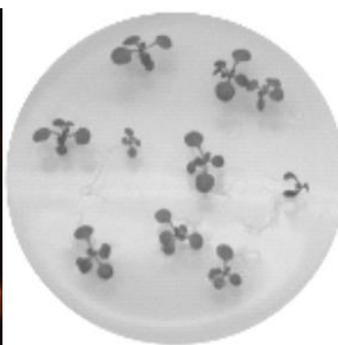
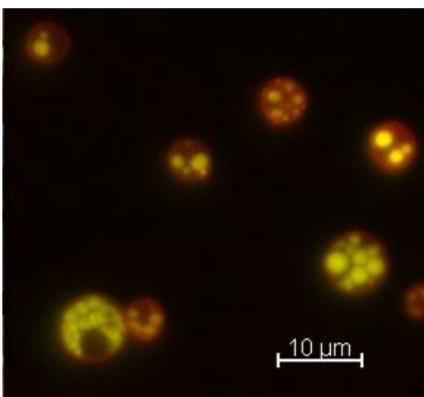


2 Renewable Energy (RE): Bioenergy -Feedstocks and Pretreatment-

Contact: Prof. Dr. Georg Müller

The Department for Pulsed Power Technology is focusing on research and development of pulsed power technologies and related applications. The applications involves the electroporation of biological cells for extraction of cell contents (PEF- process), dewatering and drying of green biomass, pre-treatment of micro algae for energetic use and sustainable reduction of bacteria in contaminated effluents. Another key research topic is devoted to the development of corrosion barriers and materials for improved compatibility of structural materials in contact with liquid metal coolants. This year's report focuses primarily on the activities and results of ongoing third-party funded projects of the department.



PEF treatment, 10ns pulses



Microalgae deposition, anode

2.1 Microalgae cultivation and processing

Contact: Dr. Wolfgang Frey

Pulsed electric field treatment maintains cell shape, does not produce cell fragments and consequently allows real cascade processing of microalgae biomass for implementing a zero-waste biorefinery. During the past period, subsequent processes for recovery and valorization of the water-soluble fraction, the protein fraction and the lipids have been further developed. Finally, utilization of the residual biomass for biogas production have been assessed. Furthermore, new activities were started on bacterial decontamination of industrial water streams and on stress-responses of *Spirulina* microalgae biomass.

2.1.1 PEF4AlgBiotics

Contact: Dr. Christian Gusbeth

Title of the bilateral project, between IHM-KIT and University of Belgrade (UB) is: *Integrated Pulsed Electric Field Extraction and Lactic Acid Bacteria Fermentation for the Production of Microalgal Extracts Fortified with Probiotics* (PEF4AlgBiotics). The objective of this project is to develop a utilization option for the water-soluble fraction which spontaneously is released after PEF-treatment of microalgae. It targeted on the production of higher-value components from aqueous microalgae fractions by lactic acid fermentation (LAF) by probiotic bacteria which were grown in the aqueous fraction separated after PEF-treatment. The UB team was responsible for growth optimization of lactic acid bacteria (LAB) exhibiting probiotic characteristics and for evaluation of the effect of LAF on antioxidant properties of the fermented suspension.

Two microalgae strains, *Chlorella vulgaris* and *Auxenochlorella protothecoides*, have been selected to meet the requirements, such as having sufficient antioxidant activity and providing a growing substrate for LAB. The impact of the PEF treatment on chemical composition of the water-soluble fraction has been elaborated. Investigations were performed with the microalgae *C. vulgaris* and *A. protothecoides*, which are able to accumulate large amounts of lipids (up to 70% of the dry biomass) as energy storage molecules. We have found that a lipid-to-protein ratio of 1: 1 is optimal for extracting proteins by PEF treatment. While in case of *C. vulgaris* up to 50% of the protein content could be recovered, we were able to extract less than 10% from *A. protothecoides*. Hence, for further investigation we used *C. vulgaris* as model organism. The LAF performed on the water-soluble fraction, which was recovered within 2 h after PEF treatment, satisfied the growth requirements of LAB and had an increased antioxidant activity of the suspension after fermentation.

Funding: DAAD PPP Serbia 2017

2.1.2 Protein Extraction: Bioeconomy Graduate Program “BBW ForWerts”

Contact: Daniel Scherer Collaboration

Pulsed electric field (PEF)-assisted protein extraction from microalgae biomass: With regard to a rising demand for protein sources, microalgae are currently being discussed as an alternative source of protein for food and feed applications.

