high cost. | Non-thermal physical process, ambient temperature, uniform treatment regardless of the geometry and size of the food, no chemical use. | Constrains of HPP equipment, high cost, less effective on spores. | (Balasubramaniam et al., 2004; Daher et al., 2017; Khadre and Yousef, 2002; Koseki and Yamamoto, 2007; Ross et al., 2003) |
| | Pressure and $CO_2$ | Pathogens are inactivated by the combination of carbon dioxide and high pressure. | At the R&D stage for food processing. Not suitable for water disinfection due to the high cost. | Non-thermal, broad spectrum, non-toxic. | Relatively high cost (than thermal treatment), constraints of equipment, concerns of global warming for the use of CO2. | (Martín-Belloso and Sobrino-López, 2011; Ross et al., 2003) |
| | Electrolyzed water | Electrolyzing a weak salt solution generates disinfecting agents, oxidation-reduction potential (ORP) stress, and extreme pH. | At the R&D stage for food processing. Electrochemical disinfection is a more direct way of this method for water. | Non-thermal chemical process, easy and simple operation, relatively low cost, low adverse impact on the environment and users. | Low stability, chlorine gas emission, and potential corrosion. | (Mikš-Krajnik et al., 2017; Park et al., 2004; Rahman et al., 2010) |

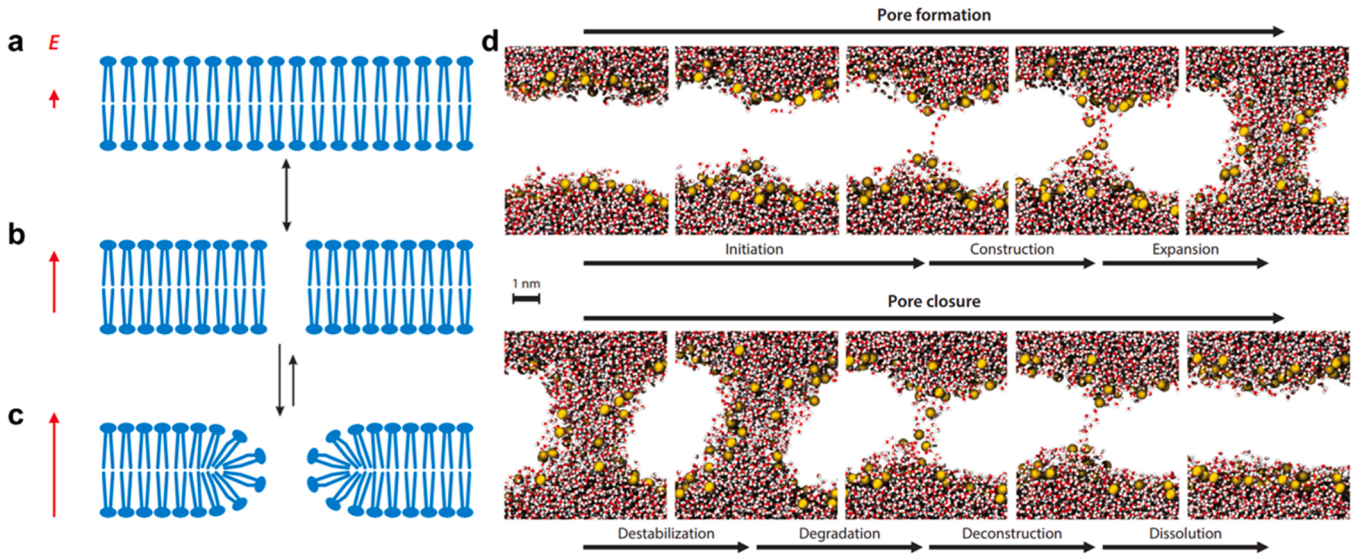



**Fig. 1.** Mechanism of electroporation. Starting with an intact lipid bilayer membrane (a). Electrically induced formation of aqueous pores in the lipid bilayer, shown here in two stages, with water molecules first penetrating the bilayer and thus forming an unstable hydrophobic pore (b), and with adjacent lipids then reorienting with their polar headgroups toward these water molecules and thus forming a metastable hydrophilic pore (c) (Kotnik et al., 2019). (d) The life cycle of an electrically induced pore in the lipid bilayer. Stages of pore formation and closure are displayed in their order of appearance but disregarding the differences in their characteristic timescales. Formation begins with the onset of the electric field, and closure begins as the field ceases. For clarity, only water molecules and phosphorus atoms from the lipid headgroups are shown (Levine and Vernier, 2012).

electric field ($E$) is estimated by Eqs. (1) and (2): (Kinosita et al., 1988; Weaver and Chizmadzhev, 1996)

$$\mathrm{TMP} = f_s \bullet R \bullet E \bullet \cos\theta \bullet \left[1 - \exp\left(-\frac{t}{\tau}\right)\right] \tag{1}$$

$$\tau = \frac{R \bullet C_m}{\left(\frac{2\lambda_e \bullet \lambda_i}{2\lambda_e + \lambda_i}\right) + \frac{R}{d} \bullet \lambda_m} \tag{2}$$

where $f_s$ is a function relating to the electric and geometric properties of the cell and the medium; $R$ is the radius of the cell; $\theta$ is the angle between a tangent at the studied point on the surface of cell and the direction of the electric field; $t$ is the duration of the external electric field; and $\tau$ is the membrane charging constant, which is related to the surface capacitance of the membrane ($C_m$), the membrane thickness ($d$), and the conductivity of intracellular cytoplasm ($\lambda_i$), extracellular medium ($\lambda_e$), and membrane ($\lambda_m$).

Depending on the treatment conditions and the breakdown TMP threshold for specific microorganisms, electroporation can be reversible or irreversible (Weaver and Chizmadzhev, 1996). For reversible electroporation, microbial cells reseal the pores and heal themselves, and thus maintain their activities (Kotnik et al., 2015). When the external electric field strength further increases, the TMP increases and at a certain point, irreversible electroporation happens and the microorganisms are inactivated (Kotnik et al., 2015).

Generally, EFT shows multiple advantages against other competing methods. EFT is independent of chemical disinfectants due to its physical nature of inactivation (Weaver and Chizmadzhev, 1996). EFT has been applied for the inactivation of multiple pathogens, including *Cryptosporidium*, a protozoan that is highly resistant to chlorination (Haas, C. and Aturaliye, D., 1999; Haas, C.N. and Aturaliye, D.N., 1999). Pathogens develop little resistance to EFT after survival, while ultraviolet radiation suffers from bacterial regrowth and resistance development (Gusbeth et al., 2009). Additional advantages of applying non-thermal EFT for food processing include sustaining the flavor, texture, and nutrient compositions (McAuley et al., 2016).

EFT has been well-studied for liquid food preservation, and some pilot- to full-scale equipment is already commercially available on the market. Sitzmann et al. summarized the development history of EFT from its early beginning around the 1940 s until the 1990s in various countries (Sitzmann et al., 2016) Jeyamkondan et al. reviewed how the processing parameters affected the EFT of food and pointed out that the high initial cost was the major obstacle in the industrial applications (Jeyamkondan et al., 1999). Yang et al. and Buckow et al. critically summarized the applications of EFT for alcoholic beverages and dairy products, respectively (Buckow et al., 2014; Yang et al., 2016). Wang et al. looked at the sub-lethal effects on cells after EFT, while Huang et al. provided an overview of the state-of-art EFT treatment chamber design (Huang and Wang, 2009; Wang M.-S et al., 2018).

The major obstacle of EFT is the high cost. To achieve desired inactivation performance, a strong electric field and a long treatment time are usually required, which thus results in an intensive consumption of energy (Barbosa-Cánovas and Zhang, 2019; Jeyamkondan et al., 1999). Meanwhile, unfavorable processes, such as overheating, electrochemical reactions, and electrode corrosion, take place in the treatment chamber, which further hinders the application of EFT (Goettel et al., 2013; Morren et al., 2003). Due to the same economic consideration, the application of EFT for water disinfection is relatively less developed. Therefore, in this review, we focus on the engineering aspect of EFT for pathogen inactivation, and summarize the current and potential solutions for the above obstacles.

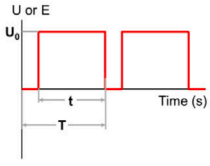
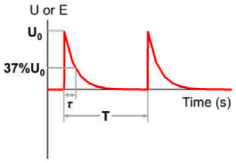
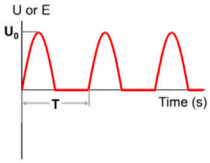
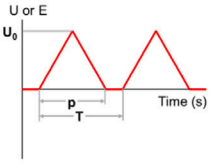
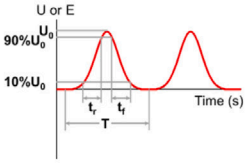
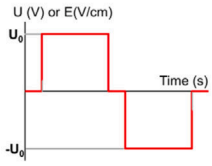
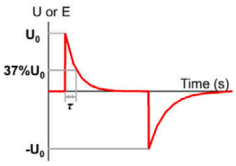
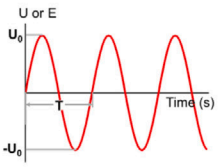
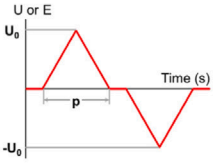
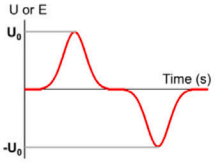
In the following Sections 3–6 of this review, the influence of key operating parameters (in three domains: process, product, and microorganisms) on EFT performance, the novel design, and materials are examined. The recent advances in how the operating parameters affect the EFT performance are summarized in Table 3. In most previous studies, liquid food or artificial water samples were used to test the performance of microbial control. The research outcomes and engineering experiences gained from both water disinfection and food pasteurization will hugely benefit each other in the adoption of real-world applications.

