



Newsletter No. 67

Emerging Pulsed Electric Field Processing for Single-Cell-based Applications

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A growing world population and anthropogenic climate change call for novel resources and technological innovation to develop more sustainable value-chains in the bio-based domain. Here, single-cells such as microalgae have attracted attention owing to the sustainable notion of their connected value-chains. For instance, microalgae (i) are cultivable on non-arable land offering production concepts even under highly urbanized constraints whilst showing (ii) 5-10 times higher bioconversion efficiencies than terrestrial crops at an (iii) exceptional nutritional profile. Efficiency enhancement remains a main target in the bio-based domain for rendering the value-chains of single-cell biorefineries into economically viable scenarios. For example, the economic viability of microalgae feedstock production remains hampered by low upstream productivities, leading to an increase in total biomass production costs.

Pulsed electric field (PEF) processing is an energy efficient approach for single-cell process innovation both for up- and downstream applications. Upstream, nsPEF processing emerged as a promising technological innovation as it enhances single-cell bioconversion efficiencies. nPEF processing applies pulses high in an electric field and short in duration (1-100 ns), where the rise time of the pulse and its duration are below the time required to fully charge the plasma membrane. Thereby, the transmembrane potential of an organelle exceeds that of the plasma membrane predominantly targeting subcellular membranes, without causing irreversible damage to the plasma membrane. In microalgal cultures nsPEF processing can induce effects including selective inactivation or growth stimulation depending on the processing window applied. For instance, applying a specific energy input of $3 \times 256 \pm 67.5 \text{ J kg}^{-1}$ was shown to significantly leverage the biomass yield of the cyanobacterium *Arthrospira platensis* ($+13.1 \pm 1.6\%$). Simultaneously, the content of the high-value added component phycocyanin ($+19.2 \pm 5.8\%$) could be increased. Additionally, nsPEF processing enhanced biomass yields of eukaryotic heterotrophic and phototrophic *Chlorella vulgaris* (up to $17.5 \pm 10.5\%$).

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Although the exact treatment mechanisms of nsPEF-based bioconversion efficiency enhancement remain elusive, congruency exists about an involvement of transient cytosolic Ca^{2+} hubs and reactive oxygen species formation culminating in the onset of cellular response pathways.

On the other hand, it is widely known that μ sPEF treatments can cause reversible and irreversible membrane electropermeabilization, depending on the applied electric field strength $E(t)$. Downstream, harnessing the concepts of μ sPEF processing enables gentle component recovery that maintains the structural and techno-functional properties of the extracted components at low energy demand. Currently protein extraction relies on energy-intensive processes (harvesting, cell disruption, depigmentation, compound drying), resulting in high operating costs. μ sPEF processing however allows for cyclic component extraction maintaining cellular viability. Here, increasing protein extraction rates were observed with increasing electric field strengths (up to $96.6 \pm 4.8\%$ of the free protein in *C. vulgaris*). A free protein extraction rate up to $29.1 \pm 1.1\%$ without a significant influence on microalgal growth after 168 h was achieved.

In the bio-based domain nsPEF and μ sPEF processing bear potential as key processing steps towards a more sustainable and efficient value-chains. However, continuous treatments are currently often based on heterogeneous treatments conditions. Therefore, incorporating flow and electric field distributions into modified treatment chambers for improving treatment homogeneity will be necessary for a more targeted and, thus, more reproducible effect within cellular cultures. This could ameliorate the effects induced by μ sPEF and nsPEF highlighting the potential of the technology as a high-impact processing tool to resolve current challenges of single-cell processing in the bio-based domain.

Further/Suggested readings:

Haberkorn, I., Siegenthaler, L., Buchmann, L., Neutsch, L., Mathys, A. (2021c). Enhancing single-cell bioconversion efficiency by harnessing nanosecond pulsed electric field processing. *Biotechnol. Adv.*, doi: [10.1016/j.biotechadv.2021.107780](https://doi.org/10.1016/j.biotechadv.2021.107780)

Haberkorn, I., Buchmann, L., Hiestand M., Mathys, A. (2019). Continuous nanosecond pulsed electric field treatments foster the upstream performance of *Chlorella vulgaris*-based biorefinery concepts. *Bioresour. Technol.*, 293, 122029. doi: [10.1016/j.biortech.2019.122029](https://doi.org/10.1016/j.biortech.2019.122029)

Buchmann, L., and Mathys, A., (2019). Perspective on pulsed electric field treatment in the bio-based industry. *Front. Bioeng. Biotechnol.*, 7, 1–7. DOI: [10.3389/fbioe.2019.00265](https://doi.org/10.3389/fbioe.2019.00265)

Buchmann, L., Brändle, I., Haberkorn, I., Hiestand, M., Mathys, A. (2019). Pulsed electric field based cyclic protein extraction of microalgae towards closed-loop biorefinery concepts. *Bioresour. Technol.*, 291, 121870. doi: [10.1016/j.biortech.2019.121870](https://doi.org/10.1016/j.biortech.2019.121870)

Forthcoming events

8th School on Pulsed Electric Field Applications in Food and Biotechnology

Compiègne, May 30 – June 3, 2022

<https://pefschool2022.electroporation.net>

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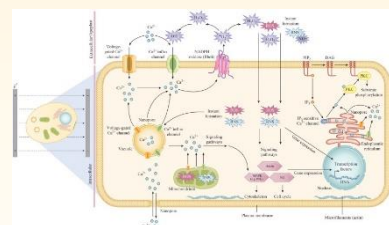
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Response of cells to nsPEF. From <https://tinyurl.com/jbiotadv>, hi-res photo: <https://tinyurl.com/hqcell>.

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