# MOSFET-Based Pulse Power Supply for Bacterial Transformation

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***Abstract*—In this paper, a MOSFET-based pulsed power supply capable of supplying square pulses of up to 3000 V and widths from nanoseconds to milliseconds is presented and used for an investigation into electroporation-mediated delivery of a plasmid DNA molecule into the pathogenic bacterium *Escherichia coli* O157:H7. It was concluded that increasing the electric field strength and pulse amplitude resulted in an increase in the number of transformants. However, increasing the number of pulses had the effect of reducing the number of transformants. In all the experiments, the number of cells that were inactivated by the exposure to the pulsed electric field were also measured.**

***Index Terms*—Bacterial transformation, DNA transfusion, electroporation, *Escherichia coli*.**

## I. Introduction

THE USE of high-voltage pulsed electric fields in biotechnology and medicine has led to new methods of cancer treatment, gene therapy, drug delivery, and nonthermal inactivation of microorganisms. Regardless of the application, the objective is to open pores in the cell membrane, and hence, either facilitate the delivery of foreign materials into the cell, or to kill the cell completely. Pulsed power supplies that are capable of producing high-voltage controllable pulses are needed for electroporation. Electroporation is the process of applying pulsed electric fields to the living cells to induce permeability in the cell membrane. The process can be reversible if the permeability is temporary and able to reseal or irreversible if the permeability is permanent, causing the membrane to rupture. The pore opening during electroporation allows for the insertion of drugs or genes into the cell [1]. Pulses in the microsecond range, with electric field intensities in the tens of megavolts per meter, have been reported to kill bacteria in water and liquid foods [1]. Pulses having widths of 100 $\mu$s and electric field strengths of 100 kV/m have been effective in delivering drugs to mammalian cells, and 20-ms, 5-kV/m pulses have been effective in gene delivery [2]. Recently, the ability to control the pulse width in the nanosecond region has received increased attention due to the fact that pulses in the range of nanoseconds with electric field intensities in the range of several megavolts per meter are able to manipulate the inner structures of the biological cell, a process known as intracellular electromanipulation [3]. Also, nanosecond pulses could be used for applications ranging from gene therapy to selected apoptosis induction and tumor growth inhibition [4]. A comprehensive review of medical applications of electroporation is available in [2].

There are many pulse parameters such as electric field strength, pulse width, pulse shape, rise time, number of pulses per second, and time interval between the pulses that influence the process of electroporation. However, the most important parameters for effective electroporation are pulse width and the electric field strength [1], [2]. In addition, a fast rise time is also beneficial [5]. The key point is that the pulse parameters must be highly controllable to get the optimal pore size and pore population, and avoid rupturing the cell membrane causing the cell to be irreversibly damaged. The shape of the pulse also influences the results in the applications of pulsed electric fields [6]. Therefore, pulse generators that are capable of controlling many of the important pulse parameters over a wide range and provide certain pulse shapes regardless of the medium conductivity are highly desirable. Semiconductor switches have controllable ON and OFF, which allows them to produce square-wave pulses of controllable widths. With a Thyratron switch, only the ON is controllable, leaving the OFF dependent on the *RC* time constant of the load resistance and the energy storage capacitor, which produce an exponential decay pulse. A comparison between semiconductor-based and thyratron-based pulse generators is provided in earlier studies [7]. Semiconductor switches also have the advantage of higher repetition rates and long lifetimes. In this regard, a recent trend has been to use power semiconductor devices, mainly the MOSFETs and insulated gate bipolar transistors (IGBTs) for pulse generators to be used for biotechnology and medical applications. Hickman and Cook [8] tested several MOSFETs and IGBTs for use in pulse applications and noticed that MOSFETs were capable of faster ON and OFF times, but had lower voltage and current ratings than IGBTs. A single IGBT-based pulse generator was used to investigate the viability of *Escherichia coli* (*E. coli*) after exposure to 1.3 and 1.5-MV/m pulsed electric fields of 4–32 $\mu$s in duration [9]. A single MOSFET-based pulse generator capable of producing nanosecond pulses up to 400 V was designed and built in [5]. The design emphasis was focused on a compact device that had control over the pulse shape. A similar 400 V nanosecond pulse generator for use with electroporation-mediated drug and gene delivery was presented in [10]. In order

