Intermittently Delivered Pulsed Electric Fields for Sterile Storage of Turbid Media

Alex Golberg, Judith Kandel, Michael Belkin, and Boris Rubinsky

***Abstract*—This paper introduces a new concept and method for long-term sterile storage of turbid product, which is potentially subject to microbial contamination. The method uses intermittent delivery of pulsed electric fields (IDPEF) throughout the storage at time intervals that are prescribed according to microorganisms' growth kinetics. This new approach facilitates sterile storage without the need for chemical preservatives, additives, radiation or the complex infrastructure demanded by refrigeration. Unlike ultraviolet radiation, IDPEF can be used in turbid media. The first part of this paper is a theoretical discussion on the growth kinetics of microorganisms treated by IDPEF. We then provide a preliminary experimental study on the kinetics of microorganism growth in a turbid microbial growth media as a function of the IDPEF delivery intervals. Last, we demonstrate the use of the method using milk as a medium. IDPEF of 30 pulses, 17.5 kV/cm field strength, 40 μs long, 1 Hz delivered every 12 h was found to have the ability to preserve milk in a non-sterile environment at room temperature as effectively as refrigeration at 4 °C. The method has many obvious applications in biotechnology, the food industry, and is of particular importance with regard to geographical areas lacking refrigeration for storage of pharmaceuticals and food. This study was performed on the laboratory scale and a substantial adaptations are required in order to apply it to the industrial scale.**

***Index Terms*—Active storage, electroporation, intermittent delivery, pulsed electric fields (PEF), sterilization.**

I. Introduction

ELIMINATING pathogenic microorganisms and preventing them from infecting people, animals, and chemical solutions are major challenges to many industries, such as food, pharmaceutical, and cosmetic production. A particularly difficult situation to prevent is recontamination occurring after a sterile product leaves the factory and the sterility is compromised [1].

Traditional methods of microbial growth retardation under such circumstances include thermal methods, chemical preservation, and various modes of irradiation [2]. Several types of thermal methods of bacterial contamination control exist [3]. These methods are often insufficient due to the diversity of the microbial population. Some microbes pose a particular threat to food storage, in that they are resistant to the temperatures usually used [4], [5]. It is almost impossible to find a thermal regimen which effectively eliminates an entire diverse microbial population. Furthermore, inhibiting the microorganisms' growth by refrigeration requires a complex industrial infrastructure that is not always available, and is lacking in many areas of the world. Chemical preservatives are widely used in food, pharmaceuticals, and cosmetics; even though, it was recently observed that microorganisms can adapt to these agents [6]. Furthermore, chemical additives themselves can become health hazards [7], [8]. Ultraviolet irradiation is not effective in turbid media, and other modes of irradiation require a complex infrastructure and pose a potential safety problem. A review of several alternative non-thermal methods for microorganism inactivation can be found in [9].

The method we chose to focus on involves the delivery of short high-field strength pulsed electric fields (PEF). PEF of microsecond to millisecond duration destroy cells by damaging the cell membrane, a phenomenon known as irreversible electroporation (IRE) [10], [11]. The earliest, most fundamental study on the effect of PEF on microorganisms was performed by Sale and Hamilton in the early 1960s [12]–[14]. Reviews on the use of PEF for food sterilization can be found in several publications, e.g., [11], [15]–[17]. Some specific applications are described in [18]–[21].

The goal of this study is to introduce a different concept aimed at long-term storage of biological matter under exposure to non-sterile, potentially septic conditions, without the use of chemical additives, refrigeration or irradiation. We propose the intermittent delivery of PEF at specific, planned time intervals during the storage period. We term this technique Intermittently Delivered Pulse Electric Field (IDPEF) storage. The electric and temporal parameters of IDPEF can be determined by studies of microorganisms' growth kinetics in such a way as to maintain microbial load at an admissible level while minimizing the release of endotoxins and untoward effects in the material being sterilized. The IDPEF mediated storage concept may be of particular value in those parts of the world where refrigeration facilities are insufficient.

The paper is divided into three parts. First is a theoretical discussion of the kinetics of microbial growth treated with IDPEF and the treatment parameters. Then we discuss the results of a preliminary experimental study on the kinetics of microorganisms' growth under treatment by IDPEF. The third part of the study demonstrates the value of the concept by using

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A. Golberg is with the Center for Bioengineering in the Service of Humanity and Society, School of Computer Science and Engineering Hebrew University of Jerusalem, Jerusalem 91904, Israel (e-mail: agolberg@gmail.com).

