Statistical Analysis for Survival Fraction of E. Coli in Liquid during Inoculation by PEF Technique

Krishnaveni S*

*Associate professor, Department of Electrical and Electronics Engineering,

Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai, TamilNadu, India.

krishnavenis@ssn.edu.in

Abstract: *Since past few decades, Pulsed Electric Field as food preservation technique has drawn a lot of attention because of its potential to improve food quality and prolong its shelf life by inactivating microorganisms in it. However, there are numerous PEF parameters like pulse amplitude, pulse frequency, pulse shape and pulse nature are involved in determining the PEF efficacy in food processing methods. In this work, experiments are conducted to inactivate E.coli present in orange juice by applying rectangular pulses of widths 0.62, 1.2, and 7μs at 5 and 10 kV/cm. The pulse frequency is increased from 1, 10, 20 and 51 kHz. The survival fraction of E.coli is observed as good at 51 kHz and 10 kV/cm. But the explicit study could not be possible in this method to deal the effectiveness and importance of each individual parameters. So, the study is performed by modelling the survival fraction of microorganisms when subjected to PEF treatment. The comparison is made from Bigelow Model, Geeraerd Tail Model, Biphasic Model and Weibull Model. It is observed that the Weibull model can be highly recommended in this type of studies because of low residuals when compared to other models.*

Keywords: **Pulse width; pulse frequency; Bigelow model; Weibull Model**

1. INTRODUCTION

Commercial liquid food preservation involves the use of a high-temperature, short-time method. Even while the heat treatments increase the liquid food's shelf life, they have an adverse effect on its flavor, chemical makeup, and nutritional value. Therefore, non-thermal food processing technology is needed, and among all these non-thermal food processing techniques, the Pulsed Electric Field (PEF) method has been the attractive technique [1-4]. The researchers have been encouraged to continue their interest in PEF technology. Following that, several studies on the PEF method's ability to inactivate microorganisms are published. Even though it is somewhat challenging to compare the contrast outcomes obtained by various study teams, the shared metrics can be thought of as the PEF treatment's efficiency characterizing variables. On the other hand, the PEF approach at the research level itself is limited by technical reasons. In fact, one of the main reasons for the PEF system to be retained in research level itself is due to the absence of dependable industrial equipment [5]. Other important factors are inadequate operating procedures, control, and treatment status monitoring. Therefore, additional information is needed for the commercial stage of PEF technology to increase treatment repeatability.

Several robust models have been developed to account for the varying trends of the numerous factors involved in the PEF process [6–9]. Users can objectively assess the impact of PEF processing on the microbiological safety and quality of food through the application of quantitative science in predictive microbiology. The modeling of microbial inactivation by PEF is a challenging procedure because to the large number of parameters involved, making it challenging to disentangle the effects of various parameters. Thus,

among food microbiologists, predictive modeling of microbe survival is a key area of study [10–13]. So, Predictive modeling of survival of microorganism is an important research topic among food microbiologists [10-13]. The fundamental models are divided into two primary groups: probability models based on microbiological occurrences and kinetics models. The PEF parameters are analysed by both traditional models (Bigelow model, Geerared Tail model, Biphasic model) and an evolving mathematical model (Weibull model) for comparing the effect of induvial PEF parameters. In this study, the influence of frequency, pulse width, treatment time along with the electric field intensity is considered on the shape of the survival curves.

2. Methodology

Orange juice mixed with E. coli is treated using a laboratory scale PEF system described in [14]. A maximum of 1.2 kV, 1 µs pulses with a configurable frequency range of 1 kHz to 51 kHz can be produced by using the developed PEF system. The PEF treatment is used to investigate how pulse frequency affects the percentage of E. Coli that survives after the PEF treatment. The liquid sample is prepared in accordance with the requirements by keeping the liquid sample at room temperature. Then, PEF treatment is applied to a 200µl E. Coli sample every time.

Small cuvettes with copper electrodes are utilized, with the electrode spacing maintained at 1 mm to provide a homogeneous electric field intensity of 5 kV/cm and 10 kV/cm. An Agilent digital storage oscilloscope is used to measure the pulse amplitude and wave shape. The temperature is also measured before and after the PEF treatment. After a 30-second process period, it is seen that the temperature has reached 39°C at 51 kHz. Thus, the experiment is carried out at different frequencies of 1 kHz, 10 kHz, and 20 kHz for a maximum process duration of the 30s. Every time, a new sample is obtained, and three duplicates of the experiment are run. The kinetic and probability models are applied to the inactivation curve fitting in this work.

