



Journal of Applied Sciences

ISSN 1812-5654

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Non-Thermal Lethal Effects of Low-Voltage Alternating Current on *Bacillus cereus*

¹K. Thamaphat, ²C. Oopathump and ³B. Panacharoensawad

¹Department of Physics, Faculty of Science,

King Mongkut's University of Technology Thonburi, Bangmod, Bangkok 10140, Thailand

²Division of Physics, Faculty of Science and Technology,

Rajamangala University of Technology Krungthep, Bangkok 10140, Thailand

³Department of Physics, Faculty of Science, Naresuan University, Pisanulok 65000, Thailand

Abstract: The bactericidal mechanisms of low-voltage alternating current (0-300 mA cm⁻²) on the bacteria *B. cereus* were investigated. The *B. cereus* cells in logarithmic phase suspended in phosphate buffer solution (0.2 M) were exposed to alternating current of 50 Hz, 120 V under aerobic conditions. The related parameters for bactericidal actions by low-voltage alternating current were measured. At the temperature 29±3°C (non-lethal temperature), the surviving fractions of cells exposed to Alternating Current (AC) were decreased with an increase in current density for a definite exposure time. At a certain current density, the surviving fractions were decreased proportionally with exposure time. The lethal effect to cells was attributed to the toxicity of hydrogen peroxide and direct effect of alternating current. These surviving fractions were closely related to the amount of H₂O₂ formed in the cell suspensions. At a definite current density, the amount of H₂O₂ in the suspension increased with increasing AC-exposure time. At a definite exposure time, the amount of H₂O₂ increased with increasing current density. The H₂O₂ was produced on the surface of carbon electrodes by AC-electrolytic reduction of dissolved oxygen. The suitable condition for efficient bactericidal by low-voltage AC at non-lethal temperature was AC-exposure at 300 mA cm⁻² for 1 h or 200 mA cm⁻² for 3 h. This study shows that the low-voltage alternating current method is a promising technology for the nonthermal pasteurization of foods and is an appropriate complement or replacement of traditional thermal. Furthermore, the mechanism of bactericidal activity by AC may offer a useful method for eradicating bacteria from catheter surfaces.

Key words: Alternating current, *Bacillus cereus*, nonthermal method, microbial control, hydrogen peroxide

INTRODUCTION

The worldwide increasing number and severity of food poisoning has considerably gained more public awareness of food safety. Control of foodborne pathogens is not always easy. Many pathogens survive in different environments for long period of time. They can be transmitted to humans by a variety of routes. Food processors currently rely on quite a few methods of preserving food to increase shelf life and maintain food safety by inactivating spoilage and pathogenic microorganisms. Preservation methods have been altered from those used in the past. Conventional methods include heating, drying, freezing and the addition of approved preservatives. Heat is the most commonly used preservation method and heat-treated foods generally have a good safety record. When properly applied, heat can eliminate bacteria, fungi, viruses, parasites and enzymes, which are the biological agents that spoil food.

Conventional technologies produce safe food but the products have less nutritional and sensory quality and consumer acceptability comparing to their fresh counterpart. Interest in alternative food processing technologies has been driven by consumer demand for food with fresh-like taste, crisp texture, high nutrient content and natural color. Alternative technologies have been advanced by both industry and academia in an attempt to meet the challenge of producing safe processed food of a high quality. Preservation of food by nonthermal methods provide as an option because during nonthermal processing, the temperature of the food is held below the temperature normally used in the thermal processing (60 to 100°C for a few seconds to minutes). Therefore, the quality degradation expected from high temperatures is minimal. The electrical treatment of foods for microbial control is called nonthermal method. The easiest applicable method, especially for industrial purposes, is low-voltage Alternating Current (AC)

method, since it is possible to control temperature below lethal temperature during processing. The microbicidal action of low-voltage alternating current (50 Hz) is based on a defined quantity of electricity applied at or above a certain minimum current density (Barbosa-Canovas *et al.*, 1999).

