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Modeling Yeast Cell Viability in Continuous-Flow Pulsed Electric Field Treatment

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Abstract

In continuous-flow pulsed electric field (PEF) chambers, the number of pulses a cell receives is the product of repetition rate and residence time, and two combinations with the same product may treat the cell differently. This study treated *Saccharomyces cerevisiae* suspensions in a parallel-plate chamber at fields of 300 to 500 kV/m, repetition rates of 150 to 250 Hz, flow rates of 10 to 20 mL/min, and 4 μ s pulses, and recorded cell viability, voltage and current waveforms, and temperature rise. At 500 V, 200 Hz, and 15 mL/min, viable-cell density fell by about 1.5 log; at 250 Hz or at 10 mL/min, it fell below the detection limit. Two conditions with the same effective pulse number differed in viable-cell density by a factor of 3.12, with no overlap between replicates ($p = 5.8 \times 10^{-4}$). An effective-dose model, which treats repetition rate and residence time as separate factors and computes the thermal contribution from literature kinetic constants, reproduced the experimental medians ($R^2 = 0.9979$, a fit expected with three coefficients estimated from five conditions with a positive median); leave-one-out cross-validation gave R_{LOO}^2 from 0.61 to 0.84, depending on the fold assignment. The computed thermal dose was more than four orders of magnitude below the electric dose. In continuous-flow PEF, repetition rate and flow rate should be specified separately, not only through the pulse number they produce.

Keywords: Irreversible Electroporation, Continuous-Flow PEF, Cell Viability, Thermal-Electrical Coupling, *Saccharomyces cerevisiae*

1 Introduction

Electroporation is induced by the application of electric pulses capable of temporarily increasing cell-membrane permeability. This response has been explored in biomedical, biotechnological, and food-processing contexts, including molecular delivery, electrochemotherapy, and pulsed-electric-field (PEF) food processing (Belehradek et al., 1993; Campana et al., 2019; Kotnik et al., 2015; Neumann, Schaefer-Ridder, Wang, & Hofschneider, 1982; Rodrigues et al., 2027; Weaver, 1993). In liquid foods and biological suspensions, PEF technology enables rapid treatments with reduced undesirable thermal load, supporting microbial inactivation while tending to preserve product-quality attributes such as color, flavor, texture, and nutritional value (Barba et al., 2015; Delso, Berzosa, Sanz, Álvarez, & Raso, 2023; Ghoshal, 2023; Lytras et al., 2024; Ortega-Rivas, 2011; Salinas-Roca, Elez-Martínez, Welte-Chanes, & Martín-Belloso, 2017).

Despite these advantages, optimizing electroporation-based processes is challenging because the biological response depends on multiple coupled factors, including cell type, electric-field amplitude, pulse width, number of pulses, repetition rate, electrical properties of the medium, exposure time, and temperature (Barba et al., 2015; Ghoshal, 2023; Huang, Tian, Gai, & Wang, 2012; Miklavčič, Mali, Kos, Heller, & Serša, 2014; Pintarelli, da Silva, Yang, & Suzuki, 2022). Many experimental descriptions and kinetic models have been developed under batch or laboratory-controlled exposure conditions, in which the treated volume, exposure time, pulse sequence, and thermal boundary conditions can be directly prescribed. In such configurations, the electrical exposure is often described from the imposed pulse protocol. However, extrapolating these descriptions to flow-through systems is not straightforward, because each cell experiences the treatment while moving through a nonuniform electrical, thermal, and hydrodynamic environment (Pae, Kim, Ri, Han, & Jo, 2025; Sastry, 2023).

For industrial implementation, PEF processing is commonly expected to operate under continuous-flow conditions. This transition introduces an additional level of complexity because electrical exposure becomes coupled with residence time, volumetric flow rate, hydrodynamic stability, and temperature rise. In addition, continuous-flow operation shifts the design problem from isolated pulse delivery to the combined effects of pulse energy, repetition rate, average power, and heat removal. In flow-through chambers, the same effective number of pulses can occur under different combinations of flow rate and repetition rate, leading to different thermal signatures, local exposure histories, current responses, and apparent conductivity changes in the treated medium. Therefore, experimental protocols for continuous-flow PEF must document chamber geometry, electrical conditions, and thermal variables (Morales-de La Peña, Elez-Martínez, & Martín-Belloso, 2011; Qin, Barbosa-Cánovas, Swanson, Pedrow, & Olsen, 1998; B. Yan et al., 2025). Microbiological assessments

must follow established analytical guidelines (Castellucci, 2010). Specifically, Resolution OIV/OENO 206/2010, issued by the Oenology Commission (OENO) of the International Organisation of Vine and Wine (OIV), details the methods for detecting, differentiating, and enumerating microorganisms in wines and musts (Castellucci, 2010).

Yeasts, such as *Saccharomyces cerevisiae*, are a biological system for investigating PEF protocols because of their importance in fermentation processes and the availability of established methods for assessing cell viability. Previous studies show that yeast inactivation depends on electric-field intensity, number of pulses, initial cell density, growth phase, treatment temperature, treatment duration, medium conductivity, and the possibility of sublethal injury or cellular recovery after exposure (Cserhalmi, Vidács, Beczner, & Czukor, 2002; Emanuel et al., 2020; Hülshager, Potel, & Niemann, 1983; Martínez, Cebrián, Álvarez, & Raso, 2016). Irreversible electroporation thresholds for yeast cells are commonly reported in the order of hundreds of kV/m (300–750 kV/m) (El Zakhem, Lanoisellé, Lebovka, Nonus, & Vorobiev, 2006; Gášková, Sigler, Janderová, & Plášek, 1996), with values depending on strain, medium composition, cell concentration, pulse protocol, and viability criterion. As a representative value, 500 kV/m (eight 100 μ s pulses at 1 Hz) reduced viable yeast density from 2.5×10^7 to 10^5 cells/mL in previous yeast-electroporation work (Pintarelli, Ramos, da Silva, Rossi, & Suzuki, 2021), measured by dye exclusion. As microbiological context, Loureiro and Malfeito-Ferreira (2003) report that bottled white wine appears limpid only with a load below 10^4 – 10^5 colony-forming units (CFU) per mL for powdery-sediment yeasts, and below 10^2 – 10^3 CFU/mL for flocculent-sediment ones. The two scales are not interchangeable, and the comparison between them is read as a one-sided bound. Thresholds near 750 kV/m are reported for dense suspensions, in which cell clustering raises the required field (El Zakhem et al., 2006).

Although electroporation has been widely studied, continuous-flow PEF still poses modeling and process-optimization challenges. Mathematical descriptions based only on pulse number, treatment time, delivered energy, or electric-field intensity may not distinguish operating conditions that produce the same nominal exposure but different chamber residence times, repetition rate effects, and pulse signatures. This distinction is important because electroporation is a dynamic membrane-level phenomenon, and the biological outcome may depend not only on the total applied electrical dose, but also on how the pulses are distributed in time and experienced by cells moving through the treatment zone. Formulations that decouple repetition rate and flow rate can therefore provide a more consistent interpretation of continuous-flow experiments and support model-based selection of operating conditions that balance cell inactivation, temperature rise, and treatment capacity.

This study addresses this issue through an experimental assessment of PEF in continuous flow applied to *Saccharomyces cerevisiae*. The protocol combines microbiological viability analysis, voltage and current waveforms, temperature-rise measurements, and an effective-dose mathematical model. The proposed model organizes the dominant effects observed within the evaluated experimental range and provides a framework for comparing voltage, repetition rate, and volumetric flow rate.

The main contribution is the integration of experimental data and mathematical modeling in a controlled flow configuration: repetition rate (RR) and mean residence time (t_{res}) enter the model as separate factors, and the experiment shows that conditions with equivalent effective pulse numbers differ in viability; the thermal contribution is kept as a term of its own, computed from literature kinetic constants (Török & King, 1991) and not fitted to the data. This approach adopts a cell-exposure perspective: the effective treatment is defined from the moment a cell enters the chamber until it leaves the active treatment region. Because electroporation occurs at the cellular membrane level, this perspective allows operating conditions with similar effective pulse numbers ($N_p = RR \cdot t_{res}$) to be compared while preserving their distinct exposure histories.

2 Materials and Methods

2.1 Strain Preparation and Cell Suspension

Saccharomyces cerevisiae strain TB10 (Levteck Tecnologia Viva LTDA, Florianópolis, Brazil) was used as the biological model in all *in vitro* experiments. Cell preparation followed the cultivation routine used in previous yeast-electroporation studies conducted in the laboratory (Pintarelli et al., 2021), with adjustments for the continuous-flow protocol adopted in this work. Cells were grown in liquid *Yeast Peptone Dextrose* (YPD; 2% peptone, 1% yeast extract, and 2% glucose), previously sterilized by autoclaving. After incubation for 24 h at 30 °C, the suspension was homogenized and its initial viability was determined using the methylene-blue procedure described in Section 2.3.

