Journal Pre-proof

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Please cite this article as: M. Gavahian and B.K. Tiwari, Moderate electric fields and ohmic heating as promising fermentation tools, *Innovative Food Science and Emerging Technologies* (2020), https://doi.org/10.1016/j.ifset.2020.102422

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Moderate electric fields and ohmic heating as promising fermentation tools

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 Abstract

Fermentation is an important bioprocess in food production and its improvements can bring profits to the food industry. Therefore, researchers are exploring the feasibility of applying emerging process technologies such as moderate electric field (MEF) and ohmic heating. This study demonstrated the current status, potential benefits, mechanisms, and limitations of innovative MEF- and ohmic-assisted fermentation. Research showed that these techniques can positively affect *Lactobacillus*, *Streptococcus*, and *Saccharomyces* fermentations that are involved in the production of bakery (*e.g.,* leavened breads), dairy (*e.g.,* yogurt), and alcoholic products. Also, volumetric ohmic heating can accelerate fermentation by providing optimum fermentation temperatures quickly. MEF-induced stress-response conditions can affect microbial metabolism and fermentation products. Electrical fields may affect the fermentation process by altering the substrate such as releasing its micronutrients. These approaches can be considered prospective industrial fermentation tools. Further economic studies and in-depth research on their effects on fermentation by-products are expected in the near future.

Keywords: ohmic heating; moderate electric field; fermentation; electroporation; emerging technologies; food industry.

1. Introduction

Fermentation can be described as chemical transformations of complex organic compounds into smaller components by microbial enzymes (Corma Canos et al., 2007). These processes are among the most traditional techniques that have been used for food processing and preservation. Nowadays, the industry employs innovative techniques to enhance production yield, decrease fermentation time, and improve product quality. Emerging process technologies are the perspective tools that may assist the food fermentation industry to meet customers' expectation (*e.g.,* producing high quality and safe products at a reasonable price) and industrial demands (*e.g.,* improving the energy efficiency, enhancing the product yield, and reducing process time) (Pereira &Vicente, 2010).

Stress-response conditions can affect metabolic processes such as fermentation and promote bioprocesses and products. Nowadays, the fermentation industry is taking advantage of this scientific concept for increasing the product yield by employing emerging techniques, such as high-pressure processing, ultrasound, pulsed electric field, moderate electric field (MEF), and ohmic heating, in the fermentation process. The applicability of many of these emerging techniques in assisting fermentation processes are well-described, discussed, and reviewed in the literature (Galván-D'Alessandro &Carciochi, 2018; Mota et al., 2018). However, to the best of our knowledge, there is no publication that specifically overviews the potential applications of ohmic heating and MEF in the fermentation-based processes. Furthermore, the lethal impacts of electrical fields on spoilage microorganisms have been explored for a long time (Ayadi et al., 2004; Eliot-Godéreaux et al., 2001; Gavahian, Chu, et al., 2019; Gavahian, Tiwari, et al., 2019; Kulshrestha &Sastry, 2003; Machado et al., 2010; Sun et al., 2008). These comprehensive studies resulted in the industrial application of ohmic heating in many regions of the world (deAlwis &Fryer, 1990; Eliot-Godéreaux et al., 2001; Ramaswamy et al., 2014; Ruan et al., 2001; Sakr &Liu, 2014) indicating the potential

industrial application of this technique in other sectors of the food industry such as fermentation. On the other hand, benefits from the concepts of MEF to promote the activity of microorganisms is a relatively new topic that necessitates elaboration on the benefits that this technology may bring to the fermentation industry. Therefore, this study overviewed the current knowledge on the MEF- and ohmic-assisted fermentation, the effects on microorganisms involved in the fermentation process, mechanisms, and challenges for commercial application, and the prospects of these innovative approaches to assist fermentation processes.

2. Ohmic heating and moderate electric field

Although the general design of an ohmic heating system and a MEF system could be quite similar, there are substantial differences between these two techniques in terms of process parameters and ways that food products and microorganisms are affected.

2.1. Ohmic heating

Ohmic heating is described as the process of passing an electric current through materials for heating purposes (Gavahian et al., 2020; Gavahian &Farahnaky, 2018; Knirsch et al., 2010). The food products that are treated inside an ohmic system act as electrical resistors that are heated through the dissipation of electrical energy (Ramaswamy et al., 2014). Unlike traditional heating methods which usually rely on thermal conduction and convection modes of heat transfer, ohmic heating relies on direct heat generation in the volume of the product, *i.e.,* volumetric heating. Hence, this heating method has a high coefficient of performance, that is, nearly all the input electrical energy is transformed into thermal energy inside the ohmic-treated materials (Gavahian et al., 2012, 2013). Several parameters affect the heating

rate in an ohmic process including electrical conductivity and electrical field strength. These are well-discussed in the literature (Gavahian et al., 2017; Gavahian, Farhoosh, et al., 2015; Gavahian &Farahnaky, 2018). Briefly, the most important equations that should be considered in designing an ohmic system are probably the power equation (Eq. 1) and Ohm's law (Eq. 2) which together infer Joule's first law (Eq. 3) (Gavahian &Farahnaky, 2018).

$$P = IV \qquad (1)$$

$$V = IR \qquad (2)$$

$$P = \frac{V^2}{R} \qquad (3)$$

Where V, R, I, and P represent voltage (volts), resistance (ohm), electric current (amps), and electric power (watt), respectively.