In order to extract proteins, it is usually necessary to disrupt the cells by mechanical force such as high-pressure homogenization (HPH), bead-milling or sonication. These methods all create cell debris and homogenize the biomass components, which makes separation of the debris from the extract a challenging task. An alternative to these mechanical disruption methods is the PEF-treatment that permeabilizes the cell membrane by electric field application. The extraction yield is lower compared to the aforementioned mechanical methods, but the idea is to extract the proteins through the permeabilized membrane without creating cell debris and thus maintaining separability of other biomass compounds. This makes it possible to embed PEF-treatment into a processing cascade in which the microalgae biomass can be fractionated and sequentially extracted for proteins and lipids. Then, the residual biomass can be used for energy-related processes such as biogas fermentation or hydrothermal liquefaction (HTL).

In the course of this research program, we established the microalgae *Chlorella vulgaris* as our model organism to investigate PEF-applications. We also established various protocols utilizing infrared-spectroscopy and modified Lowry assay to determine protein content of our algae and extraction yields respectively. The extracted proteins were visualized using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). While establishing these methods we observed striking differences in the two protein extraction processes. Unlike HPH, where proteins are immediately released into the environment, proteins are released gradually in a time-dependent manner after PEF-treatment.

In a subsequent work the factors which influence the protein release kinetics after PEF-treatment were investigated. It was found that protein release is influenced by the algae concentration, extraction time and extraction temperature and is also influenced by extreme values of pH. It was concluded that extreme temperatures and pH values are counterproductive whereas extraction under physiological conditions results in maximum yield. Our results so far indicate that PEF-treatment induces a controlled, systematic breakdown of the biomass by some intrinsic enzymatic activities. These results have also been presented at status seminars for the BBW ForWerts graduate school and microalgae subgroup meetings with collaborators of the University of Hohenheim.

Moving on from these findings we intend to investigate the characteristics of protein fractions obtained by mechanical methods such as HPH and our PEF-treatment in terms of amino acid composition and technofunctional properties (e.g. foaming, gelling, emulgation, solubility) with our collaborators from the University of Hohenheim. With our collaborators from the IKFT we want to investigate how the residual, protein-extracted biomass can be used in the energy-related HTL process and which quality the resulting crude oil has.

Funding: Bioeconomy Graduate Program BBW ForWerts / LGF scholarship

2.1.3 Pulsed Electric Field (PEF) application for biofertilizer production from wet microalgae biomass through enzymatic hydrolysis

Contact: Dr. Sahar Akaberi

It is known from literature that microalgae can contain a high amount of proteins. The strain *Scenedesmus almeriensis* exhibits a comparably high content of proteins of 30-55 % of the cell dry weight. In 2012, Romero Garcia et al., has introduced a process for the production of amino acid concentrates to be utilized as biofertilizer from *S. almeriensis* through enzymatic hydrolysis. Due to the presence of the rigid cell wall, a cell disruption method is necessary to improve enzyme access into the cell and, in consequence to increase the hydrolysis yield of algal proteins to amino acids. In the European project SABANA, Sustainable Algae

Biorefinery for Agriculture and Aquaculture, PEF-treatment was suggested as an energy efficient cell disruption method prior to enzymatic hydrolysis.

To provide biomass for process development the new strain *S. almeriensis* was transferred to the IHM lab. Since mid of 2017 *S. almeriensis* are cultivated in shaker-culture and in 25 liter photobioreactors. The latter allows obtaining a larger volume of higher pre-concentrated biomass.

For PEF-treatment of *S. almeriensis* biomass, fresh *S. almeriensis* was harvested and concentrated by centrifugation to reach at least a biomass concentration of 50 gdw/l of suspension. Continuous-flow PEF-treatment was performed with 1 μ s long pulses at an electric field strength of 40 kV/cm and at a treatment energy of 150 kJ per liter of suspension. PEF-treated microalgae were analyzed microscopically. As expected, cell structure was preserved after PEF-treatment. Other parameters, such as conductivity changes showed the efficiency of pulse parameters.

The enzymatic hydrolysis was done by adding the commercial enzymes Alcalase 2.5 L and Favourzyme 1000 L to the pretreated biomass suspension in hydrolysis reactor, equipped with pH and temperature control. Hydrolysis reaction was performed for three hours. The amino acid concentrates were separated from the residual biomass by centrifugation. Subsequently, free amino acids were detected by using o-phthalaldehyde (OPA) method. Total protein content of the microalgae biomass was determined according the Lowry method following on alkaline extraction with 1 M sodium hydroxide, using Bovine Serum Albumin (BSA) as standard.

Compared to the control, the PEF-treated microalgae biomass resulted in an increase of the degree of hydrolysis by 10% to 40%, depending on biomass cultivation and storage conditions.