2.2 Electroporation Setup

PEF experiments were conducted in a continuous-flow system comprising an inlet reservoir, a peristaltic pump (K100, OFA Ambiental, São Paulo, Brazil; 2.5–100 mL/min, silicone tubing of 3 mm inner diameter), a parallel-plate chamber, voltage and current measurement, temperature sensors, and an outlet collection tube. The working flow rates were set on the pump and verified gravimetrically at each flow-rate change, from the mass collected at the outlet over a timed interval. Figure 1 shows the general experimental arrangement. The electroporation chamber was manufactured with AISI 304 stainless-steel parallel electrodes and an acrylic-resin body. The PEF chamber was 10 mm wide, 26 mm long, and 1 mm high, with a 1 mm electrode gap. During the experiments, the suspension flowed horizontally through the chamber channel, crossed the active electric-field region, and was collected after treatment.

The cell suspension was held at 17 °C in the inlet reservoir by a Peltier stage with its own controller. Two thermoelectric modules (TEC1-12706) cooled the treatment chamber and its electrodes throughout pulse application. Negative-temperature-coefficient (NTC) thermistors of 10 k Ω , positioned in the reservoir, in the chamber/electrode region, and in the room air, served for monitoring only, with no closed-loop control of the cooling. The temperature rise used in the model, ΔT , is the difference between the temperature of the suspension collected at the outlet at the end of the run and that of the inlet reservoir before treatment; because it is

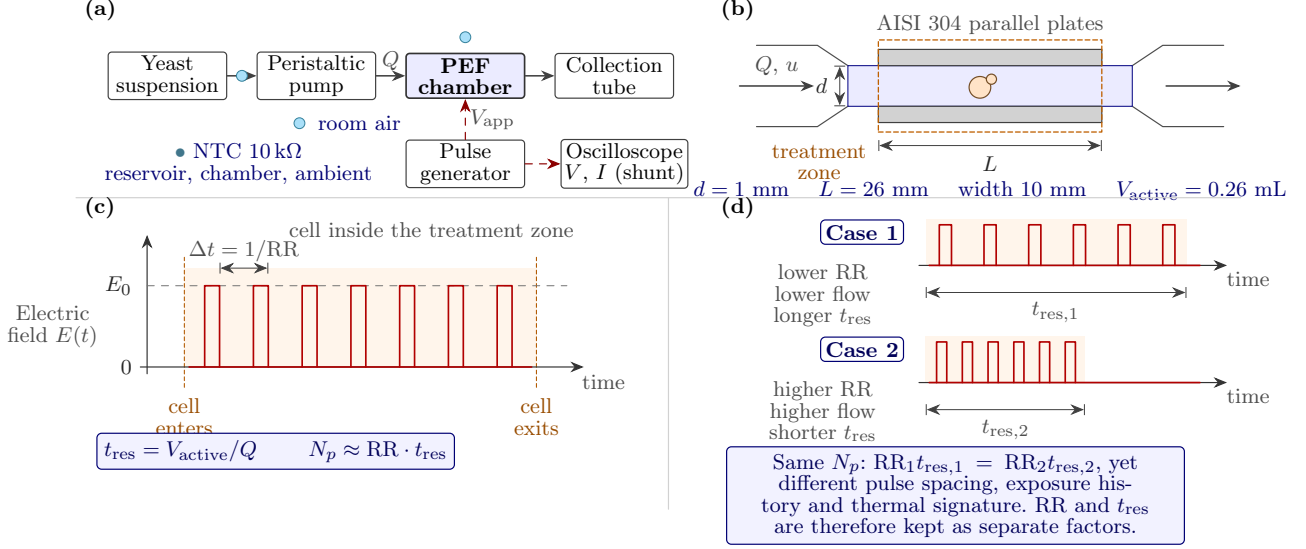


Fig. 1 Continuous-flow PEF treatment and the exposure experienced by a single cell. **(a)** Hydraulic and electrical chain: the yeast suspension is driven by a peristaltic pump through the treatment chamber and collected at the outlet, while the pulse generator drives the electrodes and the oscilloscope records voltage and current, the latter across a resistive current shunt; NTC thermistors are placed in the reservoir, in the chamber region, and in the room air. **(b)** Longitudinal cross-section of the parallel-plate chamber, showing the treatment zone and a cell in transit; the electrode gap is exaggerated relative to the channel length for legibility. **(c)** Electric field seen by one cell while it crosses the treatment zone: the cell is exposed only between entry and exit, so the effective pulse number is $N_p \approx \text{RR} \cdot t_{\text{res}}$, with $t_{\text{res}} = V_{\text{active}}/Q$. **(d)** Two conditions with the same N_p but different pulse spacing and residence time. Repetition rate and residence time are treated as separate factors, not collapsed into a single pulse count, because the exposure history and the thermal signature differ.

measured on the collected liquid, it includes passive exchange along the outlet tubing. The chamber/electrode reading served only as an indication of local heating. For each condition, the median of the seven replicates was adopted.

In each PEF experiment, the cell suspension was transferred to the inlet reservoir and driven by the peristaltic pump through the treatment chamber in a single pass, and collected at the outlet. The hydraulic circuit requires 5 mL to fill the tubing before treatment starts. Monopolar rectangular pulses of $4 \mu\text{s}$ were applied to the chamber by a custom electroporator (IEB-UFSC 3.0). The applied voltage ranged from 300 to 500 V, corresponding to 300–500 kV/m for the 1 mm electrode gap. The electric field was calculated as $E = V/d$. The repetition rate ranged from 150 to 250 Hz, the volumetric flow rate from 10 to 20 mL/min, and each run lasted from 25 to 67 s across the experimental matrix, so that the number of applied pulses corresponds to the product of duration and repetition rate. This total duration was recorded for operational control, whereas the effective cellular exposure time used in the model was defined as the mean residence time inside the active chamber volume. Therefore, the model did not use the total run duration as the treatment time, but rather $t_{\text{res}} = V_{\text{active}}/Q$, which is the residence time of a single pass.

The experimental matrix was organized into three controlled groups, each varying only one primary parameter: applied voltage (V), pulse repetition rate (RR), or volumetric flow rate (Q). In the voltage series, RR and Q were fixed at 200 Hz and 15 mL/min, respectively. In the repetition rate series, V and Q were fixed at 500 V and 15 mL/min, respectively. In the volumetric flow rate series, V and RR were fixed at 500 V and 200 Hz, respectively. Table 1 summarizes the controlled experimental matrix.

Table 1 Controlled experimental matrix used to assess the main effect of each operational variable.

Series	Variable	Fixed conditions	Range
Voltage	V, E	$RR = 200 \text{ Hz}; Q = 15 \text{ mL/min}$	300–500 V; 300–500 kV/m
RR	RR, N_p	$V = 500 \text{ V}; Q = 15 \text{ mL/min}$	150–250 Hz
Flow rate	Q, t_{res}	$V = 500 \text{ V}; RR = 200 \text{ Hz}$	10–20 mL/min

The electrical conductivity of the medium was measured using a portable conductivity meter model (HI 8633, Hanna Instruments, Woonsocket, RI, USA) equipped with a four-ring conductivity probe (HI 76301D). The measured bulk conductivity of the YPD suspension was 1.8 S/m, compensated to 25 °C with a manual coefficient of 2 %/°C. Apparent conductivity in the chamber is $\sigma_{app} = I d / (VA)$, with plateau current I , gap d , voltage V , and effective area A , and it was computed in two ways: from the nominal voltage and the campaign-recorded currents, which is the value used in the model estimation, and from the plateau means of voltage and current in the oscilloscope waveforms.

Measurements were taken with an oscilloscope (DPO2012B, Tektronix, Beaverton, OR, USA), a resistive current shunt (RS300-75, HOBUT, Walsall, UK; 300 A / 75 mV, i.e. 0.25 m Ω , accuracy class 0.5, useful range 10–120 % of the rating) and NTC thermistors (stainless-steel capsule, Novus Automation, Porto Alegre, Brazil; $R_{25} = 10 \text{ k}\Omega \pm 1 \%$). The shunt is specified for direct current, and the supplier does not state the parasitic inductance that governs the response at the pulse edge. NTC response time is 9 s to 63 % of a step, and inlet and outlet temperatures were recorded after the reading had stabilized. Voltage and current waveforms were recorded during the PEF protocol following procedures similar to those described by [Pintarelli et al. \(2021\)](#). Each record was baseline-corrected by subtracting the median of its initial samples from both channels. The pulse interval was located on the voltage channel as the samples above 50% of the positive peak, which also set the pulse width. To separate the switching-edge transients from the conduction current, the plateau was taken as the central part of this interval, discarding its leading and trailing quarters; the mean and root-mean-square (RMS) plateau currents were computed only there, whereas the edge-transient current was recorded as the channel’s maximum absolute value. Electrical energy per pulse was estimated by integrating the positive part of $V(t)I(t)$ over the pulse, edge transients included, whereas conductivity uses only the plateau. This energy is an electrical estimate, not the heat absorbed by the liquid, and it does not enter the viability model.