3. Survival fraction modelling of E. Coli

3.1. Bigelow Model

A popular first-order kinetic model for explaining the PEF process of microorganism inactivation is the Bigelow model. This can be expressed as (1), and when (1) is integrated, (2) is produced.

$$\frac{dN}{dt} = -k \times N \tag{1}$$

$$\log S(t) = \log\left(\frac{N}{N_0}\right) = -\frac{t}{D} \tag{2}$$

where, S(t) - Survival fraction after treatment time t

N_0 - Microorganisms population before the treatment time

N - Microorganisms population at treatment time t

k - Kinetic constant.

D - Time for decimal reduction (2.303/k).

The GInaFiT Microsoft excel Add-in is used for deriving the model constants. The first order kinetic constants k and the corresponding D values are tabulated in Table 1 by considering electric field strength and pulse frequency for pulses of 0.62, 1.2 and 7 µs. The D values show the time required for reducing the cells by one decimal and found less when the frequency is 50 kHz for all the pulse widths. The Bigelow model is fit with good precision for the 1.2 µs pulse with regression coefficient R2 > 0.85. However, the small value of k constant for all the treatment conditions showed the inactivation rate as prolonged. The results indicate that the experimental data are not followed the log-linear model. The smaller R2 values indicate the need for new mathematical models with better accuracy. The residual between the

observed survival and predicted survival is observed within + 1.5 from the Figure 1.

Table 1. First Order Kinetic Parameters for E.coli in Orange Juice Treated by the PEF Method

Pulse duration in µs	Electric field intensity in kV/cm	Frequency (kHz)	k (µs-1)	D (µs)	MSE*	R^2**
0.62	10	51	0.09	25.58889	0.15	0.8327
		20	0.06	38.38333	0.2	0.6206
		10	0.07	32.9	0.33	0.5586
		1	0.04	57.575	0.15	0.5184
	5	51	0.08	27.43353	0.1793	0.7644
		20	0.07	32.54761	0.1082	0.7924
		10	0.07	32.68038	0.2165	0.6543
		1	0.04	56.56169	0.0829	0.6228
1.2	10	51	0.11	21.014	0.0358	0.9651
		20	0.08	27.79151	0.0346	0.9424
		10	0.05	50.51422	0.0128	0.9308
		1	0.04	52.52236	0.0287	0.8466
	5	51	0.12	19.45873	0.0140	0.9880
		20	0.08	29.06155	0.0129	0.9757
		10	0.05	48.47163	0.0107	0.9455
		1	0.05	48.47163	0.0043	0.9764
7	10	51	0.05	42.08763	0.1239	0.6660
		20	0.05	43.93134	0.1330	0.6303
		10	0.05	43.64656	0.1305	0.6378
		1	0.04	51.95966	0.1761	0.4794
	5	51	0.05	46.42088	0.1644	0.5526
		20	0.05	42.44864	0.1897	0.5615
		10	0.06	40.61478	0.0883	0.7502
		1	0.05	45.57724	0.1652	0.5605

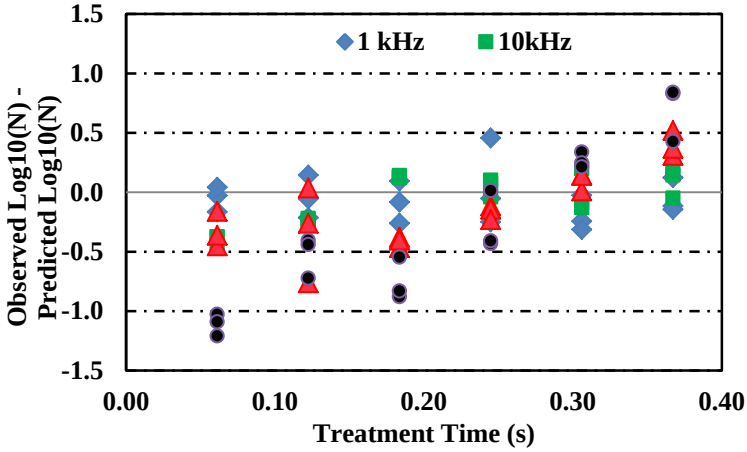

Figure. 1. Residual plot for Bigelow Model

3.2. Geeraerd Tail Model

The smaller R2 value for the Bigelow model indicates the deviation of the data derived from experiments from the log-linear method. So, a modified nonlinear model is recommended and an attempt is made. The experimental data are fit in a basic non-linear model where the effect of shoulder and/or tail could be considered. The shoulder during

