## Integration of Pulsed Electric Field (PEF) Technology into the Microalgae Biorefinery

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In recent years, many studies have shown that the use of pulsed electric field (PEF) technology to extract valuable compounds from microalgae is suitable for energetic and nutritional purposes and meets economic requirements. This was only possible through the implementation of the microalgae biorefinery concept, which involves the use of the residual biomass after the extraction of high and medium value products (e.g., pigments, polysaccharides, and proteins) for biofuel production. The International Energy Agency, IEA, defines biorefinery as "the sustainable processing of biomass into a spectrum of marketable products and energy". A major advantage of PEF treatment is the bypassing of drying and the immediate processing of the wet concentrated biomass. Moreover, PEF treatment can be used as a mild and effective method of cell disruption, facilitating recovery of unaltered constituents at low energy costs and allows cascade processing for multiple component recovery, since it does not destroy cell shape and maintains gravimetrical biomass separability after single extraction step. However, cell components are not immediately available since electroporation does not conduct to cell disintegration. It was assumed that irreversible electroporation of the cell membrane and the subsequent increased permeabilization are the main effects which allows proteins and other ingredients to pass through the membrane. We found in our studies that efficient protein extraction after PEF treatment requires an incubation step, and that the progress and kinetics of this release depend on the biomass concentration and the incubation temperature. In addition to diffusion in a chemical gradient, a second biological, enzyme-driven process within the PEF-treated biomass was confirmed to facilitate the release of more than 40 % of total proteins from the cells. This is mainly the water-soluble protein fraction, but proteins from the cell organelles such as nucleus, mitochondria and chloroplast are also released. It is not exactly known which biological processes are triggered by PEF treatment in the cell, which lead to the release of valuable components - apart from the enzymatic autolysis. It seems that the type of cell death (programmed cell death, necrosis, or apoptosis) induced by PEF treatment influences the efficiency of protein extraction. Therefore, a better understanding of cell death in response to PEF treatment could lead to possible improvements in protein extraction efficiency. This presentation will give an overview about the various factors, such as the post PEF treatment incubation parameters, which have an impact on protein release, as it could be possible to optimize the incubation to obtain higher yields. In addition, the role of an important discovery is discussed, namely a water-soluble factor that is released by PEF-treated cells and triggers cell death in untreated cells. Based on the cell death inducing factor released after treatment, a working model for the recovery of compounds from microalgae is proposed.