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Sterilization of *Escherichia coli* cells by the application of pulsed magnetic field

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Abstract: The inactivation of microorganisms by pulsed magnetic field was studied. It was improved that the application of electromagnetic pulses evidently causes a lethal effect on *E. coli* cells suspended in phosphate buffer solution Na_2HPO_4/NaH_2PO_4 (0.334/0.867 mmol/L). Experimental results indicated that the survivability (N/N_0; where N_0 and N are the number of cells survived per milliliter before and after electromagnetic pulses application, respectively) of *E. coli* decreased with magnetic field intensity B and treatment time t. It was also found that the medium temperatures, the frequencies of pulse f, and the initial bacterial cell concentrations have determinate influences in destruction of *E. coli* cells by the application of magnetic pulses. The application of an magnetic intensity $B = 160$ mT at pulses frequency $f = 62$ kHz and treatment time $t = 16$ h result in a considerable destruction levels of *E. coli* cells ($N/N_0 = 10^{-4}$). Possible mechanisms involved in sterilization of the magnetic field treatment were discussed. In order to shorten the treatment time, many groups of parallel inductive coil were used. The practicability test showed that the treatment time was shortened to 4 h with the application of three groups of parallel coil when the survivability of *E. coli* cells was less than 0.01%; and the power consumption was about 0.2 kWh/m^3.

Keywords: *Escherichia coli* bacteria; pulsed magnetic field; induced current; cell membrane

Introduction

Various chemical treatments have been predominantly used to inactivate or kill microbes in water. However, the use of disinfectants changes the water composition and causes contamination of the treated medium from toxic chemical by-products. Therefore, physical treatments can be used because they do not change the quality of water. New physical techniques to inactivate bacteria in water are being explored as potential alternatives to the chemical methods, including the utilization of pulsed electric fields, magnetic field, intense light pulses and so on. In particular, magnetic treatments have attracted much attention for over 100 years, and inactivation of microorganisms in water using pulsed magnetic field has been studied in recent years (Guo, 1996; Lei, 1994; Luo, 2001). Its efficiency is still a controversial question. In general, people agree on the fact that electromagnetic field can cause changes for cells on configuration, formation, and function, etc. Even magnetic field of low intensity can strongly influence biologic system (Bassett, 1993). For example, magnetic treatment causes the activity of blood platelet, the breakdown of cell, the inactivation of enzyme (Wang, 2000). Pulsed magnetic field has greater biologic effects than constant field (Zhong, 1998). Possibilities of inactivating the microorganisms in liquid media using a pulsed electromagnetic field have been considered by many researchers in an effort to develop a promising sterilization method. It have been reported that the survivability of microorganisms when exposed to a pulsed magnetic field depends on both the magnitude of the magnetic field intensity and the treatment time (Guo, 1996). Despite several studies which have examined the magnetic field effects on the death of microorganisms, the under lying mechanisms involved in the breakdown of the cell leading to cell death are not clear. In this study, a home-made electromagnetic device was developed to investigate the effect of a pulsed magnetic field on the survivability of *Escherichia coli* suspended in buffered phosphate solution both by following the changes of magnetic field intensity and treatment time. The influences of pulse frequency, medium temperature, initial bacterial cell concentrations in killing *E. coli* cells were investigated also.

1 Materials and methods

1.1 Biological and chemical

The pure culture of the bacteria *Escherichia coli* used in this study was kindly provided by the institute of microbiology, Chinese Academy of Sciences. The *E. coli* cells were grown in *Escherichia* LB broth in an incubator for 24 h at 37℃. The cells were harvested from the broth by centrifugation before reaching their stationary growth phase. Centrifugation was carried out at 10000 r/min for 5 min at 4℃. The cells were washed and resuspended in phosphate buffer solution. The concentration Na_2HPO_4/NaH_2PO_4 was (0.334/0.867 mmol/L). The pH of the buffer was 7.4. The dilution of cells in the suspension was chosen to produce approximately 10^5 or 10^6 cells/ml before treatment.