2.3 Cell-Viability Assessment

Cell viability was assessed by methylene-blue staining, a rapid method widely used to estimate yeast viability (Kwolek-Mirek & Zadrag-Tecza, 2014; Painting & Kirsop, 1990). Viable cells reduce or exclude the dye and remain unstained, whereas nonviable cells remain blue. Samples were diluted 1:2 with methylene blue, manually homogenized, and held for 2 min before counting. Stained samples were evaluated with a microscope (LC1600BA, Biocentrix, São Paulo, Brazil) using a 40 $\times$ objective coupled with a 2.0 MP USB eyepiece camera (NXM-EP200, Microscope, China; 1600 $\times$ 1200 pixels at 15 frames/s). Counts were performed in a Neubauer chamber hemocytometer with $N = 7$ replicates per experimental condition, counting viable and nonviable cells. The counting procedure and reporting of cell concentration were aligned with reference microbiological procedures, including OIV/OENO Resolution 206/2010 (Castellucci, 2010). Percentage viability was calculated as $100 \cdot N_v / (N_v + N_{nv})$, where N_v and N_{nv} represent viable- and nonviable-cell counts, respectively. The viable-cell density was expressed in cells/mL and was used as a response variable in the mathematical model. Dye exclusion and colony formation are not the same quantity: cells that exclude methylene blue include cells that no longer divide, so a dye-based density is an upper bound on the colony-forming density of the same sample (Kwolek-Mirek & Zadrag-Tecza, 2014). Reference levels reported in CFU/mL are therefore compared in one direction only — a density below such a level in cells/mL is below it in CFU/mL as well — and no condition is classified here as lying above a CFU-based level. The initial viable-cell density was used as the experimental reference for the mathematical model, and ranged from 5.4×10^5 to 6.7×10^5 cells/mL across the three control campaigns.

2.4 Mathematical Viability Modeling

Mathematical models have been used to describe microbial response to electric field, treatment time, and temperature. Classical formulations applied to thermal and PEF processes include Weibull-type models (Huang et al., 2012), Hülshager-type descriptions (Hülshager & Niemann, 1980; Hülshager, Potel, & Niemann, 1981; Hülshager et al., 1983), and Peleg-type models (Peleg, 1995). These formulations provide the conceptual basis for representing cellular reduction under treatment.

The proposed model describes the effective electric dose received by cells during passage through the parallel-plate chamber. The formulation extends the logic of microbial-survival models to a continuous-flow PEF condition. Pulse repetition rate and mean residence time are represented by independent factors because conditions with the same product $RR \cdot t_{res}$ may produce different biological responses. The final viable-cell density was described by:

$$C_v = C_{v_0} \cdot e^{-(\Omega_T + \Omega_E)} \quad (1)$$

In Equation (1), C_v is the final viable-cell density, C_{v_0} is the initial viable-cell density, Ω_T is the thermal contribution, and Ω_E is the electroporation contribution.

The thermal contribution is not an adjustable parameter; rather, it is determined from published kinetic constants (Török & King, 1991). Thermal inactivation of vegetative yeast cells is described by first-order kinetics whose rate increases log-linearly with temperature, conventionally parameterized by the decimal reduction time:

$$D(T) = D_{ref} \cdot 10^{(T_{ref}-T)/z} \quad (2)$$

In Equation (2), $D(T)$ is the heating duration that reduces the viable population by a factor of ten at a given temperature (T), D_{ref} is its value at the reference temperature (T_{ref}), and z is the temperature change that shifts D by one decade. As the suspension heats while crossing the chamber, the accumulated lethality is the integral of $dt/D(T(t))$ over the residence time. For a temperature rising linearly from the inlet value T_{in} to the outlet value $T_{out} = T_{in} + \Delta T$, that integral has the closed form:

$$\Omega_T = \frac{z t_{res}}{D_{ref} \Delta T} \left[10^{(T_{out}-T_{ref})/z} - 10^{(T_{in}-T_{ref})/z} \right] \quad (3)$$

In Equation (3), $\Delta T = T_{out} - T_{in}$ is the liquid temperature rise. The three kinetic constants were taken from published measurements on vegetative cells of *Saccharomyces cerevisiae* (Török & King, 1991), which reports $D_{ref} = 2.67$ min at $T_{ref} = 55$ °C with $z = 6.23$ °C for the more heat-resistant of its two strains; that strain was adopted as the conservative choice, because it underestimates rather than overestimates the thermal effect. The thermal term is therefore fully determined by literature constants, without consuming any degree of freedom of the estimation.

Integrating along the temperature ramp is not an optional refinement. Because $1/D$ grows exponentially with T , the mean of the rate along the ramp is much larger than the rate evaluated at the mean temperature, and evaluating Equation (3) at the mean chamber temperature instead of integrating underestimates Ω_T by a factor of 96.6 in the most severe condition. The ratio is $2 \sinh(a/2)/a$, with $a = \ln(10)\Delta T/z$, using $\Delta T = 39.2$ °C and $z = 6.23$ °C under the linear-ramp assumption.

With the chamber actively cooled during pulse application, ΔT is not a free input: it is set by the steady-state balance between Joule dissipation and the heat removed by the cooling path. That balance equates the power absorbed by the liquid, $\rho c_p Q \Delta T$, to the difference between the mean Joule power, $VI\tau RR$, and the removal, $UA_{cool}(\bar{T} - T_{cool})$. In it, ρc_p is the volumetric heat capacity of the medium, Q is the volumetric flow rate, the product $VI\tau RR$ is obtained from the measured pulse voltage, plateau current, pulse width, and repetition rate, $\bar{T}$ is the mean liquid temperature in the chamber, and T_{cool} is the cold-side set point, taken at the inlet. The effective thermal conductance of the cooling path, UA_{cool} , is a lumped quantity: it aggregates the thermoelectric modules, the stainless-steel electrodes, the thermal interface, and the mechanical contact into a single effective value. Taking $\bar{T} = T_{in} + \Delta T/2$, and $T_{in} = T_{cool} = 17$ °C, which the temperature-controlled stage held within 16.5–17.6 °C across the 63 replicates, the balance becomes explicit in ΔT :

$$\Delta T = \frac{VI\tau RR}{\rho c_p Q + UA_{cool}/2} \quad (4)$$

Solved for UA_{cool} , Equation (4) gives one value per campaign from the measured ΔT ; the median of these values, excluding the most discrepant campaign, was adopted, $UA_{cool} = 0.83$ W/K, and this single value is used for all conditions. The viability-model coefficients were estimated with the measured ΔT ; Equation (4) serves to estimate ΔT where it was not measured, as in the operating map. The effective electric contribution was defined as:

$$\Omega_E = k_E \cdot \sigma \cdot \tau \cdot \left(\frac{RR}{RR_0} \right)^{\eta_R} \cdot \left(\frac{t_{res}}{t_0} \right)^{\eta_t} \cdot \left(\frac{\max(0, E - E_c)}{E_0} \right) \quad (5)$$

In Equation (5), k_E is the effective electric-coupling coefficient, σ is the effective electrical conductivity, τ is the pulse width, RR is the pulse repetition rate, RR_0 is the reference rate, t_{res} is the mean residence time, t_0 is the reference time, E is the reported electric field, E_c is the effective irreversible electroporation threshold, E_0 is the reference electric field, fixed at 1 kV/cm, that is, 100 kV/m, η_R represents the effective sensitivity to repetition rate, η_t represents the effective sensitivity to residence time. The field factor enters linearly above the threshold, which corresponds to holding the field exponent at unity (Table 2). The operator $\max(0, E - E_c)$ ensures that fields below E_c produce no electric contribution, so that Ω_E becomes active only above the threshold. We interpret E_c as an effective irreversible-electroporation threshold, specific to this chamber, medium, and pulse form, and not as a universal biophysical constant. Normalized by E_0 , the field factor is dimensionless, and the coefficient k_E carries the unit $(\text{S/m})^{-1}\text{s}^{-1}$ obtained from the dimensional analysis of Ω_E ; it is an effective coefficient, without a named SI unit.

The mean residence time was estimated as $t_{res} = V_{active}/Q$, where V_{active} is the active volume of the treatment chamber (the volume between the electrodes, $A \cdot d \approx 0.26$ mL) and Q is the volumetric flow rate. The effective number of pulses received by a cell during one passage through the chamber was calculated as $N_p = RR \cdot t_{res}$. Table 2 lists the estimated coefficients with their confidence intervals, and Table 3 collects the nomenclature and units of every quantity used in the formulation.

