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論　　文

Optimal Condition of Waveforms for Electrical Sterilization of *Escherichia coli* by HV Impulse

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This report describes effects of the cell survivability is influenced by changing L values in HV impulse circuit. Two different discharging newworks, RC and RLC circuit, were employed to investigate the voltage waveform effect for the sterilization and compared the effects of voltage waveforms being treated. And then, in RLC discharging circuit, we investigated whether RLC parameters are the critical factor on the condition of sterilization. When the L value is changed from 0 to 20 mH in this case, there is showed more sterilization on the 4 mH and over. Details of the results are described. Though further studies are necessary, the results obtained with the sterilization effects was related on number of threshold voltage waveforms (Ne) being applied and the survivability seems to be proportion to number of Ne.

1. Introduction

Electrical sterilizations of biological cells have been studied by many researchers. Sale and Hamilton have reported that the degree of kill of a population was determined by the product of the pulse length and number of pulses, and by the electric field strength in the suspension[1,2] and Lin Qin *et al.* have compared with the exponentially decaying pulses and monopolar square pulses, bipolar pulses provided more efficient inactivation of microorganism.[3] Exponentially decaying voltage waveforms obtained by discharging a capacitor are widely used in the sterilization apparatus. Chang[4] have observed the mechannical oscillation of cell membranes exposed to applied oscillating fields using a planar lipid bilayer as a model system. In this work, we have evaluated the difference RC and RLC circuit for sterilization effect. RLC circuit with oscillating parameters, which consists of a capacitive energy storage source and a inductive pulse duration and a resistance cell suspension chamber has been used for a discharging network. This work deal with oscillating impulse waveforms for sterilization effect of *Escherichia coli* (*E.coli*) that is, oscillating waveforms obtained by adding an inductance L to the CR circuit are used to investigate effect of the multiple peak waveforms on the survivability of *E.coli*.

2. Experimental Apparatus and Procedure

HV regulated DC power supply, a product of Pulsed Electronic Engineering Co., Japan, has a 50 kV, 3 mA max. Figure 1 shows pulsed high voltage network was used in this study. Co (=0.1 μF) of condencer was charged from dc high voltage source and discharged into the electrode system (=chamber) by rotary spark gap switch. In this case the pulsed voltage was decayed by time constant (=CoR). In this experiment, R and C values are fixed, while only L value is changed from 0 to 20 mH respectively. Gap distance of electrode system in chamber is adjusted to 1 mm. The shot number is changed from 10 to 40. The shot number means the period to take charging and discharging on the Fig. 1. In this process, we counted shot number. In Fig. 2, the chamber resistance of full suspension is about 100 Ω. Figure 2 shows details of the electrode chamber system used in this study. The chamber is filled with 0.1 mL of diluted *E.coli* suspension and 0.9 mL of NaCl (0.9%) solution. The concentration of *E.coli* was about 1×10/mL. These *E.coli* was grew for 24 h with 36°C on the TS broth. We picked up the 0.1 mL of solution which is the diluted solution of *E.coli* with 1/100 and then adulterated *E.coli* solution with the 0.9 mL NaCl solution also the cell suspensions, which 0.1 mL of diluted solution, which dilutes to 1:100 (10^{-2}) was added to 9.9 mL of NaCl solution were injected into the treatment chamber. After we performed electrical shock, the sample which picked up 0.1 mL from chamber diluted again to 1/1,000. And

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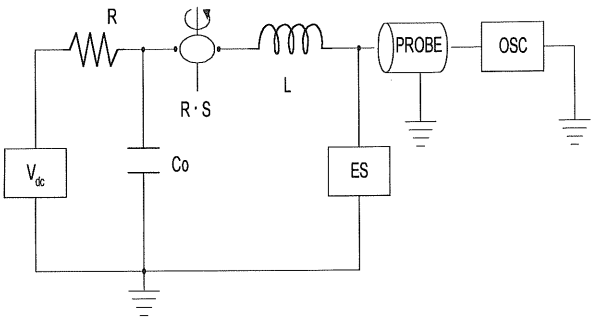


Fig. 1　Pulsed high voltage generator.
V_{dc}: dc high voltage source, Co: condencer, R.S: rotary spark gap.