The survivability of the *E. coli* cells following the treatment was measured by means of colony counting in an

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agar dish. The treated sample was serially diluted with deionized and sterilized water, and 1 ml of the dilutions was plated on agar medium and incubated for about 48 h. The dilutions for the viable count were carried out in such a way that the number of colonies on the agar plate was 30—300. Each reading was calculated from an average of eight plates, and for each experimental condition measurements were repeated 3—4 times.

In addition to the colony counting as a measure of survivability of microorganisms, scanning electron microscopy (SEM) observations, chromatographic and atomic absorption spetrophotometry analysis to detect the presence of anions and cations were carried out. For electron microscopy the microbial cells were fixed for 2 h at 4℃ with 2.5% glutaraldehyde in (0.334/0.867 mmol/L) phosphate buffer followed by filtration (0.2 mm millipore polycarbonate filter paper). The filter paper was washed with phosphate buffer and dehydrated in graded acetone. Further drying was done using a critical point dryer. The filter containing the microbes was mounted on copper stubs and coated with gold. The scanning electron micrographs were taken using a Hitachi model S-570 (SEM). Before and after treatments the concentrations of anions, chlorine(Cl^-), phosphate(PO_4^{3-}), nitrate (NO_3^-), and sulfate(SO_4^{2-}) ions were measured using a Dionex-4500 ion chromatograph(IC); the concentrations of cations, natrium(Na^+), kalium(K^+) were measured using a Hitachi model Z-6100 atomic absorption spectrophotometry.

1.2 Electrical

The circuit configuration used to generate direct current pulse was based on the complete discharge of a capacitor producing an exponentially decaying voltage waveform. The capacitor was charged using a DC power supply. Its working principle is shown in Fig. 1. The frequency of the pulse application was variable between 400 and 6000 Hz. Four high levels were used for the tests. The magnetic field treatment set-up is shown in Fig. 2. The magnetic field used to inactivate or kill microorganisms was generated by inductive coil group linked to the direct current pulse generator, which were wrapped around the outer wall of the pipe. The magnetic field intensity was varied by the output power of the direct current pulse generator. The length of each inductive coil was 10 cm. The total length of the pipeline was 1.0 m, which was sterilized before treatment. The test sample was added to a glass cuvette in a totally aseptic condition. A metric pump was used to make the sample recycle in the pipeline. The flow velocity of 0.2 m/s was used for all measurements.

2 Results and discussion

2.1 Principal on pulsed electromagnetic field treatment of microbial cells

Biologic effects of electromagnetic field include the thermal and nonthermal effects. The thermal effects refer to the biologic function changes due to temperature rising when cells absorb the energy of electromagnetic waves. The nonthermal effects involve the strong biologic responses without obvious temperature rising including changes on physiology, biochemistry, and function, which are related to the frequency and power of electromagnetic field. The nonthermal effects often happen in the state far away from balance. For the nonthermal effects, the responses of cells to electromagnetic waves are nonlinear, i.e., the inducements from outer small energy can give rise to the release of intracellular great energy. Using electromagnetic pulse to inactivate microorganisms depends on the nonthermal effects primarily(Zhou, 2000).

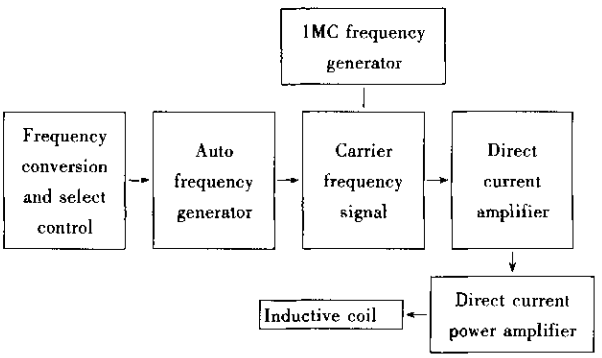


Fig. 1 Diagram of direct current pulse generator

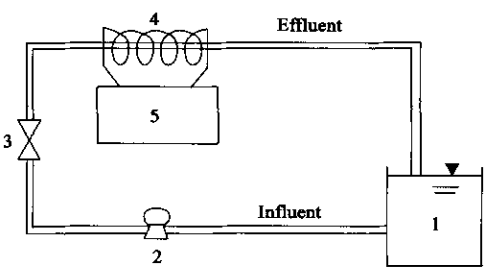


Fig. 2 Sketch map of magnetic field treatment set-up
1. influence container; 2. metric pump; 3. discharge valve; 4. iductive coil group; 5. direct current pulse generator