Table 2 Estimated mathematical-model coefficients and confidence intervals.

Estimated coefficient	Value	CI95	Unit
k_E	$\approx 7.14 \times 10^5$	$5.91 \times 10^5 - 7.95 \times 10^5$	$(\text{S/m})^{-1}\text{s}^{-1}$
η_R	≈ 1.86	0.92–2.33	dimless
η_t	≈ 4.89	0.95–5.76	dimless

CI95: 95% *bootstrap* confidence interval ($B = 300$), with E_c and the field exponent held at their adopted values. Full nomenclature, fixed parameters, and thermal kinetic constants are listed in Table 3.

2.5 Data Analysis and Parameter Estimation

Data were organized and verified in spreadsheets and processed using Python routines. The three free coefficients $\{k_E, \eta_R, \eta_t\}$, all in the electric term, were estimated

Table 3 Main nomenclature and units used in the effective-dose model.

Symbol	Definition	Unit
C_v	Final viable-cell density	cells/mL
C_{v0}	Initial viable-cell density	cells/mL
V	Voltage applied to or measured in the chamber	V
d	Plate-to-plate distance	m
A	Effective electrode area	m ²
V_{active}	Active volume of the treatment chamber, $A \cdot d$	mL
E ; E_c	Reported electric field and effective irreversible electroporation threshold	kV/m
E_0	Reference electric field, fixed at 1 kV/cm	kV/m
ΔT	Validated thermal variation of the process, $T_{out} - T_{in}$	°C
T_{in} ; T_{out}	Liquid temperature at the chamber inlet and at the outlet	°C
$D(T)$	Decimal reduction time at temperature T	min
D_{ref}	Reference thermal decimal reduction time	min
T_{ref}	Reference temperature for thermal model	°C
z	Thermal sensitivity constant of yeast (z -value)	°C
ρc_p	Volumetric heat capacity of the medium	J/(m ³ K)
UA_{cool}	Effective thermal conductance of the active cooling	W/K
T_{cool}	Cold-side set point of the thermoelectric cooling	°C
σ	Effective electrical conductivity of the medium	S/m
τ	Pulse width	s
Q	Volumetric flow rate	mL/min
t_{res}	Mean residence time in the chamber	s
RR	Pulse repetition rate	Hz
RR_0	Reference repetition rate	Hz
N_p	Effective pulses during t_{res}	dimless
t_0	Reference residence time	s
k_E	Kinetic coefficient of electrical inactivation	(S/m) ⁻¹ s ⁻¹
η_R	Repetition-rate sensitivity exponent	dimless
η_t	Residence-time sensitivity exponent	dimless
Ω_T	Effective thermal dose, computed from literature constants	dimless
Ω_E	Effective electric dose	dimless

by global nonlinear least squares, within admissible ranges fixed a priori, from the experimental medians of the voltage, repetition rate, and volumetric flow rate series, restricted to the protocol limits evaluated in this work: 300–500 V, 10–20 mL/min, 150–250 Hz, and 4 μ s monopolar rectangular pulses. The threshold E_c and the field exponent were held fixed throughout: with only five distinct conditions of nonzero median, the data do not separate these two constants from k_E , which compensates for them without changing the sum of squares. E_c was fixed at 335 kV/m (3.35 kV/cm), within the 2.0–3.5 kV/cm range reported for the inactivation of *S. cerevisiae* (El Zakhem et al., 2006; Gášková et al., 1996) and between 300 kV/m, the level that produced no measurable inactivation in this work, and 400 kV/m, the first that did; the field exponent was set to unity, so that the field response is linear above the threshold. The estimation was carried out in the dose domain: the observed dose was obtained from the viability reduction as $\Omega_{obs} = -\ln(C_v/C_{v0})$, and the coefficients minimized the sum of squared differences between $\Omega_{obs} - \Omega_T$ and the predicted electric

dose Ω_E . The thermal dose Ω_T was computed from Equation (3) with the literature constants and subtracted from the observed dose before estimation, so that it does not compete with the electric term for the residual. Conditions with a zero median were treated as censored lower bounds and kept out of the logarithm. The coefficients were then held fixed, and the model was evaluated on the original cell-density scale, $C_{v,pred} = C_{v0} \exp[-(\Omega_T + \Omega_E)]$.

Model predictions were compared with the experimental medians using the root-mean-square error (RMSE), the coefficient of determination (R^2), the mean absolute percentage error over positive medians only (MAPE₊), and the symmetric mean absolute percentage error (sMAPE), which remains defined for the conditions with a zero median. Replicates with zero counts were kept in the analyses. The within-condition association between temperature rise and viable count was quantified by the Spearman rank correlation (Spearman, 1904). Bootstrap confidence intervals (Efron, 1979) came from $B = 300$ resamples, drawing the seven replicates of each condition with replacement, recomputing the medians, and re-estimating the three free coefficients; the reported CI95 are the 2.5th and 97.5th percentiles. Leave-one-out cross-validation (LOOCV) (Arlot & Celisse, 2010) removed one operating condition at a time, re-estimated the coefficients on the remainder, and predicted the held-out condition. The three central-condition campaigns were consolidated before forming folds, yielding seven distinct conditions (two censored and five evaluated folds). Each fold was re-estimated with 600 random multi-start initializations and seed $7000 + i$, where i is the index of the withheld condition. The restarts mitigate local minima; the sum of squares of the estimation is reproducible, but R_{LOO}^2 depends on the fold seed and is reported with that spread in Results and Discussion.

Large language models, Codex (OpenAI, San Francisco, CA, USA) and Gemini (Google LLC, Mountain View, CA, USA), assisted in writing the Python code for data processing and figure generation and in language revision. The authors reviewed the routines and their outputs and take full responsibility for the analyses and the text.

3 Results and Discussion

3.1 Microbiological Response to Operating Parameters

The microbiological response was assessed in three independent series, varying applied voltage, repetition rate, or volumetric flow rate according to the experimental matrix in Table 1. Figure 2 combines, in each panel, the viable-cell density of the series (left axis) and the apparent log reduction relative to the corresponding control median (right axis). Experimental densities use as a comparative reference the viable-yeast level reported for limpid bottled white wine (Loureiro & Malfeito-Ferreira, 2003). The highest repetition rate (250 Hz) and the lowest flow rate (10 mL/min) reduced the viable density below the counting threshold (zero observed median), an apparent log reduction near 2.0 relative to the control median; the voltage series reached about 1.5 at 500 V.

Oscilloscope records from the nine tested groups indicated pulses consistent with the nominal protocol, with pulse width between 3.76 and 4.46 μs when estimated using the 50% voltage-peak criterion. Table 4 summarizes peak voltage, mean plateau