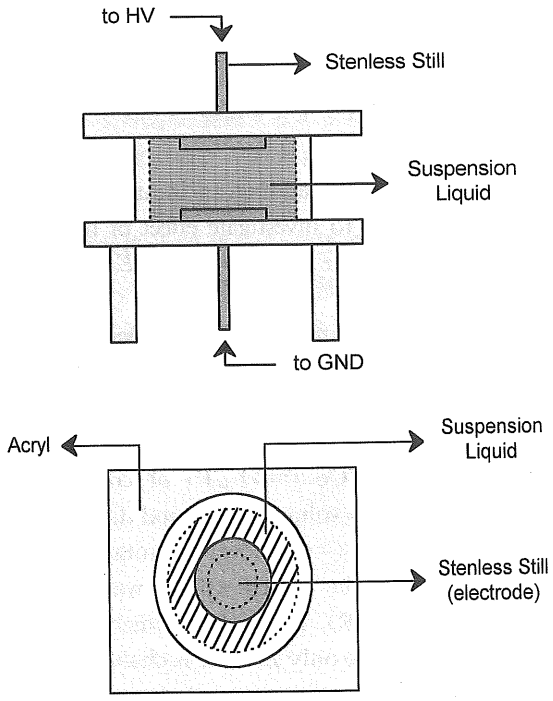


Fig. 2　Structure of the electrode chamber system.

then, pickup again, the sample of 0.1 mL which diluted to 10^{-7} was spreaded on to the surfaces of Desoxy cholate agar plates. For the enumeration of colony, dilutions in the 10^{-7} range were used. Then the plates are incubated at a temperature of 35°C for 24 h and the numbers of colonies are counted: Cell survivability after electrical shock treatments was determined by means of usual cultivation methods[5–7]. Therefore the influence of HV impulse on the *E.coli* has been measured as a survival ratio (S=N/No, where N and No are a number of active microbes per unit volume after and before the voltage treatment, respectively). The aim of this study is not to evaluate "true" survivability, but to examine effects different voltage waveforms on electrical sterilization. This can be realized as long as all tests are conducted using an identical chamber.

3. Experimental Results and Discussion

In this experiments the energy stored in the capacitor ($1/2\,C V_0$) is used as the energy input to the suspension by one pulse. The energy input was 20 J at V_0=20 kV

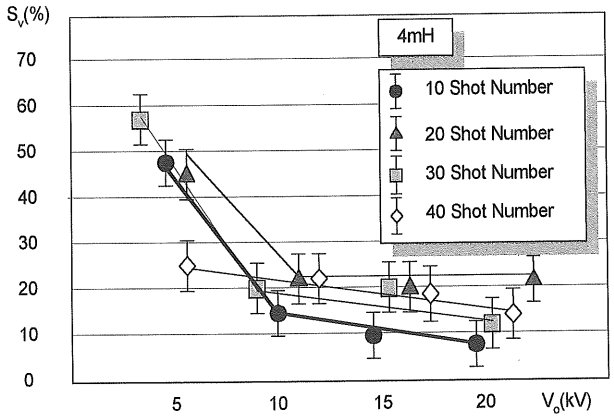


Fig. 4　Survivability of *E.coli* for each inital voltage and various applied pulse number with 4 mH.