Cells can be penetrated by magnetic field because their magnetic conductivity is similar with that of vacuum. As a result, the induced current will be produced in cells for pulsed magnetic field because of its great variable rate of magnetic field due to short pulse delay. Studies show that the biologic effects of pulsed magnetic field lie on the category and size of cells. Cells with different category and size have different endurable capability to pulsed magnetic field intensity.

The mechanisms for pulsed magnetic field biologic effects on cells are explained as follows: For pulsed magnetic field, a transient magnetic flux must be produced in cells resulted from the instantaneous appearance and disappearance of magnetic field. The induced electromotive force E due to the changes of magnetic flux Φ is given as:

$$E = -\mathrm{d}\Phi/\mathrm{d}t = -S\,\mathrm{d}B/\mathrm{d}t, \qquad (1)$$

the corresponding induced current I is as follows:

$$I = E/(SR) = -R^{-1}\,dB/dt, \qquad (2)$$

where S is the cell sectional area penetrated vertically by the magnetic field; R is the cell circuit resistance; dB/dt is the average variational ratio. The induced current size, direction, and form are primary factors to determine the biologic effects of magnetic field on cells. The higher the induced current is, the more obvious the biologic effects are. The force density of the interaction between the induced current and magnetic field can destroy normal physiologic functions of cells. Large cells are easier to be inactivated than small cells because the force density is proportional to the diameter of cells. Otherwise, the induced current gives rise to an electric potential difference between two sides of cell membrane because of its electric resistance. As then, this potential difference changes the transmembrane potential, which bring destruction to cell membrane. On the other hand, the movement of electric ions especially electrons and ions with little qualities is limited within a restricted area. They can not transfer through membrane normally, and normal physiologic functions are not available for cells. For biomacromolecules such as enzyme their conformations are twisted or transformed. As a result, the normal physiologic functions of cell are destroyed.

2.2 Lethal effect on *E. coli* cells

2.2.1 Effect of electric field

The viability of *E. coli* as a function of field at room temperature with a different treatment time is shown in Fig. 3. At each level of treatment time a finite rate of cell death increased exponentially as the field intensity increased. As a function of field, survivability declined rapidly at low strengths and more gradually at higher strengths. With a field intensity as low as 160 mT, it was possible to reduce the viable cell numbers by above 85%. With further increase in field intensity, larger number of cells were killed; however, the rate gradually declined.

The survivability of *E. coli* as a function of field intensity at different frequencies f is shown in Fig. 4. At all four test frequencies the death rate rapidly increased with a field intensity up to 120 T; whereas at slightly higher field intensities (> 160 T), the death rate remained relatively constant. It was possible to reduce the viable cell count to 10^{-2} ml^{-1} at 200 mT with application of 62 kHz frequency. The treatment time applied was kept constant (t = 6 h) for all these tests. However, actual exposure time to magnetic field was different as the pulse number varied with the frequency. At elevated pulse frequencies, the pulse number increased for each discharging of the capacitor. Hence, the frequency effects cause higher death rate due to an increased exposure time (Hulsheger, 1981). With the present setup it was therefore not possible to test the effects of frequency on the lethality of microorganisms above 70 kHz.

2.2.2 Effect of treatment time

Survivability as a function of t and T at field intensity

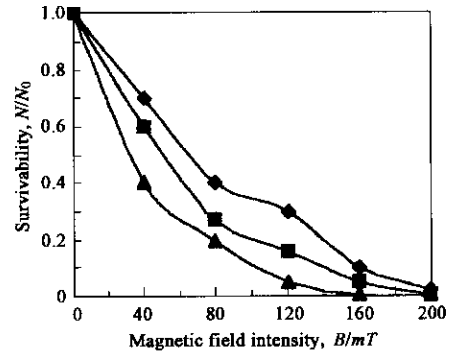


Fig. 3 Survivability of *E. coli* cells as a function of magnetic field intensity B with different reatment times
◆ t = 6 h; ■ t = 12 h; ▲ t = 16 h; T = 25℃; pH = 7.3; f = 56 kHz