Accordingly the passage of low-voltage AC through the cell suspension at non-lethal temperature has been known to exert a killing effect. This action was found to be primarily due to toxic substances formed in the suspension by electrolysis. By using Ag-metal electrodes, Fritz believed that silver salts and free chlorine generated by electrolytic action of alternating current were responsible for the killing effect of yeast cells (Shimada and Shimahara, 1981). Tracy (1932) proposed that the formation of temporary toxic substances like free chlorine might cause the killing effect. After that, Rosenberg *et al.* (1965), by using certain group of VIII transition metal electrodes, demonstrated that the metal complexes, e.g. Pt (IV) complexes or irons like Ni²⁺ produced in the medium at the level of about 1-10 ppm by electrolysis, caused inhibition of *E. coli* cell division or resulted in bacterial death. Using stainless-steel electrodes in suspension containing chloride, Pareilleux and Sicard (1970) reported that the toxicity was due mostly to labile compounds, whose effect on *E. coli* K-12 cells could be reduced by the addition of cysteine or albumin to the suspension. In order to get rid of minimal electrolysis by-product production and examination the genuine causes of bactericidal, carbon electrodes were employed in this work because carbon electrode is gas-ion electrode. It acts as only receptor or the passing way of charges. It dose not have any role in chemical reaction produced because of chemical inertness (Rieger, 1987).

The aim of the present investigation was to study the lethal effect of *Bacillus cereus* following exposure to low-voltage AC (120 V, 50 Hz) at non-lethal temperature (29±3°C during the treatment) in relation to the ratio of viable count at that moment to initial viable count (surviving fraction) and inspect the effect of low-voltage alternating current on stimulates sporulation of *B. cereus*. Current densities were varied from 0 to 300 mA cm⁻². For the reason that lethal effect could be observed and growth of cells was eliminated, phosphate buffer solution (0.2 M)-the solution that prevents abrupt changes in acidity or alkalinity-was utilized as solution treatment instead of culture media. Moreover, chlorides that were described to form toxic substances in cell suspensions during exposure to alternating current were not used to be the composition of treated solution.

MATERIALS AND METHODS

Bacillus cereus was used throughout the experiment. The test apparatus of AC-exposure to cell suspension consisted of four fundamental components: (1) regulator that comprised variable voltage transformer (input 110/220V, 50/60 Hz and output 0-260 V) and variable resistor, (2) treatment chamber, (3) cooling device and (4) current, temperature measurement devices as demonstrate in Fig. 1.

The current was measured by true-rms multimeters (Fluke, Model 179), which connected in series to a variable resistor. The treatment chamber was designed to hold cell suspension during alternating current application and to house the electrodes. Materials selected to construct a treatment chamber need to be washable and able to use with autoclavable at 121°C for 15 min. Additionally the materials should appreciably transfer heat because the purpose of experiment was to investigate the effect of low-voltage alternating current to the cell suspension at non-lethal temperature. Glass was used to construct static chambers. Figure 2 shows a glass chamber for AC-exposure. The glass chamber is constructed to form a vertical pipe with four holes connecting with four vertical arms. The chamber is 14 cm long and has a volume of 25 mL. The chamber cross sectional area is 1.77 cm². The inner diameter of all arms is 1.5 cm. The first and fourth arms are used to accommodate the electrodes. The second arm is provided to support a mercury thermometer for temperature measurement during

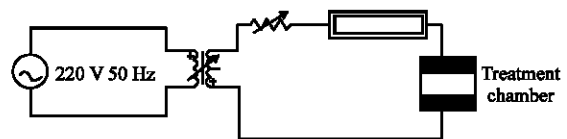


Fig. 1: Fundamental components of the low-voltage alternating current method

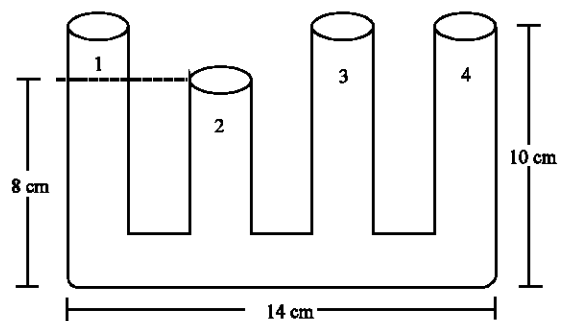


Fig. 2: A glass of chamber for AC-exposure

the treatment of cell suspension with alternating current. The third arm serves as a passage to introduce and remove a sample.