Funding: Horizon 2020, SABANA

2.1.4 Post-PEF-treatment incubation as a strategy for reduction of energy input

Contact: Dr. Aude Silve

In the past years, successful lipid recovery from the fresh microalgae *Auxenochlorella protothecoides* has been demonstrated using PEF pre-treatment and subsequent solvent extraction with hexane/ethanol blends. Performing immediate extraction, an energy of 1.5 MJ per kilogram of dry weight of microalgae (1.5 MJ/kg_{dw}) was required to achieve total lipid extraction, i.e. already lower than the lowest reported values for conventional techniques such as bead-milling or high pressure homogenization.

Recent results have shown that adding an incubation period after having applied PEF-treatment to the microalgae can considerably reduce the energy demand for PEF-treatment. All investigations were performed on fresh microalgae biomass cultivated at our institute. Directly after harvesting, microalgae were concentrated to 100g of dry weight per liter and treated with PEFs in a continuous flow setup in order to be as close as possible to real-time industrial processing.

Following PEF-treatment, the microalgae suspension was incubated under inert conditions, i.e. covered with nitrogen and kept in the dark. After a given incubation time, ranging from 10 minutes to 20 hours, the microalgae were centrifuged. Conductivity and carbohydrate content of the supernatant were quantified and the microalgae pellet was further submitted to lipid extraction. For all PEF-treatment energies tested, the amount of ions and carbohydrates released into the supernatant increased with incubation time.

Additionally, incubation had an overwhelming effect on lipid extraction yield (figure xxx). For the previously applied standard energy input of 1.5 MJ/kg_{DW}, maximum lipid yields are obtained after about 1 hour of incubation. When incubating the samples for 20 hours, similar yields can be obtained using only 0.25 MJ/kg_{DW} i.e. about 6 times lower than the lowest reported values in the literature so far. By allowing significant reduction of operating costs, the strategy which was develop in our institute increases the potential benefit for industrial applications. Future work will focus on clarifying how the biomass evolution during incubation facilitates extractions processes. This will enable further optimization of the complete microalgae downstream processing chain.

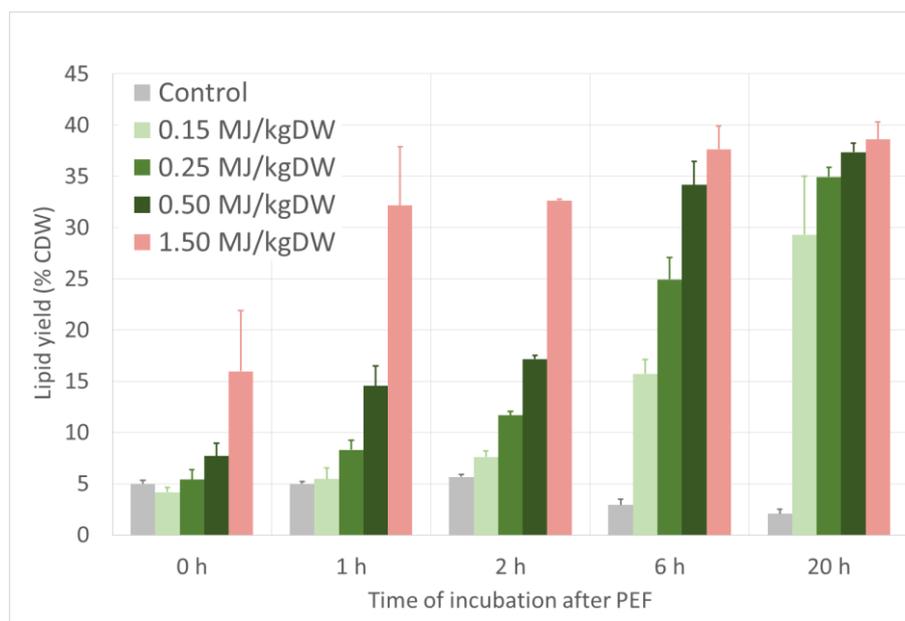


Fig. 2.1.1: Lipid extraction yield as a function of the duration of incubation after PEF-treatment and for different specific PEF treatment energies. Incubation was performed in an inert atmosphere with samples covered with nitrogen, and stored in the dark at 25°C. For each sample, time zero corresponds to the end of the PEF-treatment. Results are the average + std of 3 independent experiments.

Funding: H2020 SABANA

2.1.5 Biogas production from residual microalgae biomass fractions

Contact: Dipl.-Ing. Ralf Straessner

On this topic we investigated the possible energy gain from residual microalgae fractions by anaerobic digestion (AD). Different residual fractions of microalgal biomass from *Auxenochlorella protothecoide* (A.p.) were evaluated for methane yield and fermentation kinetics: a) untreated microalgae, b) PEF-treated microalgae, c1) PEF-treated with replacement of the supernatant, directly after treatment by deionized water, c2) PEF-treated with replacement of the supernatant, after two and a half hour diffusion time, by deionized water and d) PEF-treated and subsequent lipid extracted microalgae. After treatment, the mentioned samples were transferred to Umweltforschungszentrum (UFZ) in Leipzig. The different microalgae samples were subjected to appropriate microorganisms (anaerobic bacterial communities) in biogas reactors. The continuous production of biogas, which consists of 55-90% of methane, in the AD-reactors was recorded, until biogas generation tend towards zero, which happened approximately after 6 weeks.