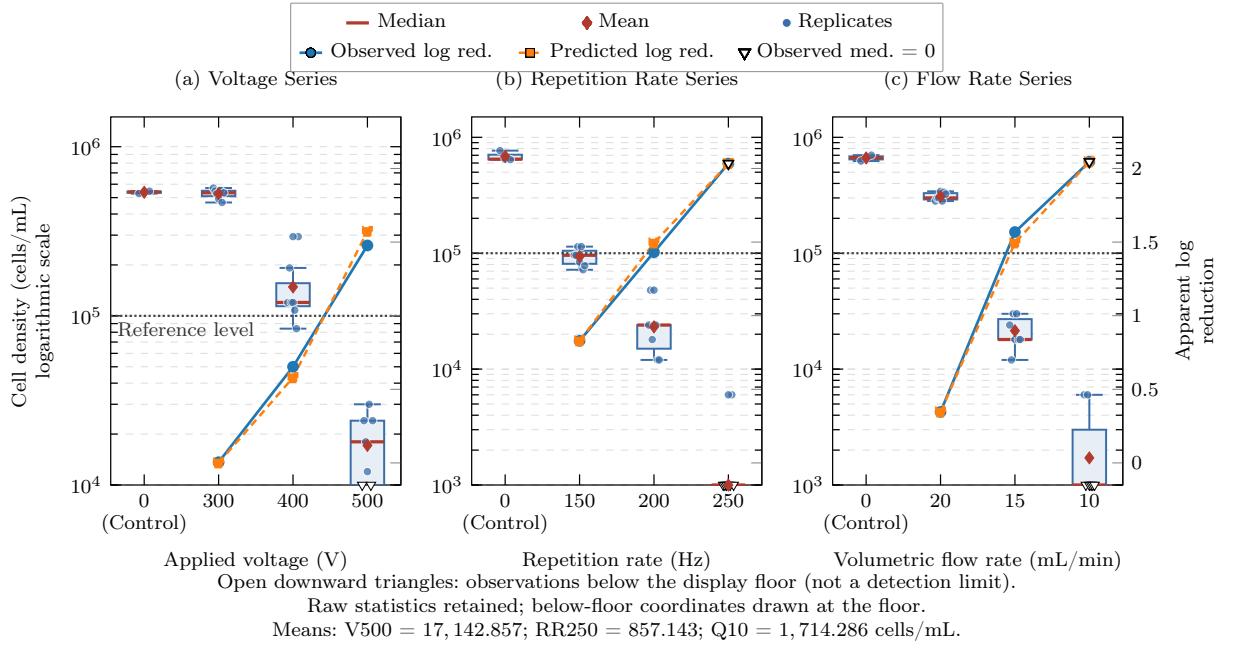


Fig. 2 Microbiological response organized by operational series. In each panel, the left axis shows the experimental cell density in cells/mL, on a logarithmic scale, with boxplot, individual replicates, median, and mean; the right axis shows the apparent log reduction relative to the control median of the series, comparing observed medians and predictions from the effective-dose model. In the flow rate series the horizontal axis is in decreasing order, so that treatment severity increases from left to right in all three panels. The reference level is the one associated with white wine that still appears limpid (Loureiro & Malfeito-Ferreira, 2003); counting follows the procedures of OIV/OENO Resolution 206/2010 (Castellucci, 2010). Conditions with zero median are drawn at the floor of the logarithmic axis, 6×10^3 cells/mL, which is the detection limit of Neubauer-chamber counting (one counted cell); a zero median means that no viable cell was observed in the sampled fields, not that the population was extinguished.

current, and pulse width for each experimental series. The complete waveforms are presented in Appendix A.

Table 4 Electrical metrics extracted from the oscilloscope records. Currents are apparent values from the oscilloscope-shunt chain. The plateau current is the quantity used by the model.

Series	V_{pk} (V)	$I_{pl,mean}^{app}$ (A)	τ_{50} (μs)
Voltage	271.49–466.85	49.02–109.51	4.08–4.15
RR	389.96–460.98	84.00–123.38	3.76–4.32
Flow rate	473.90–531.00	95.75–136.66	3.96–4.46

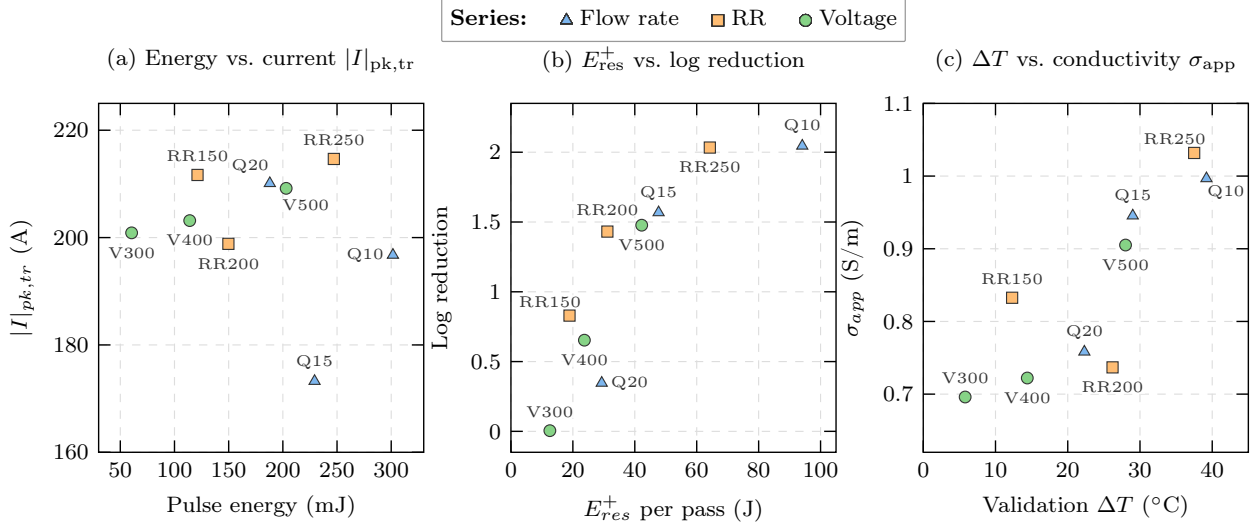


Fig. 3 Relationship among reconstructed electrical response, temperature, and apparent inactivation. **(a)** Transient peak current as a function of the estimated positive electrical energy per pulse. **(b)** Apparent log reduction as a function of the positive electrical energy accumulated by one cell during a single passage, $E^+_{res} = E^+_{pulse} \cdot N_p$, with $N_p = RR \cdot t_{res}$; this is the exposure the effective-dose model is written on, and not the energy delivered over the whole run. **(c)** Apparent conductivity estimated from the mean plateau current as a function of the ΔT adopted in the quantitative validation. A shared legend identifies the series with the groups *Flow rate*, *RR*, and *Voltage*. Condition abbreviations are: *V300*, *V400*, and *V500* for the voltage series, with $RR = 200$ Hz and $Q = 15$ mL/min fixed; *RR150*, *RR200*, and *RR250* for the repetition rate series, with $V = 500$ V and $Q = 15$ mL/min fixed; and *Q10*, *Q15*, and *Q20* for the flow rate series, with $V = 500$ V and $RR = 200$ Hz fixed. Within each group, the abbreviation indicates the modified variable, whereas the remaining parameters were kept fixed.

Figure 3 summarizes auxiliary process descriptors extracted from the electrical and thermal measurements, including estimated pulse energy, peak current, log reduction, temperature rise, and apparent conductivity estimated from the plateau current. These quantities were used to support data interpretation, but they were not treated as independent predictors in the viability model. For readability, points were identified using abbreviations for the main conditions: *V300*, *V400*, and *V500* for the voltage series; *RR150*, *RR200*, and *RR250* for the repetition rate series; and *Q10*, *Q15*, and *Q20* for the flow rate series.

In panel (a), the transient peak current stays between 173 and 215 A across all nine conditions (within the useful range of the shunt), a span of approximately 41 A, while the positive energy per pulse rises from 60 to 302 mJ, a factor of five. The peaks therefore do not track estimated pulse energy proportionally. Panel (b) shows that the energy accumulated in one passage, from 12.6 to 94.1 J across the matrix, orders the response only in part. The trend is increasing. The 150 Hz condition reaches an apparent log reduction of 0.83 with 18.9 J, while the 400 V and 20 mL/min conditions reach only 0.65 and 0.35 with 23.7 and 29.3 J. Delivered energy alone therefore does not rank the nine conditions. Panel (c) shows the apparent conductivity rising with the validation ΔT , from 0.70 S/m at 5.8 $^{\circ}\text{C}$ to 1.03 S/m at 37.5 $^{\circ}\text{C}$, both being

consequences of the delivered energy; every value stays below the 1.8 S/m measured for the medium.

The seven replicates of each condition were carried out at fixed voltage, flow rate, and repetition rate, and still differ in the recorded temperature rise. At 300 V the ΔT spans 5.4 to 11.5 °C, a ratio of 2.1. The viable-cell count does not follow that variation — the Spearman rank correlation between ΔT and count, within each condition, averages +0.05 across the nine, with a minimum of −0.45. In three of the 63 replicates, the measured rise approached the adiabatic limit of the corresponding condition, that is, negligible heat removal; taking the median across replicates keeps those three out of the value adopted per condition, and their viable-cell count is indistinguishable from the median of their own condition, even though the liquid ended 5 to 8 °C warmer. The thermal dose computed from Equation (3) with the literature constants remained below 5.1×10^{-5} in every condition with measurable counts, against an electric dose of order unity.

3.2 Coupled Effects of Voltage, Repetition Rate, and Flow Rate

Figure 2 shows that the microbiological response to PEF in continuous flow was not governed by a single operational parameter. In each panel, the left axis shows the cell-density reduction of the series, and the right axis shows that the apparent log reduction increases in different ways when voltage, repetition rate, or flow rate is modified. This behavior is consistent with PEF literature, in which field intensity, pulse duration, number of pulses, temperature, conductivity, and medium properties interact in microbial inactivation (Barba et al., 2015; Delso et al., 2023; Ghoshal, 2023; Huang et al., 2012; Z. Yan, Yin, Hao, Liu, & Qiu, 2021), and supports interpreting the process as a coupled electrothermal and hydrodynamic phenomenon rather than as a sequence of independent pulses.

In the voltage series, increasing from 300 to 500 V produced the largest relative change in cell density, which is compatible with the field-threshold hypothesis represented by the $\max(0, E - E_c)$ term in Equation (5). Below a certain field range, permeabilization is weak (Martínez et al., 2016; Teissié, Escoffre, Rols, & Golzio, 2008); above it, three voltage levels cannot distinguish a linear from a nonlinear response, and the model adopts the linear form. This pattern is also consistent with classical and recent studies in yeasts, in which field intensity and accumulated exposure directly influence cell survival (Cserhalmi et al., 2002; Emanuel et al., 2020).