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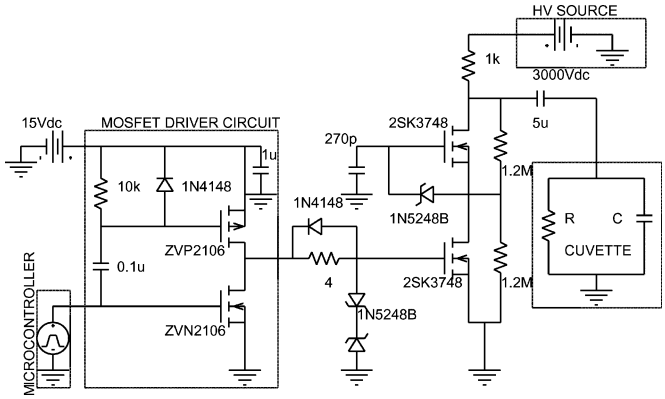


Fig. 1. Circuit diagram of the series-MOSFET pulsed power supply.

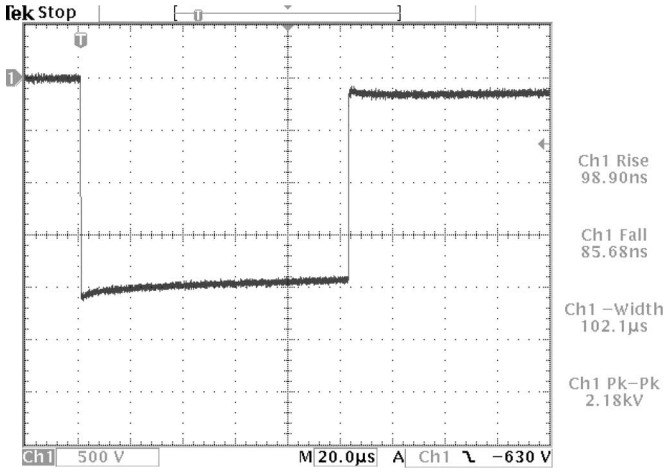


Fig. 2. Measured output voltage waveforms of the MOSFET-based pulsed power supply using the Tektronix P5100 250-MHz, 100× voltage probe and the Tektronix TDS 3044B 400-MHz, 5-GS/s oscilloscope.

to overcome the lower voltage ratings of the MOSFET-based pulse generators, a pulse generator using several MOSFETs in series to increase the voltage capabilities of the device has been designed and tested [11]. A similar device was presented in [12], which showed simulation results on the effects of varying the load resistance on the output pulse parameters.

The MOSFET-based pulsed power supply designed and built in this research is capable of producing controllable square pulses of amplitudes up to 3000 V and widths in the range of nanoseconds to milliseconds, without compromising on the waveshape with varied loads. This pulsed power supply is then used to conduct experiments on electroporation-mediated plasmid DNA molecule delivery in *E. coli* O157:H7, which was first recognized as a food-borne pathogen in 1982 [13].

## II. Experimental Setup

### A. MOSFET-Based Pulsed Power Supply

The MOSFET-based pulsed power supply that was designed and used for this research is capable of producing controllable square pulses with amplitudes up to 3000 V and widths in the range of 100 ns to a few milliseconds. The pulsed power supply, shown in Fig. 1, consists of a microcontroller, a MOSFET driver circuit, a gate protection circuitry to protect the MOSFET, two MOSFETs connected in series, an energy storage capacitor (5 $\mu$F), a high-voltage dc source (Glassman High Voltage WX5R200, 5 kV, 200 mA), and the load, an electroporation cuvette, which is modeled as a resistor in parallel with a capacitor. Fig. 2 shows a sample output pulse produced by the MOSFET-based pulsed power supply with a load conductivity of 0.7 mS/m.