**Table 3**
Summary of the recent research advances in EFT for pathogen inactivation.

| Target pathogen | Liquid | pH | Cond.* (ms $cm^{-1}$) | $Temp_{in}$* (℃) | $t_{pw}$* (μs) | f* (Hz) | Pulse shape | E * (kV $cm^{-1}$) | $Energy_m$* (kJ/kg)$^{+}$ | $LIE_m$*, # | Ref* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Salmonella Panama*, *Saccharomyces cerevisiae*, *E. coli*, & *Listeria monocytogenes* | Apple, orange, and watermelon juices | 3.5–5.3 | 2.6–3.8 | 20 & 36 | 2 | 120–964 | Monopolar | 20 | 90 | 7 | (Timmermans et al., 2014) |
| *E. coli*, *Listeria innocua*, *Saccharomyces cerevisae* & *Bacillus megaterium* | Ringer solution | NR※ | 1.25–1.5 | 45 | 1.5–10 | 1–100 | Exponential | 16 | 120 | 6 | (Toepfl et al., 2007) |
| *Bacillus cereus* spores | Milk (whole and skim) | 6.56 & 6.31 | 5.12 & 4.90 | 45–75 | 5–20 | 75–175 | NR | 30, 35, & 40 | NR | 6.6 | (Bermúdez-Aguirre et al., 2012) |
| *E. coli*, *Staphylococcus aureus*, & *Listeria innocua* | Whole milk | NR | 3.91 | 4–55 | 20 | 10–60 | NR | 23–28 | 462.5Δ | 8 | (Sharma et al., 2014) |
| *E. coli* & *Pseudomonas fluorescens* | Whole milk | NR | NR | 50–56 | 22.5 & 20 | 209–626 | NR | 30 & 35 | 150Δ | 7 | (Walter et al., 2016) |
| *E. coli* K12, *Staphylococcus aureus*, & Pseudomonas fluorescens | Milk | 6.69 | NR | 32.5 | 4–32 | up to 1k | NR | 20–42.5 | 478 | 7 | (Cregenzán-Alberti et al., 2015) |
| *E. coli* | Cranberry juice | 2.49 | 0.94 | 20, 30, & 40 | N/A | 20k | NR | 2.2–13.2 | 380.8 | 6.6 | (Rezaeimotlagh et al., 2018) |
| *Staphylococcus aureus* | Citrate-phosphate buffer | 7 | 2 | 10–40 | 4 | 0.5 | Exponential | 26 | 4.25 | 2.5 | (Cebrián et al., 2016) |
| *Pseudomonas putida* | Hospital wastewater | 6–8 | 1.1–1.7 | 25 | 0.6 | 0.3 | NR | 100 | 120 | 3.5 | (Gusbeth et al., 2009) |
| *Enterococcus faecium* & *Pseudomonas aeruginosa* | Clinical wastewater | NR | 1000–1200 | NR | 1 | 10 | NR | 80 | 190 | 5.5 | (Rieder et al., 2008) |
| *Listeria innocua* | Liquid whey protein | 4–7 | 3.7 | 20–40 | 3 | 12k | Rectangular | 32 | 160 | 6.5 | (Schottroff et al., 2019) |
| *Candida humilis* & *Saccharomyces cerevisiae* | Phosphate buffer | 6.5 | 0.5– 0.6 | 20–32 | 0.086–4 | 10k | Square | 17–71 | 48 | 3.9 | (Ou et al., 2017) |
| *Bacillus subtilis* spore | Distilled water | NR | NR | 30–75 | NR | 60 | NR | 0.3 | NR | 2 | (L.-H. Wang et al., 2020) |
| *E. coli* | Fresh carrot juice | 6.03 | 6.0 | NR | 1 | 1 | Square unipolar | 9–21 | NR | 3.5 | (Singh et al., 2017) |
| *E. coli* | Buffered peptone water | NR | NR | 30–40 | 1.2 | 1–51k | NR | 15 | NR | 4.5 | (Krishnaveni et al., 2017) |
| *Saccharomyces cerevisiae* | Fresh medium | 4 & 6 | 2.52–4.23 | 25, 50, & 60 | 2.5 | 54 | Exponential | 25 & 50 | 170Δ | 3.5 | (Montanari et al., 2019) |
| *Lactobacillus rhamnosus* | Raw milk and Ringer solution | 6.7 | 5.3 | 30 | 3–8 | 400 | Rectangular | 10–30 | 240 | 6 | (Jaeger et al., 2009) |
| *Enterococcus faecium* | Citrate-phosphate buffer | 4.0, 5.5, & 7.0 | 2 | 4 & 37 | 2.96 | 1 | Exponential | 37 | 1580 | 5 | (Fernández et al., 2018) |
| *E. coli* | DI water with KCl | NR | 0.180 | 15 | 20 | 1k | Bipolar square | 23.3 | NR | 3.5 | (Liu et al., 2017) |
| *Listeria monocytogenes* & *Staphylococcus aureus* | Citrate-phosphate McIlvaine buffer | 3.5–7.0 | 1.0 | 37 | 3 | 1 | Square | 15–35 | 3.7 | 6.1 | (Saldaña et al., 2010a) |
| *E. coli* (4 strains) | Citrate-phosphate McIlvaine buffer | 3.8–4.0 | 2 | 35 | NR | 1–60 | Exponential | 10–40 | NR | 4 | (Somolinos et al., 2008) |
| *Enterobacter sakazakii* | Citrate–phosphate McIlvaine buffer | 3.5–7.0 | 2 | 35 | 4 | 1 | Exponential | 19–37 | 8.53 | 5 | (Arroyo et al., 2010) |
| *E. coli* & *Salmonella Typhimurium* | Citrate-phosphate McIlvaine buffer | 3.5–7.0 | 1.0 | 35 | 3 | 1 | Square | 15–35 | 3.7 | 5 | (Saldaña et al., 2010b) |
| ***Range*** | | 2.49–7.0 | 0.180–1200 | 4–75 | 0.086–32 | 0.3–51k | | 0.3–100 | 3.7–1580 | 2–8 | |

*- The "Cond.", "Tempin", "tpw", "f", "E", "Energym", "LIEm", and "Ref" stands for "conductivity", "influent temperature", "pulse width", "frequency", "electric field strength", "maximum specific energy input", "maximum log inactivation efficiency", and "references", respectively.
# - For some studies, the presented maximum log inactivation efficiency reached the maximum detection limit.
+- The unit of the maximum specific energy input is normalized to "kJ/kg" by the density of the liquid.
※ - The "NR" stands for "not reported" in the original studies.
Δ - The density of the liquid was not reported in the original paper. To normalize the maximum specific energy input, the density of the liquid is assumed to be 1 kg/L.

**Table 4**
Pulse shapes applied in EFT.

| Pulse | Square | Exponential | Sinusoid | Triangle | Bell |
|---|---|---|---|---|---|
| Shape (Unipolar) |   |   |   |   |   |
| Shape (Bipolar) |   |   |   |   |   |
| Defining parameters | $U_0$ - Amplitude, t - Pulse width, T - Period. | $U_0$ - Amplitude, $\tau$ - Time constant*, T - Period. | $U_0$ - Amplitude, T - Period. | $U_0$ - Amplitude, T - Period, p - Time constant. | $U_0$ - Amplitude, $t_r$ - Rise time, $t_f$ - Fall time, T - Period. |
| Equation | $U = U_0$ | $U = U_0 \cdot e^{-t/\tau}$ where the $\tau$ defines the pulse shape | $U = U_0 \cdot \sin\left(\frac{2\pi}{T} \cdot t\right)$ | $U = \frac{2U_0}{\pi} \cdot arcsin\left[\sin(\frac{2\pi}{p} \cdot x)\right]$ | Each half bell shape follows the normal distribution equation. |

*- The time constant refers to the time to decrease the voltage/electric field strength to 37 % (1/e) of its peak value.