J. Kandel is with the Stern College for Women, Yeshiva University, New York, NY 10016 USA.

M. Belkin is with the Goldschleger Eye Research Institute, Sheba Medical Center, Tel Hashomer 52621, Israel.

B. Rubinsky is with the Department of Mechanical Engineering, University of California at Berkeley, Berkeley, CA 94720 USA.

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it for milk storage. The results were then compared to those of the standard refrigeration method.

II. Theory Of Active Sterilization By IDPEF Sequences During Storage

Chemical preservatives and refrigeration continuously inhibit microorganism growth in products throughout the period of storage. In this paper, we evaluate a method of maintaining the sterility by applying PEF intermittently during long-term storage. The concept of intermittent sterilization in a non-sterile environment is not new. It has developed, by necessity, in parts of the world lacking the industrial infrastructure needed for refrigeration or financial resources for chemical additives. For instance, in rural India, milk is boiled every 24 h to destroy the microorganisms that develop to prolong its shelf life [22]. As another example, the World Health Organization (WHO) has proposed the repeated use of solar energy for the sterilization of water [23].

It is well established that the use of PEF is effective for the reduction of the microbial load in both batch and flow systems, [11], [15], [24]. This sterilization technology is the subject of substantial research e.g., [11], [17]. However, PEF sterilization systems currently being studied are aimed at large scale industrial processes, and use a single pulse application [24]–[26]. Following treatment, products are packaged and transported to consumers, at which point they become far more vulnerable to contamination. The post-processing steps are of great concern as potential sources of contamination. The use of PEF at these points of the storage process will alleviate these concerns.

While substantial fundamental research has been done on kinetics of microorganism growth, we have found only one study on the kinetics of microorganism growth after a single application of PEF [27]. The resistance of bacteria to various stress conditions is widely reported in the literature [6]. However, we did not find a single proof about developed microbial resistivity to PEF. Much work has been done on the resistance of bacteria exposed to a single instant of PEF. Although PEF was thought to be "all or nothing event," recent studies show that large amounts of sub-lethally injured cells may be found in the product after the treatment, indicating microbial resistivity. This ability of microbes to survive after the application of PEF is related to the nature of cell walls, pH, temperature and chemical composition of the solution [28]–[30]. No translocations were detected in the research that aimed to test microbial adaptation to PEF [31]. Gusbeth *et al.* (2009) investigated whether the descendant of the PEF surviving bacteria develop resistivity to PEF. They tested 30 generations and reported no changes in the variable intergenic spacer region of the ribosomal operon of *P. putida* during the repeated PEF treatments experiments [32]. The lack of information about the changes in growth kinetics resulting from multiple PEF treatments is a vital consideration in the development of optimal IDPEF treatment protocols.

IDPEF-mediated storage has not been considered nor studied before, and no research has been done on the kinetics of microorganisms exposed to PEF multiple times. In this section, our goal is to discuss a methodology for determining optimal

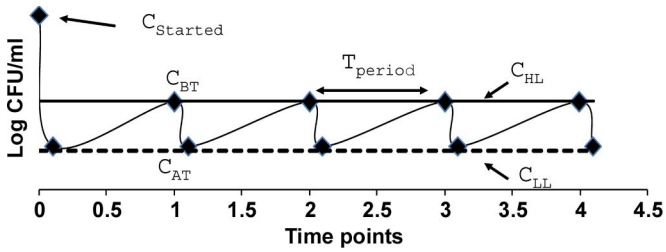

Fig. 1. Schematic diagram of microbial growth under intermittently delivered pulsed electric fields (IDPEF) mediated storage protocol. The graph describes the behavior of the simplest first order kinetic model described in Equation (1). Here, we assume that exponential growth of bacteria occurs between the PEF treatments (C_{AT} to C_{BT}). The reduction of the microbial load from C_{BT} to C_{AT} due to the application of PEF may be modeled by one of the semi-empirical inactivation kinetics curves described in the literature [33]–[38].

IDPEF protocols and the treatment parameters involved as a basis for the experimental studies in the next section. The optimal IDPEF treatment parameters will depend on the initial microbial load type and quantity, the microbial growth kinetics and the nature of the material to be sterilized. A general schematic of a possible IDPEF treatment planning protocol is shown in Fig. 1.

The various parameters in Fig. 1 are:

C_{started} (CFU/ml)—initial microbial concentration before treatment or after the recontamination.

C_{HL} (CFU/ml)—highest level of microbial concentration allowable.

C_{LL} (CFU/ml)—lowest level of microbial load allowed to be found in the product (relevant to bio active products).