The carbon rods were used as electrode in this work. They are made of commercial carbon, fine graphite and nontoxic type. They are purchased from Thai Carbon and Graphite Co., Ltd. Electrode specific resistance was $11 \mu\Omega \cdot m$. Each carbon rod dimensions are 15 cm in length by 1 cm in diameter. Distance of the electrodes is about 12.5 cm. Recirculation cooling water through the treatment chamber will control the temperature of cell suspension in the gap formed by the two electrodes in the treatment chamber.

Vegetative cells in logarithmic phase were prepared. They were suspended in NB. Cells were harvested by centrifugation ($8000 \times g$ for 10 min) at $5^\circ C$, washed twice with 0.2 M phosphate buffer solution (pH 7.0) and resuspended in the same buffer solution. The initial cell concentrations used were approximately 10^6 CFU mL^{-1} . A cell suspension of 23 mL was introduced to treatment chamber. It was exposed to varying intensities of AC 50 Hz in glass chamber with two carbon electrodes. Alternating current densities were used at 0, 50, 100, 200 and 300 $mA \text{ cm}^{-2}$, respectively. The temperature of cell suspension was maintained at $29 \pm 3^\circ C$ during AC-exposure by cooling system. For untreated (0 mA cm^{-2}) cell suspension, it must be kept under the same condition as treated cell suspension. Thus it was introduced in the same glass chamber and placed in the water bath (Eyela, Model SB-24) at temperature $29 \pm 1^\circ C$. Treated and untreated cells were AC-exposed in parallel. At the start of the exposure and at 1, 3, 5 and 7 h thereafter, 0.2 mL of cell suspension was taken out from the chamber to determined viable count instantaneously and one loop of cell suspension was smeared on slide for investigation spore forming. Viable counts of untreated cell were served as control. Additionally, cell suspension was measured the content of H_2O_2 every 1 h for 12 h by using peroxide test strips (Merckoquant[®]).

The results shown in this research were taken from the average of observation in duplicate experiments. Statistical analysis of the data from all experiments was performed using the software program Sigma Stat by SPSS Inc.

RESULTS AND DISCUSSIONS

Effect of low-voltage alternating current on survival of *B. cereus*: In this study, the bacteria *B. cereus* in exponential phase was exposed to AC of 0, 50, 100, 200 and 300 $mA \text{ cm}^{-2}$, respectively under aerobic condition. The temperature of cell suspension was held at $29 \pm 3^\circ C$.

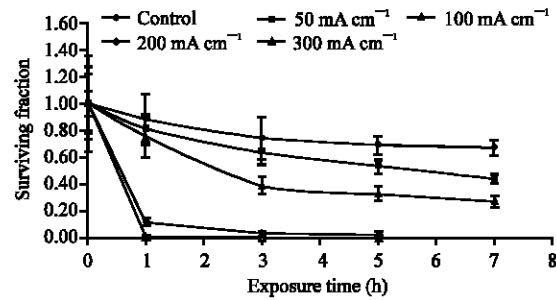


Fig. 3: Survival curve of *B. cereus* exposed to AC under aerobic conditions

Viable counts of untreated cell were served as control. Effect of electricity on surviving fraction and stimulation spore forming were elucidated.