## 3. Key processing parameters of the EFT

The processing parameters are summarized in two levels. First-level parameters are the properties of electric pulses including the amplitude, width, shape, frequency, and polarity. These parameters are extrinsic and can be adjusted on the pulse generator. The parameters at the second level include the electric field strength, effective treatment time, and specific energy input. These three parameters are determined based on the first-level pulse properties and the EFT device configuration. Meanwhile, these parameters are often used to predict the inactivation efficiency by mathematical models. In terms of the performance, we primarily focus on the pathogen log inactivation efficiency, which is calculated by Eq. 3 wherein the $c_{eff}$ and $c_{in}$ represents the effluent and influent pathogen concentrations. The unfavorable side reactions and their energy consumption are also evaluated.

$$\text{Log inactivation efficiency} = -\log_{10}(c_{eff}/c_{in}) \quad (3)$$

### 3.1. First-level pulse properties

**Amplitude.** An electric pulse is defined by its amplitude, shape, and width, while a number of pulses, *i.e.*, the pulse train, can be assembled with different frequencies and polarity. The amplitude of the pulses is the peak value of the applied voltage in a pulse train. In general, a higher applied voltage leads to higher inactivation efficiency, energy consumption, and the rate of side reactions (Jeyamkondan et al., 1999).

**Pulse shape.** The most used pulse shapes include square, exponential, sinusoid, triangle, and bell pulses (Table 4). Currently, the exponential waveform is the most widely used in commercial EFT (Cebrián et al., 2016; Fernández et al., 2018; Toepfl et al., 2007). Although the long and low-intensity tail of the exponential waveforms prolongs the treatment time and generates extra heat, generating exponential pulses is the least expensive option. When the amplitude is fixed, the square waveform is generally more effective than other waveforms, because bacterial cells are continuously subjected to EFT for an extended period (Kotnik et al., 2003). However, generating square pulses needs more complex pulse-forming electric networks (Jeyamkondan et al., 1999). In general, the study of pulse shapes is limited, partially due to the high capital cost of programable high-voltage pulse generators.

**Pulse width.** Pulse width (or pulse duration, $t_{pw}$) is the time interval that the amplitude of the pulse is higher than a specific value. According to the theory of electroporation, bacterial cells placed in the electric field function as capacitors (Weaver and Chizmadzhev, 1996). A pulse width of a few μs is generally required to build up TMP and realize electroporation (Mahnič-Kalamiza et al., 2014). The pulse width of a square waveform is the time it maintains at the maximum applied voltage, *i.e.*, the amplitude. It becomes more complicate for other pulse shapes. In some cases, the time interval higher than 37 % or 50 % of the amplitude is regarded as the pulse width. The pulse width of different waveforms can vary when the amplitude ($U_0$), threshold (0.5 $U_0$), and unit time period ($T_0$) are fixed, as the example shown in Fig. 2. Mañas et al. (2001) reported that the inactivation efficiency was independent of the pulse width (1.2–1.9 μs) if the total input energy was constan. The decrease of pulse width from 2 μs to 300 ns of a square waveform did not significantly lower the inactivation efficiency of *Lactobacillus plantarum*, either (Fox et al., 2008). Nanosecond EFT (even shorter pulse width in the range of nanoseconds) can also cause a lethal or sub-lethal effect on the pathogens (Chopinet and Rols, 2015; Perni et al., 2007). The short pulse width (*i.e.*, short charging time) may lead to the damage of organelle membrane, a potential difference across individual organelles, and thus, the inactivation of bacteria (Kolb et al., 2006; Kotnik and Miklavčič,

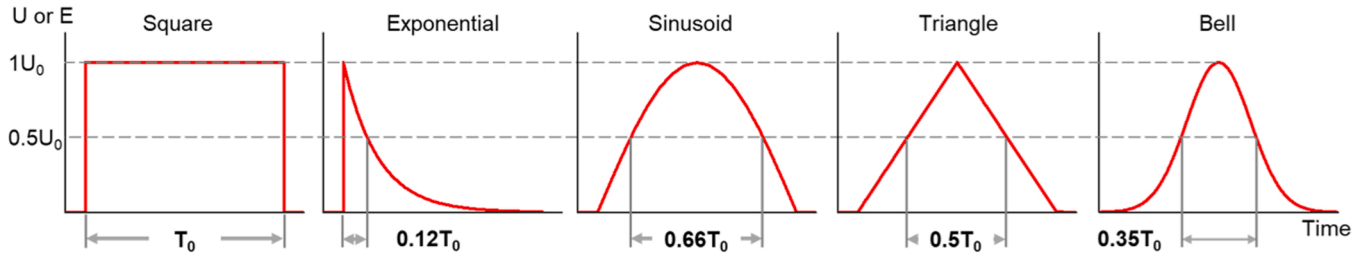



**Fig. 2.** An example of the pulse width for a given voltage/electric field to be exceeded (0.5 $U_0$) of different pulse shapes. The figure shows the case of unipolar pulses of unit $T_0$ and $U_0$ amplitude. For the exponential waveform, the τ value is $T_0$ and the $U_{T0}$ value is 0.003 $U_0$. For the sinusoid waveform, the T equals $T_0$. For the triangle waveform, the p equals to 2 $T_0$. For the bell-shape waveform, the $t_r$ and $t_f$ both equals 0.5 $T_0$ and the $U_{T0}$ value is 0.003 $U_0$.

2006). Besides, the pulse width also affects the rate of electrochemical reactions in the electrode-media interface. Typically, when the pulse width is shorter than $10^{-4}$ s, electrochemical reactions can be largely eliminated (Chang and Park, 2010).

**Frequency.** Frequency (*f*) is the number of pulses applied in a unit time and is the reciprocal of the period (T, *f*=1/T). How the frequency affects EFT is still controversial in different studies in the literature. In the case of the inactivation of *Bacillus* spores, the influence of frequency (75–175 Hz) and pulse width (5–20 μs) is not notable (Bermúdez-Aguirre et al., 2012). On the contrary, according to Giladi et al., EFT with high frequency inhibits the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The inhibition effect maximizes at 10 MHz with a test range of 0.1–50 MHz when the total treatment time is kept the same (Giladi et al., 2008).

**Polarity.** The polarity (unipolar or bipolar) of the pulses also influences the pathogen inactivation efficiency. Multiple mechanisms may be involved, including the charge movement on the cell membrane, and microbial cell movement subjected to the electric field. In terms of the charge movement, bipolar waveforms show better inactivation than monopolar ones in the parallel plate EFT device (Qin et al., 1994; Wang et al., 2018). The sudden change of the polarity leads to the movement of the charged groups, structural fatigue of the cell membrane, and thus more vulnerable cells against the electric field (Qin et al., 1994). The microbial cell movement in the electric field can be induced by the electrophoretic force. For example, negatively charged bacteria tend to move against the direction of the electric field, while positively charged bacteria along the direction of the electric field (Zhou et al., 2019). The force usually does not directly contribute to inactivation, but could be utilized to transport the bacterial cells to specific regions.

### 3.2. Second-level pulse parameters

**Electric field strength.** The electric field strength is one of the key parameters to describe the intensity of EFT. The strength is not directly set up from the pulse generator, but is collaboratively determined by the reactor geometry and the externally applied voltage. In a parallel plate configuration, a uniform electric field is generated with the strength equal to the applied voltage divided by the distance between the two electrodes (Raso et al., 2016). In another commonly used coaxial-electrode configuration, the electric field is described by Eq. (4) (where $E_s$ is the strength of the electric field at the place with a distance of *s* to the center of the chamber, *U* is the externally applied voltage, $r_{center}$ and $r_{outer}$ is the radius of the coaxial center and outer electrode, respectively), which indicates the non-uniform electric field strength in the treatment chamber (Di Bartolo, 2004; Zhou et al., 2019)

$$E_s = \frac{U}{s \bullet \ln \frac{r_{center}}{r_{outer}}} \tag{4}$$

**Effective treatment time.** The effective treatment time ($t_e$) is determined by Eq. (5) (where $t_{pw}$ is the pulse width and *n* is the number of pulses applied) after the pulse width is identified in an EFT. The total treatment time (*t*), determined by Eq. (6) (where *T* is the pulse period) is usually longer than the effective treatment time because of the existence of the low-electric-field or low-voltage time intervals. In most cases, the inactivation efficiency increases with the effective treatment time (Bermúdez-Aguirre et al., 2012; Jeyamkondan et al., 1999).

$$t_e = n \bullet t_{pw} \tag{5}$$

$$t = n \bullet T \tag{6}$$

**Total and specific energy input.** The total energy input of aanEFT always consists of the energy consumption of the applied electric pulses, the capacitor for the conversion of high-intensity pulses, and the discharge. In addition, cooling systems are usually necessary for food processing as joule heating generates along the EFT. The total energy input is critical to evaluate the feasibility of a technology to be adopted at full scale.