C_{BT} (CFU/ml)—microbial concentration before a PEF treatment.

C_{AT} (CFU/ml)—microbial concentration after a PEF treatment.

T_{period} (h)—time interval between intermittent PEF treatments.

The design goal of the IDPEF treatment protocol is to maintain a microbial concentration below a particular level C_{HL}. To this end we are seeking to find an optimal way of applying the PEF to reduce the concentration intermittently from C_{BT} to C_{AT} at prescribed time intervals T_{period}. The IDPEF treatment parameters will obviously depend on the kinetics of PEF treated microorganisms. Assuming that after a PEF treatment, the microorganisms experience exponential growth phase with a constant rate constant k (h^{-1}), and that the time interval between treatment is T (h), the following equation is relevant to treatment design:

$$C_{BT} = C_{AT} * e^{kT}$$
$$T = \ln \frac{C_{BT}}{C_{AT}} / k. \tag{1}$$

It should be emphasized that the kinetics of microorganism growth may change from one PEF treatment to another. Therefore, an optimal protocol C_{BT} may be different from C_{HL} and the growth constant k may also change [27]. Another important aspect of treatment planning is the thermal constraint. PEF produce Joule heating and the treated product temperature cannot exceed a maximum allowed temperature. Equation (2)

describes the major parameters which should be taken into account when IDPEF treatment planning is done.

$$T = f(k, final\_allowed\_Temperature, C_{BT}, C_{AT}). \quad (2)$$

Substantial research has been published on PEF treatments of microorganisms beginning with the now classic series of three papers by Sale and Hamilton [12]–[14]. Parameters affecting the outcome of a PEF treatment include: pulse voltage, electric field strength in the treated medium, pulse duration, number of pulses, and the frequency at which they are delivered [9], [11], [15], [17]. Furthermore, various kinetics models have been developed predicting the fraction of the microbial load that survived after the application of PEF [33]–[38]. These models describe what we consider to be the microbial load reduction from $C_{\rm BT}$ to $C_{\rm AT}$ due to the application of PEF. The specific pulse parameters used in IDPEF treatment planning can be drawn from irreversible PEF research, e.g., [16], [17].

In the following section, we describe experiments we performed to illustrate the IDPEF mediated storage method. It is important to emphasize that this is a primary report describing the method; therefore, much work remains to be done to develop an industrial IDPEF protocol.

III. Kinetics of Microorganisms Exposed to IDPEF

A. Materials and Methods

The study was performed using tetracycline-stable *E. coli*, kindly provided by the laboratory of Dr. Y. Tzfati, Alexander Silberman Institute of Life Sciences, Hebrew University, Jerusalem. A defined growth medium composed of 0.5 g NaCl/L, 10 g bactotryptone/L, 5 g yeast extract/L, and 0.5 g glucose/L dissolved in distilled water was used. It was heated in an autoclave to 121 °C and then cooled to room temperature before being stored at 4 °C. The bacterial culture was prepared by transferring the microorganisms from Luria-Bertani plates to 25 ml of the growth medium. The culture was allowed to grow until the stationary phase was reached, with a final concentration of approximately 10^8 colony forming units (CFU)/ml.

In the first experimental part of the study, we applied a particular PEF sequence twice to the same sample. The second PEF sequence was delivered at different time intervals after the first, at various points of the *E. coli* kinetic growth curve. Primary inoculation was done by pouring 20 μL of the bacterial inoculums into 25 ml of the autoclaved growth medium. This resulted in an initial concentration of $6.8 * 10^4$ cells/ml, which was used in all subsequent experiments with the defined growth medium. The solution was treated immediately (T_0) in 90 μL aliquots in a 1-mm gap electroporation cuvette (model 610; BTX, San-Diego, CA, USA), and the pulses were delivered by a BTX ECM 830 square-wave electroporator. We delivered 25 100-μs duration pulses by applying 1.2 kV amplitude, at 1 Hz. The treatment parameters were chosen in such a way so as to not affect the solutions' pH and not to cause a temperature rise to the level where thermal inactivation of bacteria would take place. The treated samples were divided into three groups. The samples were kept in an incubator at 37 °C before and after treatments.

The second PEF sequence, identical to the first, was delivered 3, 6 and 12 h (T_3, T_6 and T_{12}, respectively) after the first sequence. In the interim, the medium was sampled every 3 h and the total number of bacteria was counted as described later in this section. The contaminated samples were kept in plastic tubes in a non-agitating incubator at 37 °C between the treatments. Six repeats were done for each experimental group.

