Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Bactericidal contact active stainless steel via a sustainable process utilizing electrodeposition and covalent attachment in water

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Table of Contents

General Procedures:

All chemicals (4-hydroxyaniline, ethanol, isoamyl nitrite, acetic acid, diethylether, Na_2MoO_4, oxalic acid, sodium phosphate, deionised water) and stainless steel (SS316LS, 0.12 mm thickness) were purchased from Aldrich or D-Chem and used as received. Stainless steel was mechanically cleaned with steel wool, washed with deionized water and sonicated for ten minutes before being dried under vacuum, then was cut into ~0.8x4 cm flat, long strips that were used as electrodes.

Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded using a Bruker AMX-250 spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported in ppm downfield from tetramethylsilane. Abbreviations used commonly in NMR experiments: b, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; v, virtual.

Synthesis of parahydroxo phenyldizaonium tetrafluoroborate

Parahydroxo phenyldizaonium tetrafluoroborate was synthesized using a modified literature procedure.[18] 4-hydroxyaniline (4.6mmol) was dissolved in 5 mL of ethanol that was cooled to -10^{o}C. Isoamyl nitrite (5.75 mmol; 0.98mL) was added and afterwards 13.5 mmol of 48% aqueous acetic acid was added (1.76mL). The reaction mixture was stirred for half an hour and afterwards 30ml of cold diethylether were added. A viscous purple liquid formed at the bottom of the flask and the solution was decanted. The purple liquid was washed several times with cold diethylether and the washings decanted to give a solid material which was dried under reduced pressure, and finally vacuum dried overnight to give the desired salt in quantitative yield as a purple solid that was stored at 5^{o}C. ^{1}HNMR (D_2O, 300MHz): 8.40 (d, 2H, J_{HH}=7.5Hz), 7.14 (d, 2H, J_{HH}=7.5Hz). ^{13}CNMR (D_2O, 75MHz): 170.1, 136.7, 119.3, 99.5.

Electrodeposition

A stainless steel electrode prepared as above was first passivated at a potential of 0.8V with a 60mM solution of Na_2MoO_4 for 30 minutes. For this purpose a standart three electrode electrochemical setup (BioLogic, France, SP-150) consisting of a stainless steel as working electrode, platinum wire (Holland Moran Ltd., Isreal) as auxiliary electrode and a Ag/AgCl pseudo-reference (i.e., a silver wire (Holland Moran Ltd., Isreal) immersed in $FeCl_3$/HCl solution) electrode was used. After the passivation step, the electrodes were quickly rinsed and immersed in a solution containing 0.25M oxalic acid, 5.0mM diazonium salt and 1.0mM of sodium phosphate. Three CV scans were donefrom 0.6 to -0.3V vs SCE at a speed 25mV/s. The electrode was removed from the solution and rinsed with deionised water for one minute. After a number of electrodes were prepared via this procedure, they were sonicated for 10 minutes and dried under vacuum to give surfaces **1.**

X-ray photoelectron spectroscopy

X-ray photoelectron measurements (XPS) were carried out in order to gain an insight into the chemical structure of the active substrates. XPS were measured at 45° relative to the surface plane with a PHI 5600 Multi Technique System. The base pressure of the main chamber was 3×10^{-10} Torr.[1-2] Spectra were excited with Al-Kα radiation and structures due to satellites were subtracted before data processing. XPS peak intensities were obtained after Shirley background removal.[3] Spectra calibration was achieved by fixing the C 1s peak of the aliphatic C-C bond at 285.0 eV and experimental uncertainties in binding energies lie within ± 0.4 eV.[4] Core level spectra were fitted after subtraction of the background using pseudo-Voigt functions with the binding energy, peak full width at half maximum height (FWHM), relative peak area and Gaussian/Lorentzian mixing parameter all treated as variables. This process involves data refinement, based on the method of the least squares fitting, carried out until there was the highest possible correlation between the experimental spectrum and the theoretical profile. The residual or agreement factor R defined by $R = [\Sigma(F_{obs} - F_{calc})^2 / \Sigma (F_{obs})^2]^{1/2}$ after minimization of the function $\Sigma(F_{obs} - F_{calc})^2$ converged to values of 0.01.[5]

