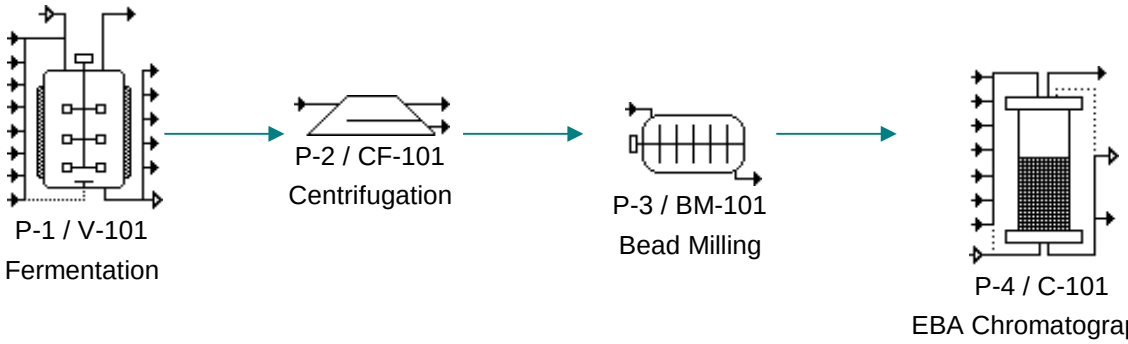
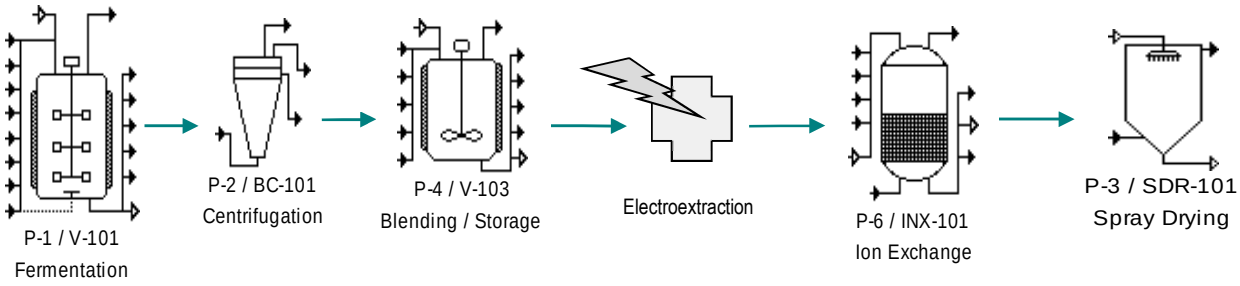
Electroextraction: The figures mentioned in the description of the main S&T results/ foregrounds