*1) Power and Control Module:* It has been established that precise control of the pulse parameters is important for the applications of pulsed electric fields, especially when it is necessary to optimize the process. For this reason, it was decided that a microcontroller should be used to send a gating pulse to the MOSFET driver circuit. The microcontroller is the PIC18LF458, which has a clock frequency of 40 MHz and an instruction cycle of four clock cycles. Therefore, the microcontroller can compute 10 million instructions per second, which means that a single instruction is completed in 100 ns. This sets the limit for the smallest pulse width and time between pulses that can be achieved. Other than the 100 ns limitation, the user has full control in specifying the pulse width, number of pulses, and the time between two successive pulses. The user can program any sequence of pulses, and the pulses do not have to be all of the same width nor does the time between the pulses have to remain constant.

To function properly, the microcontroller requires a 5 V dc power supply. This supply is built as part of the power and control module to make the device more compact and less dependent on external components. The power and control module is connected to standard 110-V rms, 60-Hz electrical outlet, and the power is converted to dc using a conventional ac-to-dc converter and a 5 V regulator. With this power supply, the microcontroller is able to send signal pulses to the MOSFET gate driver, which uses a 15 V dc power supply to drive the MOSFETs. This 15 V dc power supply was also built as part of the power and control module for the aforementioned reasons and to ensure that the supply is capable of supplying the required gate current in the required amount of time, during the ON and OFF periods of the MOSFET.

*2) Pulse Generator Circuit:* The MOSFET-based pulsed power supply uses two MOSFETs, each with a rating of 1500 V, in series to produce controllable square pulses with amplitudes up to 3000 V, theoretically. Since the components in the circuits are nonideal, it is advisable to leave a safety margin with respect to the maximum blocking voltage of the MOSFETs. Although there are two MOSFETs, this pulsed power supply still uses one driver circuit to drive two MOSFETs, which reduces the number of components and simplifies the board layout. The design of the series connection of the MOSFETs is based on extending the ideas that are presented in [11] and [14] to high-voltage power MOSFETs. It uses a gate-side technique that makes use of the MOSFET's internal capacitances to achieve synchronization of the gate signals. This technique was used in [15], and resulted in a fast and balanced ON of the series MOSFETs without any additional load-side voltage-balancing device such as snubber

circuits. This design uses a single MOSFET driver and a 270 pF capacitor that is placed between the gate of the second MOSFET and ground. The proper operation of the circuit relies on the voltage division among the effective gate–source capacitance of the MOSFET and the additional 270 pF capacitor. When the MOSFET that is directly connected to the driver circuit turns ON, the change in its drain voltage divides across the effective gate–source capacitance second MOSFET and the 270 pF capacitor.

The shunt resistors that were placed between the drain–source terminals of the MOSFETs ensure equal voltage sharing when the MOSFETs are not conducting. In order to achieve equal voltage sharing when the MOSFETs are not conducting, the current in the shunting resistors must be greater than the leakage current of the MOSFETs. In addition, the power rating of the resistors had to be considered during the design. Low resistances would have to be rated at higher power, because they would allow more current to flow through them. In addition to the increased losses of the circuit at low resistances, high-power resistors would be more costly, more difficult to obtain, and physically larger. With these design constraints, 1.2 M$\Omega$, 2 W resistors were used in this circuit. The resulting current of 1.25 mA flowing though the shunt resistors is sufficiently larger than the 100-$\mu$A zero-gate voltage drain current of the MOSFETs, to ensure equal voltage sharing.

