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Xiaojun Liu, Thomas Lendormi, Magali Le Fellic, Yves Lemée, Jean-Louis Lanoisellé. Hygienization of mixed animal by-product using pulsed electric field in a continuous treatment system: Synergistic effect with ohmic heating on the inactivation of indicator bacteria. *Waste Management*, 2020, 118, pp.18 - 26. <10.1016/j.wasman.2020.08.012>. <hal-03492048>

HAL Id: hal-03492048

<https://hal.science/hal-03492048v1>

Submitted on 5 Sep 2022

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Hygienization of mixed animal by-product using pulsed electric field in a continuous treatment system: synergistic effect with ohmic heating on the inactivation of indicator bacteria

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Declarations of interest: none.

Wordcount: 5888 with 1 Table and 6 Figures.

Abstract

Thermal hygienization of waste animal by-products (ABP) before anaerobic digestion is imposed by EU regulations in order to minimize its sanitary risk during digestate land application. This process is energy and time consuming. The present paper deals with the hygienization of ABP using pulsed electric field (PEF) in a continuous system.

Enterococcus faecalis ATCC 19433 and *Escherichia coli* ATCC 25922 were tested as indicator bacteria characterizing the microbial inactivation efficiency. Four electric field strengths (15, 20, 25 and 30 kV·cm⁻¹) were applied to the continuous treatment chamber where circulated the ABP suspension. Synergistic effect of PEF and ohmic heating (T_{ave} = 41 °C) and single effect of PEF (T_{ave} = 28 °C) on bacterial inactivation were investigated.

With the effect of ohmic heating, PEF treatment at 25 and 30 kV·cm⁻¹ for 0.9 ms could obtain 5-log₁₀ reduction of *Ent. faecalis*. This efficiency complies with the EU criteria to validate an alternative hygienization process. The time estimated by Weibull model for 5-log₁₀ reduction of both indicator bacteria (5-D value) was significantly reduced by 2 – 24.5 times when the synergistic effect of PEF and ohmic heating was present.

The increase in electric field strength from 25 kV·cm⁻¹ to 30 kV·cm⁻¹ did not amount to a further inactivation. PEF process coupling ohmic heating at 25 kV·cm⁻¹ was the most efficient for ABP hygienization among the four electric field strengths studied.

Keywords: Hygienization; Anaerobic digestion; Animal by-product; Pulsed electric field; Ohmic heating; Weibull model

1. Introduction

Animal by-products (ABP) mainly derive from the slaughter of animals (*e.g.* animal organs and blood), the production of animal-originated products (*e.g.* dairy products, animal manure of feedstock) and the disposal of dead animal bodies (*e.g.* carcasses of sick animals). Many studies have revealed that ABP might be conveyors of pathogenic agents that are dangerous to the environment. High occurrence and abundance of various pathogens were seen in animal waste, such as *Listeria* spp., *Campylobacter* spp., *Escherichia coli* O157:H7, *Salmonella* spp., *Cryptosporidium parvum*, *Giardia lamblia* (Hutchison et al., 2004; Sui et al., 2019; Ziemer et al., 2010). He et al. (2019) confirm the potential sanitary risk of introducing antibiotic resistance genes from food waste into the environment despite biological treatment. Public health and the environment may therefore be threatened by improper waste ABP management.

Many valorization methods (*e.g.* incineration, composting and anaerobic digestion) have been proposed to transform ABP into valuable resources (Moukazis et al., 2018). In the European Union (EU), the transformation and the disposal of ABP are strictly regulated by the relative legislations like EC No. 1069/2009 and EU No. 142/2011 (European Union, 2011, 2009). According to their biological risk level, the regulations divide ABP into three categories to which different processing and transformation methods could apply. One of the promising ABP valorization approaches is anaerobic digestion, a waste-to-energy technology that transforms organic matter into biogas and digestate. Biogas is used as renewable energy source or as primary material for other chemical processes. Digestate can be returned to soil as fertilizer or soil amendment. This creates a circular economy for sustainable development (Tsui et al., 2019).

Certain ABP classified as Categories II and III are authorized by the EU to be anaerobically digested for biogas production if they have been subject to the thermal pasteurization operated at 70 °C for 1 hour and the maximum particle size is reduced to 12 mm (Ware and Power, 2016). It is considered prudent of the EU to set such operational parameters (high temperature and long treatment time) (Liu et al., 2019a). Recent studies conclude that this thermal pasteurization serving as the pre-hygienization process of ABP accounts for about 6 – 25% of the primary energy produced by biogas plants in the EU (Liu et al., 2019a). As authorized by the European Commission, the thermal pasteurization can be replaced by other alternative physico-chemical hygienization processes if they are able to reduce *Enterococcus faecalis* or *Salmonella Senftenberg* by a factor of 10^5 (*i.e.* 5 log10) and to achieve a 3-log10 reduction of thermo-resistant viruses (European Union, 2011).

Electro-technologies have been widely studied for the alternative inactivation of microorganisms present in food and water, including electric discharge (Singh et al., 2019) and pulsed electric field (PEF) (Brennan and Grandison, 2012). The advantages of electrical treatment compared with conventional thermal pasteurization rely on short process time and high bacteria inactivation performance. PEF is a promising electrical technology developed during last three decades. It involves the delivery of discontinued electric field (pulses) to target products where the microorganisms can be inactivated due to the breakdown of microbial cell membrane (Brennan and Grandison, 2012). The application of PEF induced the membrane charging and polarization of microbial cell. The frequent attraction of opposed electric charges leads to thinning of cell membrane, modifying its mechanic modulus. Once electric field strength exceeds certain threshold, the additional transmembrane potential brought by PEF results in breakdown of cell

membrane (Vorobiev et Lebovka, 2020). The passage of electric current in a conductor, as described by Joule's law, can generate ohmic heat (Sakr and Liu, 2014). PEF treatment inevitably causes ohmic heating (OH) in a resistive conductor (Lindgren et al., 2002), which may give rise to a supplementary thermal effect on pathogens inactivation performance.