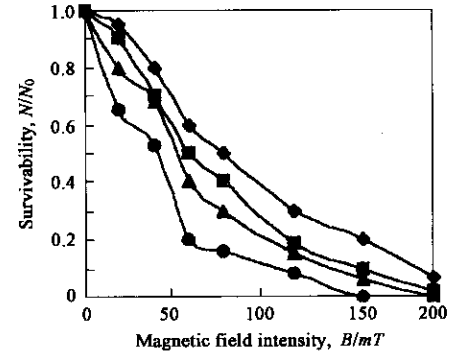


Fig. 4 Survivability of *E. coli* cells as a function of magnetic field intensity B at different pulse frequencies
◆ f = 46 kHz; ■ f = 50 kHz; ▲ f = 56 kHz; ● f = 62 kHz; T = 25℃; pH = 7.3

of 160 mT is shown in Fig. 5. As a function of treatment time the cell survivability rate fell rapidly at first and then tended to decrease, reaching a relatively constant rate at the longer treatment times. As the field intensity was higher, the survivability of *E. coli* was reduced by 80% above with applying treatment time of 10 h, when the medium was 25℃. Compared to this, at T = 60℃, it was possible to obtain a much higher reduction in survivability at corresponding treatment times. It was possible to reduce the viable cell count to 10^2 ml^{-1} at 60℃ with application of 16 h treatment time. Cells were also killed due to the temperature effect alone while increasing the medium temperature. As such, number of viable cells per milliliter before treatment at higher temperatures (N_0) was lower than initial number of viable cells per milliliter (N_i) at room temperature. When the temperature of the medium was raised to 80℃, almost all the cells were killed by the thermal effect even prior to the application of the electric field. Fig.5 shows that the killing of *E. coli* cells due to pulsed field intensity application was temperature dependent. The temperature of the medium in which cells are suspended has a significant influence in determining the membrane fluidity properties. At low temperatures, the phospholipids are closely packed in a rigid gel structure, while at high temperatures they are less ordered

and the membrane has a "liquid-crystalline" structure (Jayaram, 1991). The phase transition from gel to liquid crystalline is thermal dependent and hence can affect the physical stability of the cell membrane. A rise in temperature is known to increase the lateral diffusion rate of lipids by at least two orders of magnitude as lipids change from gel to the liquid-crystalline phase. As seen from Fig. 5, a larger reduction in survivability of *E. coli* was observed when the liquid temperature was higher (~ 60℃) at comparable magnitude of treatment time than at lower temperatures. Based on this, it is proposed that temperature related phase transition of the phospholipid molecules from gel to liquid-crystalline phase and the associated reduction in bilayer thickness may make the cell more susceptible for field effects at a relatively high temperature.

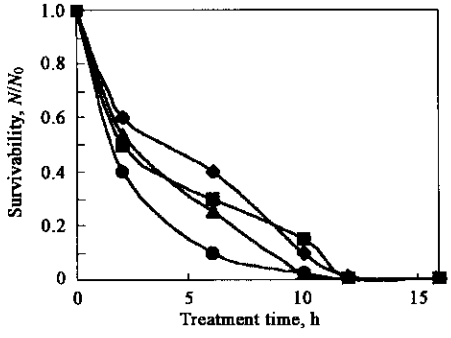


Fig. 5 Survivability of *E. coli* cells as a function of treatment time t at different temperatures

◆ $T = 25$℃, $N_i = N_0 = 1.6 \times 10^6$; ■ $T = 30$℃, $N_i = 4.6 \times 10^6$, $N_0 = 4.2 \times 10^6$; ▲ $T = 45$℃, $N_i = 5.1 \times 10^7$, $N_0 = 4.8 \times 10^6$; ● $T = 60$℃, $N_i = 7.2 \times 10^8$, $N_0 = 4.3 \times 10^6$; $B = 160$ mT; $f = 62$ kHz; pH = 7.3