Moreover, the heating rate in an ohmic process can be calculated according to the following equation (Eq. 4):

$$q = \sigma E^2 \qquad (4)$$

where q, E, and σ are the internal energy generation rate (W/m^3), the electric field strength (V/m), and the electrical conductivity (Siemens/m).

Although ohmic heating seems to be a simple process of converting electrical energy to thermal energy, designing an appropriate ohmic system requires several considerations (*e.g.,* choosing suitable electrodes and adjusting the electrical parameters) and engineering calculations. Ohmic systems usually include a power source, electrodes, and a heating chamber (Gavahian, Chu, et al., 2018; Gavahian, Farahnaky, et al., 2015). This system can be equipped with other components such as a data acquisition system, variable transformer, and safety systems (Ramaswamy et al., 2014).

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Due to the promising characteristics, such as decreasing the processing time and lowering energy consumption, ohmic heating has been proposed for various processes including cooking, extraction, distillation, and fermentation (Gavahian, Chu, et al., 2018) (**Table** 2).

2.2. Moderate electric field

Some of the previously conducted studies on ohmic heating of food material revealed that non-thermal effects can be also involved in an ohmic process (Kulshrestha &Sastry, 2003; Lima &Sastry, 1999). These observations extended the research in the area of MEF. In a typical MEF process, low levels of electric fields (*e.g.,* <1 kV/cm) are used while the product temperature remains relatively low (*e.g.,* < 60 °C) (Ramaswamy et al., 2014). The electric fields that are applied in MEF usually range from 1 V/cm to 1 kV/cm of arbitrary frequencies and waveforms.

MEF was found to have non-thermal effects on biological cells, specifically when low frequencies are applied into the interior of the cell (Sensoy &Sastry, 2004). Also, the electroporation phenomenon is involved in MEF, especially at low frequencies, which is one of the main reasons for non-thermal effects of MEF on cells (Lebovka et al., 2005; Sensoy &Sastry, 2004; Yodsuwan et al., 2018). This phenomenon can be beneficial for fermentation processes by enhancing microbial metabolism and growth (Gavahian, Chu, et al., 2018). Distinct metabolic effects, (*e.g.,* changes in the lag-phase of microbial growth and the concentration of fermentation products) can occur in a MEF process (Castro et al., 2005).

It should be noted that thermal effects of ohmic heating and non-thermal effects of MEF may occur at the same time in many processes depending on the process conditions (Gavahian, Chu, et al., 2018; Gavahian &Farahnaky, 2018; Kulshrestha et al., 2009). Although there is limited information in this area of science, the results published so far seem to be very

promising and may encourage more in-depth studies about the possible benefits that MEF and ohmic heating can bring for the fermentation industry.

3. Fermentation by ohmic heating and moderate electric field

It is believed that the electroporation effect caused by MEF at sub-lethal temperatures can assist the microorganism in fermentation processes (Knirsch et al., 2010). In addition, the ability of ohmic heating in quickly providing appropriate fermentation temperature in a fermentation batch, that is, uniformly temperature distribution, has been used to accelerate fermentation processes (Gally et al., 2017). To this date, *Lactobacillus acidophilus* was the most popular microorganism for conducting research on the application of MEF/ohmic heating-assisted fermentation considering the number of conducted studies (Loghavi et al., 2007, 2008, 2009) (**Table 2**). For example, Cho et al. (1996) assessed the effects of conventional and ohmic heating at the electric field strengths of 1.1 and 2.9 V/cm and the frequency of 60 Hz on *L. acidophilus* OSU133 cultures incubated at 30, 35 or 45 °C. The author found that fermentation temperature, the heating method, and the interaction between these two parameters affected the lag period of *L. acidophilus*. According to the authors, the lag period for fermentation at 30 °C was reduced by 18-fold at the low-intensity ohmic process, *i.e.,* E= 1.1 V/cm, as compared to that of the conventional heating method. This observation could be related to improved nutrient transport into the interior part of the cell as a result of membrane electroporation. The mechanisms involved in the leakage of materials from the cell during MEF are previously explained by Gavahian, Chu, and Sastry (2018). Furthermore, Cho et al. (1996) noted the positive effects of ohmic heating on the early stages of lactic fermentation. Moreover, ohmic heating caused a productivity decrease in later fermentative stages due to the transport of inhibitory metabolites. Ohmic treatment also caused 3-hour delay in the production of lacidin A, *i.e.,* a bacteriocin synthesized by *L.*