Figure 3 shows the relationship between the surviving fractions of exposed *B. cereus* to AC of 0, 50, 100, 200 and 300 $mA \text{ cm}^{-2}$ and exposure time under aerobic condition. Surviving fraction was ratio of viable count at that moment to initial viable count. It found that the viable counts of exposed cell by AC 200 and 300 $mA \text{ cm}^{-2}$ were undetectable numbers (<30 CFU mL^{-1}) at 7 h. From Fig. 3, it showed that surviving fractions of unexposed cells decreased gradually and tends to be constant after longer treatment time. The survival curves of cells exposed to AC 50 and 100 $mA \text{ cm}^{-2}$ decreased gradually and nearly were constant with time. On the other hand, for AC exposure of 200 and 300 $mA \text{ cm}^{-2}$, surviving fractions curve appreciably varied with time and converged to zero with longer treatment time. Conclusively exposure to a certain current density of AC, the surviving fractions of cells decreased with increased exposure time. For longer AC exposure time, the surviving fractions of cells would definitely decrease. Therefore the decrease in surviving fractions was related to the quantity of electricity, that is, the product of current density and time. For stimulation spore forming by AC-exposure did not find under observation by microscope (1,500x).

The results of this part point out that the suitable current density and exposure time for efficient bactericidal by low-voltage AC at non-lethal temperature was 300 $mA \text{ cm}^{-2}$ AC exposure for 1 h or 200 $mA \text{ cm}^{-2}$ AC exposure for 3 h. As described earlier, surviving fractions of cells exposed to AC decreased with increased exposure time at a definite current density or decreased with increased current density at a definite exposure time. Therefore the decrease in surviving fractions is assumed to relate to current density and time. These results are similarly to results of Shimada and Shimihara (1981) and Pareilleux and Sicard (1970). They exposed AC to *E. coli* cells strain B and K-12, which suspended in phosphate

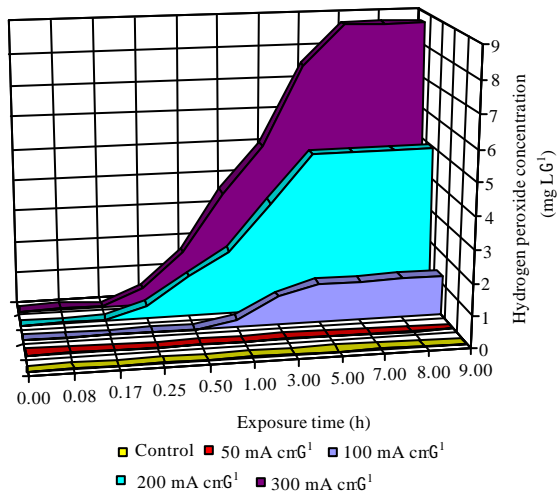
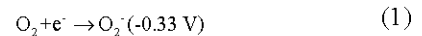


Fig. 4: Relationship between AC-exposure time and H₂O₂ concentration

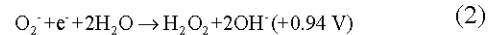
buffer solution (pH 7.0). *Escherichia coli* is Gram-negative bacteria. Carbon electrodes were used. Surviving fraction of both strains exposed to AC decreased with increased exposure time at a definite current density. Also, the surviving fraction decreased with increased current density at a definite exposure time. Although, their characteristic of survival curve is the same as the present experiment, the decreasing rate was different. The comparison suggests that exposure of AC on microorganism definitely has lethal affects however the microbial surviving fraction or decreasing rate of survival curve depends upon the types of microorganism. These inferred that, among bacteria, those that are gram-positive are more resistant than those that are Gram-negative.

Quantitative assay of hydrogen peroxide: Hydrogen peroxide concentration measurement was performed for all AC exposure experiments. Figure 4 shows the relationship between H₂O₂ concentration and exposure time in cell suspension with exposure to an AC of 0, 50, 100, 200 and 300 mA cm⁻². The formation of H₂O₂ found when AC was higher than 100 mA cm⁻². At AC of 100 mA cm⁻², H₂O₂ concentration was detectable when treatment time was over 0.5 h. The formation rate of H₂O₂ gradually increased and reached a constant after 5 h. The maximum concentration was 1.25 mg L⁻¹. On the other hand, the formation of H₂O₂ in cell suspension exposed to AC of 200 and 300 mA cm⁻² began after 10 min. Although, the H₂O₂ formation in cell suspension at AC exposure of 200 and 300 mA cm⁻² started more or less at the same time, the H₂O₂ formation rate for higher AC exposure is shown to increase faster than that for lower AC exposure.