1. Figures of the Partner Jacobs University of Bremen

1a -- Conventional bioprocess



1b -- Dovetailed Electroextraction bioprocess for SOD



1c -- Dovetailed Electroextraction bioprocess for β-GAL

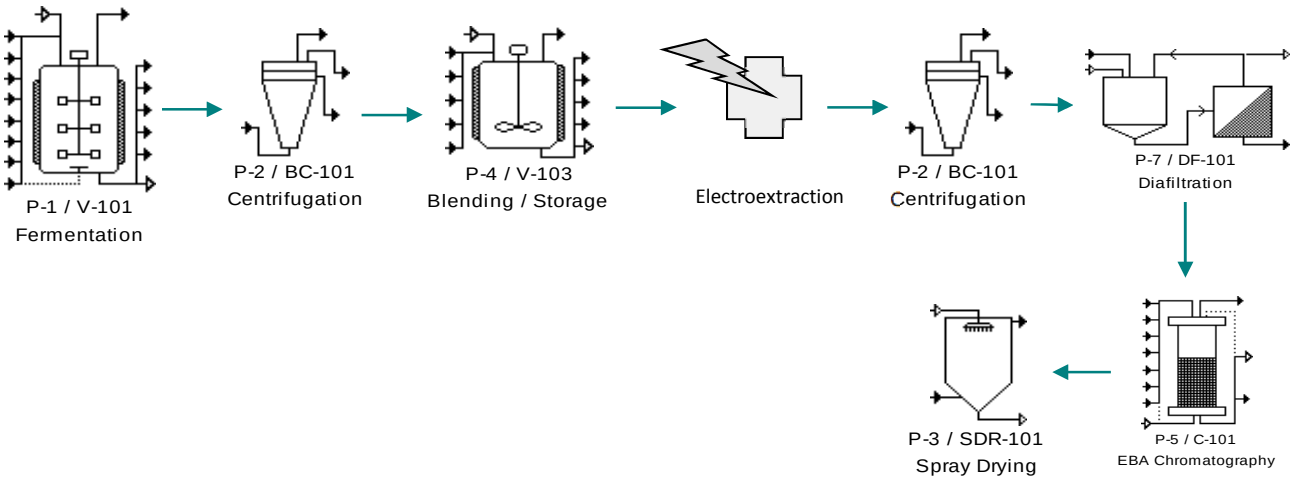


Fig. 1: Schematic flow chart of a dovetailed process

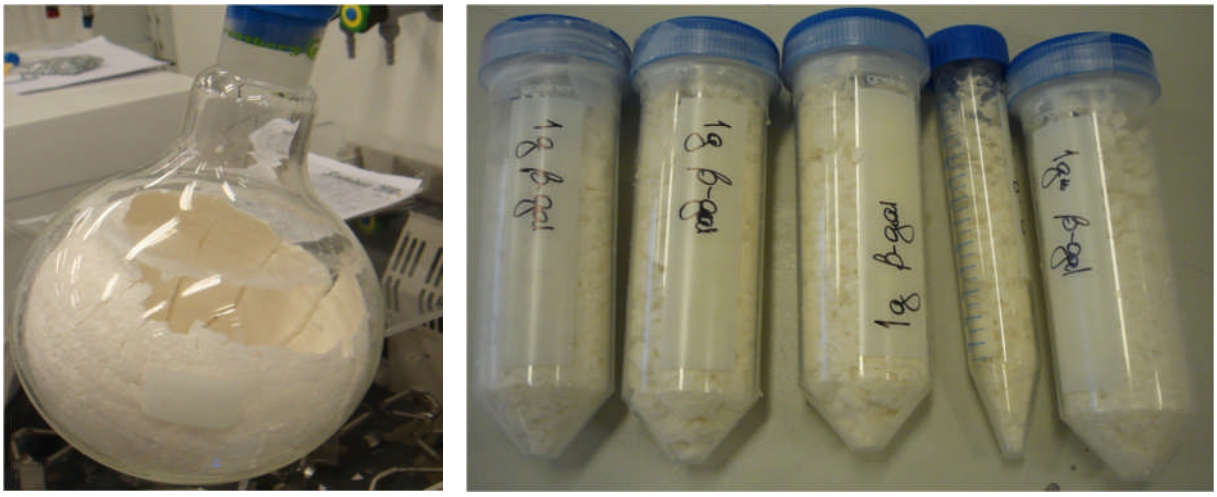


Figure 2a -- Lyophilized powder of Beta-Galactosidase (β-GAL)

Figure 2b -- Lyophilized powder of Superoxide Dismutase (SOD)

Fig. 2: Examples of obtainable products

2. Figures of the Partner CNRS

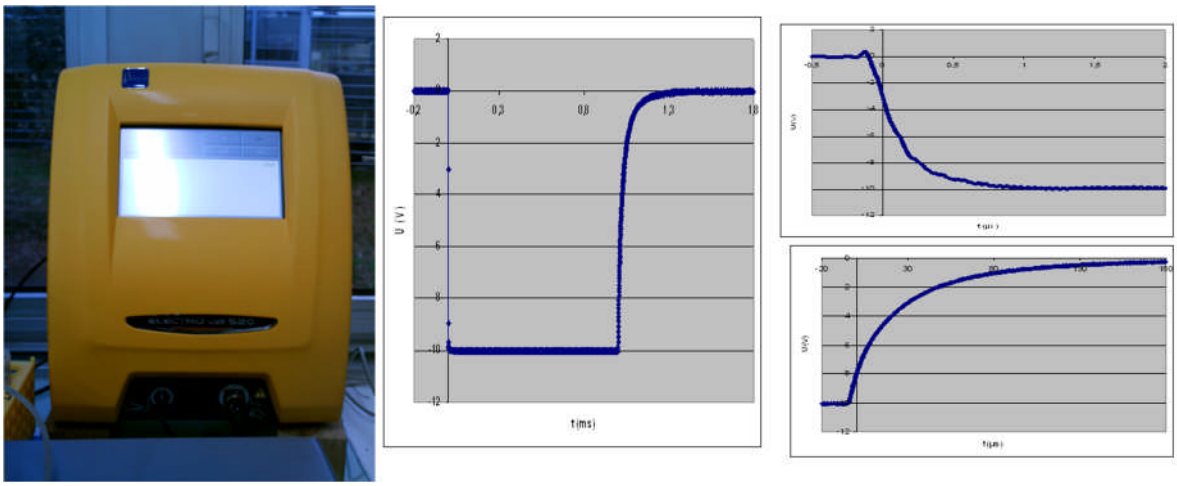


Fig. 1: The S20 electropulsator. Shape of the delivered pulse is displayed on the right

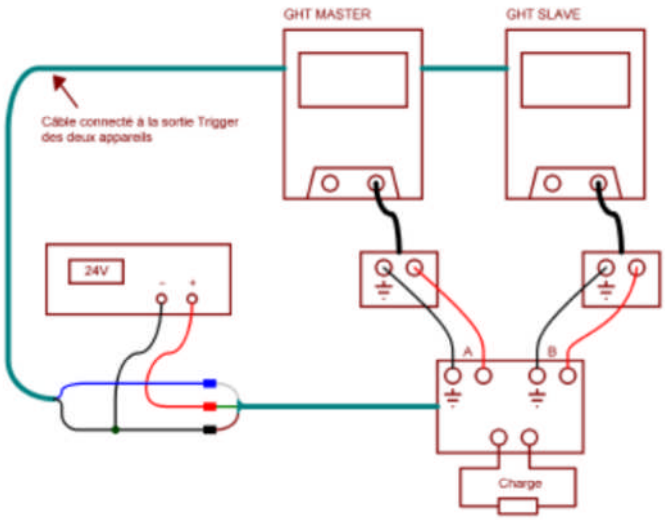


Figure 1 : Configuration pour la procédure de test.