The investigation showed differences in the behavior of biogas generation, in the final production quantity and the kinetics, as well. It turned out that untreated microalgal biomass (sample a) was fermented faster, showing the earliest start of AD- process and hence biogas production but were limited in the final production volume, showing the smallest total produced biogas volume, except the lipid extracted sample d). The lipid extracted sample d) showed comparable starting behavior of the AD-process as the untreated control sample a) but exhibited the smallest amount of final produced biogas of all investigated specimen. In this case the extracted lipid fraction cannot contribute to the biogas generation. Lipids own the highest amount of specific energy among the other microalgal components (carbohydrates and proteins) and for that reason the drop of biogas production compared to the other samples was significant.

Samples c1) and c2) showed the longest lag period at the beginning of the AD-process. Most probably this is caused by the presence of the lipids in these biomass fractions. Inhibition of anaerobic digestion with the used bacteria, caused by LCFAs (Long Chain Fatty Acid) is a well-known effect, reported in the literature (Ma et al., 2015; Montingelli, Tedesco, & Olabi, 2015; Zonta, Alves, Flotats, & Palatsi, 2013).

The PEF treated sample b) with no further modification, showed an intermediate retarded start of the AD-process, being faster than the samples with removed supernatant c1 and c2, but slower than samples a) and d) at the beginning. At the end of the AD-process the amount of produced biogas with sample b) was only slightly higher than those of the untreated control sample a).

In summury it was shown that residual biomass, after PEF treatment and subsequent product extraction, can be efficiently used for biogas production by AD process. The outcome of this investigation is specific for the used A.p. and the applied anaerobic bacterial community and may differ from other examinations with different microalgal biomass and different anaerobic microorganisms.

2.1.6 NewAlgae – nsPEF stimulation of Spirulina biomass

Contact: Dr. Christian Gusbeth

New sustainable food formulations based on algae protein NewAlgae: The main objective of the project is to facilitate process innovation in the food industry focused on algae protein. The focus of the project is on developing innovative processes along the algae value chain, leading to product development. Main task is to acquire technological insights into nsPEF-boosted cultivation and to characterize functional and bulk proteins from microalgae. In 2017 the work was focused on optimization of cultivation systems on lab-scale for the protein producing model strain *Arthrospira platensis* (commonly known as *Spirulina*) and the combination of the lab scale photobioreactor with nsPEF treatment in order to increase cell proliferation.

Cultivation experiments and the screening of suitable cultivation media allowed for a reduction of the conductivity from $18 \text{ mS}\cdot\text{cm}^{-1}$ to $10 \text{ mS}\cdot\text{cm}^{-1}$, which enabled nsPEF trials. After establishing the treatment procedure, equipment consisting in a Blumlein pulse generator and treatment chamber was build and provided to ETH Zürich for additional investigations. Now, for better statistics, parallel experiments could be carried out at ETH and KIT-IHM. The project is currently on track and first growth stimulation results were obtained for *A. platensis*.

Funding: WFSC Coop Research Program

2.1.7 DiWaL

Contact Dr. Wolfgang Frey



The aim of this project is bacterial decontamination of industrial processing water streams by pulsed electric field (PEF) treatment, in particular in electrocoating lines of the automotive industry. During the joint project, PEF technology will be integrated into a new, automated and resource-efficient water management concept for pretreatment and electrocoating lines. This will improve the circulation of water in the factory, avoid the use of biocides and reduce fresh water consumption. The joint project started in February 2017.

First task was to identify a non-impact parameter range, within which coating quality is not affected by PEF treatment. In the course of this, PEF treatment was applied for the first time to electrodeposition paints and water in anodic and cathodic electrocoating. It could be demonstrated, that PEF treatment with short unipolar pulses does not affect coating quality, even at high specific treatment energies. In the further course bipolar pulse protocols for both types of electrodeposition paints will be tested.

Second task is the development and manufacturing of a 30 kV / 0.6 kA solid state pulse generator. At current state a full-load test of 1 kV moduls was successful. An 8 kV test generator, consisting of eight moduls, is assembled and in test phase.