the initial phase of the curve showed that the inactivation is remaining unchanged by the application of PEF during the initial period of the treatment. The tail period represents the saturation or delays in inactivation of microorganisms when they are subjected to the PEF treatment. The experimental data in our study show that shoulder effect is nullified and only the tail phase is considered to derive the Geeraerd tail model (3) and (4) [15].

Table 2. Geerared Tail Model Parameters for E.Coli in Orange Juice Treated by The PEF Method

Pulse duration in µs	Electric field intensity in kV/cm	Frequency (kHz)	k (µs-1)	D (µs)	MSE*	R2**
0.62	10	51	0.16	14.39375	0.15	0.8737
		20	0.42	5.483333	0.02	0.9755
		10	0.49	4.675058	0.0067	0.9933
		1	0.42	5.52721	0.0011	0.9974
	5	51	0.37	6.28191	0.0933	0.9080
		20	0.28	8.279801	0.0716	0.8970
		10	0.38	6.095089	0.0164	0.9804
		1	0.29	8.006624	0.0027	0.9906
1.2	10	51	0.11	21.014	0.0477	0.9651
		20	0.10	22.79011	0.0403	0.9498
		10	0.06	38.95654	0.0157	0.9360
		1	0.04	52.5225	0.0383	0.8466
	5	51	0.12	18.84737	0.0178	0.9886
		20	0.08	29.06155	0.0172	0.9757
		10	0.07	31.46809	0.0092	0.9649
		1	0.06	38.38333	0.0024	0.9899
7	10	51	0.30	7.733341	0.0081	0.9836
		20	0.37	6.236383	0.0144	0.9699
		10	0.33	7.057867	0.0066	0.9862
		1	0.50	4.592673	0.0009	0.9981
	5	51	0.42	5.522189	0.0044	0.9909
		20	0.56	4.110578	0.0171	0.9703
		10	0.26	8.936898	0.0239	0.9494
		1	0.37	6.276013	0.0014	0.9973

$$N(t) = \left(N_0 - N_{res}\right) e^{-kt} + N_{res} \quad (3)$$

$$\log_{10}(N(t)) = \log_{10}\left(\left(10^{\log_{10}(N_0)} - 10^{\log_{10}(N_{res})}\right)e^{-kt} + 10^{\log_{10}(N_{res})}\right) \quad (4)$$

where, N_0 - Initial concentration of cells

N_{res} - Concentration of residual cell

Details of the Geeraerd tail model parameters which are calculated from the experimental data are provided in Table 2. The R2 > 0.9 and the low MSE prove that the inactivation rate by the PEF process is not following the log-linear model. The tail effect could be due to the cell resistance to the application of the PEF during final phase. The kinetic constant (k) shows the death rate as slow against the PEF parameters considered in the present study. So, the treatment time did not influence the inactivation rate when it is increased and the residual is observed within + 1 from the Figure 2