The specific energy input is referred as the energy consumption of the applied electric pulses, which is a part of the total energy input (Rodriguez-Gonzalez et al., 2015). The specific energy input (*P*, with a unit of kJ $L^{-1}$ or kJ $kg^{-1}$) can be determined by Eq. 7, where the energy input (*W*) is calculated by the integration of the pulse profile, voltage (*U*) times current (*I*), with respect to time (*t*) (Rodriguez-Gonzalez et al., 2015). As high electric field strength (usually >20 kV $cm^{-1}$) is required to achieve sufficient disinfection (*e.g.*, >5-log), the specific energy input of EFT is usually in the range of 40–1000 kJ $kg^{-1}$ (Timmermans et al., 2014; Toepfl et al., 2007, 2006). For comparison, the specific energy input of a thermal treatment that raises temperature from 13.62 ℃ to 78.47 ℃ is ~50 kJ•$kg^{-1}$ with a heat exchanger to recover energy (Kažimírová, 2013).

$$P = \frac{W}{Vorm} = \frac{\int U \bullet Idt}{Vorm} \tag{7}$$

Mathematical models have been established to predict the inactivation efficiency of EFT. For example, Hulsheger et al. reported a model to calculate the log inactivation from the electric field strength (*E*) and effective treatment time ($t_e$) (Eq. (8), where $E_c$ and $t_c$ are lethal electric field strength and critical treatment time, and *k* is the specific constant for each particular setup) (Hülsheger et al., 1981). Notably, both the $t_e$ and *E* should be larger than the $t_c$ and $E_c$, respectively to yield a valid estimated inactivation efficiency. Another model, Weibull distribution (Eq. (9)), has also been used in multiple studies. In Eq. (9), different parameters (including *E*, $t_e$, and specific energy input, *P*) can be taken as the independent variable (*x*) to estimate the *S* (Singh et al., 2017). The coefficient $\alpha$ stands for the *E*, *t*, or *P* required for the first log of inactivation. The coefficient $\beta$ decides the shape of the curve ($p < 1$, upward concavity; $p = 1$, linear; $p > 1$, downward concavity). A close linear relationship between the specific energy input and inactivation efficiency has been observed in several studies (Huang et al., 2012). A comprehensive review of the models for microbial inactivation by EFT can be found in the review by Huang et al. with additional functions like logarithmic and sigmoid models (Huang et al., 2012).

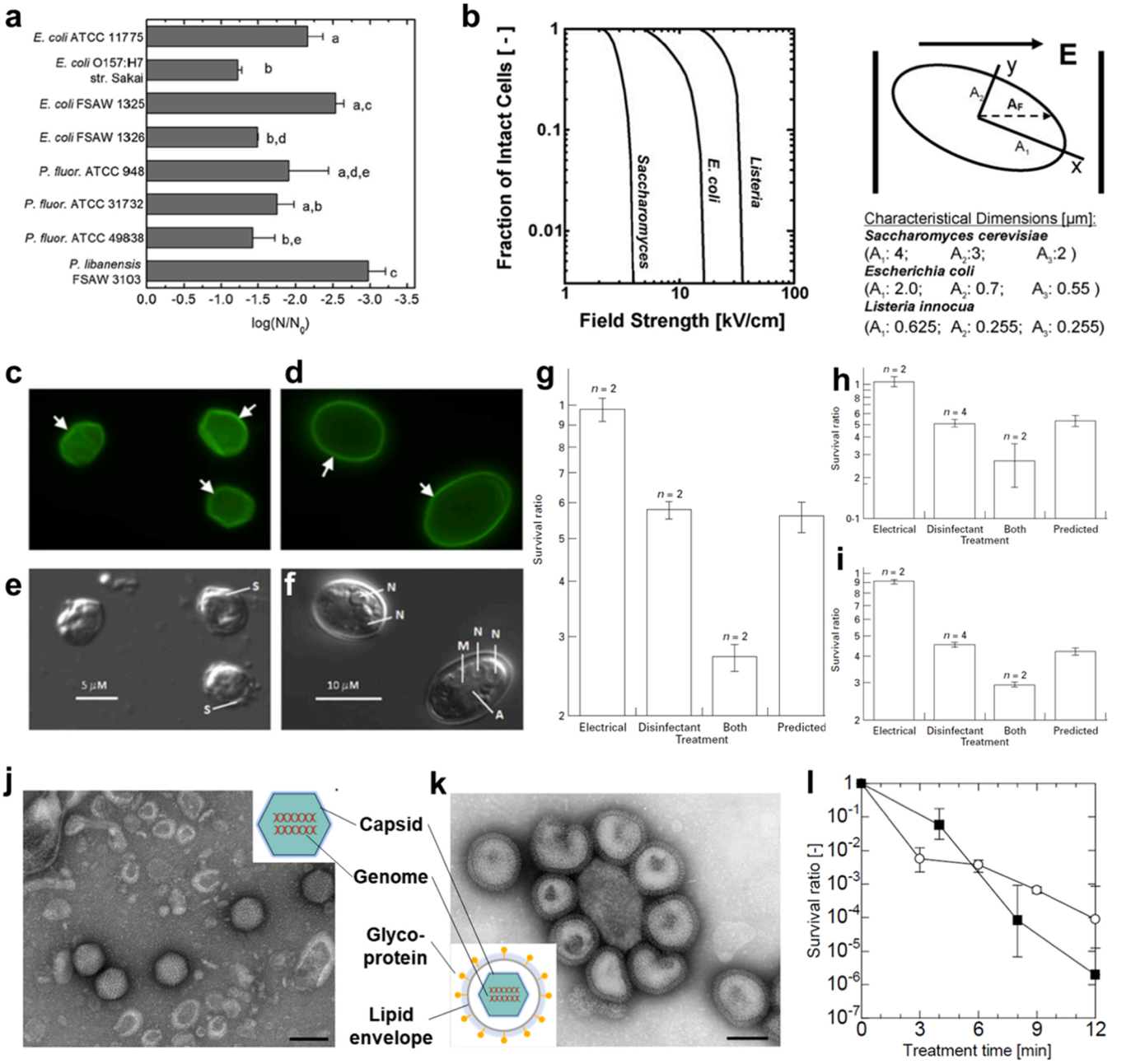



**Fig. 3.** EFT inactivation of bacteria (a&b), protozoa (c-i), and viruses (j-l). (a) Inactivation of selected strains of *E. coli* and *Pseudomonas* spp. following EFT at 35 kV/cm for 30 µs with an average temperature of 40 °C. Inactivation levels (log ($N/N_0$) of strains labelled with the same superscript letter (a-e) are not significantly different from each other ($p < 0.05$). Error bars show standard deviation ($n = 2$) (Walter et al., 2016). (b) Impact of orientation of ellipsoidal microorganisms relative to the electrical field *E*. At a cell specific threshold level, the field strength inside the cell membrane exceeds a threshold level $E_{crit}$. Those cells are electroporated which have their longer semi-axis in parallel to *E*. Other orientations require field strengths in excess of $E_{crit}$. Three organisms, different in geometry have been chosen as examples. The chart on the left shows the fraction of cells which have an orientation which does not cause electroporation in response to the given external field strength (Toepfl et al., 2007). (c)–(f) Microscopic images of *Cryptosporidium* oocysts (c) and *Giaridia* cysts (d) with brilliant apple-green FITC fluorescence of spherical objects 4–6 µm in diameter with brightly highlighted edges, (e) one to four sporozoites (S) per oocyst for *Cryptosporidium*, and (f) one or more discernable internal structure such as nuclei (N), median body (M), and/or axonemes (A). White arrows: brilliant apple green fluorescence staining *Cryptosporidium* oocysts and *Giardia* cysts walls (Rhodes et al., 2012). (g–i) Inactivation of protozoa with a combination of EFT and chemical oxidants (g) Inactivation of *Cryptosporidium* by hydrogen peroxide. Electrical: 87 J/ml; chemical: 100 mg/min (h) Inactivation of *Giardia* by potassium permanganate. Electrical: 87 J/ml; chemical: 40 mg/min. (i) Inactivation of *Cryptosporidium* by potassium permanganate. Electrical: 22 J/ml; chemical: 120 mg/min (Haas, C. and Aturaliye, D., 1999). (j) Negative stain of a large naked icosahedral virus (adenovirus). Note bead-like capsomeric structures that form flat triangular facets on the surface. Bar: 100 nm. Magnification: X100,000. (k) Negative stain of an enveloped virus with clear surface projections (influenza B virus). Bar: 100 nm. Magnification: X100,000. (l) Time courses of M13mp18 phage survival ratio in the EF treatment at 5 (open circles) and 7 (closed squares) kV (Tanino et al., 2013b).

$$\text{Log inactivation efficiency} = \frac{(E - E_c)}{k} \bullet \log_{10}(\frac{t_e}{t_c}) \quad (8)$$

$$-\log(S) = (x/\alpha)^{\beta} \quad (9)$$

## 4. Effectiveness of EFT against various pathogens

Most previous studies have used bacteria as the model microorganism and proved the high inactivation efficiency by EFT. Meanwhile, protozoa and viruses are less investigated. In terms of protozoa, studies directly demonstrating the effectiveness of EFT are lacking, but EFT can boost the inactivation efficiency of chlorination against *Cryptosporidium*, a chlorine-resistant protozoan. The occurrence of electroporation on viruses is still controversial, which may be dependent on their structures, *i.e.*, with or without envelopes.