The second experimental part of the study was aimed at evaluating the efficacy of IDPEF as a modality for long-term sterilization of an *E.coli* defined medium. This type of medium is particularly useful as a proof of principle of the capabilities of the IDPEF technology because on the one hand, it is has an optimal composition for microbial growth; while on the other hand, it is a turbid media, which cannot be kept sterile with UV irradiation. In this part of the study, the medium was treated with the same PEF sequence as in the previous experiment, i.e., 25 100-μs pulses delivered by applying 1.2 kV on 1-mm gap electroporation cuvette (model 610; BTX, San-Diego, CA, USA) at 1 Hz, repeated every 12 h for 60 h. The treated medium was transferred to plastic tubes and stored in non-agitating incubator at 37 °C between treatments. We measured both the temperature and the pH of the solution immediately after all PEF applications. Temperature was measured in the cuvette using a Neoptix Reflex signal conditioner with a 0.7-mm probe covered with polyimide (Neoptix, Québec, Canada). The pH was measured with pH indicator paper (Neutralit, pH 5.0–10.0; Special indicator). The bacterial numbers were counted before and after each the treatment by spread counting method. The samples were diluted tenfold in Dulbecco's phosphate-buffered saline (Biological Industries, Kibbutz Beit Ha-Emek, Israel) to eliminate effects of media on cell growth [39]. Additional tenfold dilutions were also performed. Samples (100 μL) of each of the tested solutions were plated on Luria-Bertani Miller (LB) agar plates supplemented with tetracycline and incubated at 37 °C for 24 h. Cells were counted using an MRC colony counter model 570 (MRC, Holon, Israel). Two samples were plated for each experimental condition. Eight repeats were done for each experimental group.

In the third experimental part of the study, a series of experiments were performed in an extremely turbid medium of high relevance to the food industry: milk. Matak (2004) reported 12000 Nephelometric Turbidity Units (NTU) for 1% skim milk, 29000 NTU for 2% reduced milk and 49000 NTU for a whole, 3.2% milk [21]. In comparison, tap water standard is no higher than 5 NTU [40]. We used a commercially available Ultra High Temperature (UHT)-treated milk with 3% fat content (Ramat HaGolan Dairies, Israel) with a shelf life of seven days at 4 °C refrigeration after the package is opened. The experiment began with the opening of the package and inoculation of the medium with 5.7×10^5 cells/ml of *E. coli*. Based on ours' and others' experience with sterilization of *E. coli* in milk [18], [19], [41], we chose to employ a PEF sterilization sequence of 30 pulses, each of 40 μs duration, 1.75 kV amplitude, and delivered at 1 Hz.1 mm gap electroporation (model 610; BTX, San-Diego, CA, USA), cuvettes

were used. We used a shorter pulse duration in this experiment to reduce the risk of the formation of byproducts [42], such as coagulated milk proteins on the cuvette's electrodes. The following groups of milk samples were tested: 1) samples subjected to the PEF sequence once at the beginning of the experiment and then stored at room temperature; 2) samples subjected to the PEF sequence once at the beginning of the experiment and then stored at 4 °C; and 3) samples subjected to the same PEF sequence intermittently (IDPEF) every 12 h and stored at room temperature. Each treated sample was stored in a electroporation cuvette, which is a closed container but is not hermetically sealed. Milk temperature and pH were measured after each PEF sequence treatment. The experiment was carried out over a period of five days, with five repeats. Milk was sampled every 24 h and live bacteria were counted by the spread plate method as described in the previous paragraph.

Statistical analyses were performed using the Microsoft Office Excel 2007 external package, as well as student t-tests with unequal variances.

B. Results and Discussion

To the best of our knowledge, this is the first work to suggest using PEF for the storage of perishable products at room temperature without chemical preservatives. Theoretical considerations discussed above emphasize the importance of identifying the kinetic growth constants exhibited by microorganisms post-PEF treatment to develop an optimal treatment protocol. In the food industry, the indicator microorganisms, i.e., the type of bacteria known to threaten a specific product, usually have known growth rate constants. Therefore, it should be possible to develop the specific IDPEF protocols for products in which indicator microorganisms are known. However, if the indicator microorganisms are not known, or a spontaneous contamination is a matter of concern, a more rigorous IDPEF protocol may be used. For instance, application of pulses may be done throughout the entire storage period, with intervals shorter than 10 min (which is the amount of time required for the division of Chlostridium perfringes, one of the fastest growing microorganism [43]). In this paper we assume that the indicator microorganisms are known and an optimal treatment protocol may therefore be developed.

