Supporting information

Impact of electrolyte solution on electrochemical oxidation treatment of *Escherichia coli* K-12 by boron-doped diamond electrodes

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1.1 Stability of the experimental setup

We characterized the experimental system before the electrolysis to minimize the influence of unknown effects. The electrolytes (physiological solution (labelled as "NaCl"), phosphate buffer saline (PBS), and phosphate buffer (PB)) were tested several times for potential toxicity against *E. coli*. As we supposed, after 120 min in the absence of the electrical current (untreated control samples), the decrease of the viability of *E. coli* was negligible in NaCl and PBS, and minor (one order of magnitude, initially 5×10^7 cells·ml^{-1}) in PB. To simplify and facilitate the comparison of untreated samples with EO treated samples, all untreated samples were averaged into a single exemplary untreated control sample value, which is further labelled as "control".

Next, we confirmed by XPS and SEM measurements that the cleaning procedure (see chapter 3.3) was sufficient to define the initial stable state of the BDDE electrodes. The successful removal of all bacterial remnants was manifested as the disappearance of all black spots in SEM images, and in the XPS spectrum as a significant decrease of relative atomic concentration of nitrogen (from 2.5% after the EO treatment to 0.3% after the cleaning procedure), together with decrease of relative

oxygen concentration (from 20% to 9%) and increase of relative carbon concentration (from 55-80% to 90%) on both electrodes.

Finally, the temperature and pH of each suspension were monitored during the 120 min experiment. The temperature increased (from 25°C) only slightly for both the control (to 27°C) and all treated samples (to 29°C) after the experiment, which was attributed to a gradual increase of the ambient temperature due to the prolonged work with a Bunsen burner in a confined space inside the UV-sterilized room for control samples, and a combined effect of increased ambient temperature and the electrolysis process itself for treated samples. Nevertheless, the temperature always stayed within the physiological range for mesophilic bacteria (*E. coli*) since it never exceeded 30°C, and thus no cooling was applied. The pH of all control samples in the PBS, NaCl, and PB remained nearly constant, while the pH stability of EO treated samples strongly depended on the composition of the electrolyte. The pH increase after the EO treatment was minor (<0.1) in both buffered solutions (from 7.11 ± 0.13 to 7.18 ± 0.13 in the PBS and from 7.17 ± 0.03 to 7.18 ± 0.03 in the PB), but comparably higher (by 0.8) in the NaCl (from 7.02 ± 0.24 to 7.77 ± 0.14) when compared to initial conditions. This pH increase in the NaCl was most probably caused by generating and gradually accumulating NaOH, as the pH of control samples was stable. However, since the pH never exceeded 8.0, it should have only a negligible effect on the cell viability, which was confirmed by our observations of only 13% decrease in the growth rate of planktonic *E. coli* cultivated in an LB growth medium with pH modified to 8.0 (data not shown), and supported by another study investigating the cultivation of E. coli in pH 7.4 and 8.0 (Kim *et al.* 2018). Therefore, it can be concluded that both the temperature and the pH variations did not have any noticeable impact on the interpretation of the evaluated data.

During the EO treatment, a constant current density of 10 mA·cm^{-2} was applied, and the voltage was recorded. The voltage fluctuation during experiments was negligible, with a slight and gradual increase by 1-5% of the initial value for PBS (from 3.70 ± 0.12 V to 3.91 ± 0.09 V), NaCl (from 3.75 ± 0.24 V to 3.93 ± 0.13 V), and PB (from 3.94 ± 0.11 V to 3.97 ± 0.13 V), which could be caused by a partial depletion of the electrolyte or by a decrease of the submerged electrode surface area due to sampling (the manual correction of the electrode immersion depth every 30 min could be slightly inaccurate). Additionally, the anode potential was measured during the EO process with a three-electrode system at a constant current of 10 mA·cm^{-2} using the Autolab PGSTAT302N (Metrohm) with BDD for working and auxiliary electrodes and Ag/AgCl as the reference electrode.

The anode voltage increased slightly by 7-14% during the 120 min, and with increasing measurement time it stabilized in all PBS (3.28 – 3.74 V), NaCl (3.62 – 3.88 V), and PB (3.31 – 3.72 V).