Additional, model calculations to integrate PEF technology into the dip coating process were carried out. It was found that in case of PEF-treatment in bypass operation mode, a rather low inactivation rate and a high flow rate is more efficient than a high inactivation rate at reduced medium flow. It was shown that an inactivation rate of 2 log reduction in bypass operation mode is sufficient to inhibit bacterial proliferation, whereas an increase of the mass-flow at 2 log reduction directly increases the inactivation performance in the entire volume of the dip coating bath. These findings provide an important approach for further energy optimization of the PEF treatment in electrodecoating lines.

Funding: Federal Ministry of Education and Research – BMBF; WavE program



2.2 Components and electroporation processes

Contact: Martin Sack

2.2.1 ZIM-Wine

In the frame of the joint research project “PEF-treatment of crushed grapes (Elektroporation von Traubenmaische)” a device for the treatment of crushed grapes by pulsed electric fields (PEF) with a flow rate of 10t/h is currently being developed in collaboration with the industrial partners ARMBRUSTER Keltertechnologie and KEA-TEC. The project is supported by the Federal Ministry for Economic Affairs and Energy on the basis of a decision by the German Bundestag.

The pulse circuit of the device comprises two Marx generators, each connected to one pair of electrodes inside the electroporation reactor. For efficient operation the design requires synchronized switching of the generators, which will be achieved by a triggered operation. For long-term operation a trigger generator operating without a separate trigger electrode has been developed. An over-voltage pulse is applied to the ground-side spark gap switch of each Marx generator causing ignition. The trigger has been tested in a test bed. Fig. 2.2.1 shows the trigger signal together with the light emitted from the triggered spark gap switch as a superposition of 20 single trigger events measured at a pulse repetition rate of 22 Hz. The trigger delay time for the selected parameter set has been measured to be approximately 130 ns and the jitter is sufficiently small.

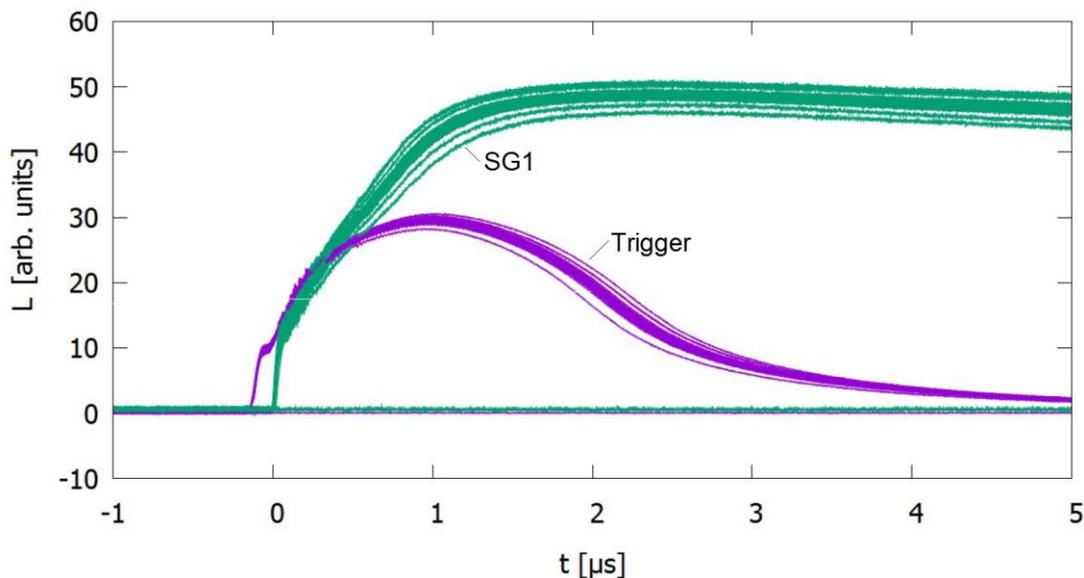


Fig. 2.2.1: Trigger signal together with a signal of the light emitted from the triggered spark gap switch as a superposition of 20 single trigger events.

In the frame of a scale-up of the existing electroporation device KEA-WEIN to a flow rate of 2 t/h in 2016, nozzles have been inserted to guide the nitrogen gas towards the gaps between the electrodes of the spark gap switches. Now, the operating performance of the device has been evaluated. Therefore, the device has been operated with different parameter settings up to the design limits. As an example Fig. 2.2.2 shows the voltage per stage when operating the device at maximum charging current of 1.4 A and approximately 50 kV ignition voltage of the spark gap switches. A pulse repetition rate of 45 Hz has been achieved.