Fig. 2 shows the concentration of microorganisms during their growth following the first and second PEF treatment sequences. It is important to note that two repeats from each of the T_3 and T_6 treatment points exhibited total inactivation, and 0 CFU were counted in all subsequent points. These data were excluded from the plots.

The data in Fig. 2 were used to calculate the basic microorganism growth kinetic parameters for the various treatment protocols. The results are plotted in Fig 3(a) and (b). The values of the growth rate constant k(h^{-1}) and g(h) [Fig. 3(a)] and generation half time (g, h) [Fig. 3(b)] were calculated from the linear region of the semi-logarithmic plot of cell number versus time (Fig. 2).

To calculate the kinetics parameters, we used the regions of growth from Fig. 2 where the exponential growth took place.

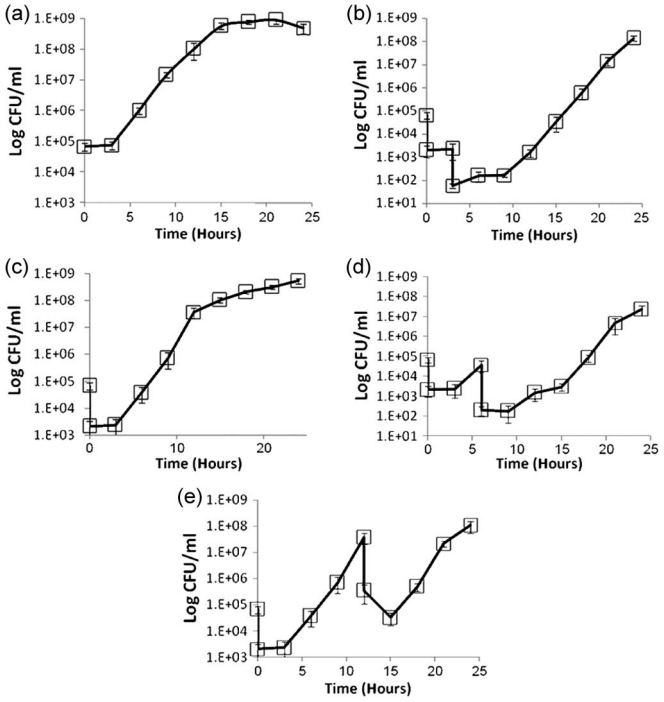

Fig. 2. Effect of PEF treatments on *E.coli* growth curves. The treatment consisted of 25 pulses delivered at 1.2 kV amplitude, 100 μs pulse duration at 1 Hz. Cuvettes with a 1 mm gap between electrodes were used. (a) untreated culture; (b) sample treated at T_0; (c) sample treated at T_0 and T_3; (d) sample treated at T_0 and T_6; (e) sample treated at T_0 and T_{12}. All samples were stored in room temperature. Error bars represent one standard deviation above and below the means.

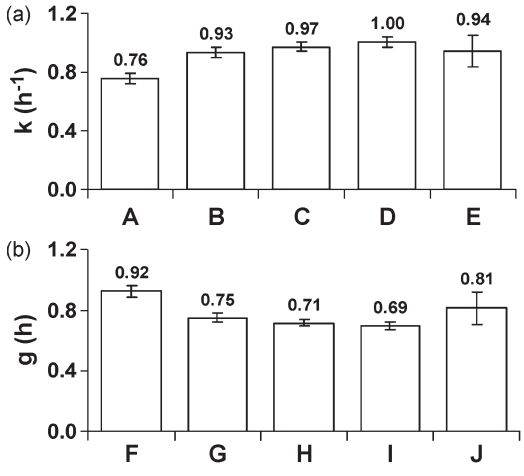

Fig. 3. Effect of PEF treatments on *E.coli* kinetics parameters. The treatment consisted of 25 pulses delivered at 1.2 kV amplitude, 100 μs pulse duration at 1 Hz. Cuvettes with a 1 mm gap between electrodes were used (a). Growth rate constant k (h^{-1}) of **A**- Untreated culture, and treated **B-** at T_0 **C-** at To and T_3; **D-** at T_0 and T_6 **E-** at T_0 and T_{12}, **(b).** Generation time of **F**- Untreated culture, and treated **G-** at T_0 **H-** at To and T_3; **I-** at T_0 and T_6 **J. -** at T_0 and T_{12}. Index of T refers to the treatment timing (h). Error bars represent 1 standard deviation.