1.2 Survival times and survival rates of bacteria

The graphical projection of dependence of a natural logarithm of the ratio ($ln(n/n_0)$) of surviving bacteria throughout the experiment (n) to the initial number of bacteria (n_0) on electrolysis treatment time [min] shows the Kinetics of dying (Figure 1a). The linear fit of the slope of Kinetics of dying represents the dying rate (k), which describes the trend of Kinetics of dying ($k = 0$ represents overall stable bacterial population, while $k < 0$ represents declining number of living bacteria, more negative for faster decline). The limited bacterial inactivation in control (one order of magnitude) and EO treated PB (two orders of magnitude) after 120 min is reflected by small values of k (-0.013 and -0.034, respectively), while rapid bacterial removal in EO treated PBS and NaCl (<10 min) is reflected by much higher k values (-3.405 and -3.298, respectively).

The theoretical survival time was calculated from the k according to formula:

$$Survival\ time = \frac{ln(n/n_0)}{k}$$

where n_0 is the initial number of $CFU{\cdot}ml^{-1}$ and n represents the final situation of sterilized suspension (<1 surviving bacteria) and was therefore set as $n = 0.5$. This allowed us to extrapolate the survival time in controls and EO treated PB, where bacteria survived longer than experiment duration (120 min) (Erkmen and Bozoglu 2016).

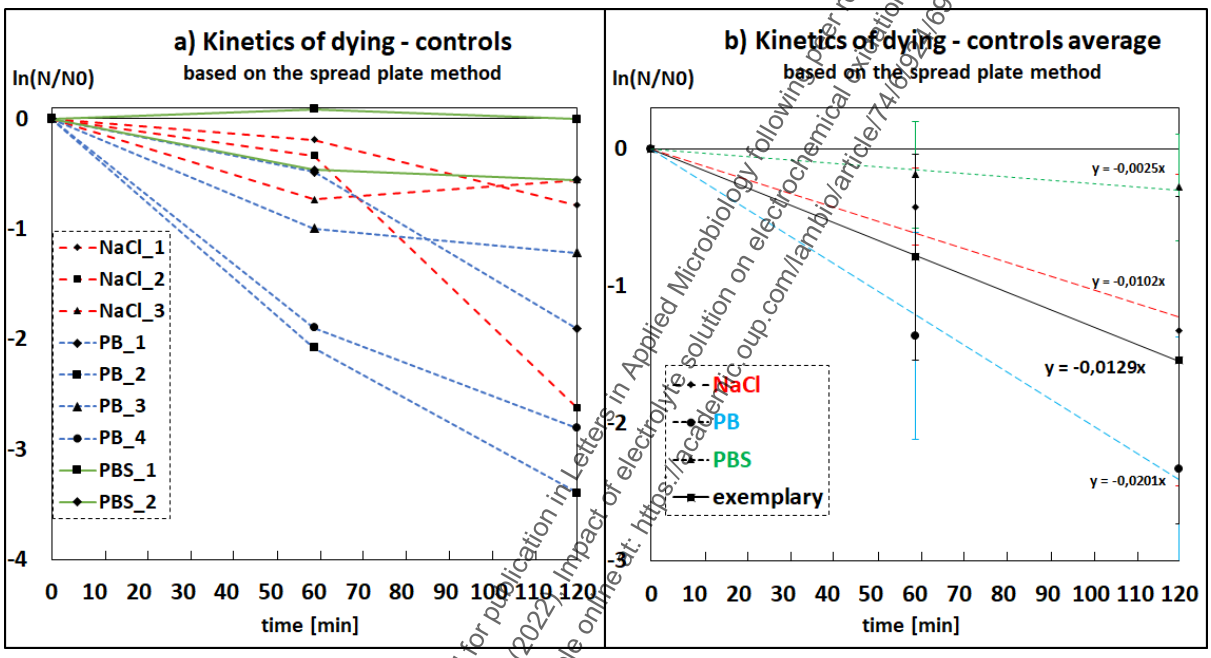
List of supporting files:

Figure S1**:** a) Kinetics of dying of *E. coli* in three untreated electrolytes measured several times (3x for NaCl, 4x for PB and 2x for PBS). b) Averaged Kinetics of dying of *E. coli* calculated for untreated samples. The "exemplary" plot was calculated as the average value from all untreated samples of all tested types of electrolyte and further was used as the "control" behavior for comparison with EO treated samples (despite all three averaged untreated samples for each electrolyte separately).