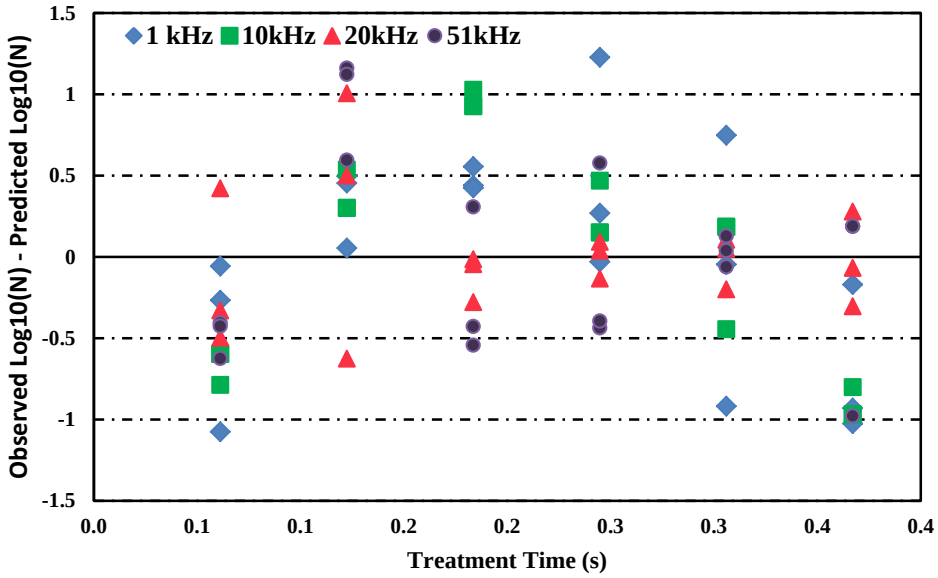

Figure. 2. Residual plot for Geeraerd Tail Model

3.3. Biphasic Model

The k and D values developed in sterilization procedures does not have the ability to deal the classical non-log linear behavior of the survival curves derived in the PEF technique. Many survival curves show tailing effect for both the thermal and non-thermal methods. This tailing effect might be due to the intrinsic resistance of some microorganisms in the cell population to the PEF parameters. As a result, under some circumstances, highly resistant microbes can live or the pace of mortality reduction would be slowed down. The Biphasic survival curves explain the combination of subpopulation of different electrical resistance that follows first-order kinetics and can be expressed as Equations (5-7).

$$\frac{dN_1}{dt} = -\mathbf{k_1} \times N_1;\ \ N_1(\mathbf{0}) = \mathbf{N_{01}};\ (\mathbf{N_{01} > 0\ at\ t \geq 0}) \tag{5}$$

$$\frac{\mathbf{dN_2}}{\mathbf{dt}} = -\mathbf{k_2} \times \mathbf{N_2}; \mathbf{N_2(0)} = \mathbf{N_{02}}; (\mathbf{N_{02} > 0\ at\ t \geq 0}) \tag{6}$$

$$\mathbf{N(t) = N_1(t) + N_2(t)} \tag{7}$$

The solution to the Equations (5-7) can be given by the Equation (8), and the corresponding integration is written as the Equation (9).

$$N(t) = N_0(fe^{-k_1 t} + (1-f)e^{-k_2 t}) \tag{8}$$

$$\mathrm{Log}_{10} S(t) = \mathrm{Log}_{10}\left(\frac{N}{N_0}\right) = \mathrm{Log}_{10}(fe^{-k_1 t} + (1-f)e^{-k_2 t}) \tag{9}$$

where, N(t) - concentrations of the entire populace

Table 3. Biphasic model parameters for E.coli in orange juice treated by PEF method

Pulse duration in µs	Electric field intensity in	Frequency (kHz)	f	k1	k2	MSE*	R2**

	kV/cm						
0.62	10	51	0.9123	2.91	0.06	0.0279	0.9841
		20	0.9497	2.67	0.02	0.0017	0.9984
		10	0.9795	0.64	0.01	0.0002	0.9999
		1	0.9293	0.52	0.01	0.0001	0.9999
	5	51	0.9357	3.14	0.04	0.0203	0.9866
		20	0.8799	2.66	0.04	0.0147	0.9859
		10	0.9618	0.48	0.02	0.0035	0.9972
		1	0.8715	0.37	0.01	0.0001	0.9998
1.2	10	51	0.5185	0.42	0.10	0.0498	0.9758
		20	0.6887	0.32	0.06	0.0212	0.9824
		10	0.6127	0.15	0.03	0.0155	0.9580
		1	0.9674	0.04	0.04	0.0575	0.9466
	5	51	0.9124	0.12	0.10	0.0498	0.9758
		20	0.9083	0.08	0.06	0.0212	0.9824
		10	0.5561	0.15	0.03	0.0155	0.9580
		1	0.8561	0.07	0.01	0.0036	0.9901
7	10	51	0.9222	0.38	0.02	0.0021	0.9972
		20	0.9112	0.76	0.02	0.0027	0.9963
		10	0.9208	0.44	0.02	0.0001	0.9998
		1	0.9394	0.77	0.00	0.0005	0.9993
	5	51	0.9197	0.44	0.02	0.0077	0.9896
		20	0.9432	0.04	0.01	0.0097	0.9888
		10	0.8611	0.68	0.03	0.0029	0.9958
		1	0.9465	0.41	0.01	0.0003	0.9996