The application of electro-technology on hygienization of ABP is rare. A large amount of research effort was put in the microbial control of sewage sludge (Astals et al., 2012; López et al., 2019). Many studies paid attention to the effect of electrical and thermal pretreatments on the intensification of biogas yield from organic waste like biosolids and pig manure (Salerno et al., 2009), slaughterhouse waste (Edström et al., 2003; Liu et al., 2018; Luste and Luostarinen, 2011) and other ABP waste (Chamaa, 2017; Safavi and Unnthorsson, 2017). Keles et al. (2010) contributed to the removal of *Salmonella* spp. in sewage sludge by PEF. Recently, Zhang et al. (2019) studied the joint effect of microwave pretreatment on antibiotic resistance genes removal and methane yield enhancement in chicken manure. A recent research primarily proved that PEF, by removing the effect of ohmic heat, was able to give the bacterial hygienization (*i.e.* 5-log₁₀ reduction of *Ent. faecalis*) of ABP at an electric field strength of 25 kV·cm⁻¹ for an effective treatment time of PEF over 30 ms during batch treatment (Liu et al., 2019b). The mentioned PEF experimental system remains in small laboratory scale. It consumes more energy than conventional thermal hygienization (Liu et al., 2019a). More studies are therefore needed to investigate alternative pretreatment of ABP for sanitary purpose.

The objectives of this paper are to upgrade the work of Liu et al. (2019b). An innovative coupling of PEF treatment and ohmic heating was realized to study its

synergistic effect on ABP hygienization. In this paper, the bacterial hygienization means a reduction of the inoculated *Enterococcus faecalis* by 5 log₁₀ in animal by-products.

The treatment system was scaled up from batch treatment in a small electroporation cuvette to the continuous treatment of ABP suspension in several liters. A newly designed treatment chamber and a more powerful high voltage generator were used.

The paper mainly deals with 1) the synergistic effect of PEF and ohmic heating on the inactivation kinetics of the indicator bacteria inoculated in ABP suspension at four electric field strengths, 2) the comparison with the single effect of PEF by removing ohmic heat, 3) the modeling of the bacterial inactivation curves and 4) the effect of the PEF energy input on the survival behaviors of two indicator bacteria.

2. Material and methods

2.1 ABP suspension

2.1.1 Animal by-products

The same mixed animal by-product as Liu et al. (2019b) was used. It was the feedstock for the local biogas plant SEM LIGER in Locminé (France), composed of fish industry waste and pig slurry. In the biogas plant, the substrates had been crushed into small particles (less than 10 mm) and then pasteurized in an industrial thermal hygienizer at 70 °C for 60 min, conforming to the EU ABP regulations mentioned above. The hygienized ABP was directly collected in hot condition at the outlet of the hygienizer and put into two containers from the pressured pipe under aseptic conditions. All of the manipulation was done with disposable nitrile gloves. The used containers (5 L buckets) were disinfected by alcohol (70% v/v) and medical grade disinfectant-detergent (Le Vrai Bactopin Extra spray, Castets, France). The samples were separated and stored in several smaller sterile containers at –18 °C for further usage.

The physicochemical, biochemical and microbial characteristics of the collected ABP samples were available in the work of Liu et al. (2019b). The ABP had a water content of 85.1%, a pH of 6.75 and an electrical conductivity of 14 mS·cm⁻¹. Despite the thermal hygienization, the aerobic mesophilic count remaining in the ABP was found at 1.07 × 10⁵ CFU·mL⁻¹ whereas neither *Enterococcus* spp. nor *Escherichia coli* was found.

2.1.2 ABP suspension preparation

The Gram positive (G+) strain *Enterococcus faecalis* ATCC 19433 and the Gram negative (G-) strain *Escherichia coli* ATCC 25922 (Institut Pasteur, Paris, France) were the indicator bacteria characterizing the bactericidal performance of PEF. It is worth noting that *Ent. faecalis* is the indicator microorganism set by the EU ABP regulations for innovative hygienization processes validation.

Two bacterial strains were respectively incubated in nutrient broth (CM0001, Oxoid, Basingstoke, UK) at 37 ± 1 °C for 24 h (*Ent. faecalis*) and 20 h (*E. coli*) to obtain their respective stationary phase of growth (Alkhafaji and Farid, 2008; Koseki and Yamamoto, 2006; Magnus et al., 1986). The ABP was filtrated by a disinfected 1-mm sieve. 100 mL of the nutrient broth with indicator bacteria and 100 mL of the filtrated ABP sample were mixed with 1 800 mL sterile distilled water. The final electrical conductivity of the suspension was 2 000 µS·cm⁻¹ and the pH was at neutral level. The prepared suspension ready for PEF treatment had an initial bacterial count of around 5 × 10⁶ CFU·mL⁻¹ and 2.5 × 10⁷ CFU·mL⁻¹ for *Ent. faecalis* and *E. coli* respectively.

2.2 Experimental PEF continuous treatment system

Fig. 1 illustrates the experimental PEF treatment system. It consisted of a high voltage PEF generator (MU-17-807-A-FR 22.5 kV, ITHPP, Thégra, France), a continuous treatment chamber where the ABP suspension was pumped by a peristaltic pump (PD5006, Heidolph, Schwabach, Germany) and a portable computer that permitted the configuration and the data collection of the PEF generator.

The high voltage generator can deliver mono-polar pulses with a configurable voltage ranging from 3.0 to 22.5 kV, a square pulse duration between 2 – 200 μ s and a maximum frequency reaching 200 Hz. The treatment chamber consists of 12 individual sets of stainless-steel alloys (**Fig. 2C**) receiving the parallel electric pulses from the high voltage generator. Each pair of the alloys sends the electricity to the corresponding electrodes (external diameter 8 mm - 316 L stainless steel, ISO parallel 1/2", Swagelok, France) as shown in **Fig. 2B**. The electrodes are separated by the PTFE ring spacers serving as insulator (**Fig. 2A**), which gives an inter-electrode distance of 2.58 ± 0.04 mm.

During the treatment, a cooling system composed of a water bath, mechanical fans and heat sinks was used when the effect of ohmic heating induced by PEF was to be minimized. Details of treatment chamber are given in the **Supplementary materials**.

2.3 Experiment protocol

2.3.1 PEF treatment

The kinetics of the indicator bacteria inactivation in the prepared 2 L ABP suspension were studied at four electric field strengths ($E = 15, 20, 25$ and $30 \text{ kV}\cdot\text{cm}^{-1}$). The effective treatment time (t_{PEF}) was studied up to 1 ms, which can be calculated using **Eq. (1)**:

$$t_{\text{PEF}} = f \cdot t \cdot \tau \quad \text{Eq. (1)}$$

where t_{PEF} is the PEF effective treatment time (ms), f is the frequency of pulse repetition ($f = 200 \text{ Hz}$), t is the real treatment time for 1 trial of the experiment (s) and τ is the duration of one pulse ($\tau = 10 \mu\text{s}$).