The repetition rate series indicates that the response cannot be reduced only to the effective number of pulses $N_p = RR \cdot t_{res}$. The formulation in Equations (1)–(5) separates RR and t_{res} , allowing conditions with similar N_p to produce different predicted densities. This is important in continuous flow because pulse spacing can affect membrane recovery between pulses, local heating, and the temporal distribution of exposure (Emanuel et al., 2020; Guedert, Pintarelli, Mena Barreto Silva, & Suzuki, 2023). The physical scale for this separation is the membrane recovery time. Resealing after permeabilization is a first-order process on a scale of seconds to minutes, and it is by exploiting this slow resealing that successive pulses can be delivered at kilohertz rates (Teissié et al., 2008). The series applied here, from 150 to 250 Hz, corresponds to intervals of 6.7 to 4 ms, within that window, so that each pulse meets the membrane

in the state left by the preceding one. [Rodrigues et al. \(2027\)](#) describe this regime in yeast under multi-pulse protocols: the permeabilization left by one pulse conditions the response to the next, so that cumulative transport depends on this electroporation memory rather than on the number of delivered pulses alone. It is this memory effect that justifies keeping repetition rate and residence time as separate factors: within the resealing window, repetition rate ceases to be interchangeable with pulse count.

[Mendes-Oliveira, Jin, and Campanella \(2020\)](#) evaluated continuous-flow PEF inactivation in liquid food; even within a grouped energy description, the estimated critical electric field decreased from 2450 to 1670 kV/m in *Escherichia coli* and from 2470 to 1660 kV/m in *Salmonella* Typhimurium as the repetition rate increased from 500 to 1500 pulses/s. Treating repetition rate and residence time as independent factors is therefore consistent with the biological dynamics, and N_p alone is not an adequate descriptor of the biological dose.

In the flow rate series, the lower flow rate increased residence time and intensified the apparent reduction in viable-cell density, which the model carries through the term $(t_{res}/t_0)^{n_t}$. The effect is not purely hydrodynamic. Flow rate simultaneously sets the temperature rise, the heat removed per pass, and the spread of residence times, and all three move together across this series. Flow also acts on the treatment in a way the residence time alone does not express. El Zakhem *et al.* pulsed *Saccharomyces cerevisiae* suspensions in a static cuvette. Stirring the sample between pulse trains raised the degree of damage and broadened the aggregate size distribution, an effect they associate with the breakup of adhesion and sedimentation layers without claiming a complete explanation for it ([El Zakhem et al., 2006](#)). A flowing channel imposes that renewal continuously and by construction, which is one reason a batch result does not translate directly into a flow-through one. In real liquid-food systems, viscosity, conductivity, solids content, and bubble formation modify these effects further ([Barba et al., 2015](#); [Morales-de La Peña et al., 2011](#); [Ortega-Rivas, 2011](#)).

3.3 Interpretation of Electrical and Thermal Descriptors

The current waveforms show transient spikes at the pulse edges and a stable plateau in the central part (Appendix A). Computed from the nominal voltage and the campaign currents, the apparent conductivity ranged from 0.64 to 0.92 S/m, with a mean of 0.75 S/m; computed from the oscilloscope waveforms, it ranged from 0.70 to 1.03 S/m, with a mean of 0.85 S/m, which are the values in Figure 3(c). Both lie below the conductivity of the YPD medium, 1.8 S/m: the apparent resistance from the waveforms, about 4.5 Ω , is roughly twice the 2.1 Ω expected for the electrolyte. This difference is consistent with electrode–solution interface impedance and with acquisition-chain limitations, which these measurements do not separate ([Pintarelli et al., 2021](#)). The rise of apparent conductivity with ΔT in panel (c) has the sign expected for an electrolyte, whose conductivity increases with temperature; because the plateau current also carries the acquisition-chain limitation, the panel is not used to estimate that coefficient. The distinction matters because medium conductivity modulates the efficiency of electroporation inactivation ([Gášková et al., 1996](#)).

Computed from the measured ΔT , the cooling removed on average ≈ 11.2 W, or 31.5% of the Joule heat generated over the nine conditions, and the liquid temperature

rise stayed between 5.8 and 39.2 °C, with 26.2 to 29.0 °C in the three central-condition campaigns. With a single UA_{cool} , Equation (4) reproduces these ΔT with a root-mean-square error of 2.9 °C, and the fraction of Joule heat it predicts as removed falls from 0.37 at 10 mL/min to 0.23 at 20 mL/min, because a larger mass flow leaves less time for heat extraction. The thermistor in the electrode region recorded a post-treatment mean of 24.3 °C across the 63 replicates, close to the ambient 23.9 °C and about 7 °C above the inlet, which is the cold-side reference; UA_{cool} thus lumps the thermoelectric pumping with passive exchange with the surroundings. The computed thermal dose is more than four orders of magnitude below the electric dose, which is consistent with a limited thermal contribution to inactivation in the bulk liquid, as also indicated by the replicate-level record in the first subsection, which does not rely on the kinetic constants. Because temperature was not resolved inside the chamber, local heating near the walls cannot be excluded (Lindgren, Aronsson, Galt, & Ohlsson, 2002; Pae et al., 2025; B. Yan et al., 2025).

Because the applied voltage, repetition rate, and flow rate simultaneously dictate both the electric-field dose and the Joule heating rate (Equation (4)), the electrical and thermal descriptors are collinear within the experimental design. This collinearity precludes independent empirical estimation of thermal kinetic constants from the present dataset. Adopting validated kinetic parameters from literature benchmarks for vegetative *S. cerevisiae* ($D_{ref} = 2.67$ min at 55 °C, $z = 6.23$ °C) (Török & King, 1991) keeps the thermal term independent of the present data.

3.4 Effective-Dose Model and Operational Sensitivity

Table 5 summarizes retrospective agreement of the effective-dose model with the experimental medians used in the estimation. The formulation achieved a global $R^2 = 0.9979$ on the linear scale and $R_{log}^2 = 0.9889$ on the $\log_{10}$ scale, with an overall RMSE of 7.91×10^3 cells/mL (or ≈ 0.059 in $\log_{10}$ units). Condition-wise cross-validation, over five folds and with E_c and the field exponent fixed, returns R_{LOO}^2 from 0.61 to 0.84 depending on the fold seed (standard deviation 0.10).

Table 5 Retrospective agreement metrics of the effective-dose model against experimental medians. The Global row summarizes model agreement with all evaluated points; the series-wise rows indicate error distribution among operational series. $MAPE_+$ considers only positive experimental medians; sMAPE is complementary when a zero median is present. R_{log}^2 is computed on $\log_{10}$ of cell density across positive medians.

Series	RMSE (cells/mL)	R^2	R_{log}^2	$MAPE_+$ (%)	sMAPE (%)
Voltage	1.34×10^4	0.9964	0.9866	13.12	13.32
RR	1.93×10^3	0.9978	0.9783	6.72	71.40
Flow rate	1.93×10^3	0.9998	0.9926	9.30	5.67
Global	7.91×10^3	0.9979	0.9889	10.20	30.13

Both linear and logarithmic scales are reported. The linear R^2 reflects the wide dynamic range (5.3×10^5 to zero cells/mL), whereas the $\log_{10}$ metrics weight relative errors across all positive medians in accordance with the additive logarithmic dose formulation. The elevated sMAPE in the repetition rate series (71.40%) is an arithmetic artifact of the metric when observed medians approach the detection threshold (250 Hz), and does not by itself indicate poorer agreement in that series, whose R^2 is 0.9978. Absolute error is not evenly distributed among the series. The voltage series, which contains the highest median of the matrix (300 V), carries the largest RMSE (1.34×10^4 cells/mL) and the largest MAPE₊ (13.12%), whereas the flow rate series has the smallest sMAPE (5.67%). The two conditions with equivalent effective pulse number — 150 Hz at 15 mL/min and 200 Hz at 20 mL/min, both $N_p \approx 156$ — differ in viable-cell density by a factor of 3.12 between medians (9.6×10^4 against 3.0×10^5 cells/mL), with non-overlapping replicate ranges. The two-sided exact Mann–Whitney test (Mann & Whitney, 1947) gives $U = 0$ and $p = 5.8 \times 10^{-4}$, the smallest p attainable with seven replicates per group. Because the two model exponents are estimated on these same conditions, the model reproduces this difference but does not test it. The three campaigns of the central condition, each with its own control, gave reductions of 1.43 to 1.57 log, a spread smaller than the gap between the two equal- N_p conditions (0.83 and 0.35 log).