acidophilus, and reduced its activity. These results introduced ohmic heating as a useful tool for industrial fermentation processes, especially in the early stages of fermentation. However, it seems that the presence of ohmic heating in the stationary phase of fermentation should be avoided (Loghavi et al., 2007, 2009). In an interesting study, Loghavi et al. (2007) assessed the application of MEF at the electric field strength of 1 V/cm and the frequency of 60 Hz on *L. acidophilus* OSU 133 during the initial stage of fermentation (5 h) (at 30 or 37 °C) followed by conventional fermentation for the rest of the fermentation time (35 h). The authors reported that this combined treatment, particularly at 30 °C, increased bacteriocin activity as compared to that of the conventional fermentation process. On the other hand, the continuous application of MEF reduced bacteriocin activity compared to conventional and combined fermentation techniques. Furthermore, the authors observed that MEF did not affect growth parameters such as the maximum specific growth rate, lag time, and biomass production. The positive effects of MEF on bacteriocin activity may be correlated with electric field-induced stress because the stress conditions usually enhance the production of defensive molecules such as bacteriocins (Verluyten et al., 2003).

In an outstanding study on the application of MEF for fermentation processes, Loghavi et al. (2008) designed and developed a MEF-assisted fermenter apparatus (**Figure 1**). These researchers noted that the frequency (45, 60, or 90 Hz), the electrical waveform, and the electric field strength affected both bacteriocin production and lag-phase extension. According to the authors, the maximum bacteriocin activity was increased (6500 AU/mL) compared to the control (4000 AU/mL) when a sinusoidal field at the frequency of 60 Hz with harmonics was applied during the early stage of the growth (5 h at a total of 40 h). It is likely that bacteriocin concentration in the environment reached the level required for triggering pre-bacteriocin production (which needs further activation) under the above-mentioned process conditions. On the other hand, if the electric field is applied during the

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entire growth time (40 h), the transport of pre-bacteriocins may happen because of temporary electroporation, thereby bypassing the critical translocation process by adenosine triphosphate (ATP)-binding cassette transporter proteins, which is required for activation of bacteriocin. Hence, some of the bacteriocin molecules may be released into the environment without becoming activated. Investigations on the effects of MEF at the electric field strength of 2 V/cm and at the frequencies of 0.045-10 kHz and the temperature of 30 C on the cell membrane of *L. acidophilus* OSU 133 showed that using a dye-staining technique can identify the population of intact and permeabilized cells in MEF studies (Loghavi et al., 2009). The authors observed that treatments at 45 Hz exhibited the maximum permeabilization followed by treatments at 60 Hz (**Figure 2**). The stage of microbial growth also affected the permeabilization of the cell membrane. According to the authors, cells at the lag-phase showing the greatest susceptibility to permeabilization, followed by those at the exponential phase. No sign of electroporation was identified throughout the stationary phase of fermentation. This finding was previously reported in the literature by Zimmermann et al. (1974) who reported that the critical dielectric cell membrane breakdown voltage increased about 30 % at the stationary phase (Zimmermann et al., 1974). This can be illustrated by the changes in the lipid and protein content during the cell growth proceeds from lag to exponential phase, leading to different dielectric properties of the cell membrane. Another reason for this observation can be the decline in the pH during cell growth and its possible effects on the permeabilization of the cell membrane. Application of ohmic heating at the electric field strengths of 0.5–2 V/cm at 30 °C during the entire fermentation process, affected the fermentation profile of a recombinant *Saccharomyces cerevisiae* strain which was growing on lactose for ethanol and ß-galactosidase production (Castro et al., 2005).

A reduction in the lag-phase and an increase in the yield of biomass suggested that ohmic heating at the electric field intensity of 2 V/cm improved the early stages of fermentation. On

the contrary, the application of milder electrical fields, *i.e.,* 0.5 and 1 V/cm prolonged the lag-phase by about 21 % and 53 %, respectively. Investigating the effect of electric field on the conversion of substrate to ethanol and ß-galactosidase revealed that greater electric field Intensities decreased the yield of ethanol but enhanced the yield of ß-galactosidase. This observation could be because of a shift from ethanol production toward pure respiratory metabolism where biomass build-up is favored. Also, researchers showed that MEF can accelerate the fermentation process of bread (Gally et al., 2017). For example, Gally et al. (2017) reported that ohmic-assisted proofing of bread dough allows the yeasts to quickly reach the optimum temperature for their activities (Gally et al., 2017) (**Figure 3**). According to the authors, ohmic heating can increase the heating rate and decrease the time needed to reach the desired expansion ratio in a proofing process of the studied bread by about 50 % by decreasing of the lag-phase at the beginning of the fermentation process of the bread (from one hour to 20 min for traditional and ohmic-assisted fermentation, respectively). However, further microbiological investigations are required to explore this innovative application of ohmic heating. It should be mentioned that a similar ohmic-assisted fermentation system was developed recently for proofing of gluten-free bread dough which was found to be a useful tool to study the formation of crumb structure and to control the fermentation temperature (Masure et al., 2019)