As a function of treatment time, while the field and temperature were constant, the degree of death declined rapidly at first and then more slowly, as shown in Fig. 6. It was observed that an increase in the killing rate of *E. coli* with the increase in the initial bacterial cell concentrations. The following is a possible explanation for the relationship between the number of live cells present prior to pulse application and the surviving cells based on the appearance of induced current due to the variation of magnetic flux (Kaler, 1983). The induced current is a dominant factor for the breakdown of cells involved in the application of magnetic field, which gives rise to an interactional force with magnetic field. This force acting on the cell surface causes irreversible breakdown of membrane, whose magnitude depends on the diameter of the cell. Consequently, the cluster of cells will experience a higher force effect due to increased cell dimensions. A greater change of transmembrane potential develops across the group of cells than across an individual cell. Therefore, cells can be killed more easily when their number density is high. As the cell concentration decreases with each pulse application, the death rate remained relatively constant in spite of increased treatment time (> 12 h). As then, a relatively higher intensity would be required to effectively kill the singled-out cells with a less force acting.

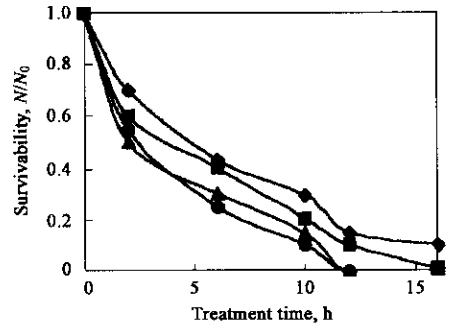


Fig. 6 Survivability of *E. coli* cells as a function of treatment time t at different cells concentrations

◆ $N_0 = 1.4 \times 10^5$; ■ $N_0 = 5.5 \times 10^6$; ▲ $N_0 = 2.5 \times 10^7$; ● $N_0 = 2.9 \times 10^8$; $B = 160$ mT; $f = 62$ kHz; $T = 25$℃,

The concentrations of ions Cl^-, PO_4^{3-}, Na^+, and K^+ in test samples subjected to various test conditions are given in Table 1.

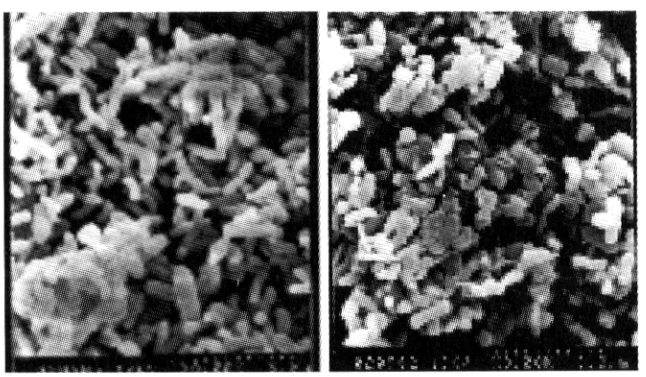
Table 1 Concentration of ions in liquid medium under different test conditions

	Experiment	Concentration of ions, mg/L			
No.	Sample	$[Cl^-]$	$[PO_4^{3-}]$	$[Na^+]$	$[K^+]$
1	Deionized water	0.0	0.0	0.0	0.0
2	Deionized water with *E. coli*	8.6	3.1	7.9	9.3
3	Phosphate buffer	1.2	186.5	78.4	1.7
4	Phosphate buffer with *E. coli*	3.4	192.3	83.7	3.5
5	Sample 3 treated with field, $B = 160$ mT, $t = 12$ h, $T = 25$℃	1.3	171.6	65.5	1.8
6	Sample 3 treated with field, $B = 160$ mT, $t = 24$ h, $T = 25$℃	1.5	179.2	73.2	2.0
7	Sample 4 treated with field, $B = 80$ mT, $t = 12$ h, $T = 25$℃	4.6	138.3	57.8	4.9
8	Sample 4 treated with field, $B = 80$ mT, $t = 24$ h, $T = 25$℃	6.7	127.4	50.3	7.3
9	Sample 4 treated with field, $B = 160$ mT, $t = 12$ h, $T = 25$℃	5.8	123.5	52.6	6.5
10	Sample 4 treated with field, $B = 160$ mT, $t = 24$ h, $T = 25$℃	7.9	130.1	57.9	8.4