Figure 4 presents the contour map of predicted viable-cell density at 500 V as a function of flow rate and repetition rate. The surface is computed by the model, with the temperature rise given point by point by Equation (4); only the circles are data. The grid spans the measured ranges of Q and RR , but not of dose: 49% of it lies beyond the largest effective dose observed with measurable counts ($\Omega = 3.5$), reaching $\Omega = 37.9$ at the low-flow corner, about eleven times the largest datum, and the contours there are extrapolation. The thermal term changes the surface by less than 0.2% over the region above one cell per millilitre. The effective conductivity weighs more: held at the central condition through a reference plateau current of 95 A, it spans 0.64 to 0.92 S/m across the nine campaigns, a range that moves the predicted density by a factor of about 3.8 at the central point. The map is therefore to be read as an operating boundary, not as a density forecast; over the same conductivity range, the fraction of the grid below the reference level varies only between 64% and 77%.

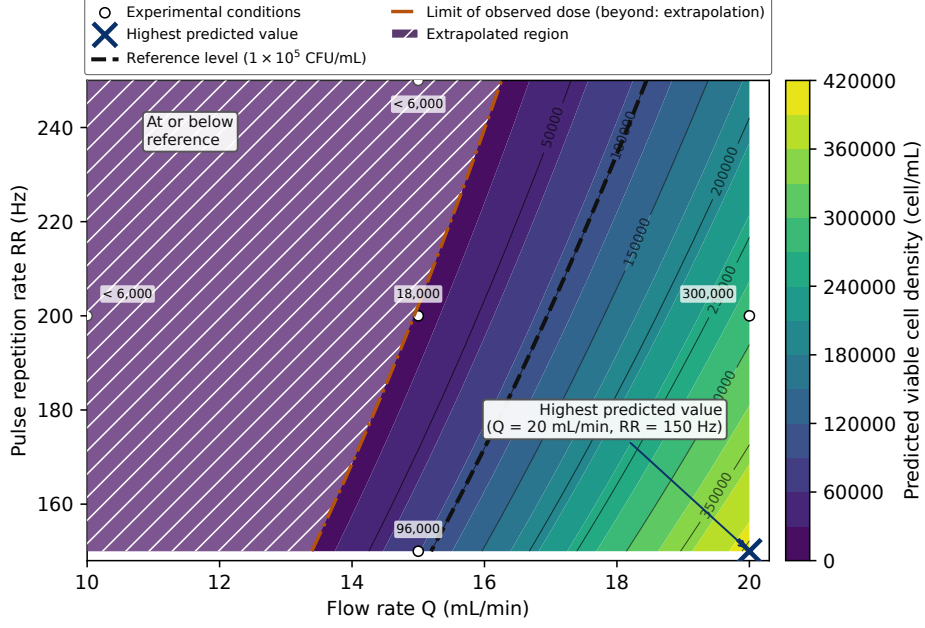


Fig. 4 Contour map of viable-cell density predicted by the effective-dose model as a function of volumetric flow rate Q (mL/min) and pulse repetition rate (Hz) at a fixed voltage of 500 V. Circles are the experimental conditions (seven distinct conditions, two with censored zero median), and the “x” marker indicates the highest predicted density on the grid. The dashed line indicates 1×10^5 CFU/mL, the upper end of the yeast load of white wine that still appears limpid (Loureiro & Malfeito-Ferreira, 2003), read as a one-sided bound (Section 2.3). The dash-dotted line marks the largest effective dose observed with measurable counts ($\Omega = 3.5$); the hatched region beyond it is model extrapolation.

3.5 Scope and Meaning of the Effective-Dose Model

The formulation represents four features of industrial flow-through PEF systems: confined chamber geometry, convective fluid transport, repetitive microsecond pulsing, and controlled temperature rise. In continuous flow, cell inactivation deviates from classical static batch models. Qin et al. (1998) found that the survival of *Saccharomyces cerevisiae* in a continuous-flow system did not follow Hülshager’s model and that continuous treatment inactivated more efficiently than batch treatment, which supports treating repetition rate (RR) and mean residence time (t_{res}) as separate factors.

The bootstrap intervals in Table 2 describe uncertainty in the three estimated coefficients, with E_c and the field exponent held at their adopted values. The nine campaigns, seven with positive medians, reduce to *five* distinct nominal conditions, because the three central campaigns repeat the same operating point; with so few conditions, a high R^2 follows from the count of points per coefficient and does not provide evidence of agreement. When E_c is moved across the admissible range of 300 to 400 kV/m, R_{LOO}^2 goes from -2.15 to 0.95 . The threshold operator $\max(0, E - E_c)$ is kept because the 300 kV/m condition produced no measurable inactivation and

because the literature reports an inactivation threshold for the species (El Zakhem et al., 2006). The fixed threshold, $E_c = 335$ kV/m, lies below the value usually quoted for the species, about 750 kV/m in static cuvettes (El Zakhem et al., 2006; Gášková et al., 1996), and the difference has a physical explanation. Gášková et al. (1996) report, in single-pulse mode, that amplitudes below about 2 kV/cm produce no appreciable inactivation of *S. cerevisiae* even with 300 μ s pulses, and that 1 μ s pulses remain ineffective at 28 kV/cm; taken as a single-pulse rule, that condition would forbid the inactivation observed here, at 4 μ s and 3–5 kV/cm. El Zakhem et al. (2006) indicate why the rule does not carry over: the critical field of about 7.5 kV/cm commonly quoted for the species is not a rigid threshold, and damage occurs below it in incomplete form, under long cumulative treatment time. The protocol adopted here is that regime, since a cell receives from 156 to 312 pulses in a single pass through the chamber. The E_c of this model therefore belongs to a cumulative exposure, and is not the same quantity as a single-pulse threshold, which also provides an independent reason to expect $\eta_t > 1$.

The residence-time exponent, $\eta_t \approx 4.89$, indicates a steeper-than-proportional sensitivity to transit time. Constraining $\eta_t = 1$ lowers R^2 on the dose scale from 0.995 to 0.708, but the bootstrap interval (0.95–5.76) includes unity: the data are compatible with that sensitivity without establishing it. It is consistent with cumulative damage (Rodrigues et al., 2027), without separately identifying membrane sensitization. Fluid delivery by a three-roller peristaltic pump also introduces flow pulsatility and a residence-time distribution (RTD) around the nominal mean $t_{res} = V_{active}/Q$, which may contribute to the observed exposure sensitivity (Sastry, 2023).

The operating map (Figure 4) distributes the predicted reduction between flow rate and repetition rate and can guide the choice of conditions for future tests. The 10^5 CFU/mL level marked on it is the one Loureiro and Malfeito-Ferreira (2003) report for spoilage yeasts forming powdery sediments; for flocculent-sediment yeasts, the same source gives 10^2 – 10^3 CFU/mL, below the resolution of this assay, in which one countable cell corresponds to 6×10^3 cells/mL. The level is therefore shown for context, and no compliance with it is claimed; the treatment outcome is reported as log reduction against the untreated control.

3.6 Limitations

The electrical and thermal descriptors are effective quantities. Current was read across a shunt of accuracy class 0.5, within its rating, but without characterization of its response at the pulse edge; the edge peaks and every quantity derived from the current — apparent conductivity, pulse energy, and UA_{cool} — are therefore treated as effective descriptors. The thermistors describe only the established regime, and temperature was recorded at the chamber inlet and outlet, not inside it (B. Yan et al., 2025). Neubauer-chamber counting is quantised at 6×10^3 cells/mL per counted cell. Medians near the detection limit therefore rest on few counted cells and carry a large relative counting uncertainty. The pH and viscosity of the medium, which influence PEF efficacy (Barba et al., 2015; Ghoshal, 2023; Morales-de La Peña et al., 2011), were not measured, and the tests used one strain, one medium, and one chamber geometry; transfer to other matrices will require further experiments (Lytras et al., 2024).

Voltage, repetition rate, and flow rate were varied one at a time around the central condition, which does not allow interactions to be estimated, and the residence-time distribution in the chamber was not measured (Sastry, 2023). The nine campaigns reduce to seven distinct nominal conditions, five of them with a positive median: E_c and the field exponent were fixed, η_R and η_t each rest on a single experimental contrast (150 Hz and 20 mL/min), and condition-wise cross-validation does not establish predictive performance. The model interprets the measured set over 300–500 kV/m, 150–250 Hz, and 10–20 mL/min; outside it, as in the extrapolated region of the operating map, new tests are needed (Alvarenga, Brito, & Lacerda, 2022).