Besides, Reta et al (2017) explored the effects of ohmic heating in reducing the acidity of coffee during fermentation of fresh Arabica coffee beans. The authors observed that at the constant temperature of 30 °C reduced total acidity (mL of 0.1N NaOH required for neutralization of coffee sample) of coffee beans from 0.53 to 0.18 during 18 hours of fermentation at the constant temperatures of 30 °C. They also observed that variations in moisture content and acidity of the coffee beans during fermentation depended on the fermentation time and fermentation temperature. It was hypothesized that an optimized

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ohmic fermentation process can be considered as an effective tool for the production of high-quality coffee Arabica in terms of total acidity (Reta et al., 2017). Besides, a study conducted at Ohio State University assessed the effects of MEF at a constant electric field strength of 1 V/cm on yogurt starter cultures, that is, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Costello, 2012). According to the results, fermentation by *S. thermophilus* at 35 °C with 45 Hz MEF showed a reduced lag time as compared to the control. In contrast, the application of the same MEF did not affect the growth parameters of *L. bulgaricus*. These differences in response to the electrical field could be related to the shape of microorganisms: *L. bulgaricus* is a Gram-positive rod *S. thermophilus* is a Gram-positive coccus. This research showed that the stimulation of growth under MEF could be strain-dependent but as to why and how the type and specifications of microorganisms play roles in response to MEF is needs further investigations.

Despite the limited published reports on the applicability of MEF and ohmic heating for enhancing/modifying the fermentation processes, the current understanding of this emerging technology and the promising results in this area of the research indicate the exceptional potential of MEF and ohmic heating for accelerating the initial stage of fermentation.

4. Mechanisms involved in MEF- and ohmic-assisted fermentations

Despite the limited fundamental research for fully understanding the ways that ohmic heating and MEF can assist conventional fermentation methods, it seems that ohmic heating can affect the fermentation process through three main ways (**Figure 4**). First, electrical treatments can affect the substrates that microorganisms converting to the microbial metabolic products (Gavahian, Munekata, et al., 2019; Karim et al., 2019; Kim et al., 2019). Second, electrical fields can affect the permeability of cells, resulting in higher performance of the microorganism in converting the substrate to the product (Qu et al., 2020). Third, MEF

and ohmic heating can provide the optimum fermentation temperature at a short time, reducing the lag-phase of fermentation and accelerating the fermentation process (Gally et al., 2017; Gavahian, Munekata, et al., 2019). However, it should be noted the presence of the above-mentioned mechanisms and their intensities vary, depending on several parameters (*e.g.,* the type of the microorganisms, electrical field condition, the type of substrate, and the desired fermentation products). Therefore, optimizations studies are required to verify the possibility of enhancing a fermentation process through electrical field application. Besides, recent studies revealed new information about the effects of MEF on enzymes and protein structure which may further clarify the details of the mechanism involved in MEF- and ohmic-assisted fermentation (Rodrigues et al., 2019, 2020; Samaranayake &Sastry, 2018).

5. Challenges and future perspectives

Despite the good results concerning the applicability of ohmic heating and MEF for fermentation processes, the continuous application of these emerging technologies throughout the entire fermentative process is not beneficial. Furthermore, it should be noted that the effects of ohmic heating and MEF on cells and fermentation processes can be determined by process parameters such as pH, waveform, electric field strength, and frequency. Therefore, a successful ohmic/MEF-assisted fermentation process requires process optimization and engineering calculations which makes the process more complicated than the conventional fermentation processes. Finally, the relatively high capital costs required for replacing the conventional fermentation process with ohmic/MEF-assisted process might be the biggest obstacle for the industrial adaptation of this technique. However, further engineering and economic studies may address the above-mentioned drawbacks and result in the implementation of large-scaled ohmic-assisted fermentation units in the industry. Also, information about the potential benefits of MEF and ohmic heating on the development

of several fermented products (*e.g.,* fermented dairy commodities) is still scarce. Therefore, a promising area for future research in the field of ohmic/MEF-assisted fermentation is available that is hoped to be explored in the near future. Besides, many of the previously conducted studies in this area of research were conducted at laboratory-scale and this could be the reason for relatively slow progress in the development and applications of these fermentation tools. Further academic-industrial collaborations can promote research in this area of science by considering the commercial applications and the ways that these techniques can provide benefits for the industry.

6. Conclusion

Research showed that ohmic heating and MEF are useful tools for lactic acid and alcoholic fermentation at laboratory-scale. The benefits that these technologies provide are related to their thermal and non-thermal effects on the microorganisms and fermentation media. For example, ohmic heating can quickly provide the optimum fermentation temperature and reduce the fermentation time. Also, a reduced lag-phase and higher concentrations of desirable fermentation products can be obtained because of the stress-response of the microorganisms to MEF. These potential benefits can be considered by the food and fermentation industries after further analysis of these emerging fermentation aids. This includes scalability and economic studies.

