Electronic supplementary information

Formation of ammonium in saline solution treated by nanosecond pulsed cold atmospheric microplasma: a way to fast inactivation of *E. coli* bacteria

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Methods

E. coli Suspension Preparation and Analyses

E. coli strain CIP 53.126 was grown to the exponential phase for 2 h in liquid Tryptone Soya Broth at 37°C. The cells were then washed three times by centrifugation at 3000 g for 3 min and resuspended in PBS solution (Sigma-Aldrich, St. Louis, MO, USA, NaCl 138 mM, KCl 2.7 mM, phosphate buffer 10 mM, pH 7.4), before cellular density was adjusted to 5×10^7 CFU.mL^{-1}.

Wet chemistry treatments were performed by exposing for 35 minutes the *E. coli* suspensions described above to the following chemicals: 500 µM NH_4Cl (Sigma-Aldrich, St. Louis, MO, USA), 500 µM NO_2^- (Merck, Darmstadt, Germany), 4.52 / 45.2 / 452 µM HClO (Hach Lange, Düsseldorf, Germany) and 0.485 / 4.85 mM H_2O_2 (Merck, Darmstadt, Germany); as well as the following chemical mixtures: 4.52 / 45.2 / 452 µM HClO + NH_4Cl (NH_4Cl added in a 5:1 molar ratio compared to HClO), 452 µM HClO + 500 µM NO_2^- and 485 µM H_2O_2 + 500 µM NO_2^-. During all treatments, suspensions were maintained at pH 7.4.

E. coli culturability after 30 min wet chemistry treatments and 90 s.mL^{-1} plasma treatments was assessed according to a plate counting method, right after treatment, after three days (plasma treated suspensions only) and after ten days post-treatment (plasma treated suspensions only). Briefly, treated suspensions underwent seven successive ten-fold dilutions in PBS before 100 µL of each were spread on Trypticase Soy Agar growth medium. Colony forming units were counted after overnight incubation at 37°C.

ICP-MS Analyses

5 mL high purity milliQ water samples were treated for 10 minutes (i.e. 120 s.mL^{-1}) with He, He + 0.50% N_2 and He + 0.50% O_2 plasmas, then acidified with nitric acid (nitric acid for trace analysis min. 67 %, LGC Standards, Molsheim, France). They were then analysed by inductively coupled plasma mass spectrometry (ICP-MS) performed on an Elan DRC-e (PerkinElmer, Waltham, MA, USA). Calibration was done using Ge as internal standard (PlasmaCAL calibration standard for ICP-AES & ICP-MS, SCP Science, Courtaboeuf, France).

TOF-SIMS Analyses

Silicon wafers were treated for 60 minutes with He, He + 0.50% N_2 and He + 0.50% O_2 plasmas before being analysed. Time of Flight Secondary Ion Mass Spectroscopy (TOF-SIMS) analyses were then performed on a TOF-SIMS 5 (IonTOF, Münster, Germany) equipped with a Bi^{3+} source. The applied intensity was 0.37 pA, with a primary ion dose density of 1.0×10^{11} ions.cm^{-2}. 500 × 500 µm regions of interest on Si wafers were scanned with a resolution of 256 × 256 pixels.

Temperature and pH Analyses

Temperature and pH of treated and non-treated 0.9% saline and PBS samples were measured on an Orion 261S meter coupled to an Orion 9109WP triode (Thermo Fischer Scientific, Sunnyvale, CA, USA). Three-point calibration (pH buffers 4.00, 7.00 and 10.01) and subsequent measurements were carried out under magnetic stirring.

Ion Chromatography Analyses

Treated and non-treated high purity milliQ water samples were analysed by ion chromatography performed on a Dionex ICS-5000$^+$ HPIC (Thermo Fischer Scientific, Sunnyvale, CA, USA). Anions (nitrates, nitrites and chlorites / chlorates) were separated with

IonPac AG18 and AS18 columns (eluent: 22 mM KOH, 0.25 mL.min^{-1} flow rate), cations (ammonium) with IonPac CG16 and CS16 columns (eluent: 40 mM methanesulfonic acid, 0.40 mL.min^{-1} flow rate).

Colorimetry Analyses

Hydrogen peroxide concentration in treated 0.9% saline and PBS samples was quantified by a Spectroquant Hydrogen Peroxide Test (Merck, Darmstadt, Germany) colorimetric assay, based on the reduction of Cu(II) ions to Cu(I) ions. Absorption was measured at a wavelength of 445 nm, against a blank solution, on a Lambda 950 UV/vis/NIR spectrophotometer (PerkinElmer, Waltham, MA, USA).

Figures

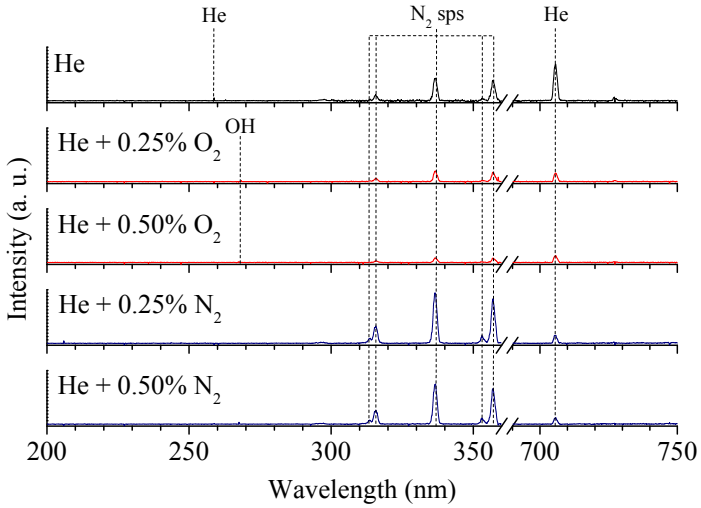

Fig. S1 Optical emission spectra of He, He / O_2 and He / N_2 nanosecond pulsed microplasmas in the 200 – 350 nm UV range.