### *B. Bacterial Transformation*

Using the pulsed power supply designed before, an investigation into the electroporation-mediated plasmid DNA molecule delivery was conducted. The goal was to deliver the 3.3-kb pGFPuv plasmid DNA into *E. coli* O157:H7 (ATCC 43888) using a pulsed electric field developed across parallel plate electrodes. The pGFPuv plasmid is commercially available from Clonetech, which contains the pMB1 origin of replication from pUC19, a mutant GFP gene from marine jellyfish *Aequorea victoria*, and a *bla* gene for ampicillin resistance; the last two properties facilitate the selection and confirmation of the transformants. Although the main goal is to deliver the plasmid DNA into the *E. coli*, inactivation of the cell is also another area of interest, as it could reveal whether low transformation efficiency is due to a high number of inactivated cells. Therefore, determining the number of inactivated cells due to the pulsed electric field was also investigated.

*1) Preparation of Electrocompetent Cells:* *E. coli* O157:H7 (ATCC 43888) was inoculated into 10 mLof Luria-Bertani (LB) broth and allowed to grow overnight at 37 °C while rotating at 200 rpm. Two milliliters of the overnight culture was transferred into 200 mL of fresh LB broth in a 500 mL flask and allowed to grow at the same conditions. Once the optical density (at 600 nm) of the culture reached 0.5–0.7, the flask was placed on ice to prevent any further growth. In the specified range of optical density, the cells are known to be in the exponential growth stage, which is optimal for electroporation. The culture was then transferred into a centrifuge tube and placed in the centrifuge at 4000 $\times$ $g$ for 10 min at 2 °C. The supernatant was discarded and the remaining cell pellet (cells brought together by the centrifugal force) was resuspended in the same volume of ice-cold 10% glycerol. This process was repeated two additional times with one-half, one-tenth 10% glycerol of the original volume, in order to adequately wash the cells, eliminating the conductive ions from the previous suspension medium. Failure to sufficiently wash the cells can result in the arcing of the electrodes in the cuvette. Finally, the cell pellet was resuspended in 0.5 mL 10% glycerol. Hundred microliter of cell suspension was mixed with 2 $\mu$l of plasmid DNA (72.5 $\mu$g/mL in 10 $\mu$M Tris-HCl, pH 8.5), and placed between the electrodes of the cuvette.

*2) Electroporation Protocol:* Once the cell suspension and plasmid DNA were mixed and placed into the 1 mm gap of the cuvette, the pulsed electric field was applied. LB broth (900 $\mu$l) was then added to the cuvette immediately after the pulse sequence was applied. It is important that the period between applying the pulse and transferring the cells to outgrowth medium be kept to a minimum. Delaying this transfer by even 1 min can cause a threefold reduction in the number of transformants [16]. The cell suspension was then transferred to a microcentrifuge tube, incubated at 37 °C for 40 min, while rotating at 200 rpm, before creating and plating the required dilutions.

*3) Plating and Counting:* In these experiments, both the number transformed and inactivated cells were measured using selective and nonselective plates, respectively. The selective plates were made of LB agar, and supplemented with ampicillin at a final concentration of 50 mg/mL (1 mL stock solution, 50 $\mu$g/mL into 1 L of melted LB agar); therefore, only the transformants, the cells that obtained the ampicillin-resistance gene on the plasmid, were able to grow and form colonies on these plates. The nonselective plates contain only LB agar, which allows all the surviving cells to grow and form colonies. To ensure the validity of the experimental results, a positive and a negative control were plated for each set of experiments. Further, to ensure the accuracy of the results, several dilutions of each sample were made in saline so that the colony count could be obtained from a plate that contained 25–250 colonies. Also, every dilution of every sample was plated twice so that the count could be averaged.