For AC of 200 mA cm⁻², H₂O₂ concentration increased with the exposure time up to 5 h and there was no concentration change thereafter. The maximum H₂O₂ concentration was 5 mg L⁻¹. For AC of 300 mA cm⁻², H₂O₂ concentration reached its maximum after 7 h of AC exposure. The maximum H₂O₂ concentration was 8.75 mg L⁻¹. It showed that the concentration of H₂O₂ increased with an increase in exposure time at a definite current density and/or with increased current density at a definite exposure time. The cause of hydrogen peroxide formation in cell suspension under AC-exposure can be explained as follows. Most of investigations (Liu *et al.*, 1997; Tadashi *et al.*, 1992; Patermarakis and Fountoukidis, 1990; Zhao *et al.*, 1998) reported that hydrogen peroxide formed in aqueous solution, if the solution is electrolyzed. It was a reduction product of dissolved oxygen at cathode. At the miliampere level of DC or AC, hydrogen peroxide was produced as a result of electrolysis. The combination of an oxygen molecule with an electron at the cathode produces the superoxide ion radical, i.e.,



More supply of negatively charge electron from the cathode and with combination with water, the superoxide becomes hydrogen peroxide and ionized hydroxide radical,



Hydrogen peroxide is a strong oxidizing agent. It can react with organic materials and alter molecular structure and then contribute to lethal and sublethal changes in living cells. So it can be referred that the lethal effect of AC exposure is mainly due to indirect action rather than direct action of AC. This consequence was confirmed by results of the third article. Surviving fractions of cells exposed to H₂O₂ decreased with increased exposure time at definite H₂O₂ concentration and surviving fraction decreased with increased H₂O₂ concentration at definite exposure time.

It is found that the concentration of H₂O₂ was related to current density at definite exposure time. However, Shimada and Shimahara (1982) found that when the exposure time was 1 h or longer, the concentration of H₂O₂ at AC of 300 mA cm⁻² was slightly lower than at AC of 200 mA cm⁻². Their explanation to the results was that because the formation of H₂O₂ was accompanied by the decomposition of H₂O₂ formed in the course of AC-exposure. Hence, the concentration of H₂O₂ was not always observed to directly relate to the current density. The present research experiment shows opposite result.

The logical explanation can be elucidated as follows. Firstly, the compound H_2O_2 can easily be decomposed by heat or the enzymes catalase and peroxidase to give the end products, oxygen and water (Branen *et al.*, 1990; Block, 1991). At room temperature, it slowly decomposes. In the quoted experiment, microbial cells exposed to AC at 200 and 300 $mA\ cm^{-2}$ were under in the same conditions thus the decomposition of H_2O_2 under the exposure of two specified levels of current density should be equal. Secondly, it is the consequent of Faraday's first law of electrolysis. Faraday's first law states that in electrolysis the amount of primary product at each electrode is proportional to the electric current. Doubling the quantity of electricity passed doubles the amount of primary product. If there is more than one primary reaction the total amount of primary products is proportional to the quantity of electric current. Accordingly, the mechanism of H_2O_2 formation was reduction product of dissolved oxygen at cathode as shown in Eq. 1 and 2. So, under the same conditions, for AC exposure of 300 $mA\ cm^{-2}$, hydrogen peroxide concentration was formed in cell suspension more than the concentration resulted from AC exposure of 200 $mA\ cm^{-2}$. These indicated that the concentration of H_2O_2 at AC 300 $mA\ cm^{-2}$ was slightly lower than the concentration at AC of 200 $mA\ cm^{-2}$ is not possible. However, it still depends on the uncertainty of H_2O_2 concentration measurement.