### 4.1. Bacteria

Most previous studies have focused on the inactivation of bacteria (Timmermans et al., 2014; Toepfl et al., 2007). The intrinsic characteristics of bacteria, including the species, strains, gram staining, size, and shape, affect the inactivation efficiency (Jeyamkondan et al., 1999). Some bacterial species were more resistant to EFT, including *Listeria monocytogenes* STCC 5672 and *Staphylococcus aureus* STCC 4459. (Saldaña et al., 2010a). Notably, even within the same species, different strains of bacteria showed a significant difference (~2-log) in inactivation efficiency (Fig. 3a) (Walter et al., 2016). Whether gram-positive or gram-negative bacteria are more susceptible to EFT is still controversial (Jeyamkondan et al., 1999; Saldaña et al., 2010a; Timmermans et al., 2014; Toepfl et al., 2007). Some researchers found gram-positive bacteria more resistant due to their thick and rigid cell wall, while others found gram-negative more resistant due to the low fluidity of the outer membrane (Saldaña et al., 2010a; Sharma et al., 2014). According to the current understanding, the observed results cannot be explained by gram staining alone, but collaboratively with the strains, size, and shape (Jeyamkondan et al., 1999).

**Size and shape.** The bacterial geometry (size and shape) affects the lethal electric field threshold and hence the inactivation efficacy. It is generally agreed that microbes with bigger sizes are more vulnerable to EFT because a higher TMP can be built (Eq. (1)) (Timmermans et al., 2014; Toepfl et al., 2014). Toepfl et al. (2007) explained this phenomenon by establishing a mathematical model and predicted the relationship between the fraction of intact cells and electric field strength. The shape of the bacteria also matters since the diameter of an "imperfectly round" bacterium is not the same in different directions. In general, a higher electric field is required to build up the same TMP for a rod-shaped cell compared with a spherical-shaped cell (Fig. 3b) (Timmermans et al., 2014). The impact of bacteria orientation in the field also plays a role. Even though the electric field was uniform in the reactor, non-uniform treatment might be possible because of the random orientation and characteristic dimensions of the bacteria. The agglomeration of bacterial cells may weaken the inactivation efficiency by reducing the local electric field distribution (Toepfl et al., 2007). Such agglomeration may be caused by the high density of bacteria, which indicates that the initial bacterial concentration also affects the EFT performance (Bermúdez-Aguirre et al., 2012).

**Culture condition and temperature.** The cultural condition of the bacteria also has an impact on the inactivation results. Arroyo et al. reported that bacteria in the exponential growth phase were more easily inactivated than bacteria in the stationary phase. (Arroyo et al., 2010) Another factor that may affect EFT is the culturing temperature. Liu et al. studied the impact of temperature in terms of the fatty acid composition on the cell membrane. (Liu et al., 2017) They observed that the fatty acid composition varied dramatically in stationary-phase bacteria at different growing temperatures. Such difference resulted in an increase of inactivation efficiency with a decrease of growing temperature from 37° to 15°C. The culturing temperature rarely affected the inactivation efficiency of the bacteria in the exponential growth phase due to the composition of membrane fatty acid maintained stable across the temperature. On the contrary, Cebrián et al. reported no statistical differences in the inactivation efficiency against *Staphylococcus aureus* when the growing temperature was 10, 20, 37, or 42 °C (Cebrián et al., 2016). The membrane fluidity measurement in this study did not match the trend of the inactivation efficiency, and thus the authors cast doubt on the existed causal relationship between membrane fluidity and inactivation performance.

### 4.2. Protozoa

EFT has been validated to inactivate protozoa, even though few studies have directly reported the influence of processing parameters (Fard et al., 2013; Haas, C. and Aturaliye, D., 1999; Slavik et al., 1993). Electroporation could occur on protozoa, since most of them possess a plasma membrane that encloses cytoplasm (Fig. 3c-f) (Yaeger, 1996). Several studies delivered foreign genes (DNA, RNA, and plasmids) into protozoa (*e.g.*, *Giardia lamblia* and *Entamoeba hitolytica*) by reversible electroporation (Nickel and Tannich, 1994; Yee and Nash, 1995). However, the effect of EFT against protozoa may be significantly different when the protozoa are in different life cycle stages. Trophozoite, the activated, feeding stage of the protozoa, was used for biomedical applications, since the purpose was for gene transfection by reversible electroporation (Nickel and Tannich, 1994). (Oo)cysts were used in pathogen inactivation experiments, since protozoa are commonly found as (oo)cysts in the environment (Erickson and Ortega, 2006; Haas, C. and Aturaliye, D., 1999). (Oo)cysts are stages with a rigid and thickened wall, which helps the (oo)cysts to survive against different disinfection agents or in the harsh environment (Yaeger, 1996). The inactivation of protozoa by EFT was reported by Haas et al. *Giardia* and *Cryptosporidium* (oo)cysts were treated with a specific energy input between 30 and 1500 kJ $L^{-1}$ (Haas, C. and Aturaliye, D., 1999; Haas, C. N. and Aturaliye, D.N., 1999). The inactivation efficiency of stand-alone EFT was not significant (<1-log). Nevertheless, the EFT demonstrated an enhancement effect against the chlorine-resistant protozoa when cooperating with disinfectants like permanganate and peroxide (Fig. 3g–i) (Haas, C. and Aturaliye, D., 1999).

### 4.3. Viruses

Viruses are bioparticles containing the viral genome packaged in a protein coat (capsid) (Goyal and Cannon, 2006; Lucas and Knipe, 2001). For some viral species (*e.g.*, Coronavirus, human immunodeficiency virus (HIV), and hepatitis B virus (HBV)), the capsid is covered by an envelope containing lipid bilayers and proteins (Goyal and Cannon, 2006; Sakudo et al., 2011). Typically, non-enveloped viruses (*e.g.*, bacteriophage MS2, norovirus, and human hepatitis A virus (HAV)) are more resistant against disinfectants than the enveloped viruses (Fig. 3j&k) (Hirneisen et al., 2010; Lin et al., 2020). When treated by EFT, the enveloped viruses could be theoretically inactivated by electroporation because of their membrane-like glycoprotein-rich lipid bilayer structure. However, stronger treatment conditions are expected because the "TMP" is more difficult to be built up on viruses of smaller size. Besides, the occurrence of electroporation on non-enveloped viruses is still unclear (Hirneisen et al., 2010) Swine Vesicular Disease Virus (SVDV, enveloped) and Equine Herpesvirus-l (EHV-1, enveloped) were inactivated (up to 9-log) by an EFT of 30 kV•$cm^{-1}$ electric field strength and a pulse number of 60–120 times (Fig. 3l) (Mizuno et al., 1990) After treatment, the shape of the SVDV capsid was maintained, while the RNA in the core disappeared and hollow particles were observed. Other studies reported the inactivation of MS2 with various processing parameters and experimental setups (Huo et al., 2019; Tanino et al., 2013a). On the contrary, the inactivation efficiency of

Rotavirus (non-enveloped) was negligible at 20–29 kV $cm^{-1}$ with a treatment time of 145.6 ms (12 pulses) (Khadre and Yousef, 2002). To conclude, though several studies have demonstrated the effectiveness of EFT against viruses (enveloped and non-enveloped), more comprehensive studies are needed and the mechanisms to cause inactivation are still unclear.

## 5. Impact of liquid properties on the performance of EFT

### 5.1. Temperature

Microbial inactivation is highly sensitive to the liquid temperature during EFT, which is different from the culturing temperature discussed in Section 4.1. Typically, the medium temperature increases during the EFT because of the Joule heat generated by the current. The unit Joule heat generation ($Q$) per time per volume is determined by the conductivity ($\delta$) of the medium and the strength of the electric field ($E$) (Eq. (10)) (Salengke et al., 2012). The heat results in a temperature increase ($dT$), which depends on the treatment time ($t$) and the density ($\rho$) and heat capacity ($c$) of the medium (Eq. (11)) (Salengke et al., 2012).

$$Q = \delta \bullet E^2 \tag{10}$$

$$\frac{dT}{dt} = \frac{Q}{\rho \times c} \tag{11}$$

The Joule heat generated during EFT is usually used as additional stress to enhance inactivation efficiency (Montanari et al., 2019). The elevated temperature of 50–75 °C assisted inactivation when the same electric pulses are applied to the system (Bermúdez-Aguirre et al., 2012; Sharma et al., 2014; Somolinos et al., 2008). Mild heating induces the increase of membrane fluidity, reduction of lipid bilayer thickness, and disorganization of outer cell membrane (gram-negative bacteria only), which thus makes the microorganisms more susceptible to the EFT (Montanari et al., 2019; Sharma et al., 2014). The lethal electric field strength threshold for bacteria also decreases with the increased temperature (Coster and Zimmermann, 1975). During the normal food processing, some liquid foods are pre-cooled in the refrigerator for storage. Researchers have found that, after exposure to the sublethal cold stress (4 °C), acid-adaptive bacteria, *Enterococcus faecium*, can be inactivated more easily by EFT (Fernández et al., 2018). According to Somolinos et al., the effect of cold shock during EFT was dependent on the strains of bacteria, the electric field strength, and the treated medium (Somolinos et al., 2008).