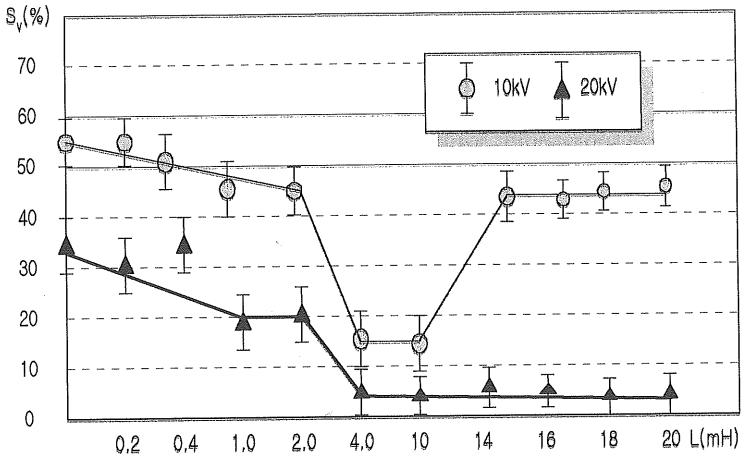


Fig. 3　Survivability of *Escherichia coli* for each inductance.

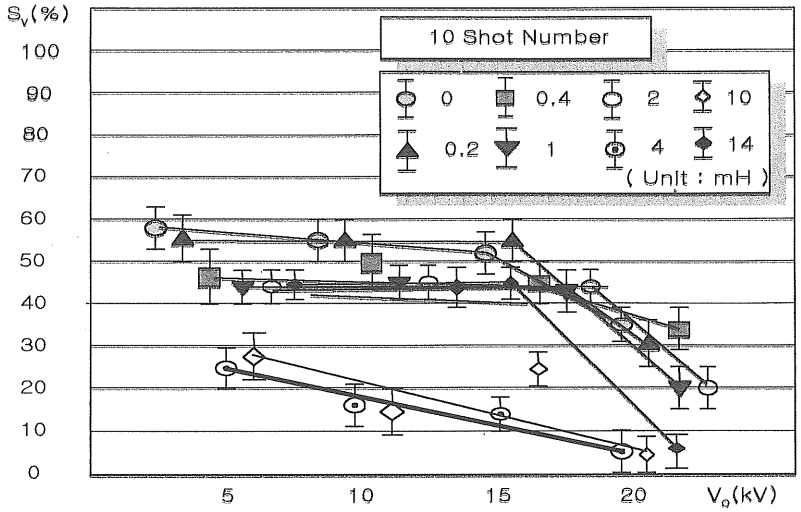


Fig. 5 Survivability of *E.coli* for each initial voltage and various L value with 10 shot numbers.

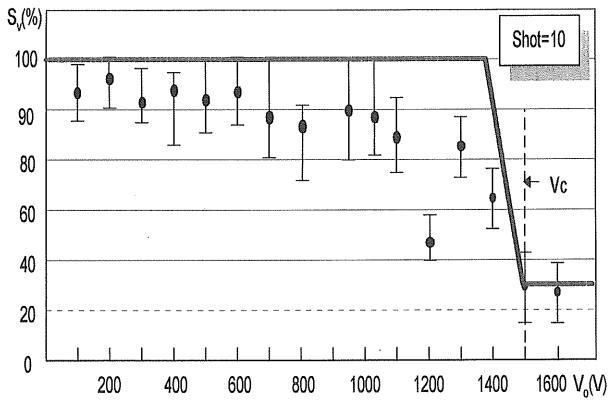


Fig. 6 Threshold voltage (V_c) for survivability.

and 5 J at V_0=10 kV. But in the same condition as in this case, the survivability decreased with the charge in the inductance values. Especially, comparing this result with the tilt in Fig. 3, the cell survivability with 4 mH inductance or more was much smaller than other L values. Figure 4 shows the survivability of *E.coli* for each initial voltage. The survival ratio decrease with the increase in shot number (the pulse application number). In this experiment, the inductance value was fixed to 4 mH, while the shot number was adjusted to 10, 20, 30, and 40, and initial voltage was changed from 5 kV to 20 kV. At V_0=10 kV, n=10, the decrease in survivability was faster than other case. The pulse duration was about 0.2 ms. At V_0=20 kV, the lowest survivability was detected with N>10, or longer than 0.6 s of pulse application time. A strong dependence of survivability on the number of pulses applied is observed within the range of N=1 to 10 shot numbers. Mazurek and Lubicki[7] reported this case is observed within the range of n=1 to 15 pulses.