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Tables

Table 1- Some of the common applications of ohmic heating for food processing

Application/process	products	Key benefits	References
Cooking	Cooked rice, vegetables, and meat	- Reducing cooking time - Saving energy consumption	(Farahnaky et al., 2012, 2018; Gavahian, Chu, et al., 2019; Wang &Farid, 2015)
Extraction	The essence of aromatic herbs	- Reducing extraction time - Saving energy consumption - Enhancing the concentration of extract's valuable components	(Gavahian et al., 2011; Gavahian, Farahnaky, et al., 2015; Gavahian, Farhoosh, et al., 2015; Gavahian, Lee, et al., 2018; Gavahian &Chu, 2018)
Distillation	Bioethanol	- Substantially reduction of the distillation time	(Gavahian, Farahnaky, &Sastry, 2016a, 2016b; Gavahian, Farahnaky, Shavezipur, et al., 2016)
Fermentation	Yogurt, alcohol, and bread	- Altering the fermentation products - Reducing the fermentation time	(Cho et al., 1996; Loghavi et al., 2007, 2008, 2009)

Table 2- Summary of the conducted studies on ohmic/MEF-assisted fermentation

Process conditions	Microorganism	Key observations	Ref.
E: 1.1,2.9 F: 1, 60 T: 30, 35, 45	*Lactobacillus acidophilus*	- The low-intensity MEF positively affected the early stages of lactic fermentation. - MEF reduced the productivity in the later stages of lactic fermentation. - MEF prolonged the production of lacidin A and reduced its activity	(Cho et al., 1996)
E: 1 F:1, 60 T: 30, 37	*Lactobacillus acidophilus*	- MEF-assisted fermentation enhanced the activity of bacteriocin - Continuous application of MEF reduced the activity of bacteriocin - Growth parameters were not affected by MEF	(Loghavi et al., 2007)
E: 1 F: 1, 45, 60, 90 T: 30	*Lactobacillus acidophilus*	- Bacteriocin production and lag-phase were affected by the frequency and waveform of the MEF - Sinusoidal waves at the frequency of 60 Hz resulted in the greatest activity of bacteriocin	(Loghavi et al., 2008)
E: 2 F:1, 45, 60, 1k, 10k T: 30	*Lactobacillus acidophilus*	- The greatest permeabilization was observed for 45 Hz MEF treatments followed by at 60 Hz MEF treatments - The maximum susceptibility of cells to permeabilization was observed at lag-phase followed by the exponential phase. - No sign of electroporation was detected during the stationary phase	(Loghavi et al., 2009)

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E: 0.5–2 T: 1, 30	*Saccharomyces cerevisiae*	- MEF improved the early stages of fermentation - An increase in lag-phase was observed when MEF was applied at low intensities (< 1 V/cm) - Increasing the electric field intensity enhanced the yield of ß-galactosidase - Increasing the electric field intensity decrease the yield of ethanol	(Castro et al., 2005)
E: 1 F: 45, 60 T: 35, 44	*Lactobacillus bulgaricus* and *Streptococcus thermophilus*	- MEF (at 35 °C and 45 Hz) reduced the lag time of S. thermophilus fermentation. - MEF (at 44 °C and 60 Hz) did not affect the growth parameters of S. thermophilus. - MEF (at all the conditions studied) did not affect the growth parameters of L. bulgaricus. - Stimulation of growth under MEF could be strain dependent.	(Costello, 2012)