## III. Results

Three sets of experiments were conducted, each consisting of five trials. In each experiment, trials $\mathrm{T}_1$–$\mathrm{T}_4$ were completed using the pulsed power supplies presented before, and trial C was conducted using the Gene Pulser Xcell electroporation system from Bio-Rad. The Gene Pulser Xcell electroporation system produces exponential pulses, which are commonly used by many recommended electroporation protocols and provides a basis upon which the results obtained using MOSFET-based pulsed power supply can be compared. For each trial, the survival ratio and the number of transformants were determined. The survival ratio is the number of cells $N$ after the exposure to the pulsed electric field, divided by the total number of cells $N_0$ before the exposure to the pulsed electric field, and the transformants are the number of *E. coli* cells in which the pGFPuv plasmid was successfully delivered. The transformants are measured in colony forming units (CFUs) per milliliter.

TABLE I
RESULTS OF INCREASING THE PULSE WIDTH OF MULTIPLE 1.35-MV/M PULSES

| Trial | Pulse Width (μs) | Electric Field Strength (MV/m) | Survival Ratio ($N/N_O$) | Transformants (CFU/ml) |
|---|---|---|---|---|
| $T_1$ | 1 | 1.35 | 0.877 | 800 |
| $T_2$ | 200 | 1.34 | 0.937 | 35000 |
| $T_3$ | 500 | 1.22 | 0.607 | 23600 |
| $T_4$ | 1000 | 1.25 | 0.510 | 51000 |
| C | 5000 | 1.28 | 0.369 | 63000 |

TABLE II
RESULTS OF INCREASING THE PULSE WIDTH OF A SINGLE 2.15-MV/M PULSE

| Trial | Pulse Width (μs) | Electric Field Strength (MV/m) | Survival Ratio ($N/N_O$) | Transformants (CFU/ml) |
|---|---|---|---|---|
| $T_1$ | 100 | 2.18 | 0.613 | 3000 |
| $T_2$ | 200 | 2.18 | 0.331 | 14500 |
| $T_3$ | 500 | 2.15 | 0.227 | 77500 |
| $T_4$ | 1000 | 2.16 | 0.260 | 152500 |
| C | 4900 | 2.12 | 0.118 | 720000 |

### A. Low-Field (1.30 MV/m) Microsecond Pulses

In an attempt to test a different strategy, $T_1$ used 255, 1.35-MV/m, 1-$\mu$s square pulses each separated by 3 $\mu$s. Trials $T_2$–$T_4$ used two increasingly longer 1.35-MV/m square pulses separated by 600 $\mu$s. Trial C used a single exponential pulse with a time constant of 5 ms. The selective plates containing the number of transformants and the nonselective plates containing the number of surviving cells for each trial were counted, and the data are shown in Table I.

For trial $T_1$, 800 CFU/mL were transformed, indicating that the technique of applying multiple short pulses was not the most effective technique for transforming *E. coli*. Trials $T_2$–$T_4$ were successful in transforming the cells, and the resulting number of transformants is of the same order of magnitude as the results of trial C, which uses the longer exponential pulse. It is worth noting that two 1-ms square pulses were able to transform a comparable number of cells as the much larger exponential pulse. This is a significant result because the two 1-ms square pulses contain less energy than the 5-ms exponential pulse. A successful transformation did not occur without a reduction in the survival ratio. The lowest cell survival ratio recorded in the experiments was 0.369, which occurred when using the 5 ms pulse. This cell survival ratio represents a 0.43-log reduction in the initial concentration of $4.05 \times 10^{10}$ CFU/mL.

### B. High-Field (2.15 MV/m) Microsecond Pulses

In the second set of experiments, trials $T_1$–$T_4$ used a single square pulse of increasing pulse width. Trial C used a single 5-ms exponential pulse from the Gene Pulser. The selective plates containing the number of transformants and the nonselective plates containing the number of surviving cells for each trial were counted, and the results are summarized in Table II.