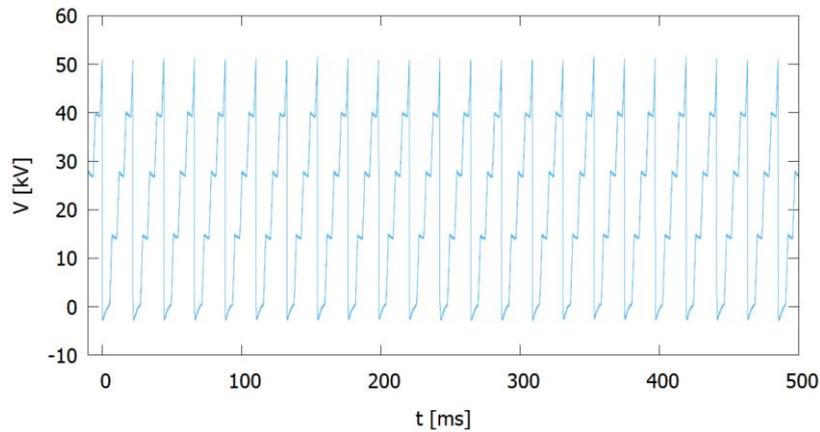


Fig. 2.2.2: KEA-WEIN: Stage voltage when operating the device at maximum charging current of 1.4 A and approximately 50 kV ignition voltage of the spark gap switches.

Collaboration: ARMBRUSTER Keltertechnologie, KEA-TEC

Funding: The project is supported by the Federal Ministry for Economic Affairs and Energy on the basis of a decision by the German Bundestag.



2.2.2 Marx-type pulse generator for stepwise arbitrary waveform generation

Contact: Dr.-Ing. Martin Sack

Currently a 150-stage pulse generator for stepwise arbitrary waveform generation to drive the Pulsed Electron Beam Device (GESA) is under development. In order to validate the design of the stage modules, an 8-stage generator has been set up. Fig. 2.2.3 shows a photo of the test setup. The stages are arranged in such a way, that the current path forms a helix. Each stage comprises a capacitor and an IGBT switch together with a bypass diode. A microcontroller at each stage enables individual toggling of each stage switch, synchronized to a trigger event common for all stages. Fig. 2.2.4 shows a stepwise arbitrary voltage shape measured at the generator's output and the current through a resistor connected to the terminals of the generator. A peak current of 600 A and a current rise time of 106 ns at 600 A have been achieved.

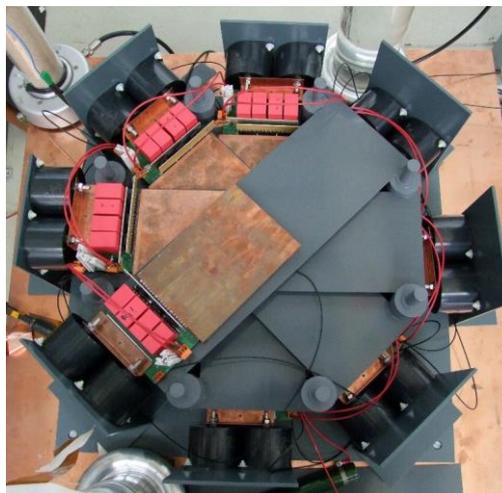


Fig. 2.2.3: 8-stage Marx-type generator for generation of stepwise arbitrary voltage.

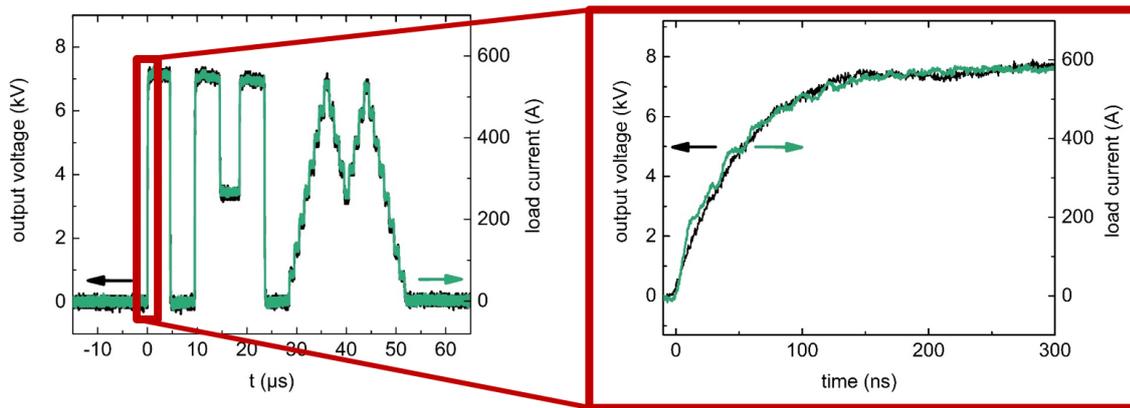


Fig. 2.2.4: Generation of a stepwise arbitrary voltage and magnified voltage- and current rise.