Fig. 2: Electric system containing two GHT unipolar generators (with a 24V alimentation), one electrodes stimulator for each generator, the inversor to deliver the bipolar pulsations to the pulsing chamber, and a picoscope connected to an laptop PC to follow and visualize the impulsions delivered in real time. The load was the flow of yeasts in the pulsing chamber. The pump is used to flow the yeasts suspension in water through the pulsing chamber.

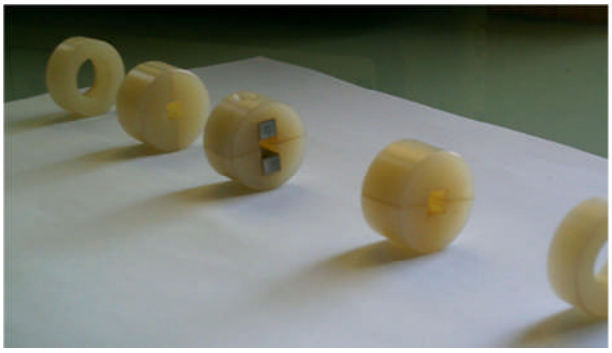


Fig. 3: The set of sub-parts of the applicator in a pulsing chamber

Fig. 4: The applicator. Tubing for the connection to the pump and to the collector can be easily fixed on both ends.

Fig. 5: Photos of the pilot containing the two Betatech unipolar generators connected to the inversor by the electrode stimulators.

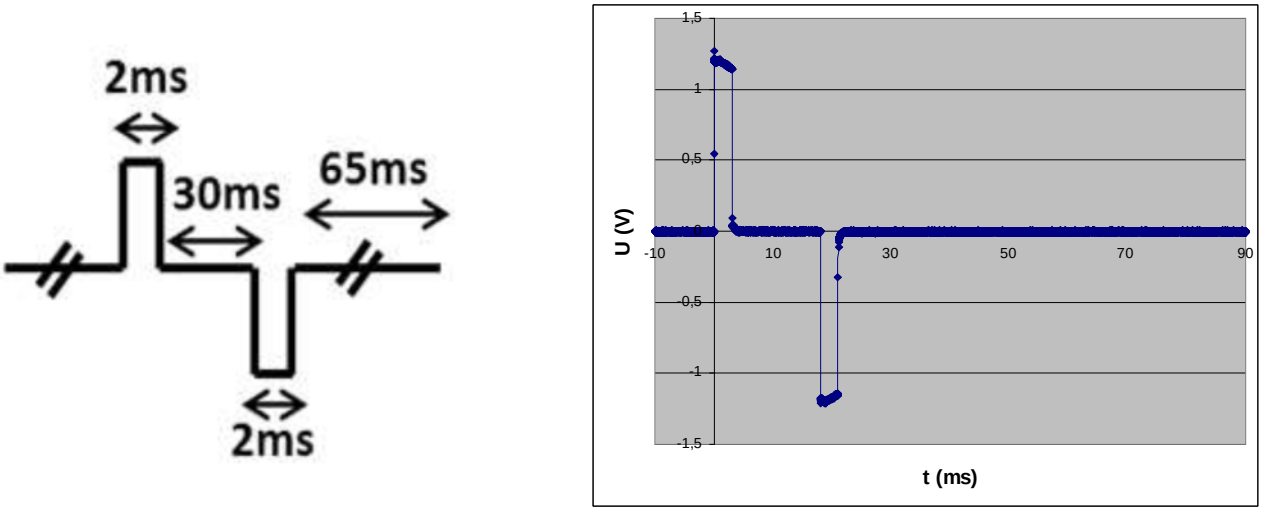


Fig. 6: Pulse sequence theoretical on the left and as stored from the Laptop PC

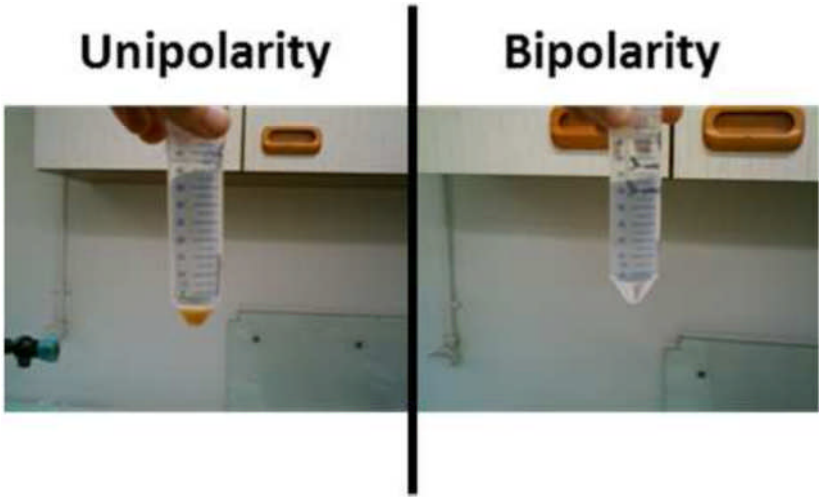
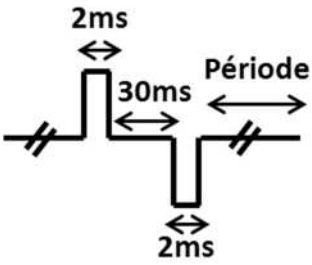