TABLE III
RESULTS OF INCREASING THE NUMBER OF 1-MS, 2.15-MV/M PULSES

| Trial | Number of Pulses | Electric Field Strength (MV/m) | Survival Ratio ($N/N_O$) | Transformants (CFU/ml) |
|---|---|---|---|---|
| $T_1$ | 1 | 2.15 | 0.231 | 70500 |
| $T_2$ | 5 | 2.15 | 0.127 | 9600 |
| $T_3$ | 10 | 2.15 | 0.135 | 4050 |
| $T_4$ | 100 | 2.15 | 0.01744 | 0 |
| C | 1 | 2.15 | 0.253 | 18000 |

The results indicate that all five of the trials successfully produced transformed cells, which increase linearly as the pulse width is increased in the range of 100 $\mu$s to 5 ms. As the pulse width is increased within the same range, the survival ratio decreased to a minimum value of 0.118 (0.93-log reduction) occurring at a pulse width of 5 ms. As in the first set of experiments, a higher number of transformants were accompanied by a lower number of surviving cells. The initial cell concentration of the samples, in this set of experiments, was $2.47 \times 10^{10}$ CFU/mL.

### C. High Field (2.15 MV/m), 1-ms Pulses

In the third and final set of experiments, multiple 1-ms, 2.15-MV/m pulses were applied across the electroporation cuvette. In trials $T_1$–$T_4$, the number of pulses used was increased from 1 to 100, with 100 ms between each pulse in order to allow the capacitor to fully recharge. In trial C, the Gene Pulser was used to generate a single 1-ms exponential pulse with peak electric field strength of 2.15 MV/m. The selective plates containing the number of transformants and the nonselective plates containing the number of surviving cells for each trial were counted, and the results are summarized in Table III.

From the results in Table III, it is clear that increasing the number of pulses decreased both the number of transformants and the survival ratio. When 100 pulses were applied, there were no transformants, and the survival ratio was decreased by two orders of magnitude to 0.0174 (1.76-log reduction), where the initial cell concentration was $1.58 \times 10^{10}$ CFU/mL.

## IV. DISCUSSION

In the first two sets of experiments, using low- and high-field microsecond pulses, respectively, it is interesting to note that the successful transformation of *E. coli* was accompanied by a reduction in the survival ratio in the range of 0.118–0.937, with more transformants occurring when the survival ratio was lower. This would suggest that the lower number of transformants is not due to a high number of cells being inactivated, but that there was not enough energy to make the cell membrane permeable. In the third experiment, when multiple pulses were applied to the cuvette, the transformation of *E. coli* was accompanied by a reduction in the survival ratio in the range of 0.0174–0.253, with more transformants occurring when the

survival ratio was higher. In this case, it appears that the lower number of transformants could be due to the number of cells that were inactivated.

In the third set of experiments, using high field, 1 ms pulses of a square and an exponential pulse of similar durations and amplitudes can be compared. In trial $T_1$, a single 1-ms square pulse was used, and in trial C, a single 1-ms exponential pulse was employed. The results show that the 1-ms square pulse yielded approximately four times more transformants than the 1-ms exponential pulse with the same amplitude. One possible explanation for the improvement in the number of transformants with the square pulse over the exponential pulse is that the average electric field strength is higher in the former. The concept of the average electric field strength was used to explain the increased inactivation with square pulses as compared with the exponential pulse [17]. Another study concluded that square pulses result in 60% more inactivation than the exponential pulse with the same energy [6].

The electric field strength, to which the cells are exposed, is critical to the resulting number of transformants and survival ratio. When the field strength of a 5-ms exponential pulse was increased from 1.28 to 2.12 MV/m, the number of cells in the initial population that were transformed was increased from 1 in 642 857 to 1 in 34 305. It is important to note that this comparison was between two different sets of experiments. This is normally not done because there are many conditions such as temperature, length of time at the different stages of the experiments, and the growth phase of the cells that could vary during the two experiments. However, in these experiments, the same procedures, solutions, and bacterial cells were used, and the difference between the results is significant enough to make this observation with less risk of error. One study reported that the transformation efficiency of *E. coli* JM109 reached a maximum at a field strength of 1.7 MV/m, which then decreased with any further increase in electric field strength [18]. An increase in the electric field strength also resulted in a decrease in the survival ratio. A study on the inactivation of *E. coli* 0157:H7 concluded that increasing the field strength in the range of 1.2–2 MV/m resulted in a decrease in the survival ratio [19]. In addition, the survival ratio of *E. coli* JM109 decreased as the electric field strength was increased from 1.1 to 2 MV/m [18], and up to a 6-log reduction in the survival ratio of *Lactobacillus brevis* by increased electric field strength was reported in [20].