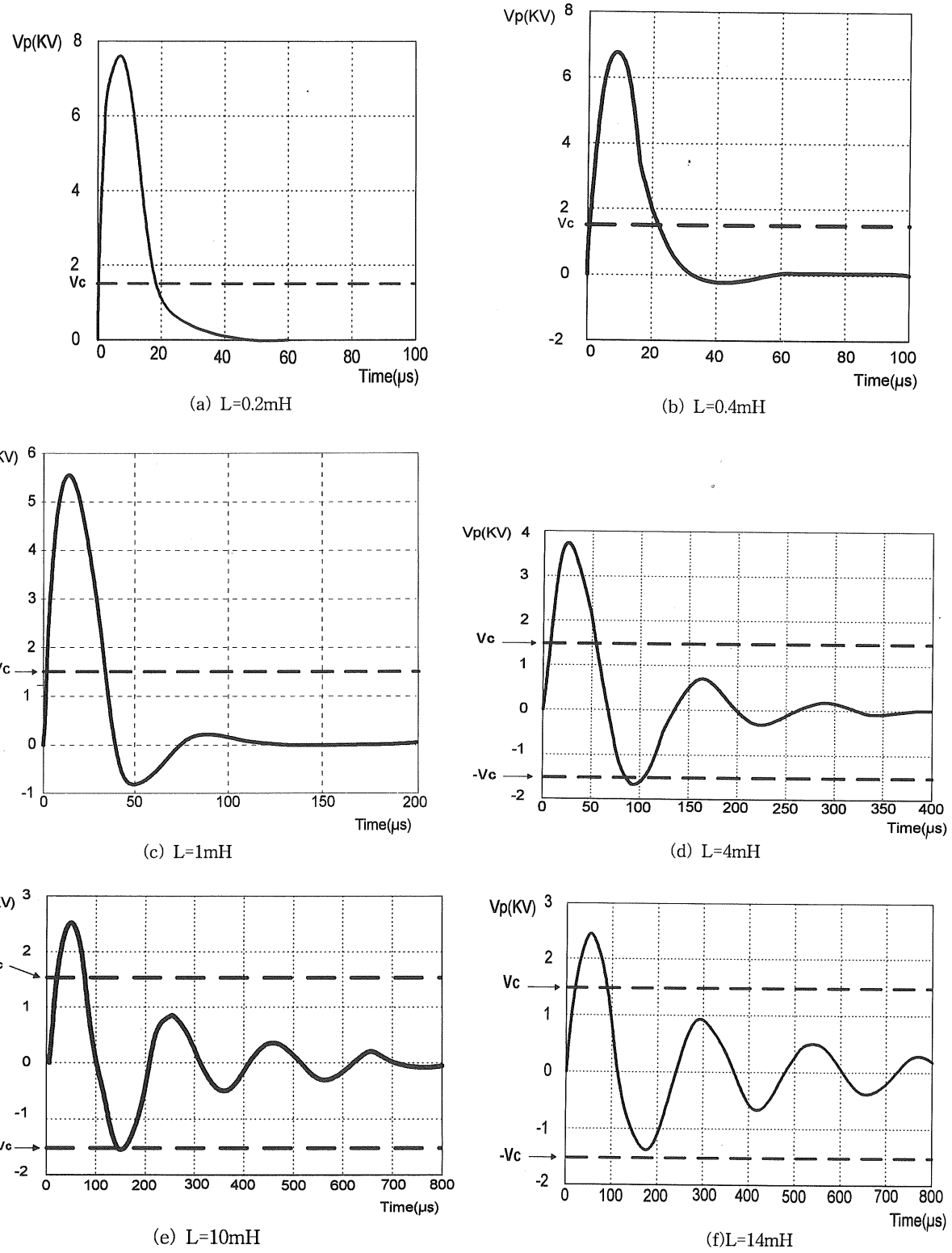
In Fig. 5 the survival ratio of *E.coli* is shown as function of initial voltage and L values. The number of applied pulses was n=10. In this experimental result, when the inductance value was changed 0 to 1 mH, variation of survival ratio was slightly poor. But, when only two inductance values of 4 mH to 20 mH are applied, a decrease of 2 orders of magnitude is observed. Figure 6 shows threshold voltage for survival ratio and means limited voltage to begin sterilization. In this experiment the number of pulses applied is fixed to n= 10 and initial voltage is changed from 0 to 1,600 V. In Fig. 6 the threshold voltage, V_c is determined as 1,500 V and survivability was about 36% at V_c.