Fig. 7: Bipolar pulses prevent the occurrence of electrochemical reactions on the surface of the electrodes

			Samples	
	Negative control	Positive control	Unipolar	
	Yeasts + water	Chemical lysis (Lyticase)	1st test R=15 T=2ms	2nd test R=30 T=2ms
µg of protein/100µL suspension	2,16	4,96	2,31	4,59

Fig. 8: Results corresponding to Bradford assays for flow processes, using the following parameters: E=3kV/cm; pulses of 2ms duration; R=15 ou R=30 unipolar pulsess/cell; Period = 36 or 72ms respectively (gap between electrodes = 6mm). Negative control is made with an unpulsed suspension of yeasts in water, and positive control with chemical lysis using Lyticase.



			Samples	
	Negative control	Positive control	Bipolar	
	Yeasts + water	Chemical lysis (Lyticase)	1st test R=25 T=2ms	2nd test R=30 T=2ms
µg of protein/100µL suspension	2,46	4,96	5,07	5,35

Fig. 9: Results corresponding to Bradford assays for flow processes, using the following parameters: E=3kV/cm; pulses of 2ms duration; N=25 or N=30 bipolar pulses/cell; Period = 79 or 65ms respectively (gap between electrodes = 6mm). Negative control is made with an unpulsed suspension of yeasts in water, and positive control with chemical lysis using Lyticase.

	Samples	
	Negative control	Bipolar
	Yeasts + water	N=30 T=2ms
µg of protein/100µL suspension	2,11	6,07

Fig. 10: Results corresponding to Bradford assays for flow process on a 500 mL volume of yeasts, using the following parameters: E=3kV/cm; pulses of 2ms duration; N=30 bipolar pulses/cell; Period = 65ms (gap between electrodes = 6mm). Negative control is made with an unpulsed suspension of yeasts in water. We verified that we had symmetrical bipolar impulsions using the Picoscope acquisition.

	Samples (C=30.10^8 cell/mL)		
	Negative control	Bipolar	
	Yeasts + water	1st test N=25 T=2ms	2nd test N=30 T=2ms
µg of protein/100µL suspension	2,95	6,05	6,19

Fig. 11: Results obtained with a 30.108 cell/mL yeast suspension, using the following parameters: E=3kV/cm; pulses of 2ms duration; N=25 or 30 bipolar pulses/cell; Period = 79 or 65ms respectively (gap between electrodes = 6mm). Negative control is made with an unpulsed suspension of yeasts in water.

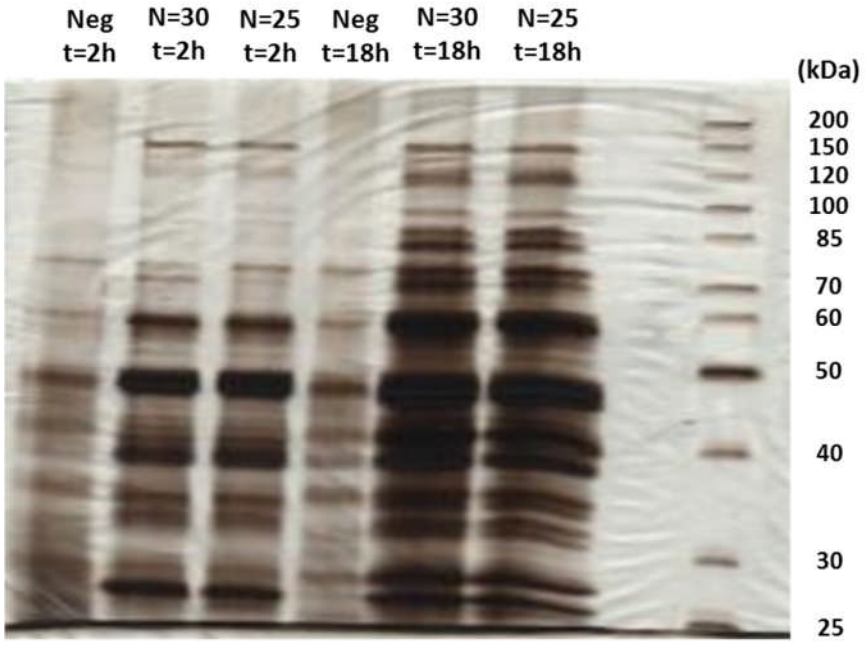


Fig. 12: Results of silver staining gel using samples from 2h and 18h after electroextraction. Negative control is made with an unpulsed suspension of yeasts in water.