Treatment of PEF can result in ohmic heating due to the passage of pulsed electric current in the ABP suspension. In the present study, only the synergistic effect of PEF and ohmic heating was examined. The pure effect of ohmic heating on bacterial inactivation is found similar to that of thermal pasteurization (Goullieux and Pain, 2014), which was therefore not studied in the present paper. The temperature of the suspension (T) was always kept under $55 \text{ }^{\circ}\text{C}$ to avoid excessive damage to the indicator bacteria from heat ($T_{\text{max}} = 55 \text{ }^{\circ}\text{C}$). The maximal temperature was set at $55 \text{ }^{\circ}\text{C}$ since the thermal resistant bacterial strain *Ent. faecalis* ATCC 19433 could be hardly inactivated by the thermal pasteurization for 60 min at $55 \text{ }^{\circ}\text{C}$ (Liu, 2019). The suspension temperature was measured distantly by an infrared thermometer (Traceable®, VWR, Leuven, Belgium). A cooling time between two trains was imposed once the suspension temperature attained $55 \text{ }^{\circ}\text{C}$. The average suspension temperature (T_{ave}) for the synergistic effect of PEF and ohmic heating was of $41 \pm 7 \text{ }^{\circ}\text{C}$. The temperature records of each experiment are available in the **Supplementary materials**.

In order to study the single effect of continuous PEF treatment with the effect of ohmic heating removed, the same experiments treated at $30 \text{ kV}\cdot\text{cm}^{-1}$ but keeping the suspension temperature not exceeding $35 \text{ }^{\circ}\text{C}$ were realized for two indicator microorganisms ($T_{\text{max}} = 35 \text{ }^{\circ}\text{C}$). Electric field at $30 \text{ kV}\cdot\text{cm}^{-1}$ was chosen because $25 \text{ kV}\cdot\text{cm}^{-1}$ has proved to be less efficient in batch system (Liu et al., 2019b). Therefore higher field strength was applied in a bid to reach a better inactivation ratio of

Ent. faecalis. The average suspension temperature (T_{ave}) for the single effect of PEF was of 28 ± 4 °C. The temperature records of each experiment are available in the **Supplementary materials**.

The ABP suspension was circulated into the treatment chamber by a peristaltic pump whose speed was set at 400 rpm, corresponding to an average fluid velocity of $15 \text{ mL} \cdot \text{s}^{-1}$ in 8-mm Tygon® tubes (E-3603, Saint-Gobain, Charny, France). Six pairs of electrodes were used, giving a real retention time equal to 31.2 ms in the treatment chamber (total working volume = $4.7 \times 10^{-5} \text{ m}^3$). Before and after every trial, the treatment chamber and its Tygon® tubes were decontaminated by P3-oxonia active solution (ECOLAB®, Minnesota, US) and then washed by sterile distilled water.

2.3.2 Culturable bacteria count

At each sampling moment, 5 mL of the treated suspension was collected under aseptic condition and cooled at 0 °C in ice water. Serial dilutions with spread plate method on selective agar were realized in duplicate, as described by Cunault et al. (2011). *Ent. faecalis* was incubated at 37 °C on Slanetz & Bartley agar (CM0377, Oxoid, Basingstoke, UK) for 48 h and *E. coli* was incubated at 37 °C on Tryptone Bile X-glucuronide agar (CM0945, Oxoid, Basingstoke, UK) for 24 h.

2.3.3 Energy input by PEF

The energy input delivered by the high voltage PEF generator (W_{PEF}) can be calculated by **Eq. (2)**:

$$W_{PEF} = \int_0^{t_{PEF}} U \cdot I / v \, dt = \int_0^{t_{PEF}} 10^{-6} \cdot E^2 \cdot \sigma \, dt \quad \text{Eq. (2)}$$

where W_{PEF} is the energy consumption of the high voltage generator ($J \cdot mL^{-1}$), t_{PEF} is the effective time of PEF treatment (s), U is the voltage applied (V), I is the intensity of electric current (A), v is the volume of the suspension subject to PEF treatment (mL), E is the electric field strength ($V \cdot m^{-1}$) and σ is the electrical conductivity of ABP suspension ($S \cdot m^{-1}$). The survival fractions of two indicator bacteria treated at the same energy levels ($W_{PEF} = 160, 294$ and $550 J \cdot mL^{-1}$ for *Ent. faecalis* and $W_{PEF} = 174, 284$ and $567 J \cdot mL^{-1}$ for *E. coli*) but at different field strengths ($E = 15, 20, 25$ and $30 kV \cdot cm^{-1}$) were investigated.

2.4 Mathematical modeling

2.4.1 Weibull model

The survival fractions of indicator bacteria against t_{PEF} were plotted and modeled by the Weibull model (van Boekel, 2002), as described in **Eq. (3)**:

$$\log_{10} N(t_{PEF}) / N_0 = - (t_{PEF} / \alpha)^{\beta} / 2.303 \quad \text{Eq. (3)}$$

where t_{PEF} is the effective time of PEF treatment (ms), $N(t_{PEF})$ is the culturable count of the given bacterium at t_{PEF} ($CFU \cdot mL^{-1}$), N_0 is the initial bacterial count ($CFU \cdot mL^{-1}$), α is the scale parameter of Weibull model (ms) and β is the shape parameter of Weibull model (–).

According to Liu et al. (2019b) and van Boekel (2002), during the hygienization of ABP in batch PEF treatment, the Weibull parameter α depends on the PEF operational conditions whereas β is an intrinsic parameter of the given bacterium and is hardly correlated with the external conditions.

2.4.2 Secondary model

The relationship between the applied field strengths (E) and the Weibull model parameters (α and β) was studied.

At first, the Weibull scale parameter α was modeled using **Eq. (4)**, a simple linear model:

$$\alpha = A - B \cdot E \quad \text{Eq. (4)}$$

where A (ms) and B (ms·cm·kV⁻¹) are the parameters of the linear model.

Another nonlinear model (shape of Gompertz model) (Gómez et al., 2005) was applied to simulate the dependency of α on the electric field strength (E):

$$\alpha = a + b \cdot e^{-e^{-c \cdot E + d}} \quad \text{Eq. (5)}$$

where a, b, c and d are the parameters of the nonlinear model.

As stated in **Section 2.4.1**, the Weibull shape parameter β might have nothing related to the external conditions and therefore the values of β for two indicators were respectively fixed.