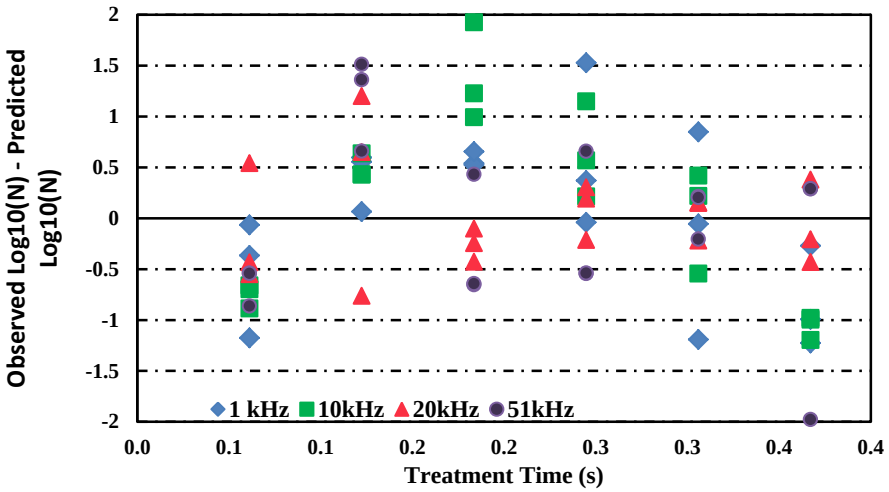

Figure. 3. Residual plot for Biphasic Model

N1(t) - Concentrations of the low resistant microorganism
N2(t) - Concentrations of the high resistant microorganism
k1 - Death rate constant for N1(t)
k2 - Death rate constant for N2(t)
f - Initial ratio of the percentage with lower resistance
(1-f) - Initial ratio of the percentage with greater resistance

The Biphasic model parameters for E.coli in orange juice are shown in Table 3. The k2 values are found to be smaller for all the treatment conditions considered in the present study indicating that the highly resistant cells in the total cell population are more reluctant to the PEF parameters of pulse frequency, electric field intensity, and process time. So, the high resistant cells could be requiring higher field strength and lengthier process time.

When the pulse width of 0.62 µs at 0 kV/cm is selected, the k1 value is found as 2.67 at 20 kHz and 2.91 at 51 kHz. Similarly, the k1 value is high as 3.14 at 20 kHz and 2.66 at 51 kHz at 5 kV/cm. This show the shorter pulses in the range of 20 to 51 kHz are inactivating the less resistant microorganisms at a faster rate. The R2 > 0.9466 and MSE < 0.06 values show the Biphasic model fits well with the experimental data than Bigelow and Geeraerd tail model. Also, it is observed from the Figure 3 that the residuals are within + 2.

3.4. Weibull Model

Applying basic kinetic theories when the inactivation process involves multiple parameters is not very realistic. A versatile model to explain microbial inactivation is the Weibull distribution. It has been effectively applied to explain the PEF method of microbial inactivation. The Weibull distribution is an empirical model that provides a statistical explanation of a failure time distribution in the main and does not relate the survival ratio to the mechanistic hypotheses.

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\delta}\right)^{p} \tag{10}$$

where,

δ - Scale parameter

p - Shape parameter

t - PEF control parameter

When p is less than 1, the Weibull distribution has a concave upward survival curve and it has a concave downward survival curve if p is greater than 1. When p = 1, the Weibull distribution reduces to an exponential distribution, and the model becomes the well-known first-order kinetic equation. When p is less than 1, the scale parameter δ grows with the time, and when p is greater than 1, it decreases. The probability of the remaining active cells dying is very low when p < 1, and it increases when p > 1, making the surviving cells more vulnerable to the PEF settings. There is no biological variation and every cell is similarly responsive at p = 1, regardless of the length of treatment [16].