4. Discussion

In impulse generator system, as the factor to have an influence on cell survivability, through it has become clear that the degree of kill of a population was determined by the product of the pulse length and number of pulses and by the field strength in the suspension[1]. We have studied the factor, to produce a powerful effect on survival ratio by analysis of impulse waveforms. In this experiment, we have analized the amplitude of impressed oscillatory decay waveform into the suspension by changing L value of network element. As this result, the condition to exert a great influence on cell survivability appeared to be from 4 mH of L value and to be average 15% of survivability. For 4 mH and over the accounted Ne numbers distribution that is the threshold voltage of sterilization on the observed oscillatory decay waveform is shown by Fig. 8. And Fig. 9 is shown survivability for each L value. In this case, it is found that the comparision of Ne numbers and survivability is to be relation of direct proportion.

5. Conclusion

The main purpose of this study was to investigate how



(a) L=0.2mH　(b) L=0.4mH

(c) L=1mH　(d) L=4mH

(e) L=10mH　(f)L=14mH

Fig. 7　Threshold voltage for each L value.

the cell survivability is influenced by changing L values. Figure 7 (d) shows the waveform for V_0=10 kV and L= 4 mH. The survivability in this case shows the lowest result. The point of threshold voltage (V_c) appears as Fig. 7. In Fig. 7 the number of Ne as a function of threshold voltage and the changed L value is calculated as Fig. 9. From Figure 7 we can evaluate number of pulse which has a peak value larger than V_c for each L value. Therefore, in Fig. 9, Ne may be indicate the number of pulses in one oscillating waveform which can be effective cell sterilization. Finally, authors found that the survivability decreased with the increase in the number of Ne.

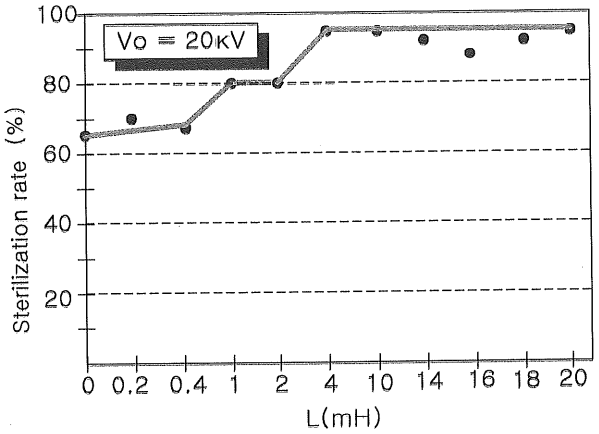


Fig. 8 Sterilization rate for each L value.

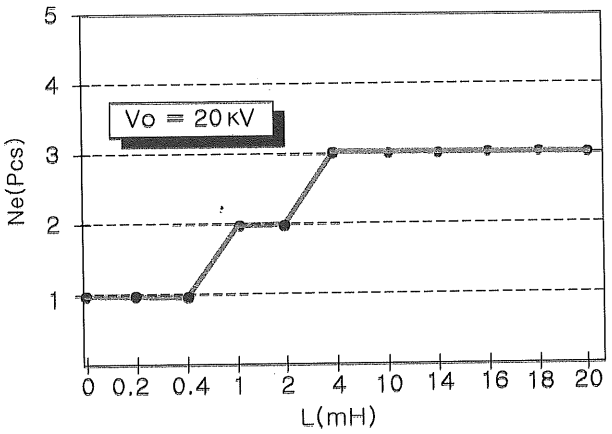


Fig. 9 Number of Ne for L value.

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Appendixes

For data supporting this manuscript, refer to the following list:

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