2.4.3 5-D values

The parameter, 5-D value, was calculated according to **Eq. (6)** in order to estimate the PEF effective treatment time required to reduce the indicator bacteria by 5 log₁₀, *i.e.* the hygienization of ABP (van Boekel, 2002).

$$5\text{-D value} = \alpha \cdot (5 \times 2.303)^{1/\beta} \quad \text{Eq. (6)}$$

2.4.4 Evaluation of modeling goodness

Curves modeling was processed by R studio (R Studio Inc., Massachusetts, US). Modeling goodness was characterized by adjusted R^2 , sum of squared estimate of errors (SSE) and root-mean-square errors (RMSE) (Huang et al., 2013).

3. Results and discussion

3.1 Synergistic effect of PEF and ohmic heating on bacterial inactivation

The reduction kinetics of two indicator bacteria treated by PEF with the effect of ohmic heating ($T_{ave} = 41\text{ }^{\circ}\text{C}$) were realized at four electric field strengths ($E = 15, 20, 25$ and $30\text{ kV}\cdot\text{cm}^{-1}$). **Fig. 3** illustrates the survival fractions of the given indicator bacteria against the effective PEF treatment time (t_{PEF}).

Fig. 3A presents the kinetics of the studied *Ent. faecalis* inactivation. The final reduction ratios at $t_{PEF} = 1\text{ ms}$ were of 0.8 ± 0.0 , 3.1 ± 0.1 , 5.0 ± 0.1 and $5.2 \pm 0.1\text{ log}_{10}$ for $E = 15, 20, 25$ and $30\text{ kV}\cdot\text{cm}^{-1}$ respectively. It is clear that the inactivation of *Ent. faecalis* could be enhanced when the applied field strength (E) was increased from 15 to $25\text{ kV}\cdot\text{cm}^{-1}$. However, slight difference could be seen between the points of $E = 25$ and $30\text{ kV}\cdot\text{cm}^{-1}$, which means that the increase in field strength will not necessarily lead to an enhanced pasteurization efficiency.

The similar inactivation kinetics between 25 and $30\text{ kV}\cdot\text{cm}^{-1}$ could be explained by the fact that the membrane electroporation of the studied bacteria achieved its physical limit at $25\text{ kV}\cdot\text{cm}^{-1}$. The electroporation level was sufficient so that the bacteria could not resist PEF stress any longer. Therefore, no further inactivation was to be obtained despite the increase in electric field strength. Similar observation was reported by Elez-Martínez et al. (2004) for PEF treatment of *Saccharomyces cerevisiae* in orange juice

and Sharma et al. (2014) for PEF treatment of *E. coli*, *Staphylococcus aureus* and *Listeria innocua* in whole milk.

It is worth noting that the innovative treatment coupling PEF and ohmic heating can give the hygienization of the ABP suspension (a reduction no less than 5.0 log₁₀ of *Ent. faecalis*) when the applied electric field strength exceeds 25 kV·cm⁻¹ for t_{PEF} over 1 ms. The same statement could also be drawn for *E. coli* as shown in **Fig. 3B** where the inactivation ratio reached 3.3 ± 0.0, 5.0 ± 0.0, 6.0 ± 0.0 and 6.4 ± 0.0 log₁₀ for E = 15, 20, 25 and 30 kV·cm⁻¹.

The studied *E. coli* ATCC 25922 is therefore more vulnerable than *Ent. faecalis* ATCC 19433. In only 0.5 ms, the viable fraction of *E. coli* was already below the detection limit (a reduction over 7.0 log₁₀) at 25 and 30 kV·cm⁻¹ while *Ent. faecalis* could only be reduced by a factor of 10³ (~3 log₁₀). Previous studies concluded that Gram positive bacteria (G+) could generally tolerate more PEF stress than Gram negative bacteria (G-) (García et al., 2007; Sharma et al., 2014) because “G+” bacteria, like *Ent. faecalis*, have thicker peptidoglycan layers that make up a more rigid and more resistant cell wall against PEF (Jay et al., 2012).

Similar observation was stated by Liu et al. (2019b) who studied the inactivation kinetics of the same strains in ABP suspension treated in a batch PEF system without the effect of ohmic heating. They found that *Ent. faecalis* was more resistant to the pure PEF exposure than *E. coli* during the first 5 ms. However, in their study, when the contribution of ohmic heating was removed, an intensive PEF treatment at 25 kV·cm⁻¹ for over 30 ms was required to achieve 5-log₁₀ reduction of *Ent. faecalis* (Liu et al., 2019b), while only 1 ms was needed when ohmic heating was integrated (present study).

3.2 PEF treatment with the effect of ohmic heating removed

As mentioned in the **Section 3.1**, the previous study Liu et al. (2019b) revealed that the batch PEF treatment removing ohmic heating could also give the hygienization of the same ABP suspension but with an effective PEF treatment time significantly extended (30 ms vs. 1 ms). In order to examine the pure effect of the continuous PEF treatment, the experiments treating ABP suspension at $30 \text{ kV}\cdot\text{cm}^{-1}$ without the effect of ohmic heating ($T_{\text{ave}} = 28 \text{ }^{\circ}\text{C}$) were realized by adding reinforced cooling devices to the treatment system.

Fig. 4 gives the inactivation curves of both PEF treatment at $30 \text{ kV}\cdot\text{cm}^{-1}$ with or without the effect ohmic heating. It can be concluded that by cooling down the ABP suspension, pure PEF had limited influence on the bacterial inactivation. Reductions of only 0.5 ± 0.0 and $3.7 \pm 0.1 \log_{10}$ were achieved for *Ent. faecalis* and *E. coli* respectively, while the ohmic heating significantly enhanced the inactivation ratios of both indicators ($5.2 \log_{10}$ and $> 7.0 \log_{10}$ respectively) in 1 ms. The survival fractions of both indicator bacteria reached a plateau ($0.5 \log_{10}$ for *Ent. faecalis* and $3.7 \log_{10}$ for *E. coli* after 0.5 ms) where no further inactivation could be achieved in spite of the extended treatment time. It is thus proved that the combined effect of PEF and ohmic heating could largely enhance the pasteurization performance and attain the hygienization of the studied ABP.

Similar phenomenon about the joint effect of PEF and elevated treatment temperature was reported by Huang et al. (2013) and Sharma et al. (2014). It could be explained by the fact that the electric potential for cell membrane rupture depends on suspension temperature. The rise in temperature results in the modification of the physico-chemical properties of cell membrane (*e.g.* electrical conductivity, viscosity

and elastic modulus), making bacteria more vulnerable to PEF exposure (Zimmermann, 1986).