### A. Effect of Pulse Width on Electroporation

The results of the first two experiments show the effect of increasing the pulse width from 100 $\mu$s to 5 ms on the number of transformants and the survival ratio. In both sets of experiments, increasing the pulse width increased the number of transformants and decreased the survival ratio exponentially. The results presented in this paper on the effect of the pulse width on the number of transformants are consistent with the results reported in the literature. One study found that increasing the pulse width of 1.15 MV/m pulses from 2.5 to 4.5 ms results in an increase in the transformation frequency of *Candida famata* L20105 [21]. However, increasing the pulse width beyond this width up to 7.5 ms resulted in a decrease in the transformation. The latter effect, showing the number of transformants decreasing as the pulse width increased, was not observed in this research, because the pulse width was not increased beyond 5 ms. The observed exponential decrease in survival ratio as the pulse width increased is consistent with other results reported in the literature. While studying the effects of pulsed electric fields on *Lactobacillus brevis*, it was reported that increasing the pulse width of exponential pulses up to 12 ms resulted in an exponential decrease in the survival ratio [20].

### B. Effect of the Number of Pulses on Electroporation

The results of the third experiment demonstrate the effect of increasing the number of pulses on the number of transformants and the survival ratio. The results in Table III confirm that increasing the number of 1-ms, 2.15-MV/m pulses from 1 to 100 resulted in a drastic reduction in the number of transformants. In fact, when the number of pulses was increased from 1 to 100, the number of transformants was decreased from 70 500 to 0. A possible explanation for the reduction in the number of transformants is the low survival ratio as the number of pulses increased, which is also shown in Table III. In all the experiments, successful transformation was accompanied by a reduction in the survival ratio; however, the survival ratio for multiple pulses was much lower (1.76-log reduction with 100 pulse) than those resulting from only a single pulse. A similar study reported a 0.82-log and 4.47-log reduction of *E. coli* O157:H7 using 50 and 300 5-ms exponential pulses with field strengths of 2.5 MV/m, respectively [22]. The results showing the number of transformations decreased as the number of pulses increased are consistent with those that were reported in [23]. Therefore, it is concluded that using multiple pulses is not effective in increasing the number of transformants; however, it is a good technique for inactivating microorganisms.

## V. Summary and Conclusion

The use of a laboratory prototype of a MOSFET-based pulsed power supply on electroporation-mediated plasmid DNA molecule delivery in *E. coli* O157:H7 has been successfully demonstrated. The pulsed power supply that is capable of producing square-wave pulses with amplitudes up to 3000 V and widths in the range of nanoseconds to milliseconds was designed and built. The microcontroller allows for a very precise control on pulse amplitude, width, and spacing between pulses, which is highly useful in electroporation applications. Since the supply can produce as high as 3000 V square pulse, a variety of microorganisms can be studied for transformation or inactivation, although the current study focused on electroporation-mediated plasmid DNA molecule delivery in *E. coli* O157:H7.

The paper also confirms that for optimum transformation, it is important to control the pulse amplitude, width, and repetition rate, simultaneously, as the cell survival rate and the number of transformants are interdependent. In these experiments, it was concluded that increasing the pulse width in the range of from 100 $\mu$s to 5 ms increased the number of transformants and

decreased the survival ratio exponentially. Furthermore, increasing the number of 1-ms, 2.15-MV/m pulses from 1 to 100 resulted in a drastic reduction in the number of transformants.

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