2.2.3 Marx-type pulse generator for bipolar rectangular pulses

For a project on the reduction of microbial contamination by applying a pulsed electric field a semiconductor-based Marx generator for repetitive generation of bipolar rectangular pulses is currently under development. The generator consists of stacked modules, thereby each module comprises an H-bridge topology enabling the generation of bipolar pulses. Each module is capable of delivering a rectangular pulse with a length in the range of between 1 μs and 10 μs at a voltage of up to 1 kV and a current of up to 600 A. In order to test the modules an 8-stage generator has been set up. Thereby, the generator has been grounded at its center to obtain ground-symmetric pulses to feed an electroporation reactor with an electrode system of parallel-plate type. Fig. 2.2.5 shows a photo of modules mounted inside the housing and an example for a pulse shape.

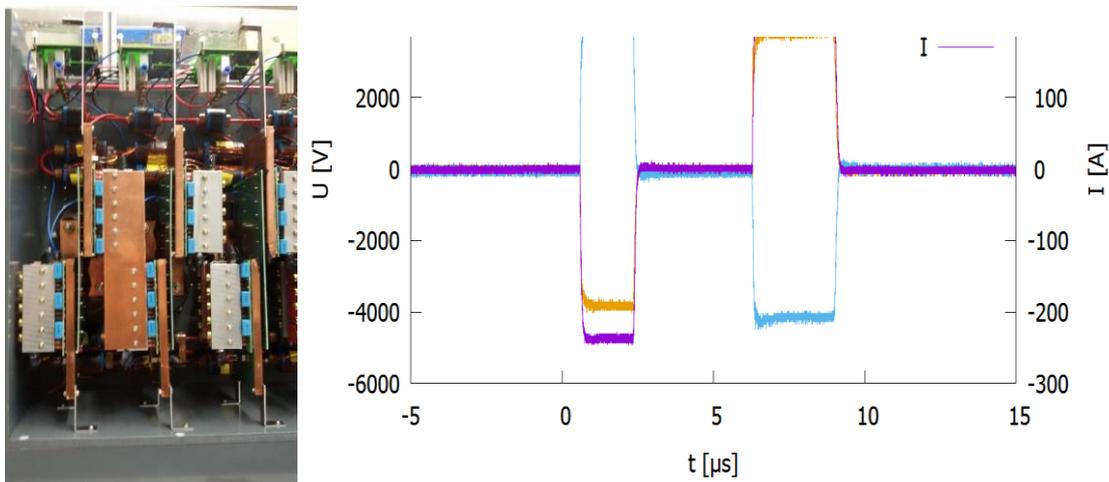


Fig. 2.2.5: Marx-type pulse generator for bipolar rectangular pulses: H-bridge modules mounted inside a housing (left) and example for a bipolar, ground-symmetric pulse shape.

2.3 Concentrating solar power (CSP)/ Liquid metal – Material research – improving the compatibility of materials for CSP

Contact: Dr. Alfons Weisenburger

Liquid metals as advanced heat-transfer- and storage media for CSP are a promising research area that will result in performance and efficiency increase and reduced costs. Different institutes and laboratories of the KIT therefore combine their long-standing experience and specific expertise in material research, system engineering, safety and thermal-hydraulics to tackle all relevant aspects of liquid metals as HTM. The IHM is especially focusing on compatibility research by surface optimization of existing materials using GESA and development of new materials that are able to form protective alumina scales.

2.3.1 Multipoint vacuum arc as cathode plasma source for sub-millisecond operation of GESA-facilities

The extension of the field of application of GESA facilities to non-adiabatic material treatment with sub-millisecond high-energy electron beams requires a comprehensive modification of the cathode. In the new cathode design, the plasma generated by vacuum arc discharges at the tips of several carbon fibers serves as electron source. At a maximum allowed load of the individual fibers of 1 A, it is possible to achieve emission current densities up to 0.5 A/cm². For a typical beam compression of $K=10-16$, this results in power densities at the target of up to 0.7 MW/cm² over pulse durations up to $\sim 450 \mu\text{s}$.

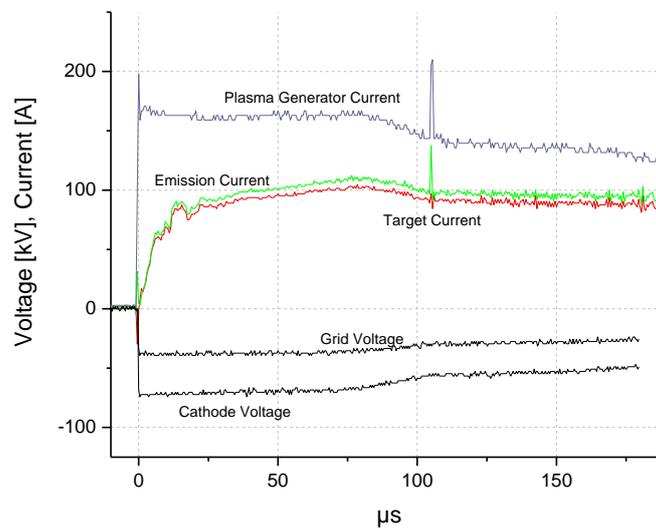


Fig. 2.3.1: Voltages and currents waveforms as function of time for typical sub-millisecond GESA pulse