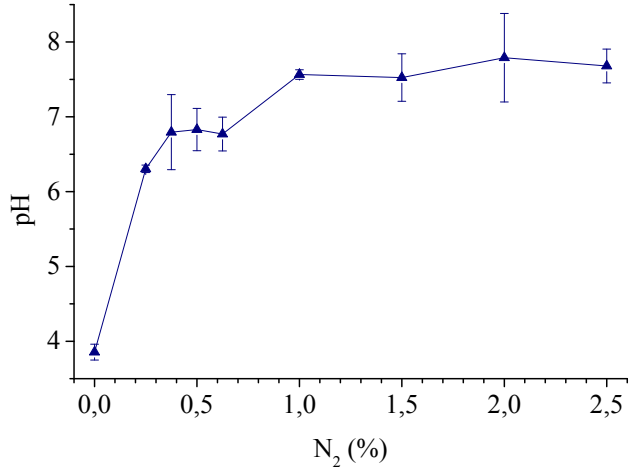

Fig. S2 pH of 0.9% saline solutions after 90 s.mL^{-1} He / N_2 nanosecond pulsed microplasma treatments with 0 – 2.5 vol. % N_2 mixed to He carrier gas (n = 3).

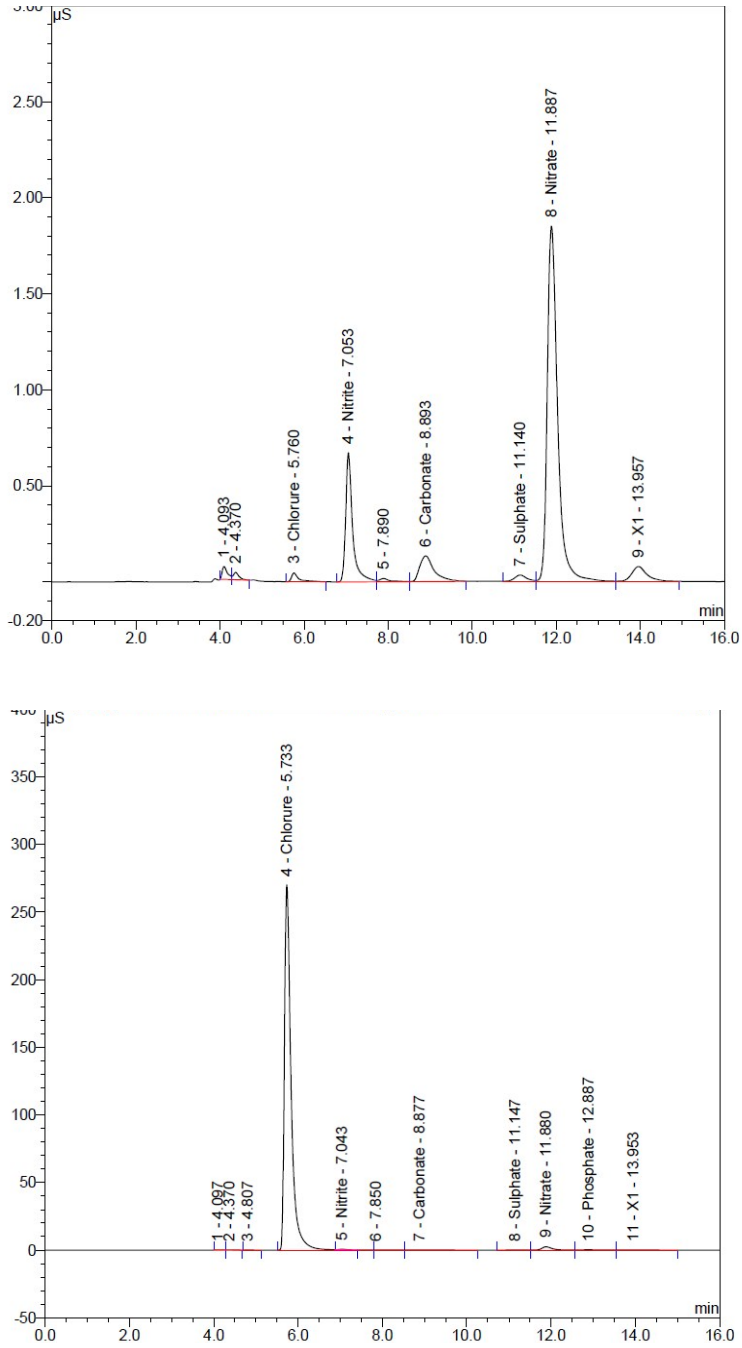

Fig. S3 Anion chromatogram of milliQ water (top) and 0.015% saline (bottom) samples after 90 s.mL^{-1} He + 0.50% N_2 nanosecond pulsed microplasma treatments. X1 elution peaks at 13.953 – 13.957 min are attributed to ClO_3^- species.