

Utilizing a combination of plasma and pulsed electric field techniques for microalgae processing

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The synergy between plasma and pulsed electric field (PEF) has been shown to exceed the cell inactivation achieved by PEF alone [1-3]. The proposed mechanism for achieving synergy involves plasma-generated radicals oxidising membrane lipids, compromising integrity, and enhancing PEF-induced pore formation. Recent studies have also demonstrated the ability of combined plasma and PEF to induce various mammalian cell death mechanisms, including apoptosis, autophagy, pyroptosis and ferroptosis [2].

Despite advancements, a research gap exists regarding the impact on microalgae and compound extraction. Our study focused on *Chlorella vulgaris* cells, exploring membrane permeability, cell wall integrity, and consequences like intracellular compound release and potential death mechanisms. To achieve our objectives, Gliding Arc Discharge plasma and PEF technologies were employed. Plasma, generated with compressed air (electrode-to-suspension distance: 30 mm, duration: 300 s, voltage: 50-250 V, frequency: 270 kHz), was combined with PEF (exponential pulses: 10 μs, 1-10 pulses, repetition rate: 1 Hz, electric field strength: 25 kV/cm).

The results showed the formation of NO₂, NO₃ and H₂O₂ radicals during plasma generation in the algal suspension, whose concentrations increased with plasma voltage. In addition to radical formation, an increased electrical conductivity and acidification of the suspension were determined. In contrast, treatment with PEF did not cause such effects. Regarding changes in *C. vulgaris*, plasma treatment alone induced changes in membrane permeability and cell walls only when the plasma generator was operated at voltages higher than 210 V. Conversely, treatment with PEF alone increased membrane permeability proportional to the number of pulses applied. In addition, an assessment of cell permeability after 24 hours showed that PEF treatment caused DNA leakage, one of the signs of programmed cell death, which was not observed in permeable cells after plasma treatment alone. Combined plasma and PEF treatment showed two different outcomes, which depended on the plasma voltage. Below 170 V, the combined treatment mirrored the effects of PEF alone: permeable cells, DNA leakage and protein release. However, above 210 V, there was increased permeability without DNA leakage, coupled with reduced protein release. These unprecedented results reveal a previously undescribed combined effect of plasma and PEF on algal cells and highlight the need for detailed analysis to elucidate mechanisms before practical application.

References:

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