### 5.2. pH

Extreme pH deviated from neutral, especially acidity, increases inactivation efficiency by exposing the microorganisms to an unfavorable environment (Rezaeimotlagh et al., 2018; Saldaña et al., 2010a; Timmermans et al., 2014). Different species of bacteria show different tolerance to pH during EFT. For example, pH affected the inactivation of *E. coli*, but not as much as *Salmonella* (Saldaña et al., 2010b). Timmermans et al. found that the reduction of bacteria with pH 3.5–3.7 was higher than that with pH 5 in juice media (Timmermans et al., 2014). When the pH was adjusted from 5 to 3.6 by HCl, the inactivation rate increased to an extent similar to that of the pH 3.5 juice. The disturbance of cytoplasmic pH by the ambient environment was concluded as the reason for the observed results. Meanwhile, the formation of non-dissociated organic acids and their transportation into the cell might also cause the enhanced inactivation efficiency at a lower pH (Timmermans et al., 2014).

### 5.3. Conductivity

The influence of media conductivity on the performance of EFT is rather complex. It is generally agreed that the microorganisms are more sensitive to the EFT when DI water is used as the media. This is due to the additional osmotic pressure caused by the large difference between the cell cytoplasm and the media. When buffer solutions or liquid foods are used with a much higher conductivity, the operating current also increases due to the reduction of the overall system resistance. This leads to an increase in the heat generation and thus overall energy consumption. In some cases, the temperature of the treated product is increased due to the heat generated, which indirectly assists the pathogen inactivation as a second stress (Gachovska et al., 2013). For water disinfection, this phenomenon can be utilized, and the cooling systems commonly used during food processing are not necessary, due to no overheating concern for drinking water treatment. However, the heat production during EFT should always be minimized since thermal treatment is an energy-intensive process.

### 5.4. Other properties

Lower water activity has a suppressive effect on the inactivation efficiency of EFT (Cebrián et al., 2012). The water activity can be adjusted from $> 0.99$ to $\sim 0.80$ by adding NaCl, sucrose, or glycerol to the media (Aronsson and Rönner, 2001). As reported by Arroyo, the water activity decreased from $> 0.99$–$0.97$ with sucrose, the inactivation rate dropped from $\sim 5$ logs to $< 1$ log (Arroyo et al., 2010). It is suggested that the reduction of water activity led to a thickening of the cell membrane, thus reducing membrane permeability and fluidity.

When EFT is applied in dairy products, the effect of protein and fat on the inactivation efficiency has been studied (Buckow et al., 2014). Jaeger et al. (2009) reported a reduction of inactivation efficiency in raw milk than in buffer solution, suggesting that protein provides protecting effect for the bacteria during EFT. The protection effect of protein is also dependent on the concentration and ratio of different proteins. In contrast, the fat components were shown not to provide protection against EFT (Bermúdez-Aguirre et al., 2012). According to Bermúdez-Aguirre et al. (2012) skim milk with a lower fat content (0.3 %) achieved better inactivation than whole milk (4 %) when *Bacillus cereus* spores were inactivated.

## 6. Novel configuration and materials for EFT devices

Configuration and materials are also critical for the application of EFT in water disinfection. To develop novel configurations and materials, both computational and experimental approaches have been applied. In terms of the computational approach, simulations of electric field distribution, temperature change, flow pattern, microbial movement, and inactivation efficiency have been conducted using the finite element method. The computational approach is usually used in well-studied systems with conventional electrode materials (*e.g.*, stainless steel). The geometry of the device can be optimized towards specific research goals. Experiments are usually employed to validate the computational results. Experimental approaches are also used independently, especially in developing new electrode materials and the studies of the complex environment.

**Towards uniform electric field.** From a conventional perspective, the electric field in the treatment chamber should be as uniform as possible to avoid overheating. This becomes remarkably important in centralized large-scale applications. Knoerzer et al. (2012) developed an iterative algorithm that could automatically modify the geometry and dimension of the treatment chamber. The optimized chamber brought a more uniform electric field distribution, and at the same time maximized the treatment volume. Employing the computational method, other researchers investigated the uniformity of the electric field by comparing the performance of different chamber designs, designing the shape of the insulator, and optimizing the geometry of the treatment chamber (Buckow et al., 2011; Masood et al., 2018, 2017). Optimizing the flow pattern can also lead to a more uniform treatment of EFT, since the dead zones are eliminated to avoid overheating. Schottroff et al. (2020)

developed a vortex-flow chamber with a non-collinear inlet and outlet. According to the simulation results, the design created a swirl flow, which enhanced the turbulence, flow mixing, and treatment uniformity. Experimental approaches were also used. Zhu et al. (2017) designed three microchips (planar comb teeth, interdigitated electrodes, and parallel plate, and found the parallel plate configuration outcompeted other designs in the inactivation efficiency due to its electric field uniformity.

**Towards the reduction of the applied voltage.** In the recent ten years, researchers gradually focus on reducing externally applied voltage by reinventing the system configuration and electrode materials, while a similar level of pathogen inactivation can be maintained. Lower applied voltage leads to lower energy consumption on electric pulses. Furthermore, when the applied voltage is reduced to lower than several hundred volts, the high capital cost of complex high-voltage pulse generator systems can be reduced. Three strategies have been developed to realize the lower applied voltage, including (1) directly reducing the distance between the two electrodes, (2) applying a co-field configuration, and (3) enhancing the electric field locally.

As the electric field strength equals the applied voltage divided by the distance between the two electrodes, reducing the distance could reduce the required voltage for sufficient inactivation. However, the minimum distance is usually around 1 mm and further decreasing the distance is not practical due to the smaller treatment capacity.

Conventional designs used a narrow channel to concentrate the electric field (some studies refer to the configuration as the "co-field" or "converged" configuration) (Fig. 4a&b) (Huang and Wang, 2009). For example, Peng et al. found that a voltage of 10 kV could generate an electric field strength of 4000 kV $m^{-1}$ in a pilot co-field treatment system, while it required 40 kV voltage in a parallel plate system of the same size (Peng et al., 2017). However, either the treatment capacity or the extent of electric field enhancement was limited (Ahmed et al., 2016). González-Sosa et al. (2014) built up a flow-through EFT device in a miniature chamber inside a needle. By applying bipolar pulses with an amplitude of 640 V, the system achieved an inactivation efficiency of 0.8-log with four consecutive treatments. The inactivation efficiency was unsatisfactory, which was due to the relatively low electric field strength in the chamber. To further take the advantages of the narrow channel, Experton et al. modified one of the electrodes by coating the pore channels of a polycarbonate membrane with gold microtubes