* E: electric field strength (V/cm); F: frequency (Hz); T: Temperature (°C); k: Thousand.
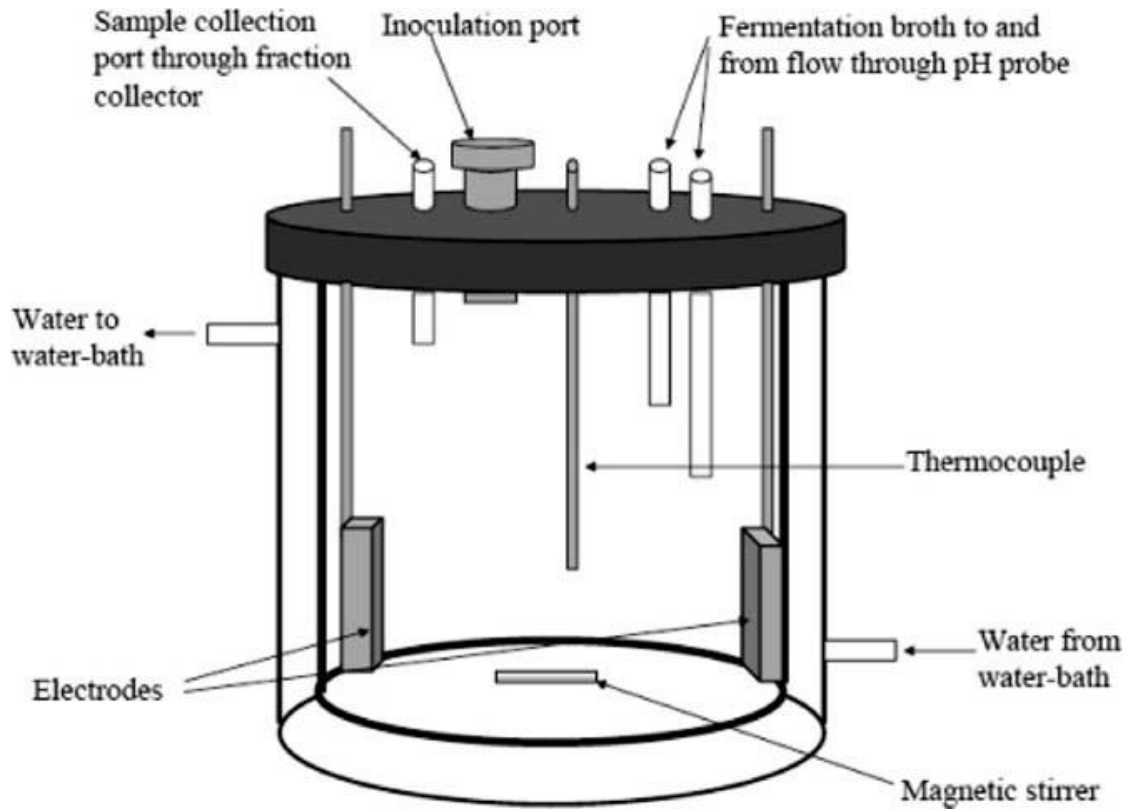
Figures