The specific time regions (T1 and T2) used for calculations appear in Table I. The calculation of k (h^{-1}) was done using

$$k = \ln\left(\frac{C(T2)}{C(T1)}\right) \Big/ (T2 - T1). \tag{3}$$

The growth rate constant [Fig. 3(a)–(e)] of the culture which was treated once at T_0 was found to be 0.93 h^{-1}. This is

TABLE I
REGIONS OF EXPONENTIAL GROWTH, USED FOR KINETICS PARAMETERS CALCULATIONS. T1 IS THE TIME AT THE BEGINNING OF THE EXPONENTIAL GROWTH PHASE, AND T2 IS THE TIME AT THE BEGINNING OF THE "STATIONARY PHASE". POINTS ARE TAKEN FROM FIG. 2 FOR CALCULATIONS

Sample	T1 (h)	T2 (h)
Untreated	3	15
Treated at To	3	15
Treated at To and T_3	12	24
Treated at To and T_6	15	24
Treated at To and T_{12}	15	24

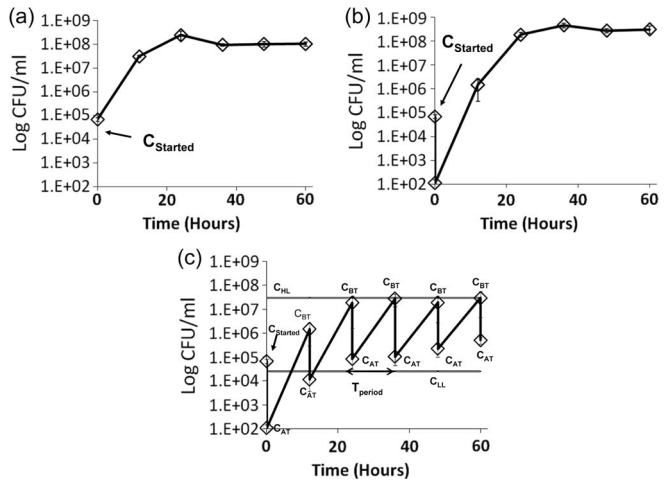

Fig. 4. Long term *E.coli* growth in defined growth medium under IDPEF. 25 pulses delivered at at 1.2 kV amplitude, 100 μs pulse duration at 1 Hz. Cuvettes with 1 mm gap between electrodes were used: (a) untreated; (b) treated only at T_0; (c) treated every 12 h. All samples were stored at room temperature. Index of T refers to the treatment timing (h). Error bars represent 1 standard deviation above and below the means.

significantly higher that of the untreated culture, which was 0.76 h^{-1} ($p < 0.0015$). The growth rate constant of the culture which was treated once at T_0 and T_3 is 0.97 h^{-1}, also significantly higher than that of the untreated culture, 0.76 h^{-1} ($p < 0.0032$).

The growth rate constant of the culture which was treated once at T_0 and T_6 is 1 h^{-1}, also significantly higher that the untreated culture, (0.76 h^{-1}) ($p < 0.0017$).

The growth rate constant of the culture which was treated once at T_0 and T_{12} was found to be 0.94 h^{-1}, also higher that the untreated culture (0.76 h^{-1}) ($p < 0.07$).

The growth rate constants of all PEF treated groups were not significantly different. The generation half time [Fig. 4(c) F-J] is directly related to growth rate constant, and is given by

$$g = \ln 2/k. \tag{4}$$

An interesting observation from the first series of experiments is that the kinetics of microorganism growth was the same after the second PEF treatment sequence regardless of when it was delivered with respect to the first sequence. It is unclear whether this phenomenon is particular to the testing

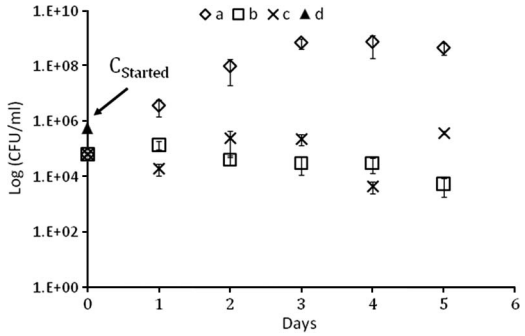

Fig. 5. Milk storage experiments, showing contamination levels. 30 pulses delivered at 1.75 kV amplitude, 40 μs pulse duration at 1 Hz, Cuvettes with 1 mm gap between electrodes were used. (a) Sample subjected to PEF treatment once at the beginning of the experiment and then stored at room temperature; (b) sample subjected to PEF treatment once at the beginning of the experiment and then stored at 4 °C; (c) sample subjected to PEF treatment every 12 h and stored at room temperature. Points show the microbial count after the PEF treatment; (d) *E. coli* concentration prior to first PEF treatment. Error bars represent 1 standard deviation above and below the means.

conditions, the microorganism, or both; however, it certainly facilitates a simple design of IDPEF treatments.