3.3 Weibull modeling

The inactivation kinetics were fitted by the Weibull model described by Eq. (3). The modeled curves at each electric field strength are shown in Figs. 3 and 4. During the modeling, we found the mean values of β were of 1.5 ± 0.4 for *Ent. faecalis* and of 2.0 ± 1.3 for *E. coli*. As mentioned in Section 2.4, the Weibull shape parameter β is related to the behaviors of the studied bacteria and is supposed to be relatively constant. Therefore, for the modeled curves presented in Fig. 3, the values of β were fixed at their respective mean values, namely 1.50 for *Ent. faecalis* and 2.00 for *E. coli*.

Table 1 gives the modeling results of the inactivation kinetics of *Ent. faecalis* and *E. coli* treated by PEF with or without the effect of ohmic heating. One can conclude from the table that by fixing the values of β , the modeling performance is rather satisfactory considering the high adjusted R^2 and the low SSE and RMSE.

The calculated 5-D values imply that, as stated in Section 3.1, the estimated time needed for the bacterial hygienization of the studied ABP could be reduced from 3.56 to 0.907 ms by increasing the field strength from 15 to 25 $\text{kV}\cdot\text{cm}^{-1}$. However, little improvement could be seen between the 5-D values of both indicators when the applied field strength was raised from 25 to 30 $\text{kV}\cdot\text{cm}^{-1}$. The 5-D value for *Ent. faecalis* (i.e. the hygienization time) with synergistic effect of PEF and ohmic heating (0.890 ms) was 24.5 times less than that with single effect of PEF (21.8 ms).

When the effect of ohmic heating was considered, the 5-D value of *Ent. faecalis* (G+) was 2 times the 5-D value of *E. coli* (G-). This confirms the previous remark that “G+”

bacteria are usually more resistant to PEF treatment than “G–” bacteria (García et al., 2007; Sharma et al., 2014).

The Weibull scale parameter (α) against the applied intensity of electric field (E) was plotted in **Fig. 5**, indicating that the “reaction rate” (*i.e.* the pasteurization efficiency in our case) was improved when applying higher field strength. However, no further efficiency enhancement could be found when the field strength was increased from 25 to 30 kV·cm⁻¹. The **Fig. 5** shows the results of the secondary model that describes the relationship between α and E using **Eqs. (4)** and **(5)**. The obtained equations of both models are presented as follows.

Linear model ($R^2 = 0.729$ and 0.911):

$$\textit{Ent. faecalis} \quad \alpha = -0.033 \cdot E + 1.067 \quad \textbf{Eq. (7)}$$

$$\textit{E. coli} \quad \alpha = -0.020 \cdot E + 0.698 \quad \textbf{Eq. (8)}$$

Nonlinear model ($R^2 = 0.999$ and 0.999):

$$\textit{Ent. faecalis} \quad \alpha = 0.172 + 276 e^{-e^{-0.050 \cdot E + 1.08}} \quad \textbf{Eq. (9)}$$

$$\textit{E. coli} \quad \alpha = 0.114 + 0.530 e^{-e^{-0.239 \cdot E - 4.82}} \quad \textbf{Eq. (10)}$$

The linear model with merely two parameters can roughly show the dependency of α on field strength but fails to present the slight difference between the values of α at 25 and 30 kV·cm⁻¹. However, the nonlinear model can safely simulate the evolution of α as function of the electric field strength E, particularly the prediction of a plateau (efficiency limit) between 25 and 30 kV·cm⁻¹, as described in **Section 3.1**. This Gompertz-shaped model has four parameters, which may cause the overfitting of the points. More points are further required to validate this model during the future study. Liu et al. (2019b) used a secondary model with Arrhenius style (log-linear equation) to

predict the values of α . In the present study, the log-linear model did not improve the modeling goodness (data not shown).

3.4 Effect of energy input

The energy input of every experimental point shown in **Fig. 3** was calculated using **Eq. (2)**. It characterizes the energy delivered by the high voltage generator. Three similar levels of energy input delivered by PEF were chosen (inactivation kinetics with the effect of ohmic heating). The corresponding survival fractions at the same energy input level were plotted against the applied electric field strength in **Fig. 6**.

The energy input (W_{PEF}) is the quadratic function of the applied field strength (E), as shown by **Eq. (2)**. The lack of the survival curves of $15 \text{ kV}\cdot\text{cm}^{-1}$ at the highest energy level ($550 \text{ J}\cdot\text{mL}^{-1}$ for *Ent. faecalis* or $567 \text{ J}\cdot\text{mL}^{-1}$ for *E. coli*) is due to the impossibility to realize the experiments for a reasonable treatment time during which satisfactory physiological state could be maintained.

From **Fig. 6**, it is clear that the microbial reductions were significantly enhanced by raising the PEF energy input. For *Ent. faecalis* treated at the same energy input, the inactivation ratios achieved its best performance at $25 \text{ kV}\cdot\text{cm}^{-1}$ rather than $30 \text{ kV}\cdot\text{cm}^{-1}$. Since the inactivation curve of *Ent. faecalis* at $30 \text{ kV}\cdot\text{cm}^{-1}$ is very close to that at $25 \text{ kV}\cdot\text{cm}^{-1}$, it is certain that more energy was consumed at $30 \text{ kV}\cdot\text{cm}^{-1}$ to obtain the same survival ratio as $25 \text{ kV}\cdot\text{cm}^{-1}$.

The influence of the electric field strength on *E. coli* is more remarkable because *E. coli* was significantly reduced when W_{PEF} raised from $285 \text{ J}\cdot\text{mL}^{-1}$ to $567 \text{ J}\cdot\text{mL}^{-1}$ in **Fig. 3B**. Therefore, *E. coli* is more sensitive to the specific energy delivered by PEF. The raise in field strength from 25 to $30 \text{ kV}\cdot\text{cm}^{-1}$ can hardly amount to the inactivation.

Liu et al. (2019b) found that the ABP suspension had protective effect on *Ent. faecalis* and *E. coli* during PEF exposure. They explained the phenomenon by the high presence of certain chemical substances in ABP, like lipids and proteins, that were beneficial for microorganisms to survive during the PEF exposure. By eliminating the Joule heat at $25 \text{ kV}\cdot\text{cm}^{-1}$, the mentioned study achieved around 3-log₁₀ reduction of *Ent. faecalis* and 4-log₁₀ reduction of *E. coli* at the energy cost of $3\,000 \text{ J}\cdot\text{mL}^{-1}$. In present paper the integration of synergistic effect with ohmic heating in PEF treatment could reduce the energy input to only $550 - 560 \text{ J}\cdot\text{mL}^{-1}$ for a similar performance.