4 Conclusion

This study presented a bench-scale continuous-flow PEF protocol applied to *Saccharomyces cerevisiae* and an effective-dose model for interpreting post-treatment cell viability, over 300–500 V (300–500 kV/m across the 1 mm gap), 150–250 Hz, 10–20 mL/min, and 4 μ s monopolar pulses. Conditions with the same effective pulse number, $N_p = RR \cdot t_{res}$, gave different viable-cell densities, with no overlap between replicates. Treating repetition rate and mean residence time as separate factors lets the model represent that difference, and the model reproduced the experimental medians, with R_{LOO}^2 from 0.61 to 0.84 in condition-wise cross-validation, depending on the fold assignment. Continuous-flow PEF viability modeling should therefore consider coupled electrical, thermal, and hydrodynamic variables, not only voltage amplitude, pulse number, or delivered energy. Within the tested matrix, 500 V, 200 Hz, and 15 mL/min reduced viable-cell density by about 1.5 log with a liquid temperature rise of 26.2 to 29.0 °C; raising the repetition rate to 250 Hz or lowering the flow rate to 10 mL/min brought the count below the detection limit, with rises of 37.5 and 39.2 °C. This trade-off between reduction and heating is the starting point for designing further continuous-flow tests.

Appendix A Complementary Electrical Waveforms

Figure 5 shows a representative waveform for the central condition (500 V, 200 Hz, and 15 mL/min), with the initial switching transient and the plateau window marked. Figure 6 gathers the voltage and current waveforms of the voltage, repetition rate, and volumetric flow rate series, one series per column. To compare conditions, each record was shifted in time so that the first rising edge of the voltage, at 50% of the baseline-corrected positive peak, coincides in all records; voltage and current received the same shift.

Current was obtained from the voltage across the shunt. The peaks at the pulse edges are switching transients; conductivity uses only the plateau current, whereas the energy per pulse integrates the whole pulse, transients included.

Statements and Declarations

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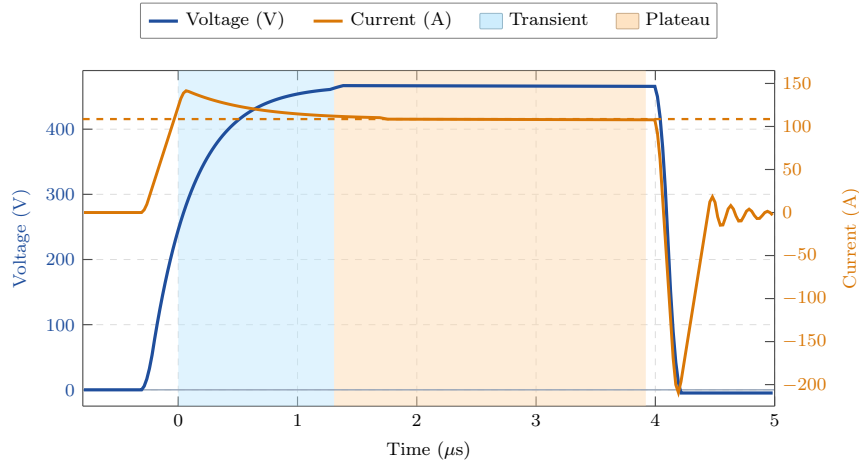


Fig. 5 Synchronized voltage (left axis, blue) and current (right axis, orange) waveforms for the central condition of 500 V, 200 Hz, and 15 mL/min, aligned by the 50% voltage rising edge. The light-blue and light-orange shaded regions mark, respectively, the initial switching transient and the plateau window used to compute the mean conduction current (horizontal dashed line).

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Competing Interests. The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate. This study does not involve human participants or vertebrate animals; experimental validation was conducted with *Saccharomyces cerevisiae* strain TB10.

Consent for Publication. Not applicable.

Data Availability. The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions. **Conceptualization:** J. Anselmo, D. O. H. Suzuki, G. B. Pintarelli, and J. Rodrigues. **Methodology:** J. Anselmo, D. O. H. Suzuki, G. B. Pintarelli, A. C. A. Junior, and J. Rodrigues. **Software:** J. Anselmo. **Validation:** J. Anselmo, G. B. Pintarelli, and J. Rodrigues. **Formal analysis:** J. Anselmo, G. B. Pintarelli, D. O. H. Suzuki, and J. Rodrigues. **Investigation:** J. Anselmo, A. C. A. Junior, and J. Rodrigues. **Resources:** D. O. H. Suzuki, G. B. Pintarelli, and A. C. A. Junior. **Data curation:** J. Anselmo. **Writing—original draft:** J. Anselmo. **Writing—review and editing:** J. Anselmo, D. O. H. Suzuki, G. B. Pintarelli, A. C. A. Junior, and J. Rodrigues. **Supervision:** D. O. H. Suzuki and G. B. Pintarelli. **Project administration:** D. O. H. Suzuki. All authors read and approved the final manuscript.

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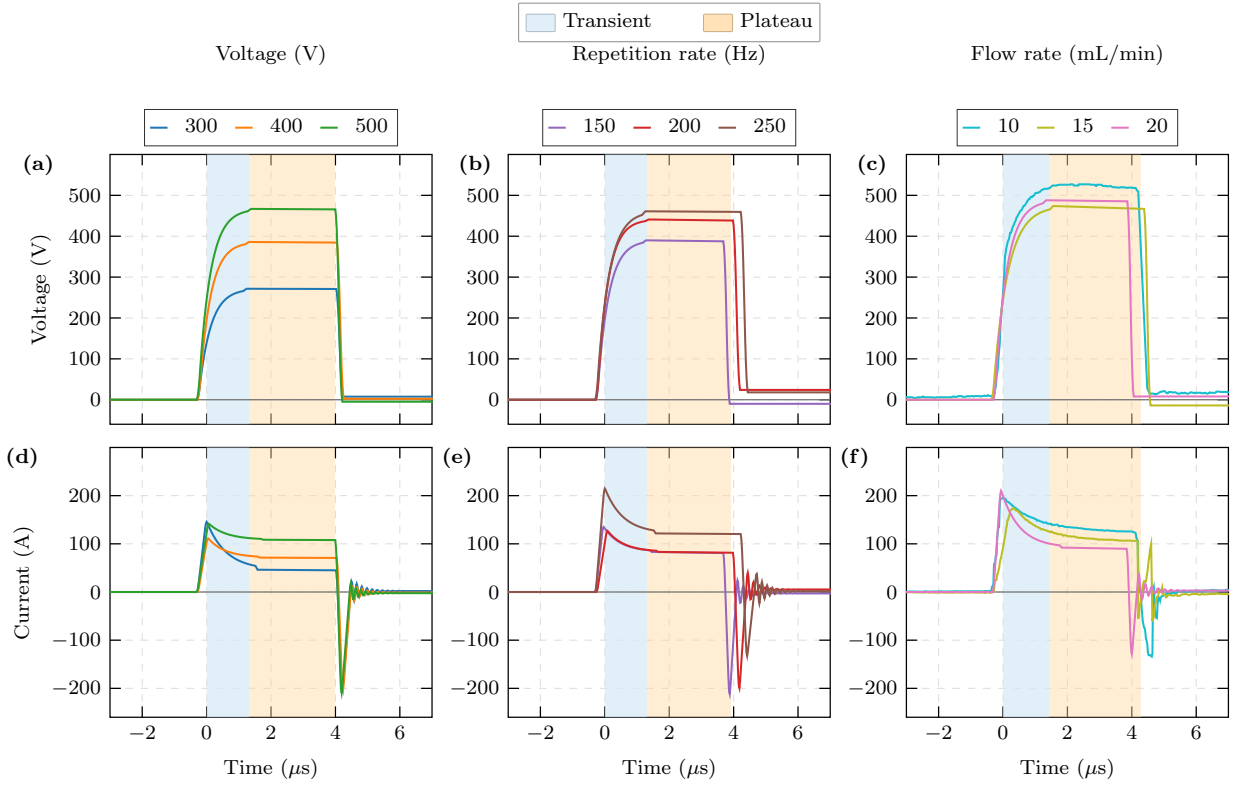


Fig. 6 Synchronized voltage (top row) and current (bottom row) waveforms for each experimental series. Column 1, voltage series: 300, 400, and 500 V, all at 200 Hz and 15 mL/min (panels (a) and (d)). Column 2, repetition rate series: 150, 200, and 250 Hz, all at 500 V and 15 mL/min (panels (b) and (e)). Column 3, volumetric flow rate series: 10, 15, and 20 mL/min, all at 500 V and 200 Hz (panels (c) and (f)). Time alignment and shaded regions (“Transient” and “Plateau”) follow Figure 5.

Use of Artificial Intelligence. During the preparation of this manuscript, the large language models described in the Methods (Codex and Gemini) were used to assist in writing the data-processing and figure-generation code, and for language review, grammar correction, translation support, technical-consistency checks, and improvement of textual clarity. The authors reviewed, verified, and approved all scientific content, equations, data interpretation, and figures, assuming full responsibility for the manuscript.

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