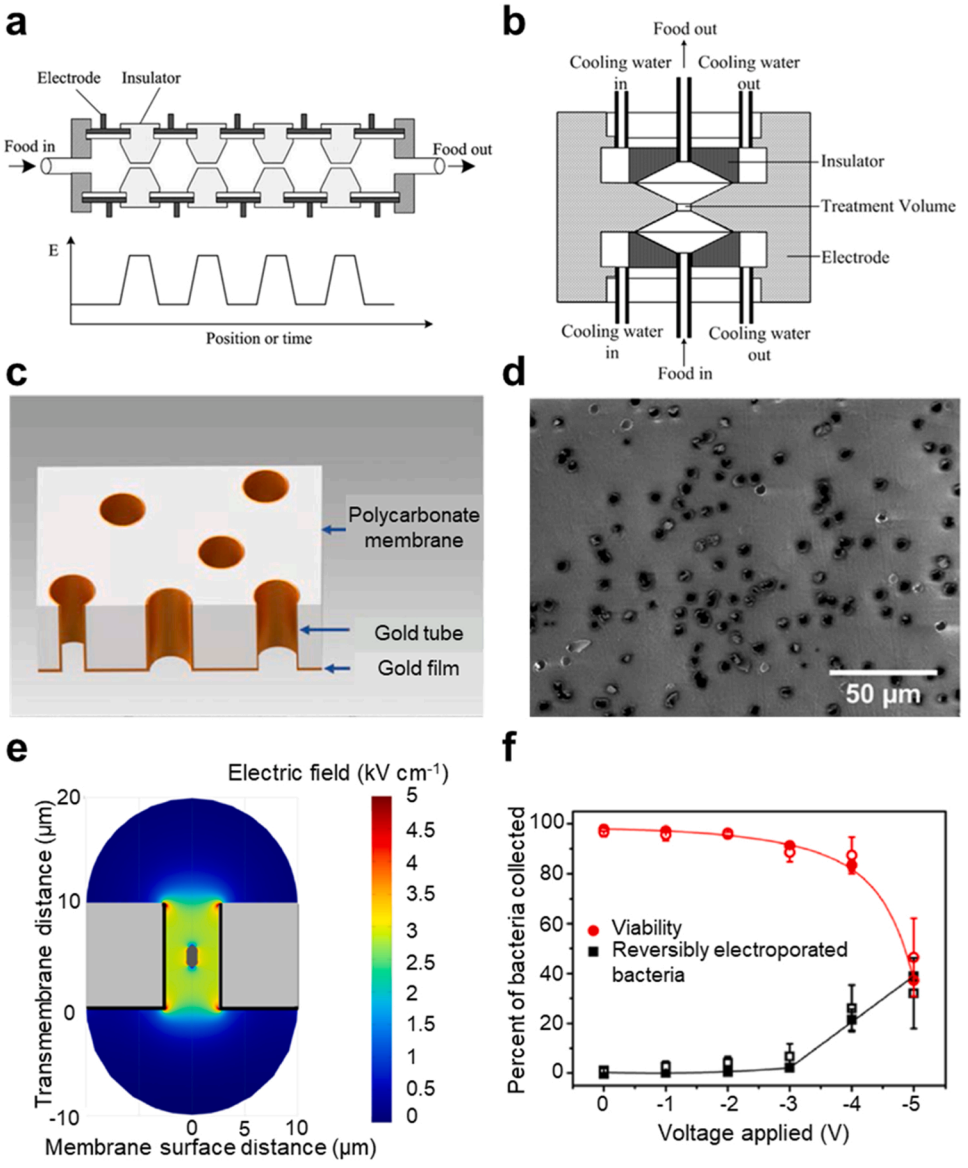



**Fig. 4.** Reducing the required voltage by confined water channels. (a) A continuous current, high electric field treatment chamber. (b) Side view of a "co-field" treatment chamber with cooling system (Huang and Wang, 2009). (c) Schematic illustration of a gold-microtube membrane. Dimensions are not to scale (Rojas-Chapana et al., 2004). (d) Scanning electron micrograph of the surface of a microtube membrane with tube diameter of 5 μm and density of $5 \times 10^5$ $cm^{-2}$ (Rojas-Chapana et al., 2004). (e) Illustration of the electric field gradients in a gold tube of diameter 5 μm and length 10 μm, obtained using COMSOL software. The voltage applied to the gold layer (black boundaries) was − 4 V. An *E. coli* is represented in dark gray inside the tube (Rojas-Chapana et al., 2004). (f) Average percentages of viable and reversibly electroporated bacteria obtained from the flow-through (solid symbols) and fluorescence-microscopy (open symbols) methods as a function of voltage applied to the membrane (Rojas-Chapana et al., 2004).

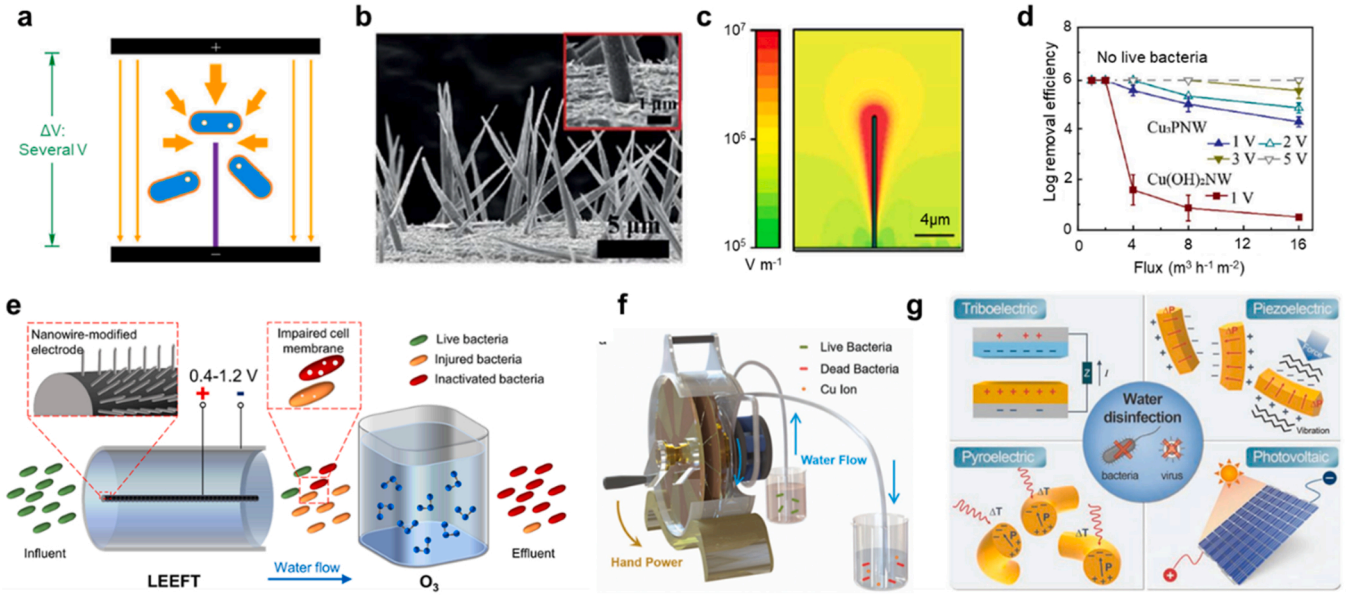



**Fig. 5.** Reducing the required voltage by the locally enhanced electric field treatment (LEEFT) (a) Schematic of LEEFT working principles. The applied voltage is reduced from several kV to serval V (Zhou et al., 2020e). (b) Scanning electron microscopy (SEM) images of the $Cu_3PNW$–Cu electrode (Zhou et al., 2020e). (c) Electric field distribution near the surface of CuONW (diameter, 100 nm; length, 15 μm) in water showing the enhancement of the electric field strength (Huo et al., 2018). (d) *E. coli* inactivation efficiency of electroporation disinfection cells (EDCs) with $Cu_3PNW$–Cu electrodes with different voltages (1, 2, 3, and 5 V) and different fluxes (from 1 to 16 $m^3 \bullet h^{-1} m^{-2}$) (Huo et al., 2018). (e) An enhancement effect was observed in a sequential treatment of LEEFT and ozonation (Zhou et al., 2020b). (f) LEEFT disinfection powered by a manual mechanical structure (Ding et al., 2019). (g) Schematics showing energy harvester-driven water disinfection systems that are based on triboelectric (motion), piezoelectric (external strain), pyroelectric (thermal cycles), and photovoltaic (solar excitation) effects (Huo et al., 2021).

(~5 μm diameter and ~10 μm length) (Fig. 4**c-f**) (Experton and Martin, 2018; Experton et al., 2016). Both reversible and irreversible electroporation was observed in the system with an external voltage of 5 V DC.

To take advantage of the localized strong electric field, researchers modified the electrode with 1D micro- and nano-structures. Electrodes with conductive nanowires have also demonstrated the ability to lower the external voltage (Fig. 5a–d) (Zhou et al., 2020e). Due to the high aspect ratio of the nanowires, electric field strength near the nanowire tips can be enhanced by several orders of magnitude, to the extent of causing irreversible electroporation of the cell (Huo et al., 2016; Pi et al., 2021; Zhou et al., 2020a). With only 1–5 V DC applied voltage, high inactivation efficiency (>6-log) of multiple bacterial species has been achieved by the nanowire-modified electrodes (Huo et al., 2018). Such advanced method with reducing voltages is termed the locally enhanced electric field treatment (LEEFT). During the past years, silver-, copper-, zinc-, and cobalt-based nanowire-modified electrodes were fabricated and demonstrated efficient in water disinfection (Pi et al., 2022; Zhou et al., 2020d). As only an external voltage of several volts is required, the specific energy consumption (1–5 $J \bullet L^{-1}$) is much lower than the conventional pulsed EFT (Zhou et al., 2020d). When LEEFT is used in combination with other methods (*e.g.*, electrochemical Cu, ozone, and electro-chlorination), an enhancement effect or synergistic effect was observed (Fig. 5**e**) (Huo et al., 2022; Zhou et al., 2019, 2020b). This indicates that LEEFT can not only act as an independent disinfection method, but also assist or be assisted by other methods to enhance its performance and relieve its limitations. As the LEEFT needs only a few volts for efficient disinfection, multiple novel energy sources including manual, solar, and smartphone-based device were developed for different scenarios and applications (Fig. 5**f&g**) (Ding et al., 2019; Huo, Z.-Y. et al., 2021; Huo, Z.-Y. et al., 2021; Zhou et al., 2020c).

**Towards more stable and versatile electrodes.** New electrode materials have also been investigated to improve the EFT. Traditionally, stainless steel is the most widely used electrode due to its low cost, light weight, and relatively low toxicity. However, when a voltage is applied, iron (and potentially chromium and nickel) ions are released from the anode, which poses secondary contamination to the treated media (Morren et al., 2003). Therefore, Takanori et al. employed carbon cloth as the electrode material in EFT (Fig. 6a) (Tanino et al., 2020). The inactivation efficiency of the carbon cloth electrode was higher than that of the stainless steel one, while the temperature rise of the two materials was of a similar level. The better inactivation efficiency of the carbon cloth was inferred from the locally non-uniform electric field produced by the irregular surface of the electrode (Fig. 6b). Roodenburg et al. proposed an "*in-pack*" EFT using conductive plastic film packaging (Roodenburg et al., 2010, 2013). The authors developed an Ethylene Vinyl Acetate (EVA) copolymer matrix with enhanced conductivity, so that the electric field pulses can be directly applied to the food inside the package (Fig. 6c). During the EFT, a prefilled food pouch is successively treated between a set of cylindrical electrodes. The authors demonstrated an efficient inactivation (~6-log of *Lactobacillus Plantarum*) by the polymer film, which is comparable with that of the conventional stainless-steel electrodes (Fig. 6d). Such design saves the cost of unpacking and packaging in food industry.