	Negatives controls		Positive control	Samples (stationary growth phase)			
	Levures + eau	Levures + PBS	Mechanical lysis supernatant t=0	1st test N=40 T=2ms	2nd test N=35 T=2ms	3rd test N=30 T=2ms	4rd test N=25 T=2ms
µg of protein/ 100µL suspension t=4h	2,08	1,96	5,8	2,71	3	2,95	2,36
µg of protein/ 100µL suspension t=7h	2,15	1,99	5,25	2,92	3,16	3,07	2,74
µg of protein/ 100µL suspension t=22h	2,18	2,17	5,54	3,82	4,98	4,28	3,36

Fig. 13: Results of Bradford assays calculated as mean of two tests in stationary growth phase using the following parameters: E=3kV/cm; pulses of 2ms duration; N=40/35/30/25 bipolar pulses/cell; Period = 39/44/65/79ms respectively (gap between electrodes = 6mm). Negative control for samples is made with an unpulsed suspension of yeasts in water, and for mechanical lysis, negative control is made with an unpulsed suspension of yeasts in PBS

3. Figures of Partner University of Sofia

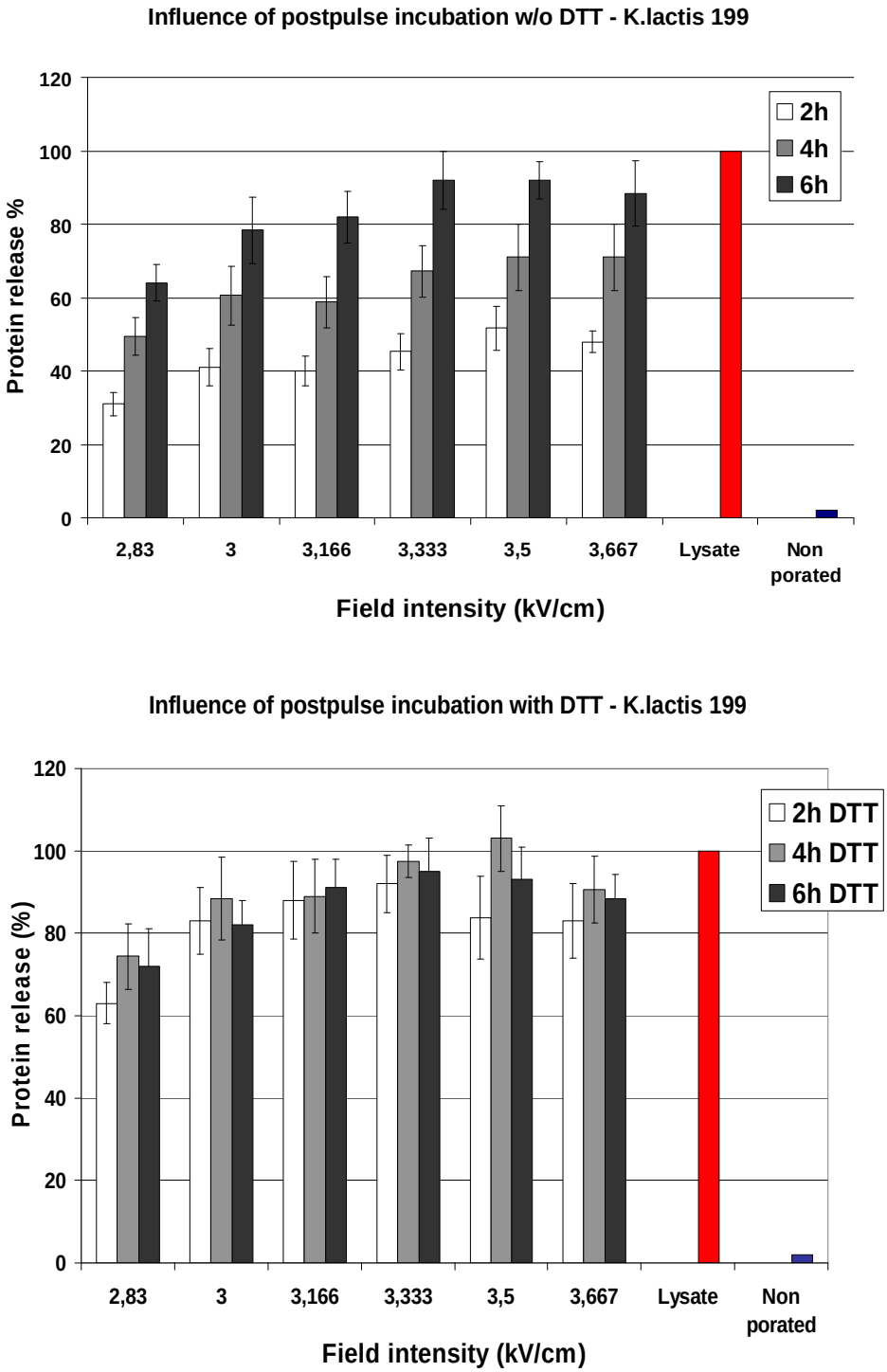


Fig. 1: Influence of reducing agent on protein liberation after pulsation.