Bacteriology

Pseudomonas aeruginosa PA14 strains were maintained as glycerol stocks and stored at -80°C. Fresh colonies were used for each experiment, following growth on either LB agar (HyLabs, Rehovot, Israel) or in LB broth (HyLabs, Rehovot, Israel) at 37°C. Starting bacterial inoculum was $55*10^6$ CFU/mL. Bacteria were inoculated onto stainless steel surfaces (EtOH pre-sterilized with) placed in multi-well polystyrene plate in LB medium for overnight at 37°C. *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922 strains were maintained as glycerol stocks and stored at -80°C. Fresh colonies were used for each experiment, following growth on LB agar (HyLabs, Rehovot, Israel) and incubation for 18–20 h at 37°C. A fresh colony was transferred to the appropriate stainless steel surface (28 mm^2), pre-sterilized with EtOH, using a sterile bacteriologic needle (Quadloop; Miniplast Ein-Shemer, Israel). Starter concentration (on loop) for *E. coli* was 6.9±0.05 and for *Bacillus cereus* was 6.6±0.05, data represented the average and SD of nine independent experiments. Bacteria were evenly spread on the surface using the sphere side of the Quadloop needle, and incubated for 30 min at 25°C. The number of viable bacterial cells associated with the surfaces was measured at time zero, immediately after surface contamination, and at 30 min, as follow: the steel was transferred into a 1.5 ml sterile eppendorf tube containing 900 μm of sterile double-distilled water (SDDW) and vortexed vigorously for 1 min at maximum speed (Mini-Gennie Vortex; Biofan, Latvia). Bacteria were serially diluted (x10) and 0.1 ml portions were plated on LB agar and the number of colony-forming units (CFU) was determined by plate counts after incubation at 37°C for 24 h. The experiments were repeated at least three times on different days and were performed in triplicates. Statistical tests were performed with the program Instat, version 3.0.6 (GraphPad Software, Inc., La Jolla, CA) using Dunn's Multiple Comparisons Test.

Fluorescent Microscopy

For fluorescent microscopy a strain (YC161 with P_{spank}-*gfp*) that produced GFP constitutively[6] obtained from the laboratory collection of Yunrong Chai (Northeastern University, USA) was used. For routine growth the YC161 strain was propagated in Lysogeny broth (LB; 10 g tryptone, 5 g yeast extract, 5 g NaCl per liter) or on solid LB medium supplemented with 1.5% agar. For adherence experiment, bacteria were grown to the stationary phase in LB liquid

medium at 30°C in shaking culture. The cultures (20 µL) were then seeded onto 24-well polystyrene multidishes plate with stainless steel substrates prepared as described above and incubated at room temperature (RT) for 20 min to allow initial adhesion. Afterwards, fresh LB medium (980 µL) was added to each well and the plate was incubated for 3h at 30 °C. To visualize the surface adhered bacteria, the substrates were removed from the wells, washed with PBS buffer, and visualized under an Olympus IX81 confocal laser scanning microscope (CLSM, Japan).

Atomic Force Microscopy (AFM)

Topographic imaging was performed using Innova AFM with a NanoDrive Controller (Bruker, California) operating in the tapping mode, in air, at room temperature. Surface images with a scan rate of 1.0 Hz were acquired at fixed resolution (512 × 512 data points). Bruker 0.01-0.025 Ohm-cm Antimony (n) doped silicon tips (model: RTESPA-CP) were used. The roughness parameter such as the root mean square (R_q) was calculated for scanned area (10 × 10 µm) using NanoScope Analysis software. The AFM images and roughness calculations were obtained for different sample places and the most typical areas are presented.

Contact angle

Contact angles were obtained using goniometer Kruss model FM40 easy drop (Germany) with a drop size of 5 µL of deionized water. The presented values are averages of six measurements reported as advancing angles.

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