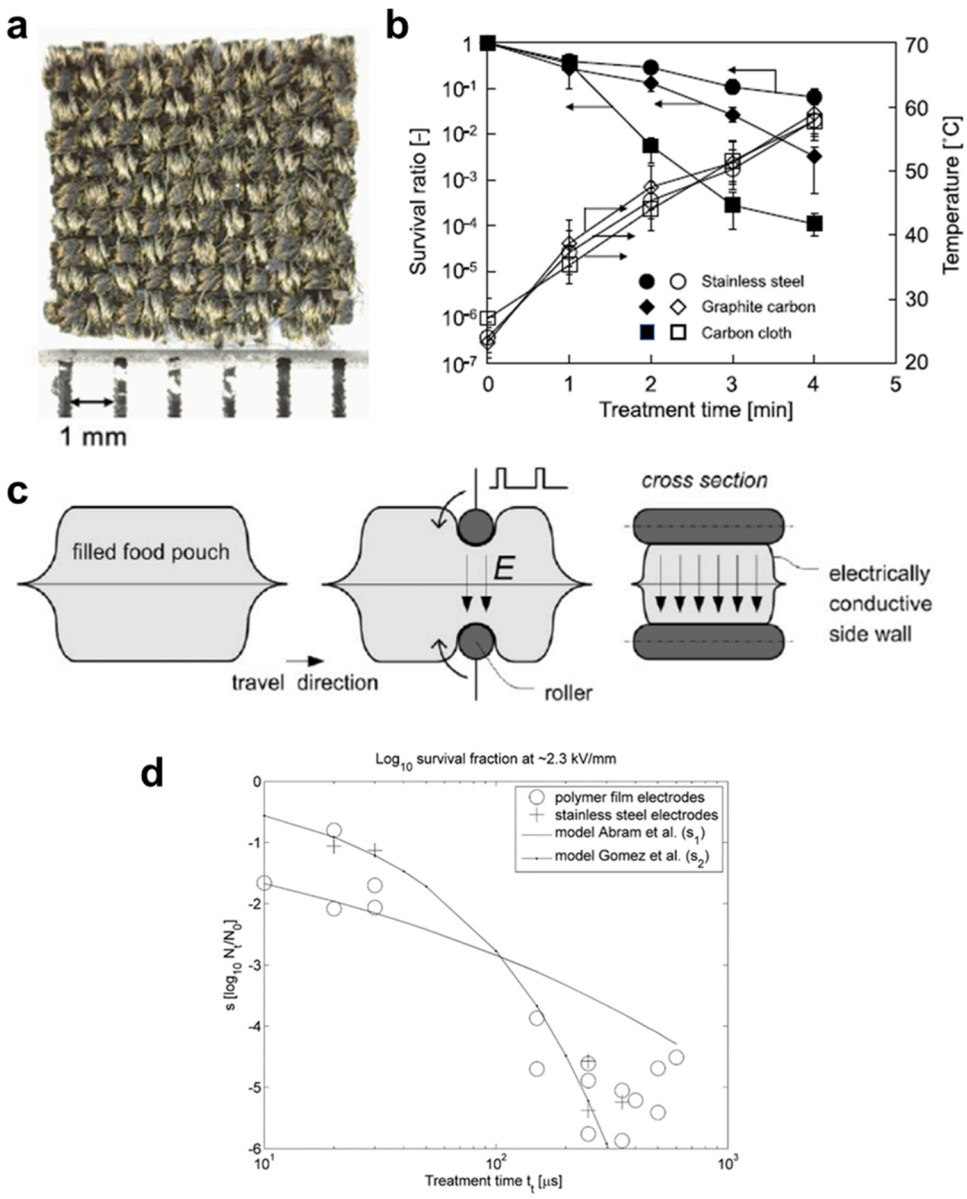



**Fig. 6.** Versatile electrode development in EFT. (a) A carbon cloth electrode for EFT disinfection with better stability. (b) Time course of the survival ratio of *E. coli* and temperature in batch EFT. Stainless steel (circles), graphite carbon (diamonds), and carbon cloth (squares) were used as the electrodes of the EFT reactor. Closed and open symbols show the survival ratio of *E. coli* and temperature, respectively (Tanino et al., 2020). (c) Pulsed electric field treatment of a prefilled electrically conductive flex. (d) Log survival numbers as function of treatment time for polymer film covered electrodes, bare stainless electrodes and predicted survival curves from literature (Roodenburg et al., 2013).

## 7. Conclusion and outlook

We critically review the recent advances of EFT for microbial inactivation in water and liquid food. Four aspects are emphasized, including the key processing parameters, the effectiveness against various pathogens, the impact of liquid properties on the inactivation performance, and novel configuration and materials for EFT devices. The basics of processing parameters and liquid properties have been well studied especially in food processing. Therefore, it is suggested that more precise control and manipulation of EFT operation should be focused on in the future research. Bacteria have been extensively investigated in the electroporation and EFT. Therefore, more attention should be paid to the inactivation of viruses and protozoa.

We summarize three main directions, *i.e.*, towards the uniform electric field, towards the reduction of the applied voltage, and towards more stable and versatile electrodes, as the key solutions to conquer the current bottleneck of EFT for wider applications. The first direction, towards uniform electric field, is specifically critical for those units that have already achieved full-scale treatment. Reducing the localized overheating with a more uniform electric field could facilitate a higher capacity and more continuous treatment. This improvement may make EFT more attractive among competing technologies. However, even with a "perfect" uniform electric field, the energy consumption during operation is still high compared with heating.

The second direction, towards the reduction of the applied voltage, is more revolutionary compared with the first direction. Successful reduction of the applied voltage by several orders of magnitude is critical to make EFT competitive in the market. The LEEFT and other innovations have been demonstrated to inactivate microorganisms by nano- or micro-structure modified electrodes at a bench scale. The major challenge is still how to scale up the treatment system, particularly new methods to fabricate high-quality functional electrodes. Meanwhile, the potential issues of all nano- or micro-materials also exist in the LEEFT, including the insufficient stability and higher toxicity when released in the liquid.

The third direction, towards more stable and versatile electrodes, is intended to improve and optimize the current EFT. The electrochemical reactions always come with electroporation, and thus cause electrode corrosion, secondary contamination, and extra energy consumption. Carbon-based and other inert electrode materials have been developed,

which limit the electrochemical reaction to only water electrolysis. Meanwhile, the "in-pack" EFT brings a new angle to reduce the overall cost of EFT by reducing the packing and un-packing processes. The above findings are critical to make EFT a more mature technology.

The properties of water and liquid food are extremely different. We speculate that it still takes some time to apply EFT for practical water disinfection, and the time is closely related to the progress of the above three directions. The difficulties of adopting EFT in water disinfection not only include its higher cost, but also the mature and prevalent operation of chlorination in large- and small-scale water treatment facilities. Nevertheless, we still believe that EFT ispotential to act as the next-generation water disinfection technology because it eliminates the concerns of harmful DBPs.

The EFT is more versatile in functions when being applied in the food industry. Apart from inactivation pathogens, EFT can be used to recover valuable compounds from different foods, enhance the juice production with higher purity, and promote drying of fruits and vegetables. The higher energy consumption and large capital cost are also identified as the major restriction of EFT in all these applications. Therefore, we are expecting solutions to reduce the complexity of EFT from both environmental and food scientists and engineers.

To summarize, it is exciting to witness the rapid development of EFT in both water disinfection and food pasteurization. There are lots of similarities and knowledge to exchange, and thus a review to connect these two media together is presented. We identify the major obstacles and three potential directions to work on. We expect to see the breakthrough findings delivered by the collaboration of environmental engineering and food science, which promote the prosperous adoption of EFT in the next 5–10 years.

## Environmental implication

Pathogenic infection is a major threat to human health in both water and liquid food. Conventional water disinfection with chemicals is limited by the inconvenience of their transportation and storage and the production of carcinogenic DBPs. Thermal treatment destroys the nutrients, color, and the taste, and thus significantly reduces the value of the food. Therefore, it is critical to look for the next-generation microbial control methods for water and food. This review establishes a knowledge bridge of EFT between water disinfection and food pasteurization, and identifies the major obstacles and research directions of EFT for wider and larger-scale real-world applications.

## Declaration of Competing Interest

The authors declare no conflict of interest for this study.

## Data Availability

No data was used for the research described in the article.

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