Effect of hydrogen peroxide on survival of *B. cereus*: As experimental evidence, under AC exposure, H_2O_2 was formed in a cell suspension under aerobic conditions that a part of lethal effect of AC exposure on *B. cereus* cells. In order to find the survival percentage due to effect of alternating current alone, the lethal action of the H_2O_2 on *B. cereus* cells was compared with that of reagent H_2O_2 added to the cell suspension directly. H_2O_2 concentrations of 2, 5 and 10 $mg\ L^{-1}$ were chosen as treatment condition. Three levels of hydrogen peroxide; 2, 5 and 10 $mg\ L^{-1}$ in cell suspension were prepared as follows. Hydrogen peroxide (Siribuncha and Co., Ltd.); 3% w/v, was diluted to 0.03% w/v by 0.2 M phosphate buffer solution. Cell suspension and hydrogen peroxide were mixed in the mixer. Hydrogen peroxide concentrations in mixture were confirmed by peroxide test strip. The temperature of cell suspension was kept at $29\pm 1\ ^\circ C$. At 30 min, 1.5, 3 and 6 h after mixed, 0.2 mL of mixture were pipetted to determined viable count by using spread plate method instantaneously.

Figure 5 shows surviving fraction of *B. cereus* that was suspended in 0.2 M phosphate buffer solution (pH 7.0) after treatment by hydrogen peroxide

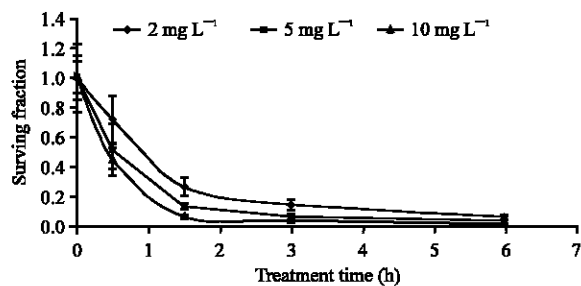


Fig. 5: Effect of hydrogen peroxide concentration of 2, 5 and 10 $mg\ L^{-1}$ on viability of *B. cereus*

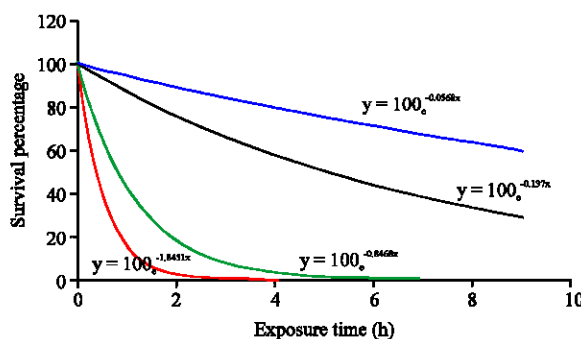


Fig. 6: The sole effect of AC of 50 (blue line), 100 (black line), 200 (green line) and 300 (red line) $mA\ cm^{-2}$

concentration of 2, 5 and 10 $mg\ L^{-1}$ at $29\pm 1\ ^\circ C$. The results indicated that surviving fractions of cells exposed to H_2O_2 decreased with increased exposure time at a definite H_2O_2 concentration and surviving fraction decreased with increased H_2O_2 concentration at a definite exposure time. Therefore the decrease in surviving fractions was related to H_2O_2 concentration and treatment time.

In order to find the sole lethal effect of AC on *B. cereus* cells, the effects of natural death and H_2O_2 are analytically subtracted out. It is clear that besides the effect of hydrogen peroxide produced as a result of electrolysis, lethal effect of AC is revealed as shown in Fig. 6. The lethal effects of AC directly to microbial cells have been reported that the cells died because surface charges and physiological properties of cells, e.g., respiratory rate and stainability with crystal violet, vary when the cells were exposed to AC and inferred that the permeability of the cell membrane is modified on AC-exposure. AC causes the release of the intracellular content of cells together with changes in the electron micrographic appearance of cellular materials located in the nucleus region within cells (Liu *et al.*, 1997; Shimada and Shimahara, 1985a, b).