The energy consumed for a 5-log₁₀ reduction of *Ent. faecalis* (i.e. the hygienization) in ABP was $831 \text{ J}\cdot\text{mL}^{-1}$ at $25 \text{ kV}\cdot\text{cm}^{-1}$ for 1 ms combined with ohmic heating. It corresponds to an electricity consumption of $5.58 \text{ kJ}_{\text{electricity}}\cdot\text{g TS}^{-1}$. On one hand, this value is comparable to the electricity inputs ($3.60 - 11.4 \text{ kJ}_{\text{electricity}}\cdot\text{g TS}^{-1}$) of other alternative non-thermal hygienization technologies such as microwave and ultrasound (Liu et al., 2019a). On the other hand, it is not proper to simply compare the electrical consumption of the electro-technology with the heat input of thermal pasteurization (between 5.23 and $7.85 \text{ kJ}_{\text{heat}}\cdot\text{g TS}^{-1}$) since they do not have the same recovery efficiency from primary energy. The electro-technology would be favored on the condition that the electricity is produced more from the renewable energies (Liu et al., 2019a). Additionally, the effect of PEF on the bio-methane potential (BMP) enhancement of treated substrates is remarkable (around 50% increase in BMP) (Liu et al., 2019a), which should be taken into account when calculating the overall energy balance of PEF. The subsequent anaerobic digestion process, especially thermophilic anaerobic digestion, plays a role in pathogens reduction as well (Lloret et al., 2013; Schnürer and Schnürer, 2006). All of these factors are to be considered when one

examines the overall interest of applying PEF-assisted hygienization process in biogas plants.

Other emerging pathogen agents like African swine fever virus (ASFV) are drawing increasing concern for sanitary aspect of swine ABP valorization. In fact, the mechanism of pure PEF treatment is attributed to the cell membrane electroporation of microorganisms, which is not the case for virus like ASFV. However, it was reported that ASFV could be inactivated with pH 7.5 – 8 at 50 – 55 °C in pig slurry (Turner et al., 1999). Whether a PEF treatment combined with ohmic heating could perform an equivalent inactivation of ASFV in swine by-products needs to be further investigated before sound conclusion could be drawn.

Compared with sewage sludge, animal by-products are generally less homogeneous, more concentrated and more electrically conductive (*e.g.* 14 mS·cm⁻¹ for animal by-products in this paper vs. 0.69 – 3.06 mS·cm⁻¹ for dewatered sewage sludge, according to Wong et al., 2001). These properties would lead to the complexity for technical maintenance and the investment of high-voltage generators. Pulses of electric field were charged in resistive mode in the present study. The high voltage generator is also able to deliver capacitive charge of PEF, which can minimize the passage of electric current, thus reducing the electric power consumed by generators. The modification of the treatment chamber is inevitably required to realize this capacitive charge (Sack and Mueller, 2016). It would be a future perspective of research focus since this innovative treatment method consumes less energy and is more flexible in dealing with the technical constraints of PEF (*e.g.* electrical conductivity, maximum electric field strength). Less current intensity implies less ohmic heat generated. Therefore, in order to realize the synergistic effect of elevated temperature in PEF hygienization process,

pre-heating of suspension is necessary. Biogas plants combined with heat and power cogeneration are generally able to offer large amount of waste heat to pre-heat substrates in hygienizers and anaerobic digesters. The coupling of capacitive PEF treatment and waste heat valorization in ABP hygienization would therefore be more feasible and profitable during its scaling up in biogas plant.

A profound research into the numerical modeling of the process is also the perspective of the project in order to obtain a clearer understanding of the mechanisms behind the experiments. The findings will help to improve the treatment efficiency as well as to optimize the configuration of treatment chambers.

4. Conclusions

The continuous treatment of PEF at over $25 \text{ kV}\cdot\text{cm}^{-1}$ for an effective treatment time (t_{PEF}) of 1 ms, combined with the effect of ohmic heating ($T_{\text{ave}} = 41 \text{ }^{\circ}\text{C}$ and $T_{\text{max}} < 55 \text{ }^{\circ}\text{C}$), could safely reduce 5 log10 of *Ent. faecalis* and *E. coli* inoculated in the ABP suspension. This pasteurization efficiency complies with the EU regulations to validate an alternative treatment that replaces the thermal pasteurization of ABP at $70 \text{ }^{\circ}\text{C}$ for 60 min. The synergistic effect of PEF and ohmic heating could significantly save the estimated 5-D values (0.9 ms) as compared with the treatment excluding the effect of ohmic heating (21.8 ms). The analysis of iso-energy curves revealed that $25 \text{ kV}\cdot\text{cm}^{-1}$ would be the optimal electric field strength for the hygienization of ABP.

Future research lies in the improvement of the PEF treatment through the numerical modeling of the process, the delivery of PEF in capacitive mode and the integration of waste heat generated by biogas plants into hygienization system.

Acknowledgement

This work was jointly financed by the French Regional Council of Brittany and the French Departmental Council of Morbihan [grant reference: ARED-HYDATE]. Thanks are extended to the mixed economy company LIGER (Locminé, France) for their financial and technical contribution [grant reference: 2017_00212]. The authors would like to thank the French government and the European Regional Development Fund (ERDF) for the investment in the high voltage PEF generator [project: CPER SMARTAGRI-APIVALE 2015-2020].

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658 **Table 1**
659 Modeling results and goodness of the inactivation kinetics of *Ent. faecalis* and *E. coli* treated by PEF with the synergistic effect of ohmic
660 heating (+) or without the effect of ohmic heating (O).