The difference between the kinetics of the PEF treated and non-PEF treated microorganisms kinetics is consistent with a previous paper proposing that leakage of intracellular contents into the media following PEF treatment may actually cause a change in the growth rate of the surviving microorganisms [27]. This observation, which is yet to be further investigated, may be related to either the difference in initial concentration or the culling effect of the PEF, which may affect primarily the weaker organisms or to the impact of PEF on gene expressions [44] The impact, if any, of PEF on microbial genome, however, is controversial in literature see [32] versus [44].

We used the kinetic data from the experiments in Figs. 2 and 3 to design an IDPEF storage experiment and demonstrate its feasibility as a storage technology. The experiment involved first contaminating the growth medium and applying the same PEF treatment as in the previous experiments every 12 h for 60 h. The contamination levels before and after the PEF treatment was measured and plotted in Fig. 4(c). This was compared to the case of contamination without sterilization [Fig. 4(a)] and contamination with one PEF sterilization [Fig. 4(b)]. Both the sample without sterilization and the one with a single PEF sterilization reached the same level of contamination after 60 h in the incubator at 37 °C. This illustrates the well known fact that if the sterilization is not complete, the microorganisms will continue to grow, reaching a steady state plateau. In contrast, the IDPEF method can maintain the level of contamination below a designated value, even when the first sterilization is not complete. The dynamics of the microorganism growth in Fig. 4(c) are identical to the theoretical design in Fig. 1, which proves the feasibility of the IDPEF concept. To our knowledge, this is the first work which models and experimentally illustrates the concept of food storage using IDPEF. Therefore, at this stage we used the basic first-order kinetic model for description of bacteria growth kinetics. Future research should reveal the additional parameters that impact the interaction between growth and inactivation kinetics.

The results of the third experimental study for contaminated milk are shown in Fig. 5. This study measured the level of contamination in milk as a function of time for three cases: a) a contaminated sample treated once with PEF and left at room temperature for five days; b) a contaminated sample treated with PEF once and stored at refrigeration temperatures for five days; c) a contaminated sample stored at room temperature subjected to IDPEF every 12 h during five days. After five days, the contamination level of the samples treated with IDPEF and kept at room temperature were comparable to those of the samples kept at 4 °C refrigeration temperatures. In both storage methods the contamination levels before the would-be consumption (directly after the PEF treatment) were lower by several orders of magnitude than contamination levels of the samples kept at room temperature after a single PEF treatment.

While refrigeration reduces the growth rate of microorganisms by altering in their metabolism, PEF directly kills them. Therefore, it presents a reasonable solution to the problem of recontamination.

Although continuous refrigeration effectively prevents the growth of the majority of known pathogens, it allows for and accelerates the growth of cryophiles such as *L. monocytogenes*, *C. botulinum*, and *Y. enterocolitica*. These organisms are capable of growth at regular refrigeration temperatures, and were detected in both raw and pasteurized milk, ice cream and cakes [2]. Refrigeration provides optimal growth conditions for these microorganisms, leading to such food borne diseases as Listeriosis [45]. On the other hand, the implementation of PEF for microbial load reduction may provide a universal method for the elimination of all bacteria. This is because electric fields affect the cell membrane, a vital cell organ to all organisms. In fact, *L monocytogenes* [46]–[49] and *Y. enterocolitica* [50] have been successfully inactivated by PEF.

Another benefit of IDPEF sterilization is the amount of energy it consumed in comparison to refrigeration. The IDPEF concept introduced in this study should be economically feasible in parts of the world with inadequate electrical power infrastructure and limited access to refrigeration. We assume that local sources of electrical power, such as car batteries or mechanical (dynamo type) electricity generators will be available in such areas. Unlike refrigeration, which needs to be applied continuously and necessitates relatively expensive machinery, PEF can be applied relatively infrequently (every 12 h in this study) and its energy can be supplied by the discharge of a charged capacitor. The treatment may be applied directly before consumption. The capacitor can be recharged using either manual power or alternative energy at any time between the pulse applications. While the voltage required for the PEF sequences in this study was high, the actual amount of energy discharged during each pulse was low. In the milk storage experiments we described, we used about 47.5 J/cc for every pulse to reduce microbial load in the treated fluid. It should be emphasized that other groups have successfully developed more efficient PEF systems than the basic one used here. Sato *et al.* (2001), for example, used a ring-mesh cylinder electrode capable of achieving a decrease of 4 orders of magnitude in microbial survival with a PEF sequence of 100 J/cc [51], and Narsetti *et al.* (2006) used a flow-through PEF system that can produce a 4-log inactivation with only 40 J/cc [52]. Given the possibility of a 4-log microorganism inactivation with about 100 J/cc per PEF sequence, and given that, as in our study, application of one sequence every 12 h is sufficient, the power required for 24 h of storage with active PEF will be about 200 J/cc. In comparison, energy-efficient refrigerators (i.e., new appliances with no dead space inside) require power of about 0.005 W for a storage volume of 1 cc [53], which translates to 308 J/cc for a 24-h period of continuous refrigeration.