The ionic concentrations detected for those samples containing microorganisms indicated that the Cl^- and K^+ concentrations increased with treatment time and field intensity while PO_4^{3-} and Na^+ decreased. There was no noticeable increase in the concentration of Cl^- and K^+ ions when buffer solutions without microorganisms were subjected to test conditions similar to those with microorganisms, thus eliminating the test medium as the source of Cl^- and K^+. This provides direct evidence for the killing *E. coli* cells due to magnetic pulse application, which is linked to the irreversible breakdown of cell surface and release of intracellular components to the outside medium. This is further confirmed from higher concentrations of Cl^- and K^+ in test samples containing microbes exposed to magnetic field

for relatively longer treatment times, higher intensities. The release of Cl^- and K^+ ions was quantified in the test medium subjected to magnetic treatments as a possible measure of damage to cell, since Cl^- and K^+ ions represent major ionic species in the normal cell. Further, the concentration of Cl^- and K^+ in deionized water was nondetectable; whereas deionized water containing *E. coli* cells had the highest concentration of Cl^- and K^+. This large difference in Cl^- and K^+ confirms that the release of Cl^- and K^+ into the liquid medium is from the lysis of *E. coli* cells in hypoosmotic medium due to rupturing of the cell membrane. The NO_3^- and SO_4^{2-} were nondetectable. The source of PO_4^{3-} and Na^+ were mainly from the buffered phosphate solution used as the treatment to suspend the *E. coli* cells. The pH of the buffered test medium remained relatively constant following the magnetic pulse treatment.

The scanning electron micrographs of untreated and treated *E. coli* cells with magnetic field intensity 160 mT for 16 h are shown in Fig.7. Fig.7a shows the untreated *E. coli* cells. It indicated that the surface of most of the untreated cell is integrity and slick, while dents, breaks, and pores were found on that of the treated cell, as shown in Fig. 7b. These disruptions result in the irreversible breakdown of cell membrane and release of intracellular components to the outside environment.



(a) (b)

Fig. 7 Scanning electron micrographs of *E. coli*
(a) untreated; (b) treated with B = 160 mT, t = 16 h, T = 25℃

2.3 Practicability of sterilization by the pulsed magnetic field

The application of a magnetic field intensity B = 160 mT at pulses frequency f = 62 kHz and treatment time t = 16 h resulted in very high destruction levels of *E. coli* cells ($N/N_0 = 10^{-4}$). The sterilization effects of the pulsed magnetic field achieved the sterilization demand of industry circular cooling water. In order to shorten the treatment time, many groups of parallel inductive coil were linked to the direct current pulse generator. Under the same test conditions above, the treatment time was reduced to 4 h with the application of three groups of parallel inductive coil when the survivability of *E. coli* cells was less than 0.01%; and the power consumption was about 0.2 kWh/m^3.

3 Conclusions

The destruction of *E. coli* in (0.334/0.867 mmol/L) phosphate buffer solution was achieved using electromagnetic pulse treatment. The survivability of *E. coli* declined with field intensity and treatment time. For the more, the death rate increased with the medium, the frequency of pulse, and the initial concentration. Of the different conditions tested, a combination of B = 160 mT, t = 16 h, and pulses frequency f = 62 kHz was most effective in killing *E. coli* cells ($N/N_0 = 10^{-4}$). The practicability test showed that the increase of number of coil group could shorten the treatment time and a survivability of *E. coli* cells less than 0.01% was achieved. The Cl^- and K^+ concentrations of the test medium increased following pulse treatment only when the test medium contained *E. coli* cells. Hence, the increased Cl^- and K^+ concentrations in liquid medium were due to the breakdown of cell surface and release of intracellular components to the outside environment. This was corroborated by the electron micrographs of *E. coli* cells before and after treatment. The destruction of *E. coli* cells was primarily due to pulsed field induced rupture of the cell wall.

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