Shimada and Shimahara (1983) found that electron microscopic observation revealed some interesting

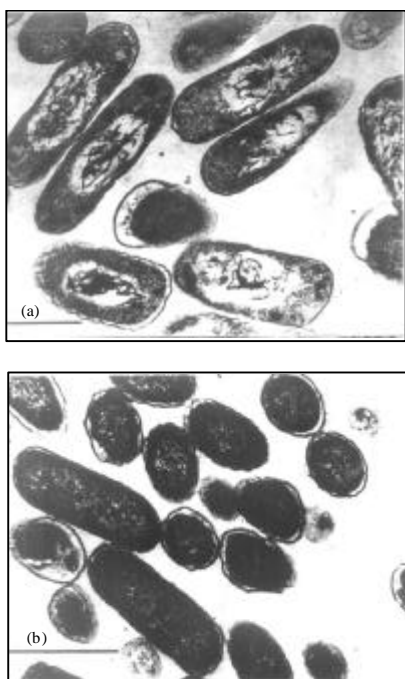


Fig. 7: Thin section of *E. Coli* cells treated and untreated with AC (Shimada and Shimahara, 1983). (a) Cells with AC-exposure of 00 mA cm^{-2} for 5 h and (b) Cells untreated with AC

differences between cells treated and untreated with an AC as shown in Fig. 7. Electron micrographs of thin sections showed that *E. coli* cells exposed to AC for 5 h in a phosphate buffer (Fig. 7a) possessed more organized materials in the central areas within cells than unexposed (Fig. 7b). In the unexposed cells, nucleus areas within cells are fewer electrons dense than the surrounding cytoplasm and membranes. These areas are presumably densely packaged DNA. When cells were exposed to AC, arrangement of the material in the nucleus areas varied from diffuse granular inclusions to irregularly dense aggregates. The electron transparent portions occurred within the areas as shown in Fig. 7a. Bacterial chromatin aggregates into compact masses under a variety of circumstances such as on exposure to a high salt concentration, low temperature, UV irradiation, metabolic inhibitors or starvation. These suggested that AC-exposure enhances the aggregation of DNA related materials within cells following the leakage of cellular contents from cells.

CONCLUSIONS

Low-voltage alternating current (50 Hz) of 0, 50, 100, 200 and 300 mA cm^{-2} affected the viability of *B. cereus*

cells, however no spore formation after effect was found. Surviving fractions of cells decreased with increased exposure time at a definite current density and with increased current density at a definite exposure time. Therefore the decrease in surviving fractions was related to a quantity of electricity and duration of applied alternating current. The decrease of living bacterial cells was attributed to the toxicity of hydrogen peroxide and direct effect of alternating current. Hydrogen peroxide was produced in cell suspension during AC-exposure. Hydrogen peroxide was formed by electrolytic reduction of oxygen on activated carbon cathodes in a neutral phosphate buffer solution. Under aerobic conditions, the amount of hydrogen peroxide increased with increased exposure time or current density. Other AC direct lethal effects to cells are the oxidation of enzymes and coenzymes such as NADH, membrane damage leading to release of the intracellular contents together with changes in the electron micrographic appearance of cellular materials located in the nucleus region within cells and decreasing respiratory rate.

These results inferred that the low-voltage alternating current method is able to be utilized as a nonthermal processing method for preservation of food. It can inactivate *B. cereus*, which is foodborne pathogen, or other microorganisms. It can be applied to the disinfection of large amounts of contaminated water or liquid food without antimicrobial substance residual. Hydrogen peroxide that produced during treatment is considered so safe that it has been approved to use in foods in many countries. It can be removed by an appropriate means, typically by addition of catalase. Then hydrogen peroxide decomposes into oxygen and water. Therefore the low-voltage alternating current method helps produce reliability safe food, which is defined as a product that is free of biological, chemical or physical hazards. However, many of microbial can form spore that can survive in long-term under unfavorable conditions. So, lethal effect of AC-exposure on spore should be further investigated to confirm that the low-voltage of alternating current is really applicable for food preservation.