The distribution parameters of Weibull model in inoculation of E.coli in orange juice are tabulated in Table 4. The shape parameter p was found to be less than 1 indicating the inactivation of E.coli not following log-linear curve except under the treatment conditions of 1.2 µs pulse width at 5 kV/cm. Therefore, the existence of microbe resistance, which progressively changes because of the application of electric field intensity, may be the cause of the upward concavity of the survival curves.

The cumulative distribution of microbial inactivation is represented by the scale parameter (δ) of the Weibull distribution. An increase in the value of δ for a given shape parameter (p) indicates the wide distribution of microbial response against the PEF parameters among the individual cells of the population. In this instance, the microbial resistance probability density function is extended. In the present study, the scale parameter (δ) was higher for the pulse width of 1.2 µs, and its value is changed from minimum of 16.8 to maximum of 50.64. But the parameter δ decreased for the other two pulse widths of 0.62 and 7 µs. This relatively small value of δ represents that the majority of the microorganism as having been killed at a specified process time. The R2 number indicated how well the model fit the data and it is found as > 0.9701 for all the treatment conditions in case of Weibull distribution model.

Table 4. Constants for the Weibull distribution for inactivation of E.coli in orange juice treated by the PEF method

Pulse duration in μs	Electric field intensity in kV/cm	Frequency (kHz)	δ	p	MSE*	R^2**
0.62	10	51	5.63	0.38	0.0365	0.9688
		20	1.06	0.14	0.0039	0.9945
		10	0.72	0.13	0.0221	0.9777
		1	1.33	0.07	0.0001	0.9997
	5	51	3.53	0.28	0.0273	0.9731
		20	10.49	0.34	0.0208	0.9701
		10	1.44	0.18	0.0016	0.9981
		1	25.69	0.15	0.0002	0.9993
1.2	10	51	16.89	0.81	0.0385	0.9719
		20	18.42	0.63	0.0175	0.9781
		10	47.41	0.63	0.0089	0.9639
		1	50.64	2.83	0.0104	0.9584
	5	51	20.45	1.05	0.0179	0.9885
		20	31.10	1.13	0.0154	0.9783
		10	45.24	0.68	0.0071	0.9729
		1	48.67	0.81	0.0035	0.9855
7	10	51	7.07	0.19	0.0013	0.9973
		20	4.90	0.15	0.0040	0.9917
		10	5.67	0.16	0.0002	0.9997
		1	37.96	0.00	0.0220	0.9511
	5	51	1.18	0.09	0.0012	0.9975
		20	0.46	0.09	0.0101	0.9825
		10	12.54	0.26	0.0037	0.9921
		1	1.27	0.08	0.0017	0.9967

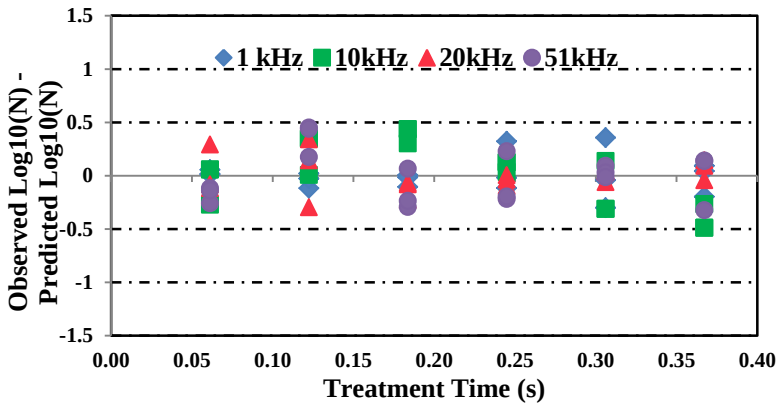

Figure. 4. Residual plot for Weibull Model

4. Summary

The present study shows the survival rate of E.coli in the orange juice when subjected to 0.62, 1.2, and 7μs pulses at 5 and 10 kV/cm. The pulse frequency is varied as 1, 10, 20 and 51 kHz. The transient and frequency analysis of the biological cell demonstrate in the suitability of the shorter pulses along with the high frequency.