Figure 1- A MEF-assisted fermenter designed and developed at the Ohio State University. Adapted from Loghavi et al. (2008) with permission.
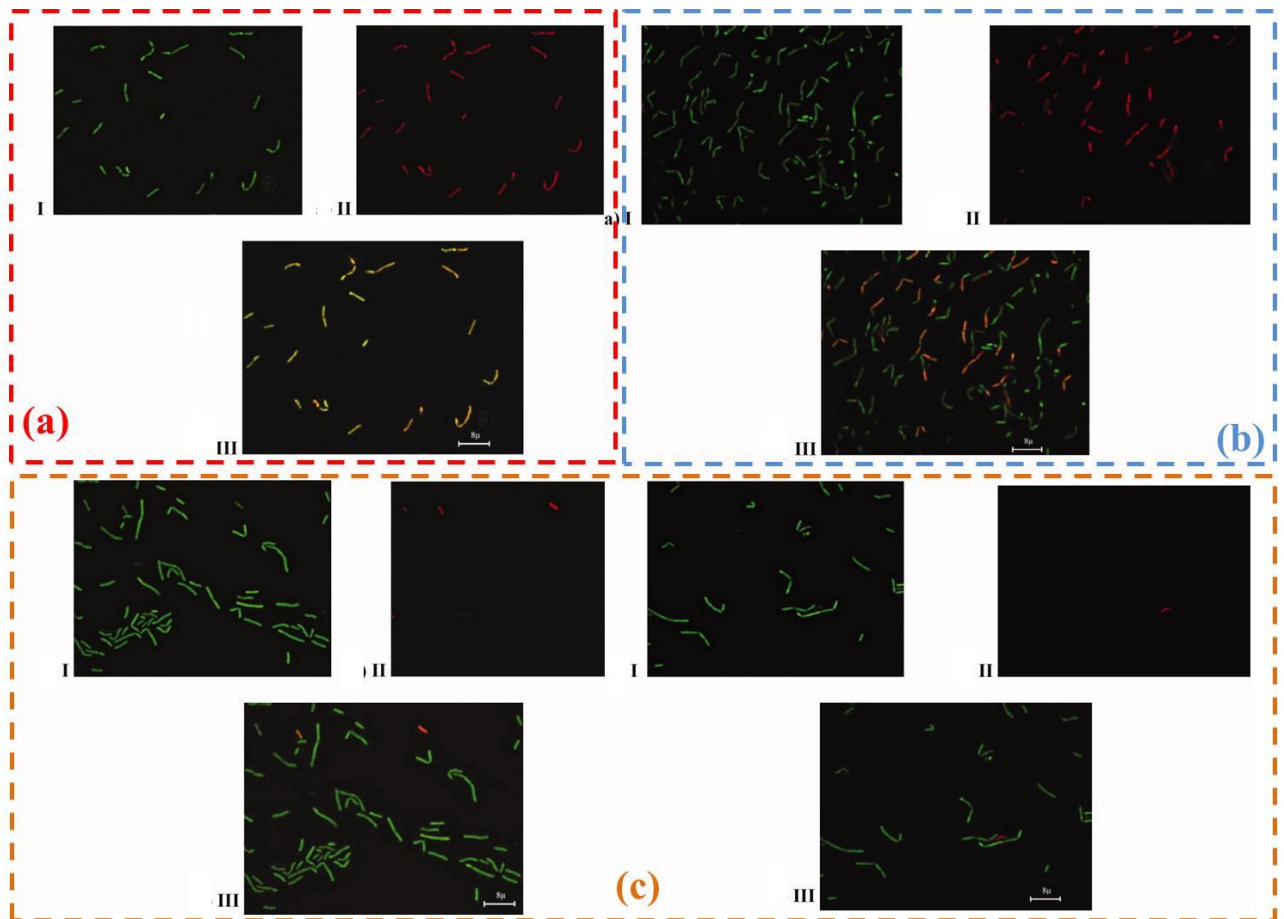
Figure 2- Fluorescence microscopy of *Lactobacillus acidophilus* during the lag-phase: Treated cells with MEF at 45 Hz (a); Treated cells with MEF at 60 Hz; Control treatment (presented below the counterpart treatments) (c). I: green fluorescent image; II: red fluorescent image; III: combined red and green fluorescent images. Adopted from Loghavi et al. (2009) with permission.
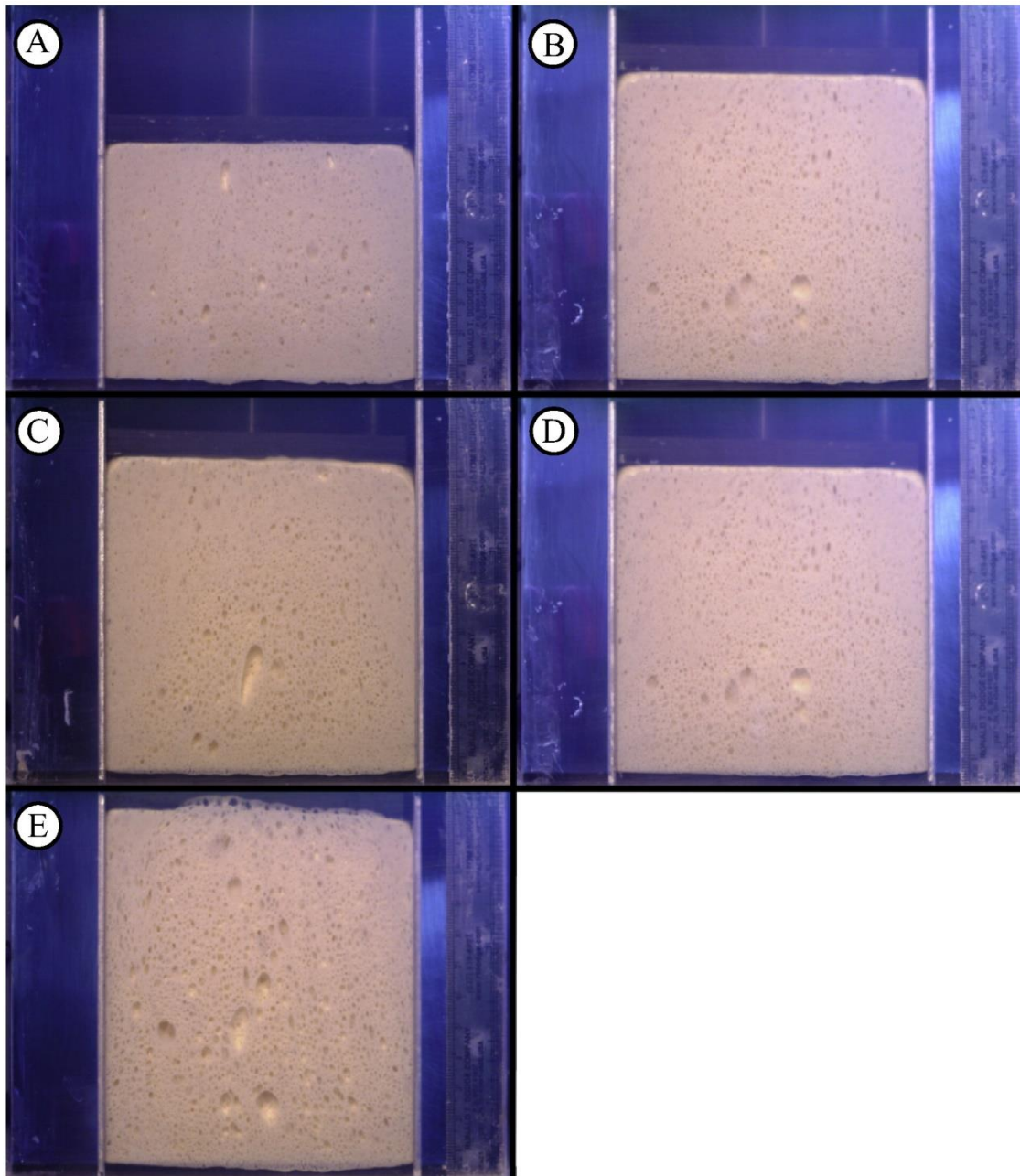
Figure 3. Side views of bread dough after proofing for 1.5 h of by ohmic-assisted proofing technique at the heating rates of 0.2 (A), 0.5 (B), 1 (C), 5 (D), and 10 °C/min (E). Higher heating rates assisted yeast to reach the optimum temperature for their activities and accelerated the proofing process. Adopted from Gally et al. (2017) with permission.