Figure S2**:** Comparison of average survival times of *E. coli* in three EO treated electrolytes and in untreated control samples in a logarithmic scale evaluated by drop plate method. The number of repetitions N ≧ 3.

***Figure S3*:** Representative SEM images of *E. coli* bacteria at selected times (0, 10, 60 and 120 min) of EO treatment in all three buffer solutions (NaCl, PB and PBS) together with a typical control untreated sample after 120 min.

***Figure S4*:** A schematic representation of the setup for electrochemical oxidation (EO). The BDD electrodes with an active area of 10 cm^2 are driven by a constant current (100 mA) in 1 L of the bacterial suspension.



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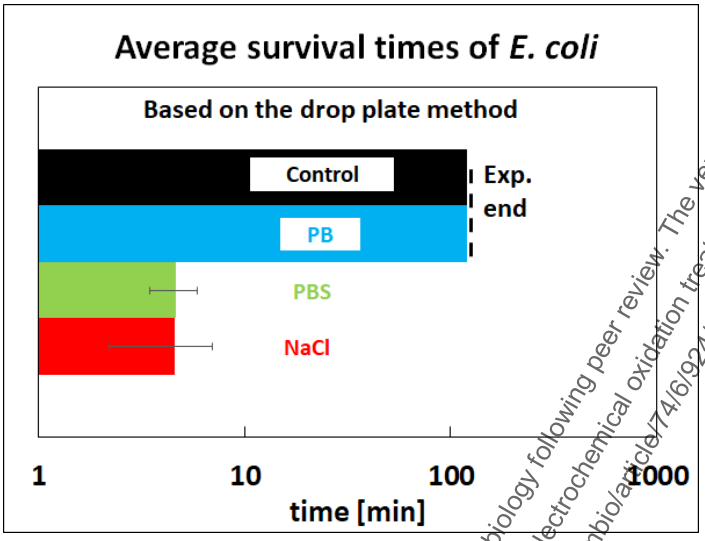
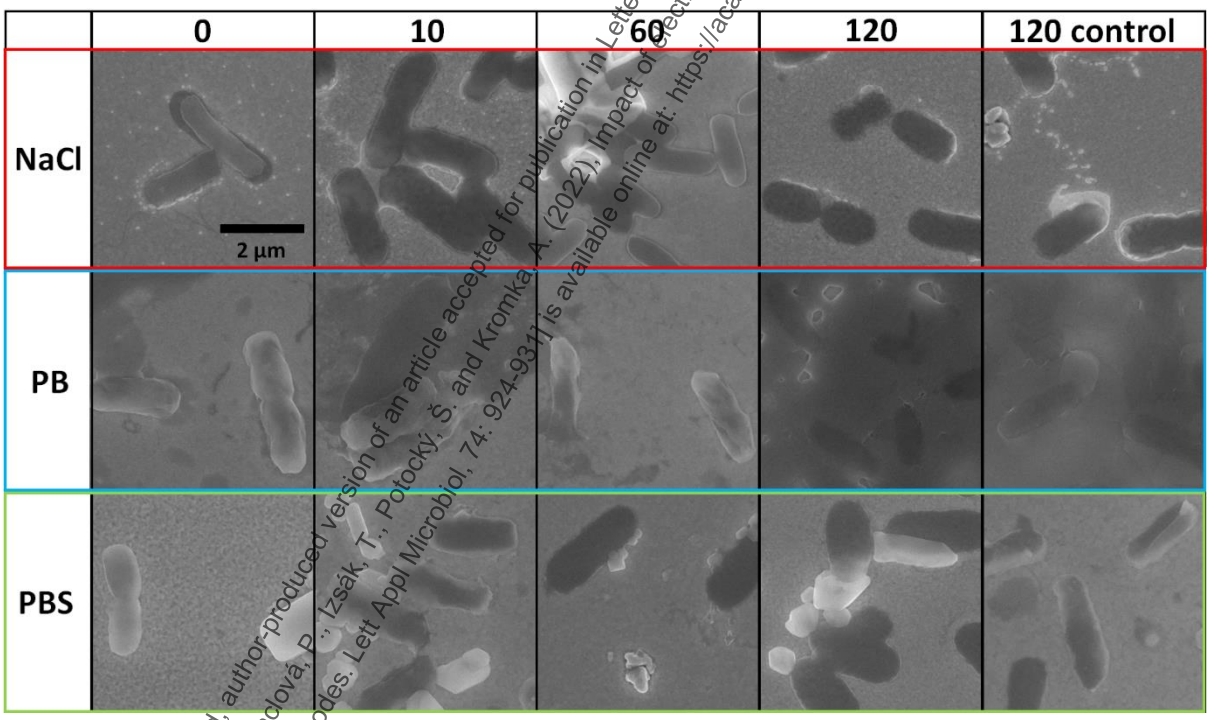