The mechanism of bactericidal activity by AC may offer a useful method for eradicating bacteria from catheter surfaces. The future research should be extended to study other factors, e.g., initial cell concentration and dissolved oxygen quantity affecting the surviving fraction of *B. cereus* exposed to alternating current. Dissolved oxygen concentration should be determined because from many evidences, oxygen quantities correlate with hydrogen peroxide formation. Varying dissolved oxygen quantities can be performed along with AC-exposure under anaerobic conditions. Although, low-voltage

alternating current application to food preservation shows satisfactory results in laboratory scale, the industrial scale implementation needs further detail investigations in order to solve enormous unseen engineering problems.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude and appreciation to Dr. Surang Suthirawat and Prof. Dr. Busaba Yongsmith, Department of Microbiology, Faculty of Science at Kasetsart University for their invaluable assistance and King Mongkut's University of Technology Thonburi for the financial support.

REFERENCES

- Barbosa-Canovas, G.V., M.M. Gongora, U.R. Pothakamury and B.G. Swanson, 1999. Preservation of Foods with Pulsed Electric Fields. Academic Press, San Diego, USA.
- Block, S.S., 1991. Disinfection, Sterilization and Preservation. 4th Edn., Lea and Febiger, Philadelphia.
- Branen, A.L., P.M. Davidson and S. Salminen, 1990. Food Additives. Marcel Dekker Inc., New York.
- Liu, W.K., M.R.W. Brown and T.S.J. Elliott, 1997. Mechanisms of the bactericidal activity of low amperage electric current (DC). *J. Antimicrob. Chemother.*, 39: 687-695.
- Pareilleux, A. and N. Sicard, 1970. Lethal effects of electric current on *Escherichia coli*. *Applied Microbiol.*, 19: 421-424.
- Patermarakis, G. and E. Fountoukidis, 1990. Disinfection of water by electrochemical treatment. *Water Res.*, 24: 1491-1496.
- Rieger, P.H., 1987. *Electrochemistry*. Prentice-Hall, Oxford.
- Rosenberg, B., L.V. Camp and T. Krigas, 1965. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature*, 205: 698-699.
- Shimada, K. and K. Shimahara, 1981. Factor affecting the surviving fractions of resting *Escherichia coli* B and K-12 cells exposed to alternating current. *Agric. Biol. Chem.*, 45: 1589-1595.
- Shimada, K. and K. Shimahara, 1982. Responsibility of hydrogen peroxide for the lethality of resting *Escherichia coli* B cells exposed to alternating current in phosphate buffer solution. *Agric. Biol. Chem.*, 46: 1327-1337.
- Shimada, K. and K. Shimahara, 1983. Sublethal injury to resting *Escherichia coli* B cells aerobically exposed to alternating current. *Agric. Biol. Chem.*, 47: 129-131.
- Shimada, K. and K. Shimahara, 1985a. Change in surface charge, respiratory rate and stainability with crystal violet of resting *Escherichia coli* B cells aerobically exposed to alternating current. *Agric. Biol. Chem.*, 49: 405-411.
- Shimada, K. and K. Shimahara, 1985b. Leakage of cellular contents and morphological changes in resting *Escherichia coli* B cells exposed to alternating current. *Agric. Biol. Chem.*, 49: 423-438.
- Tadashi, M.A., N. Satoshi, T. Toshihiro, B.J. Takamuka, B. Grant, N. Noriyuki and S. Koji, 1992. Disinfection of drinking water by using a novel electrochemical reactor employing carbon-cloth electrodes. *Applied Environ. Microbiol.*, 58: 686-689.
- Tracy, R.L., 1932. Lethal effect of alternating current on yeast cells. *J. Bacteriol.*, 24: 423-438.
- Zhao, Z.H., Y. Sakagami and T. Opaka, 1998. Toxicity of hydrogen peroxide produced by electroplated coatings to pathogenic bacteria. *Can. J. Microbiol.*, 44: 441-447.