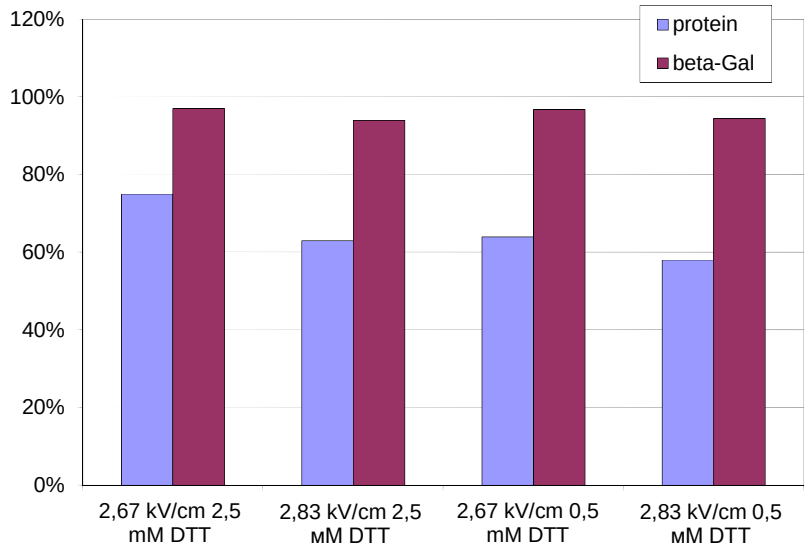


Fig. 2: Liberation of beta-galactosidase and total protein from cells incubated after pulsation for 3 h in 100 mM PPB, 350 mM glycerol, pH=7.5 Pulsing condition: 10 pulses, 15 Hz, 27 ml/min, cell concentration corresponding to 15% wet weight.

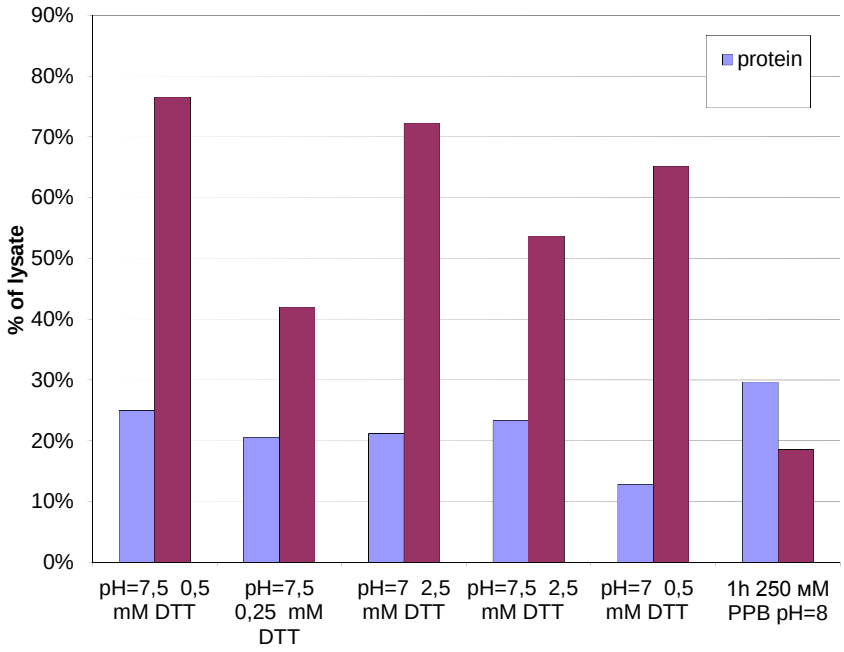


Fig. 3: Beta-galactosidase and total protein after two consecutive incubations with different buffer. Cells incubated for 1 h after pulsation in 250 mM PPB pH=8, centrifuged and supernatant removed. Then cells were resuspended in 100 mM PPB, 350 mM glycerol pH=7 – 7.5, and incubated again for 2 hours.

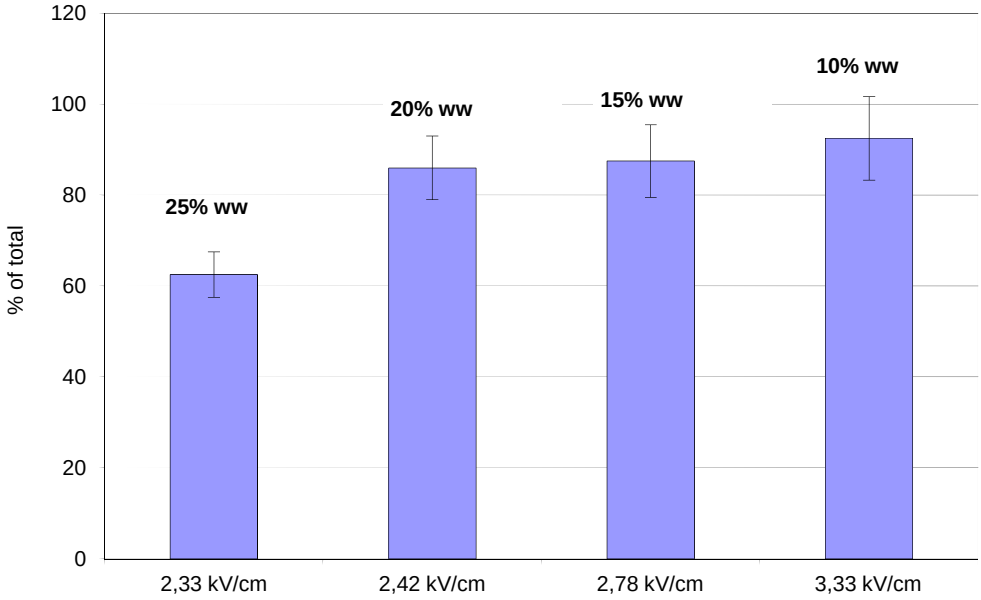


Fig. 4: Optimal field intensity for beta-galactosidase extraction at different cell concentration corresponding to 10, 15, 20 and 25 % wet weight.