Electric Field (kV·cm ⁻¹)	Ohmic heating	Weibull Model					
		α values (ms)	β values (–)	adjusted R ² (–)	SSE (–)	RMSE (–)	5-D values estimated (ms)
<i>Enterococcus faecalis</i> ATCC 19433							
15	+	0.698 ± 0.007	1.50	0.894 ± 0.033	0.069 ± 0.022	0.099 ± 0.016	3.56 ± 0.04
20	+	0.261 ± 0.004	1.50	0.957 ± 0.009	0.403 ± 0.011	0.272 ± 0.021	1.33 ± 0.02
25	+	0.178 ± 0.000	1.50	0.930 ± 0.004	1.86 ± 0.06	0.515 ± 0.009	0.907 ± 0.003
30	+	0.175 ± 0.001	1.50	0.937 ± 0.007	2.41 ± 0.17	0.491 ± 0.018	0.890 ± 0.005
30	O	1.04 ± 0.05	0.806 ± 0.045	0.735 ± 0.067	0.062 ± 0.019	0.083 ± 0.013	21.8 ± 2.6
<i>Escherichia coli</i> ATCC 25922							
15	+	0.405 ± 0.002	2.00	0.864 ± 0.001	1.13 ± 0.06	0.434 ± 0.011	1.38 ± 0.01
20	+	0.295 ± 0.001	2.00	0.988 ± 0.002	0.331 ± 0.058	0.217 ± 0.019	1.00 ± 0.00
25	+	0.126 ± 0.000	2.00	0.792 ± 0.046	5.51 ± 0.24	1.27 ± 0.16	0.429 ± 0.001
30	+	0.120 ± 0.002	2.00	0.873 ± 0.044	5.14 ± 2.31	1.00 ± 0.23	0.407 ± 0.007
30	O	0.041 ± 0.001	0.804 ± 0.016	0.979 ± 0.005	0.198 ± 0.057	0.221 ± 0.032	0.847 ± 0.024

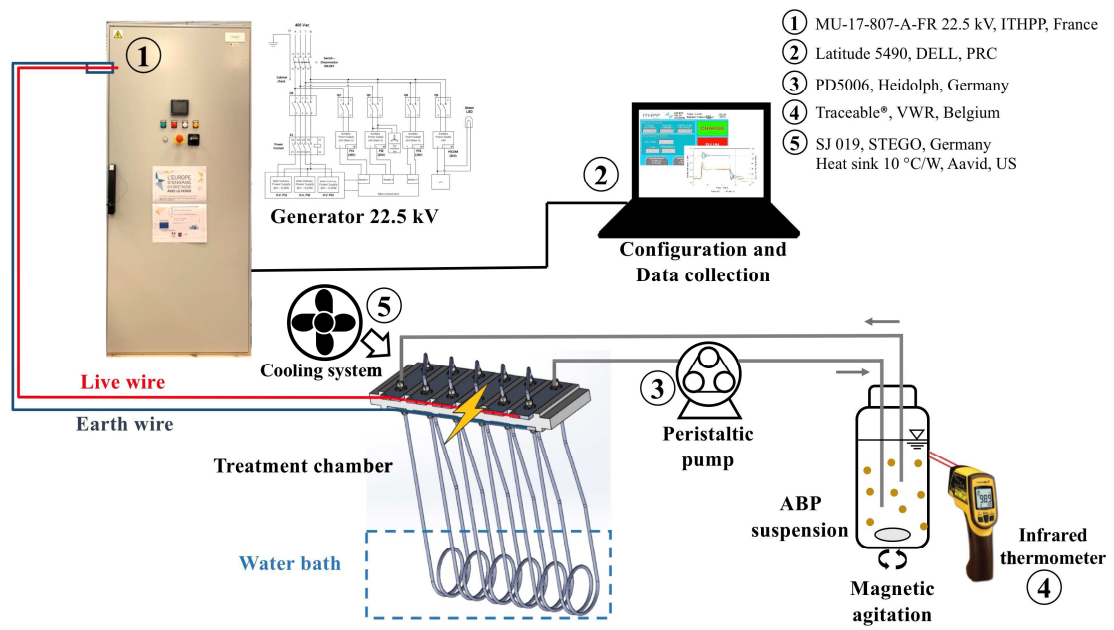


Fig. 1. Diagram of the experimental continuous PEF treatment system.

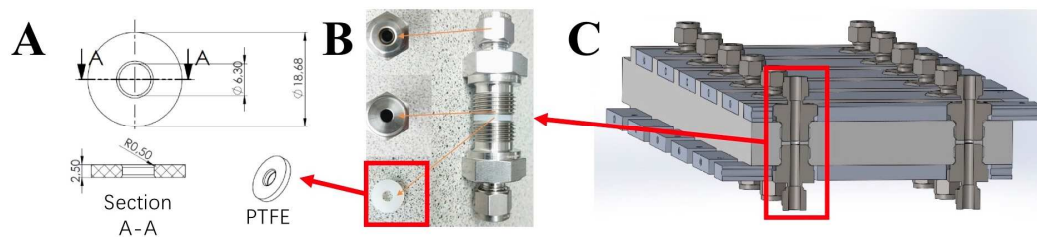


Fig. 2. Detailed illustration of the continuous treatment chamber. A) PTFE ring spacer, B) electrodes and C) treatment chamber with stainless-steel alloys.

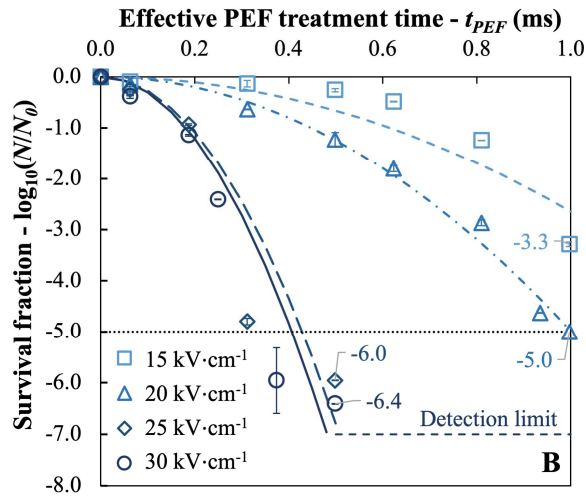
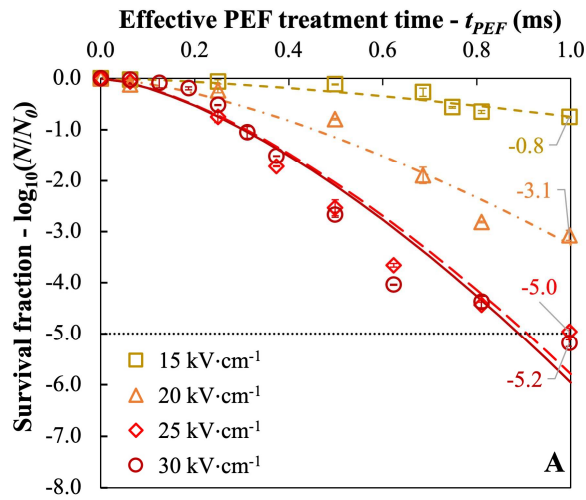


Fig. 3. Synergistic effect of PEF and ohmic heating ($T_{ave} = 41\text{ }^{\circ}\text{C}$) on the inactivation kinetics of A) *Ent. faecalis* and B) *E. coli* in the ABP suspension using different electric field strength (E). Open symbols represent the experimental survival ratios; error bars are the standard deviations of duplicate results; curves represent the modeling results using Weibull model.

