Biological response of *Chlorella vulgaris* to pulsed electric field treatment for improvement of protein extraction

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INTRODUCTION

Pulsed electric field (PEF) treatment provides an energy-efficient and gentle alternative compared to mechanical extraction methods. However, biological processes involved are poorly understood. A recent study [1] conducted with the unicellular green microalga *Chlorella vulgaris* was able to detect proteins of nuclear, chloroplastic, and mitochondrial origin in the water-soluble extract after PEF treatment, indicating a breakdown of these cell organelles. Additionally, the DNA of the cellular residue was analysed and found to show clear DNA laddering from as soon as 2 h after PEF treatment. These are indications for a cell death that is not necrotic but regulated and these observations suggest that it might be a biotechnological strategy to administer PEF treatment at lower energy as a signal to induce cell death, rather than to use energy-intensive PEF treatments to breach membranes electrically. For this purpose, the cellular mechanisms behind cell death after PEF treatment must be understood.

METHODS

A method was established to monitor cell viability after PEF treatment in *C. vulgaris* by using cell sorting based on fluorescein diacetate (FDA). Next, the experimental parameters of PEF treatment were calibrated to a point, where a set ratio of cells undergoes cell death after treatment and the other part stays viable. With these tools in hand, the cell-death response to PEF treatment can be analysed on a quantitative base.

RESULTS AND FIGURES

PEF treatment can be applied at different specific energies. Very low energies like 0.8 $J \cdot ml^{-1}$ only cause a small portion of cells to die after treatment. PEF treatment as extraction method requires high biomass concentrations to make the technology cost effective. This experiment aimed at analysing the influence factor of cell density on mortality after PEF treatment by diluting high cell density suspensions directly after PEF treatment to low cell density suspensions (Figure 1). When monitoring mortality at low cell density after PEF treatment, these low energies result in a constant mortality of around 30 %. However, at higher cell density an increasing mortality over a period of 24 h can be observed. Since the cells had been separated only after pulsing, the difference cannot come from physical parameters during the PEF treatment itself but must arise from biological processes occurring after the pulse.

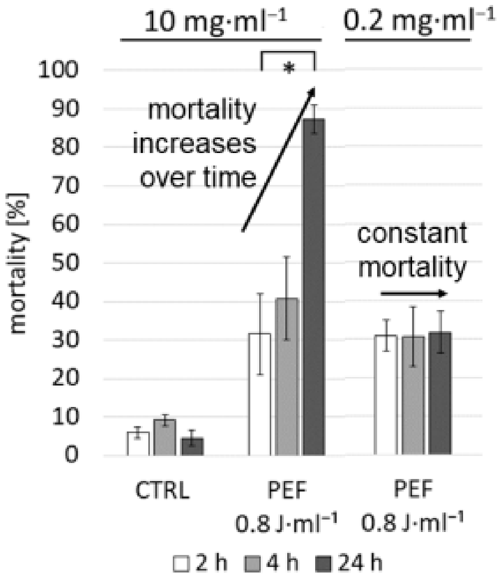

Figure 1: Influence of cell density on the mortality induced by PEF treatment. *C. vulgaris* were concentrated to high cell density of 10 $mg \cdot ml^{-1}$ and immediately after PEF treatment, the sample was divided. One set remained at high density, while the other set was diluted by adding sterile medium. CTRL: control without PEF treatment. Bracket indicates significant difference at $P \leq 0.05$ (*), using two-sample t-test.

CONCLUSION

A water-soluble factor released by PEF treated cells induces untreated cells to die. This cannot be merely a physical phenomenon but must involve a biological process. The high specificity is consistent with a model, where PEF treatment deploys active signalling culminating in the observed induction of programmed cell death while specifically releasing a cell-death inducing factor. Low-energy PEF treatment with subsequent incubation period could be a novel biotechnological strategy.

REFERENCES

[1] D. Scherer, D. Krust, W. Frey, G. Mueller, P. Nick, and C. Gusbeth, "Pulsed electric field (PEF)-assisted protein recovery from Chlorella vulgaris is mediated by an enzymatic process after cell death," *Algal Research*, vol. 41, p. 101536, 2019, doi: 10.1016/j.algal.2019.101536.