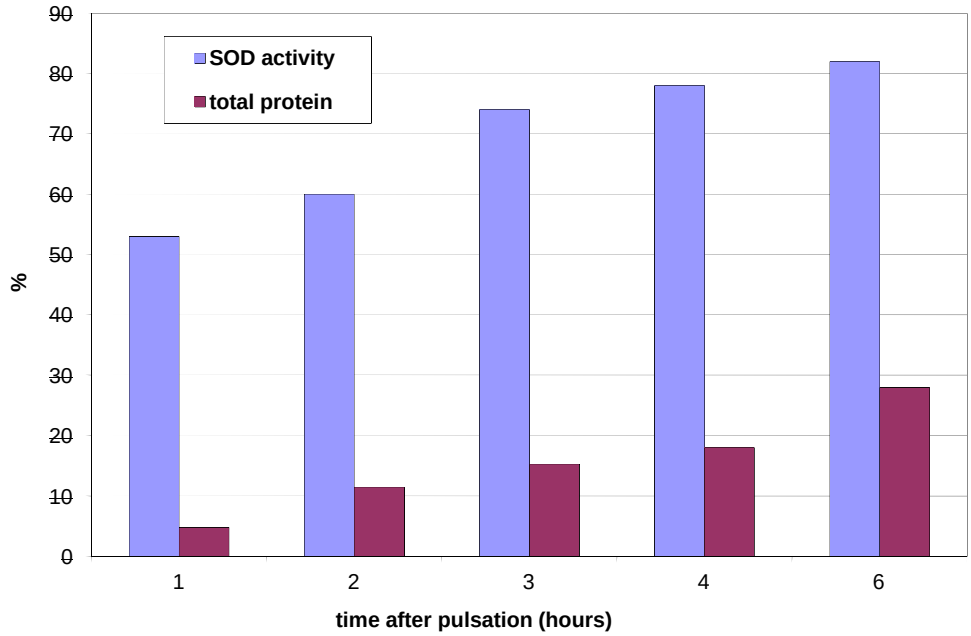


Fig. 5: Kinetics of SOD and total protein release. Experimental conditions: 4.17 kV/cm, 10 pulses, 1.5 ms, 27 ml/min. Cells incubated after pulsation in 100 mM PPB, 350 mM glycerol, pH 7, 27ml/min, 15 Hz , 10 % wet weight.

A B C

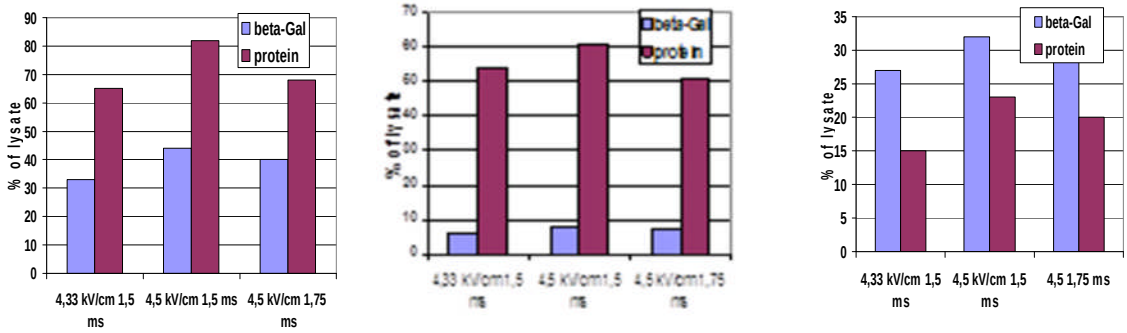


Fig. 6: Electroinduced liberation of beta-galactosidase and total protein from S. cerevisiae W303-1A. A – enzyme/protein liberation 20h after pulse application and incubation in PPB pH 7; B – enzyme/protein liberation after 2 h incubation in PPB pH 8; C enzyme/protein liberation obtained after the second 18h incubation in PPB pH 7,5