The experimental data are fitted with the well-known models to enable a study of the effect of the the pulse frequency width and. Though Bigelow model is simple, the kinetic constant,

MSE and R2 values show that the experimental data are not following the log-linear model. Geeraered tail model also proves that the PEF inactivation is not following the first order kinetic model. But Biphasic model kinetic parameters (k1 and k2) are used for distinguishing the inactivation rate in log-linear phase and tail phase. The variations in these two parameters indicate the absence of uniformity in cell resistance when the cells were exposed to the PEF parameters. However, Weibull model as the highly recommended model in the food industry shows inactivation rate as uniform due to several inextricable PEF parameters.

REFERENCES

[1] *S. Haberl, D. Miklavcic, G. Sersa, W.Frey, and B. Rubinsky, "Cell membrane electroporation – Part 2 The applications, "IEEE Electrical Insulation Magazine, vol. 29,* ***(2013)****, pp. 29–37.*

[2] *T. Kotnik, W. Frey, M. Sack, S. Haberl-Meglic, M. Peterka, and D. Miklavcic, "Electroporation-based applications in biotechnology," Trends in Biotechnology, vol. 33,* ***(2015)****, pp. 480–488.*

[3] *E. Puertolas,E. Luengo, I. Alvarez, and J. Raso, "Improving mass transfer to soften tissues by pulsed electric fields: Fundamentals and applications," Palo Alto: Annual review of food science and technology, vol. 3,* ***(2012)****, pp. 263–282,*

[4] *S. Toepfl, "Pulsed electric field food processing-Industrial equipment design and commercial applications," Stewart Postharvest Review, vol. 2,* ***(2012)****, pp. 1–7.*

[5] *G. Donsi, G. Ferrari, and G. Pataro, "Inactivation kinetics of Saccharomyces cerevisiae by pulse electric field in a batch treatment chamber: The effect of electric field unevenness and initial cells concentration," Journal of Food Engineering, vol. 78,* ***(2007)****, pp. 784–792.*

[6] *Z. Zhang, "Parametric regression model for survival data: Weibull regression model as an example. Ann Transl Med, vol. 4(24),* ***(2016)****, pp. 484.*

[7] *Z. Zhang, "Model building strategy for logistic regression: purposeful selection," Ann Transl Med, 4(6),* ***(2016),*** *pp. 111.*

[8] *Z. Bursac, C.H. Gauss, D. K. Williams, and W. Hosmer, "Purposeful selection of variables in logistic regression," Source Code Biol Med, vol. 3, (2008), pp. 1-8.*

[9] *N.J. Hoboken, "Applied logistic regression", Edited by D.W. Hosmer, S. Lemeshow , R. X. Sturdivant, John Wiley & Sons,* ***(2000)****, pp.1-63.*

[10] *Z. Zhang, K. Chen, H. Ni H, and F. Haozhe, "Predictive value of lactate in unselected critically ill patients: an analysis using fractional polynomials," J Thorac Dis, vol. 4(6),* ***(2014)****, pp. 995-1003.*

[11] *Z. Zhang, and H. Ni, "Normalized lactate load is associated with development of acute kidney injury in patients who underwent cardiopulmonary bypass surgery," PLoS One, vol. 10(3),* ***(2015)****, e0120466.*

[12] *Z. Zhang, and X. Xu, "Lactate clearance is a useful biomarker for the prediction of all-cause mortality in critically ill patients: a systematic review and meta-analysis", Crit Care Med, vol. 4(42),* ***(2014)****, pp. 2118-2125.*

[13] *D.W. Hosmer, and N.L. Hjort, "Goodness-of-fit processes for logistic regression: simulation results," Stat Med, vol. 21,* ***(2002)****, pp. 2723-38.*

[14] *S. Krishnaveni, and V.Rajini, "Diode clamped gate driver-based high voltage pulse generator for electroporation, Turkish Journal of Electrical Engineering and Computer Sciences, vol. 26,* ***(2018)****, pp. 2374 – 2384.*

[15] *A. H. Geeraerd, C. H. Herremans, and J. F. Van Impe, "Structural model requirements to describe microbial inactivation during a mild heat treatment," Int J Food Microbiol, vol. 10(59(3)),* ***(2000)****, pp. 185-209.*

[16] *M. A. J. S. van Boekel, "On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells," Int J Food Microbiol, vol. 25(74(1-2)),* ***(2002)****, pp. 139-59.*