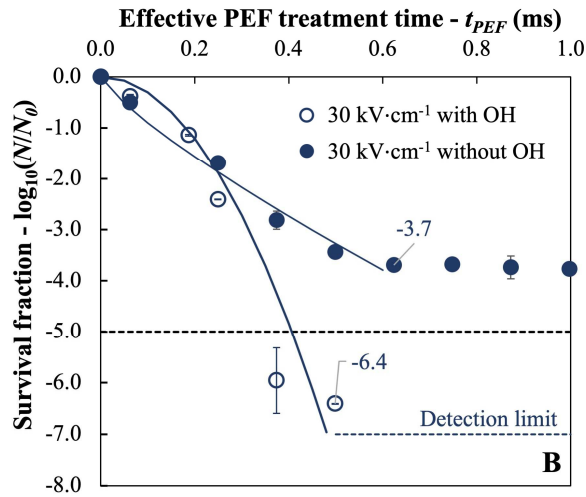
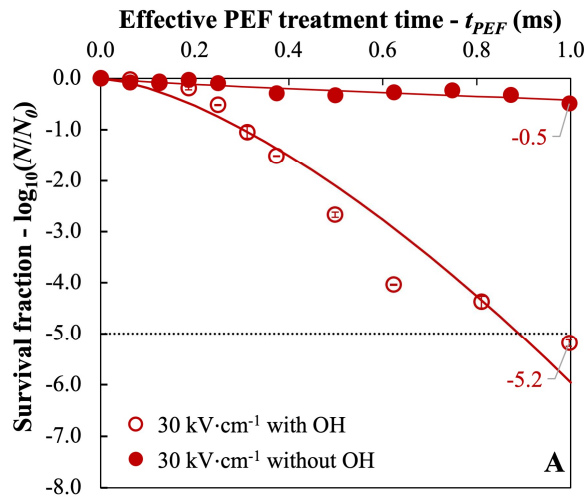


Fig. 4. Inactivation kinetics of A) *Ent. faecalis* and B) *E. coli* in the ABP suspension caused by the synergistic effect of PEF and ohmic heating (open symbols) or the single effect of PEF (closed symbols) at $E = 30 \text{ kV} \cdot \text{cm}^{-1}$. Error bars are the standard deviations of the duplicate bacterial counts; curves are the modeling results using Weibull model.

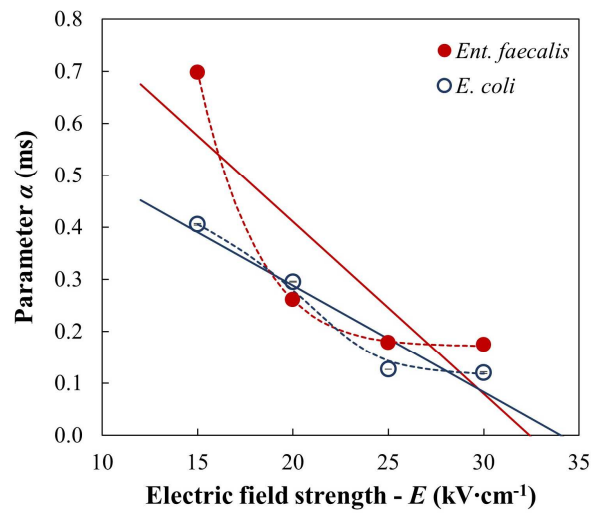


Fig. 5. Linear regression (dash lines) of the Weibull scale parameter α as a function of electric field strength (secondary model) for *Ent. faecalis* (red) and *E. coli* (blue). Solid curves: linear model. Dashed curves: nonlinear model (Gompertz shape).

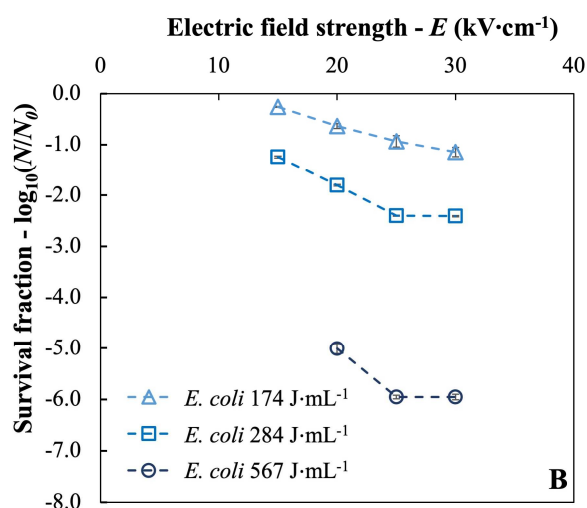
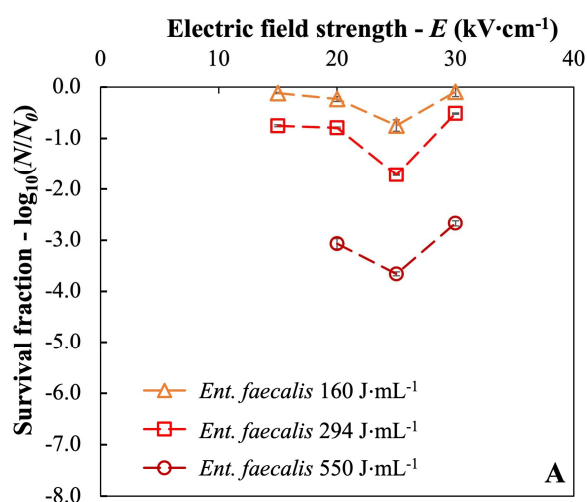


Fig. 6. Inactivation of A) *Ent. faecalis* and B) *E. coli* at three levels of energy input delivered by PEF generator, obtained with the synergistic effect of PEF and ohmic heating.

Synergistic effect of PEF and ohmic heating

