Karlsruhe Institute of Technology

Monitoring of Inactivation Efficiencies and Regeneration Potential of Antibiotic Resistant *E. faecium* during Pulsed Electric Field Treatment of Wastewater

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Problem

Dissemination of hygienically relevant multi-resistant pathogens from clinical wastewaters into the downstream wastewater systems and the aquatic environment

Conventional disinfection techniques demonstrate disadvantages like toxic by-products (chemical disinfection) or reduced efficiency due to high particle load (UV disinfection)

Wastewaters contribute to horizontal gene transfer of resistance plasmids or virulence factors due to the high number of multi-resistant pathogens

Aim

Alternative wastewater disinfection technique

- efficient reduction of microbial contaminants at local points upstream of municipal wastewater treatment plants
- no application of chemicals so no toxic technique derived by-products are generated
- no affection of the natural occurring nuclease activity being important for the break down of extracellular DNA like resistance genes on transferable plasmids

➡ **Pulsed electric field treatment (PEF)**

PEF

Mode of function: directed against membranes of biological cells; the bi-electrical breakthrough of the phospholipid double layer induced by pulsed electric fields causes cell disruption

Present application areas: biotechnology (cell disruption, extraction of intracellular products), food industry (sterilization, wine production)

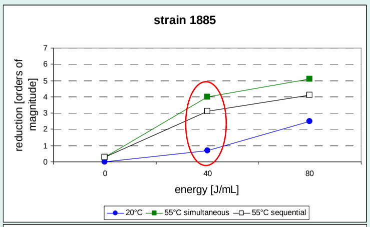
New application area ➡ **Disinfection of microbial charged wastewater**

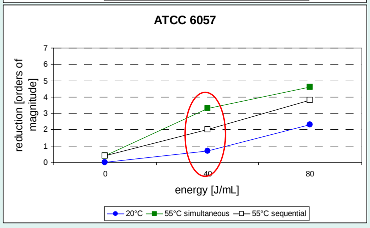
RESULTS

Inactivation efficiencies of PEF treatment and synergistic effects

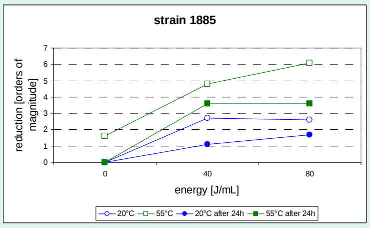
	reduction [orders of magnitude]					
	ATCC 6057	strain 1319	strain 39/05	strain 67/08	strain 1435	strain 1885
simultaneos treatment (PEF treatment and heat together)						
40J/mL	3,3	3,4	2,9	3,7	4,2	4
80J/mL	4,6	4,7	4,1	4,5	5,9	5,1
sequentiell treatment (heat, then PEF treatment)						
40J/mL	2,0	3,9	1,4	2,2	2,9	3,1
80J/mL	3,8	5,2	3,2	3,6	4,4	4,1
control						
	0,4	1,5	0,2	0,8	2,1	0,3

- antibiotic resistant strains were inactivated with up to 6 decimal orders of magnitude reduction at sublethal temperatur (55°C)
- complete reduction was determined at 60°C
- significant synergistic effects between PEF and sublethal temperature could be detected and were strain-dependent





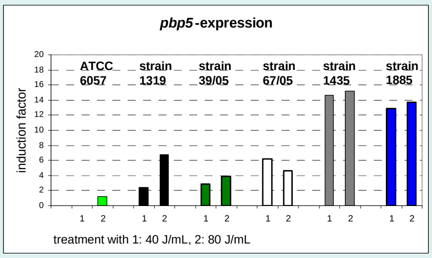
Regeneration and stress responses



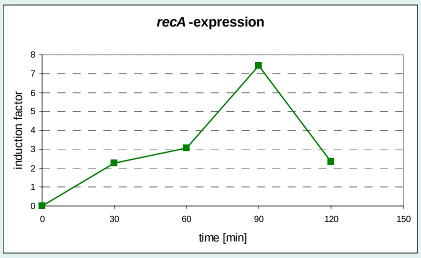
repair mechanisms

stress response

24 h after PEF treatment inactivation efficiency was declined about one order of magnitude (40 J/mL) and two orders of magnitude (80 J/mL) during simultaneous treatment at 55°C



the *pbp5* gene playing a major role in peptidoglycan synthesis was induced in all antibiotic-resistant bacteria, but not in the reference strain



the *recA* gene involved in repairing DNA injuries was induced by time

CONCLUSION

➡ **novel technology for the treatment of microbial charged wastewater like clinical wastewater**

- PEF treatment is efficient to eliminate natural occurring wastewater bacteria, including antibiotica resistant strains
- synergistic effects are strain-dependent
- bacteria are able to repair some damages resulting in a lesser inactivation rate, probably by inducing *pbp5* and *recA*