This is the first paper which presents the possibility of controlling the microbial load in perishable products using IDPEF. Our proposed first-order kinetic model does not include environmental and cell specific factors which may impact the kinetics of microbial growth under IDPEF. Indeed, medium conductivity, pH, water activity and chemical composition have been shown to be crucial factors the recovery of microorganisms after PEF treatment [28]–[30]. In addition, bacterial size, membrane type, growth stage and concentration play important roles in PEF efficiency and cell recovery [17]. However, these additional parameters do not undermine the main importance of the proposal given in this paper, namely, the fact that this method may provide a food storage solution in places where electricity supply is not continuous. Moreover, the IDPEF method may solve the problem of recontamination within the supply chain.

In summary, this study has introduced the concept of IDPEF and demonstrated its feasibility and potential utility as a method for the sterile storage of liquid media, without resorting to thermal, chemical or radiation means. Much research remains to be done to develop this laboratory demonstration into an industrial process.

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Alex Golberg received the B.Sc. degree in biotechnology and food engineering from the Technion—Israel Institute of Technology, Haifa, Israel, in 2007. He is currently working toward the Ph.D. degree, with focus on the biological effects of pulsed electric fields, in the Center for Bioengineering in the Service of Humanity and Society, Hebrew University of Jerusalem, Jerusalem, Israel.

Since 2007, he has been with the Center for Bioengineering in the Service of Humanity and Society. His research interests include irreversible electroporation and renewable energy sources.

Judith Kandel graduated *summa cum laude* from Yeshiva University, New York, NY, in 2010 with a B.A. degree in biochemistry and Judaic Studies.

In the summer of 2009, she worked as a Researcher in Professor Boris Rubinsky's bioengineering lab in the Hebrew University of Jerusalem, Jerusalem, Israel. She is beginning a doctoral program in bioengineering at the University of Pennsylvania.

Michael Belkin receive a Master's degree in science from Cambridge University, England, and received a doctorate in medicine from the Hebrew University of Jerusalem, Jerusalem, Israel.

He is the Sidney A. Fox Professor of Ophthalmology in Tel Aviv University, Tel Aviv, Israel and the director of the Ophthalmic Technologies Laboratory at the university's Eye Research Institute at the Sheba Medical Center. He is also Senior Consultant of the Singapore Eye Research Institute. He is an author of over 200 scientific publications and 19 patents and is an internationally recognized eye researcher who received various research awards. His laboratory is dedicated to originating and enabling the transfer of technologies from university-level research to clinical practice by providing expertise and facilities for laboratory, preclinical and clinical studies. He is involved in several such projects at various stages of development in lasers, optics, ophthalmic devices, pharmaceutics and biotechnology and in recent year he participated in establishing a few startup companies.

Dr. Belkin served previously as: Director of research, development and non-conventional warfare medicine in the Israel Defense Forces Medical Corps; first full-time Director of the Tel Aviv University Eye Research Institute; Chairman of the Tel-Aviv University Department of Ophthalmology, President of the Israel Society of Eye and Vision Research.

Boris Rubinsky received the Ph.D. degree in mechanical/bioengineering from Massachusetts Institute of Technology, Cambridge, in 1980 and has been a Professor at the University of California at Berkeley.

He is a Professor of the Graduate School at the University of California at Berkeley. His current research is in the field of bioengineering in particular bioelectric devices and medical imaging. He has published over 250 peer reviewed papers, holds well over 20 issued patents, published several books and has supervised the M.Sc. and Ph.D. thesis of close to 100 students. Among his more notable accomplishments are the development of the first imaging monitored minimally invasive surgical technique, directional solidification microscopy, cellular micro-electroporation technology, the field of non-thermal irreversible electroporation and recently, cellular phone based medical imaging.