***Figure S2*:** Comparison of average survival times of *E. coli* in three EO treated electrolytes and in untreated control samples in a logarithmic scale evaluated by drop plate method. The number of repetitions N ≥ 3.



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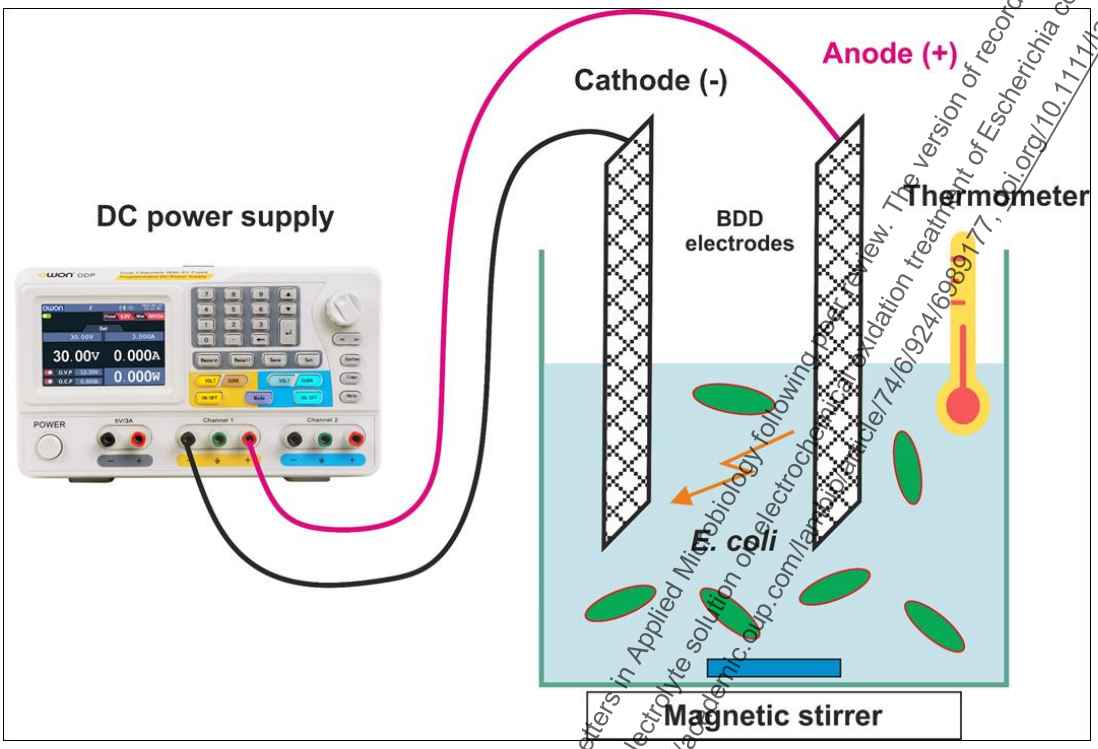

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References:

Erkmen, O., Bozoglu, T.F. (2016). *Food Microbiology: Principles into Practice.*, John Wiley & Sons: Chichester.

Kim, C., Wilkins, K., Bowers, M., Wynn, C., Ndegwa, E. (2018). 'Influence of PH and Temperature on Growth Characteristics of Leading Foodborne Pathogens in a Laboratory Medium and Select Food Beverages', *Austin Food Sci.*, *3*.