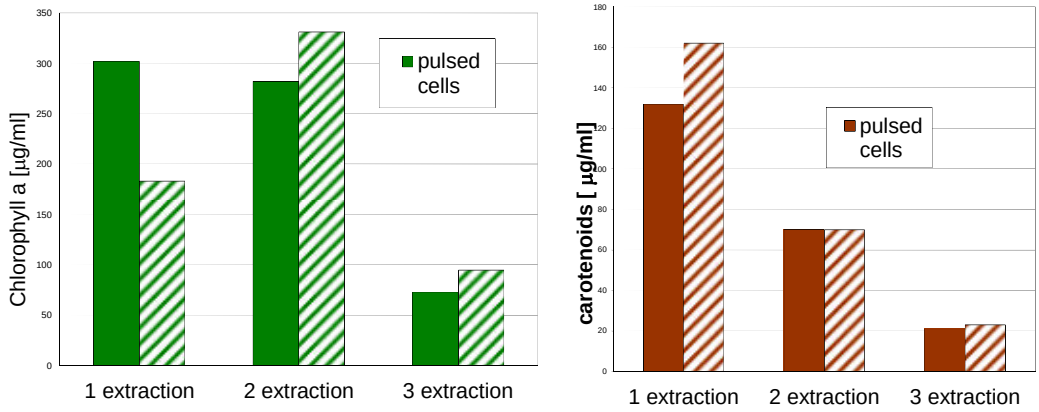


Fig. 7: Electric field effect on ethanol extraction of chlorophyll and carotenoids. Experimental conditions: Fiedl intensity 7 kV/cm, 9 Hz, 11 pulses, pulse duration 1.5 ms, 14.4 ml/min. 100 mg pulsed cells diluted in 400 □l 100% ethanol. First and second extractions were performed for two hours at 4oC. Third one was of 15 min duration.

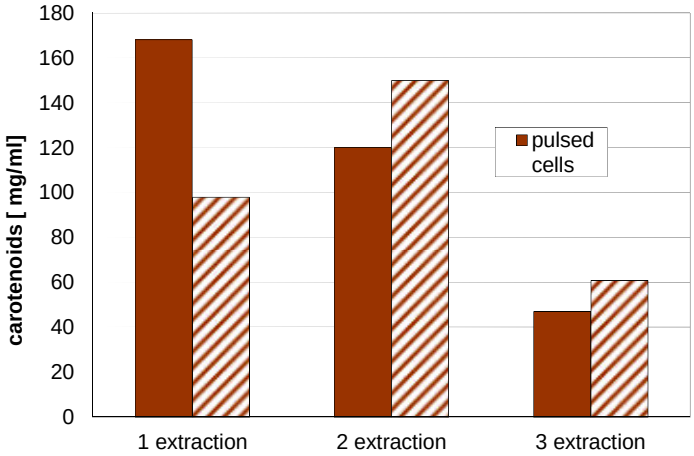


Fig. 8: Electric field effect on carotenoids extraction. Experimental conditions: cells treated with 7 kV/cm, 9 Hz, 11 pulses, pulse duration 1.5 ms, 14.4 ml/min. 150 mg pulsed cells diluted in 400 □l 100% ethanol. First and second extractions were performed for two hours at 4oC. Third one was of 15 min duration.

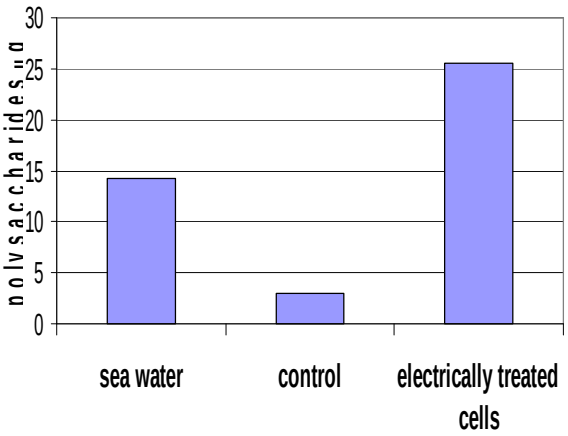


Fig. 9: Electroinduced release of polysaccharides from P.cruentum. Experimental conditions: 6 kV/cm, 1.75 ms duration, 10 pulses, 18 ml/min. Control – intact cells in distilled water.

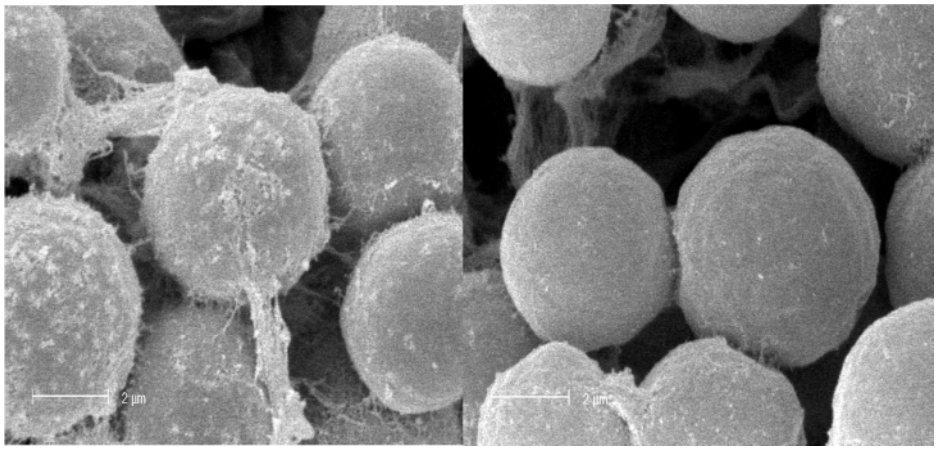


Fig. 10: Electron micrographs of intact cells (left) and electroporated cells (right)