The GESA concept with relatively low current density at the cathode and strong beam compression has the advantage that the cathode can be operated at relatively low plasma density. Thus, the formation of cathode spots at the metal surfaces that are in contact with the plasma can be controlled more easily. Using optical diagnostics, the ignition behavior of the vacuum arcs and the dynamics of the plasma was investigated. The findings enabled an optimization of the electrode geometry and potential distribution in order to generate a homogeneous cathode plasma.

2.3.2 Material development

Safe and economic operation of a CSP system requires enhanced compatibility of the selected liquid metal (Sn, LBE, or Na) with existing and new advanced materials for both targeted temperature regions (650 °C and higher). Beside the construction of experimental facilities that allow thermal cycling and corrosion tests in stagnant and flowing Na, the focus on material development was on high entropy alloys (HEA) and alumina forming austenitic (AFA) steels. The latter will be reported in section 3.1 of this report. HEA are a promising new class of materials for high temperature application in extreme environments like expected in liquid metal CSP systems.

In order to form FCC structure alloys with different compositions were calculated and finally 9 HEAs were produced. Six model HEAs contain four elements: Al, Cr, Ni and Fe, and three others contain five elements, Al, Cr, Fe, Ni with addition of either Cu or Ti or Nb. Most of these alloys obey the rules of FCC formation.

The model HEAs from FeCrAlNiX system (X: Ti, Nb, Cu) were prepared using high purity elements (99.95% or more) by arc-melting process with a non-consumable tungsten electrode under argon atmosphere (~0.9 atm). The prepared alloy ingots were flipped over and remelted at least five times in a water-chilled copper mold to facilitate alloy homogenization.

All the HEA alloys contain higher amounts of Al and Cr compared to the AFA. HEA with Al content higher than 4 wt% and without Cu addition showed good compatibility even the content of the highly soluble Ni is above 35 wt%.

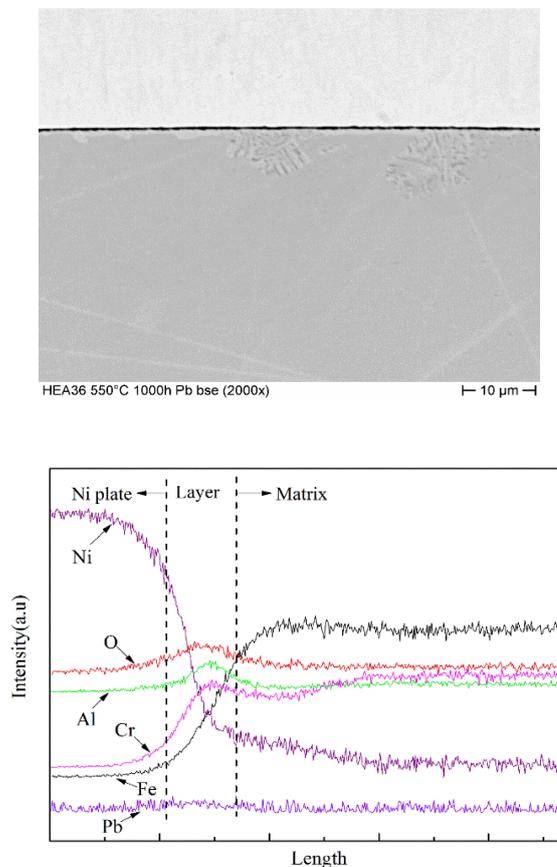


Fig. 2.3.2: SEM cross section (above) and elemental depth profile (below) of HEA exposed to Pb at 550°C for 1000h showing the formation of a protective Al-oxide scale

Involved Staff:

Dr. S- Akaberi, DP W. An, Dr. R. Fetzer, J. Fleig, **Dr. W. Frey**, Dr. Ch. Gusbeth, Dr. A. Heinzl, D. Herzog, M. Hochberg, DI (Fh), F. Lang, K. Leber, **Prof. Dr. G. Müller**, N. Nazarova, A. Neukirch, **M. Sack**, MSc. D. Scherer, Dr. G. Schumacher (Gast), H. Shi (SCS-PhD student), Dr. A. Silve, A. Sivkovich, DI R. Sträßner, Dr. A. Weisenburger, R. Wüstner, W. Zhen (CSC-PhD student)

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