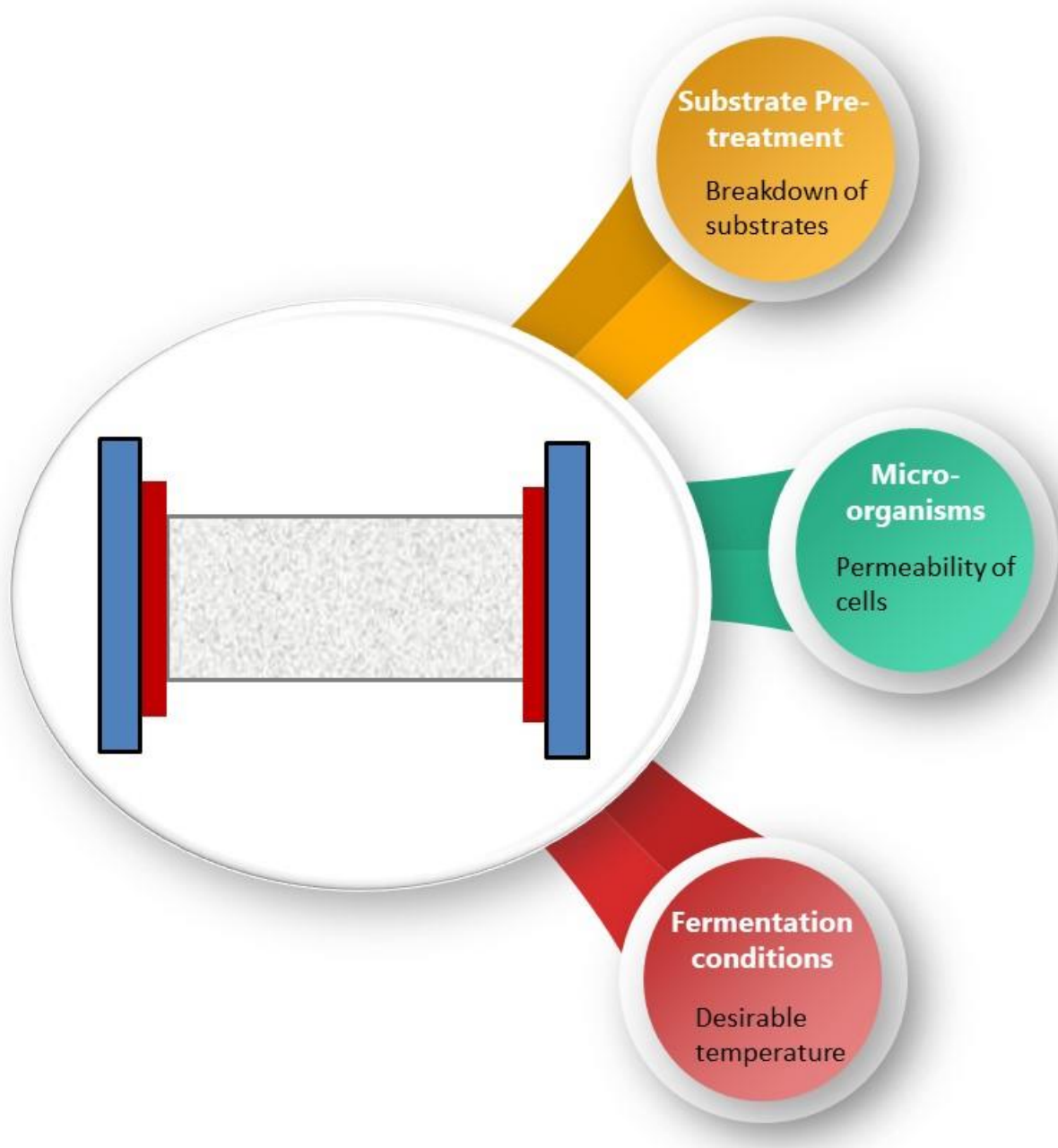
Figure 4. A generic illustration summarizing the ways that fermentation processes can benefit from ohmic heating and moderate electric field.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be
considered as potential competing interests:

Highlights

- Ohmic heating and moderate electric fields can assist fermentation processes through thermal and non-thermal effects.

- Electricity can reduce the lag-phase *Lactobacillus*, *Streptococcus*, and *Saccharomyces* fermentation.

- Electricity can alter the type and concentration of fermentation products.

- This technique applies to some dairy (e.g. yogurt) and bakery (e.g. bread) products.

- Main mechanisms are quick temperature adjustment, micronutrients release from substrates, and cell's permeability enhancement.